TO MY LATE MOTHER

ESAN

With eternal gratitude.

HERPES TYPE-2 VIRUS AND CARCINOMA OF

THE CERVIX UTERI IN NIGERIANS:

IMMUNOVIROLOGICAL STUDIES

BY

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ABSTRACT

In the search for the etiology of carcinoma of the cervix uteri, various factors have been implicated by various workers. The disease has been shown to have a venereal origin, following on its significant association with coital characteristics. These include early initiation into hetero-sexual acts and frequency of coitus, multiplicity of coital partners, multiparity, low socio-economic standards, venereal diseases and circumcision. Extensive epidemiologic studies have indicated that a "venereally transmitted factor" might be responsible for the induction of the squamous cell varity of the malignant disease. Recently, a strain of Herpes Simplex virus, antigenically distinct from the strain commonly associated with oral lesions, and designated Genital Herpes or Herpes Type-2 (HT-2) virus, was shown to be venereally transmitted, and might have oncogenic potentialities on the cervix.

At the time the present studies were contemplated, little was documented about the clinical and histopathologic presentation of Carcinoma of the cervix in Ibadan. There was also no knowlege of the prevalence of Herpes Type-2 virus antibodies in the population, nor of the precise relationship, if any, of the virus to carcinoma of the cervix uteri in Ibadan. It was clear however, as a result of the work of Edington and Maclean (1965) that carcinoma of the cervix uteri is very common in Ibadan, where it was shown to form the commonest female malignancy.

Clinical, cytologic, histopathologic and immuno-virologic studies were undertaken to see if indeed carcinoma of the cervix uteri has a venereal origin, and to ascertain whether or not there is any association between the malignancy and HT-2 virus infection in Ibadan. In addition, sero-epidemiologic studies were also undertaken to determine the prevalence of HT-2 virus antibodies in various sectors of the population.

Evidence was provided to show that coital practice was a significant correlate of carcinoma of the cervix in Ibadan. Furthermore, Immuno-fluorescence and complement fixation tests were two parameters used to provide evidence that Herpes Type-2 virus is associated with the disease, in that carcinoma of the cervix patients possessed signifi-cantly higher levels of antibodies against HT-2 virus as compared with patients having extra-cervical pelvic, and extra-pelvic malignancies and healthy controls.

In addition, HT-2 virus antigens were detected by immunofluorescence tests in the exfoliative cervical cells from all patients with carcinoma of the cervix, whereas no such virus antigens were found in exfoliated cervical cells from healthy controls. In was concluded, that the observed association between HT-2 virus and carcinoma of the cervix, in agreement with other studies, and the fact that the virus was not associated with other extra-cervical malignancies in this environment, indicate a significant relationship. Even though this may not necessarily be an etiologic one, the precise relationship of the virus and the malignancy would have to await further investigation.

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We certify that this work was carried out

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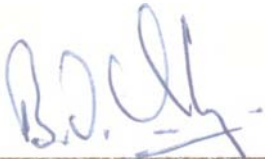
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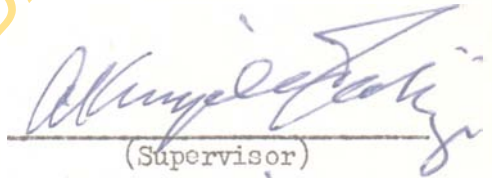
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INTRODUCTION

Cancer is a disease which like any other in the past,
taxed and baffled the skills and ingenuity of Scientists
and Clinicians. Like them it, too, will yield its secrets,
but not without a fight.

                    Victor Anomah Ngu (1964).

In the development of knowledge about the cause of a disease, the
first and most difficult stage is the search for clues on which
to base an hypothesis which can be tested. In this search, no
road can be guaranteed to lead to success. However, one of the
most rewarding is likely to be that which leads to a comparison of
the frequency with which the disease occurs in different communities,
in different areas and at different times. In other words, it is
from the study of the geographic pathology of disease, especially
cancer which relates the incidence and frequency of various tumour
types to the environmental conditions in different areas that
insight might be gained into their etiology. Examples of this
include the frequency of carcinoma of the Scrotum in Chimney
sweepers, and carcinoma of esophagus in South Africans who drink
distilled "illicit" gin.

Attempts to use this approach in Ibadan was first made by
Edington and Maclean (1965). In a cancer rate survey in Ibadan
during a three year period (April 1960 to March 1963), it was found
that carcinoma of the cervix uteri was by far the commonest malignant
tumour seen in women. This finding has recently been confirmed again
by Edington & Hendrickse (1972) who found the Relative Ratio Frequency
(R.R.F.) for this cancer to be 19.9%, being the commonest female
malignancy in Ibadan, excluding the tumours of reticulo-endothelial
system. It thus superceeds in incidence other malignancies such as
carcinoma of the Breast (10.2%), Ovary (6.4%), Trophoblast (6.1%)
and Liver (3.1%).

The reason for the high frequency of cervical cancer is not
clear. Numerous studies have been conducted elsewhere, during the
past two decades, on the social and environmental factors related
to the genesis of carcinoma of the cervix uteri. These factors
include early initiation into sexual life (Terris & Oalman, 1960;
Rotkin, 1967); frequency of intercourse (Rotkin, 1967); early
marriage (Martin, 1967); repeated child bearing with trauma to the
cervix (Martin, 1967); multiplicity of sexual mates (Rotkin, 1967(b));
sociologic customs regarding intercourse (Kennaway, 1948; Kmet
et al, 1963); low socio-economic status and sexual hygeine (Dorn &
Cutler, 1959; Pereyra, 1961); polygamy and unstable marriages
(Wynder et al, 1954); and circumcisional habits in the male
(Kmet et al, 1963).

Indeed, it has become increasingly evident that this cancer

has an epidemiologic pattern very similar to that of venereal diseases
(Martin 1967; Josey et al, 1968; Beral, 1974). Thus, the suspicion
has arisen that carcinoma of the cervix uteri may be totally or
partially dependent on an infective agent which is transmitted at
sexual intercourse (Christopherson & Parker, 1965; Rotkin, 1967;
Terris et al, 1967; Nahmias & Dowdle, 1968; Smith & Jenkins, 1969;
Josey et al, 1972).

    Early marriage is common in Nigeria, especially among the lower
socio-economic groups, and in the Northern and Eastern parts of
the country where child-marriage is a custom. Polygamy is equally
widely practised. In a 3-year rate incidence study utilising the
method of indirect standardisation, Edington & Maclean (1965),
showed that the number of cancers seen in Ibadan was almost 50%
less than that expected in the United States White population, and
only 25% of that expected in the United States non-whites. Yet the
age specific rate incidence of carcinoma of the cervix uteri in
Ibadan (Edington, 1970) is higher than that recorded in the State
of New York, U.S.A. (Handy & Burnette, 1966), has a somewhat similar
incidence to that recorded in Kyadondo, Uganda (Davies et al, 1965;
Davies & Knoweldon, 1966), although it is much less than that recorded
in the South African Bantu (Higginson & Oettle, 1966). In the age
specific rate studies carried out in Ibadan however, the onset of
carcinoma of the cervix was shown not to occur at an earlier age
than in the New York population (Edington, 1970).

    Similarly, in Ibadan, approximately all males are circumcised

in the first few days of life. In the 6-year survey of malignancies
in Ibadan (Edington & Hendrickse, 1970), only 8 penile cancers were
diagnosed in 4503 malignancies, 2266 of which were in males (giving
a R.R.F. of 0.4%). However, the incidence of carcinoma of the cervix
was found to be similar to that recorded in Uganda where less than
10% of males are circumcised, and penile cancer is one of the most
frequent tumours seen (Dodge et al, 1963; Edington, 1970).

The incidence of carcinoma of the penis has been found to be
five times higher in Kyadondo (Uganda) than in the South African
Bantu, whereas the incidence of carcinoma of the cervix was two to
three times higher in the latter group (Dodge et al, 1963). These
would tend to confirm the fact that there is indeed no relationship
between the incidence of carcinoma of the penis and cervix, nor
would the incidence of the latter appear to be related to male circum-
cision at least in Africa, South of the Sahara. It is probable
therefore, that the accepted low incidence of cervical cancer in
Jewish women may not be due to the practice of male circumcision
by that race (Stewart et al, 1966).

Several workers have reported an association between cervical
cancer and genital infections such as Moniliasis (Koss & Wolinska, 1959;
Bertini, 1970). In a survey of the incidence of genital infection
in Uganda, (Trussell et al, 1968), nearly half of their patients
had microscopic evidence of Trichomoniasis, and over 10% showed
evidence of Moniliasis. In their cytologic . screening programme
among gynaecologic, , ante-natal and family planning clinic patients
3.5% of 10,965 patients required biopsy for definitive diagnosis.

In a similar study in the Barbados, (Vaillant et al, 1968), 5.5%
of the patients required cervical biopsy, whereas, only 1.4% of
10,000 patients required this in Britain (McLaren, 1963). In a
similar screening programme in Ibadan (Ojo & Adekunle, 1976), only
0.16% required cone biopsies. Inspite of the low figure from Ibadan
however, carcinoma of the cervix is as common as in Uganda.

Hormonal status of women has been implicated as a possible
etiologic factor in carcinoma of the cervix elsewhere (Williams
et al, 1953: Stern et al, 1970). There is however, no information
regarding this in Nigeria. There are many areas regarding the causa-
tion of carcinoma of the cervix therefore that still need to be
explored in this environment and elsewhere.

Recently, a strain of Herpes Simplex virus, antigenically
distinct from the strain commonly found in association with oral
lesions was isolated from smegma samples and cervical lesions
(Dowdle et al, 1967; Figueroa & Rawls, 1967; Ejercito et al, 1968;
Nahmias & Dowdle, 1968; Rawls et al, 1968; Scheneweis, 1968;
Nahmias et al, 1969(c)). The Herpes virus, designated Herpes
Genitalis or Herpes Type-2 (HT-2) virus was shown to be venereally
transmitted (Barile et al, 1962; Hutfield, 1967; Parker & Banatvala,
1967; Hutfield, 1968; Nahmias & Dowdle, 1968; Rawls et al, 1968;
Nahmias et al, 1968(b); Rawls et al, 1969; Nahmias et al, 1969;
Adelusi et al, 1975(b)).

It was then suggested that the venereally transmitted HT-2
virus may have oncogenic properties regarding carcinoma of the Cervix

(Rawls et al, 1968(b); Josey et al, 1968; Naib et al, 1969; Rawls et al, 1969; Nahmias et al, 1969(b); Nahmias et al, 1970; Nahmias et al (1970(b); Rawls et al, 1970; Melnick & Rawls, 1970; Royston & Aurelian, 1970; Spreecher-Goldberger et al, 1970; Aurelian et al, 1970; Nahmias et al, 1971(b); Catalano & Johnson, 1971; Plummer & Masterson, 1971; Skinner et al, 1971; Adam et al, 1972; Feorino & Palmer, 1973; Ory et al, 1974; Adelusi et al, 1975). However, the problems confronting investigators who have been attempting to prove the role of HT-2 virus in human cervical cancer, are similar to those investigating the role of Epstein-Barr (EB) virus in human cancer, and indeed, any virus infection causing tumours in man. It has not been possible to obtain any direct proof for the role of viruses in causing cancer in man, as most of the evidences so far produced are circumstantial.

Hitherto, there has been no study in Nigeria on the role of HT-2 virus in the etiology of carcinoma of the cervix. The only other work on this topic in Africa, to my knowledge, was that by Adam et al (1972(b)) carried out on Ugandan patients. Antibodies to the oral strain of the virus, Herpes Type-1 (HT-1) virus, was found to occur in 100 per cent of Nigerians (Fabiyi, 1972) but there has been no prevalence studies to date on the Genital strains of the virus, in Ibadan.

In fact, most of the work done on the problem of carcinoma of the cervix generally in Ibadan up to date has been limited (for review, see Hendrickse, 1972). Apart from the works of Edington and

co-workers (Edington & Maclean, 1965; Edington & Hendrickse, 1970;
Edington, 1970; Edington & Hendrickse, 1972) on the frequency of
carcinoma of cervix, the only other published work was the study by
Lawson et al (1964), in which a review of 246 patients was presented.
It was shown in the latter study that only 20% patients presented in
Hospital with clinical Grades 1 or 2 as compared with 60% recorded
in developed countries (Wentz & Reagan, 1959; Wentz, 1961) and that
only 10% were considered likely to survive for 5 years after surgical
treatment. It was also mentioned that radiotherapy was available in
only few centres in Africa, South of the Sahara. Hence, among the
majority of women suffering from carcinoma of the cervix in Africa,
there was little possibility of cure, or even paliative therapy.
There is the need for research which may provide insight into the
cause of the disease in this locality, therefore, to argument the
only available procedure for reducing its incidence, that is, early
diagnosis and treatment.

        This study was initiated therefore to determine the prevalence
of antibody to Herpes Type-2 (HT-2) virus in patients suspected or
diagnosed as having pre-invasive or invasive cervical neoplasm in
Ibadan, and to ascertain whether these women show any difference
in the titre of virus antibody compared with controls. In addition,
the antibody titre of patients with carcinoma of the cervix was
correlated with the grade and stage of the disease, and the histologic
type of tumour. Experimental studies were also undertaken to raise
antibodies against the virus in animals, and current techniques in
immunology and virology were employed to undertake serologic studies.

In addition, a retrospective clinicopathologic review of 594 patients seen over a period of 10 years and a prospective study on another 150 patients and 120 controls over a period of 2 years were undertaken, to define more clearly the features of the malignancy as seen in Ibadan.

CHAPTER 2

REVIEW OF THE LITERATURE

The initiation of cervical cancer by viruses has not been demonstrated directly, although, such possibility of cause and effect relationship has been suggested over a decade ago by morphologic (Ayre, 1960; Dougherty, 1960) and epidemiologic (Louw, 1956; Pereyra, 1961) studies. The search for the cause or causes of cervical cancer has since led to the implication of several non-specific factors. However, in spite of the large body of data available, as of today, the causes and pathogenesis of cervical carcinoma have eluded mankind.

2.1   Coital characteristics and Cervical Cancer

The tendency to limit epidemiologic studies in chronic disease to a few promising variables has been displaced by a growing interest in multidimentional explorations. Investigation of a comprehensive set of variables serves to distinguish those areas where relation-ships are not likely to be found and thereby avoid waste of effort in designing follow up studies. For cancer of the cervix uteri, patterns of sexual practice can hardly be overlooked in a programme designed to investigate causation.

Three epidemiologic findings have been identified as being elucidating in that they stand in a near perfect relationship with

the occurence of cervical cancer (Gagnon (1950); Taylor (1959); Martin (1967); Moghissi et al (1968); Masubuchi & Nemoto (1972);

   (i)   the near-absence of the neoplasm among spieces other than humans;

  (ii)   a near-absence of the disease among Nuns, and

(iii)   a near-absence of the disease among virgins.

These and other observations (Wynder et al, 1954; Boyd & Doll, 1964; Terris & Oalmann, 1960; Stern & Dixion, 1961) have led to the conclusion that cervical cancer is uniquely a human cancer and that coital experience is pre-requisite to the squanous cell form of the disease.

Frequency of Coitus and Cervical Cancer:

Claims have been made that the frequency of intervourse can increase the risk of developing cervical cancer (Wynder et al, 1954; Terris & Oalmann, 1960). These claims have been based upon the notion of trauma to the cervix from direct encounter with the glans penis (Martin, 1967). However, Masters (1960) and Rotkin (1967) do not believe that the mechanical aspects of coitus has any influence upon increased cervical cancer. It is believed that cancer of the cervix is initiated by the introduction of an agent by the penis into the milliou of the cervix rather than by the mechanical effect on the frequency of coitus itself (Wynder et al, 1954; Terris & Oalmann, 1960; Rotkins (1967(b)).

Early Coitus and Cervical Cancer:

Another factor which seems to be important in the relationship

between coitus and cervical cancer is the age at which coitus
starts. Rotkin (1967) has shown that every patient in his study
had experienced coitus with a male at sometime in her life, and this
most often began during early life when the cervical epithelium is
said to be more susceptible to infection (Moghissi et al, 1968). This
view has also been supported by the works of Wynder et al, (1954);
Stocks, (1955); Terris & Oalmann, (1960); Rotkin & King, (1962);
Christopherson & Parker, (1965) and Masubuchi & Nemoto, (1972).

Coitus with multiple partners and cervical cancer:

Apart from frequency and early onset of coitus, a heavy excess
of multiple coital mates has been shown for cervical cancer patients
as compared with controls (Kinsey et al, 1953; Rotkin, 1967(b)).
It was shown that the risk of cervical cancer rises as there were
more marital or sexual mates (Rojel, 1953; Levin et al, 1960;
Greene et al, 1965) since multiple coital mates would increase the
probability of contact with a male who may contribute the presumptive
carcinogenic contaminant to initiate the cancer (Rotkin 1962;
Christopherson & Parker, 1965; Moghissi et al, 1968). This therefore
implies a host-agent-donor hypothesis.

The fact that multiplicity of sexual partners and instability of
marriage is related to the occurence of cervical cancer has also been
shown by the characteristics of groups of women found to have usually
low or high rates of the cancer. It is known that membership of
certain religious sects such as the Amish women (Cross et al, 1968),
Jewish women (Haenszel and Hillhouse, 1959) Protestant and Catholic

women who regularly attend religious services (Naguib et al 1966),
and women of high socio-economic status (Dorn & Cutler, 1959) is
associated with a low risk of the disease. On the other hand, the
high risk is known to be dominant among groups of low socio-economic
status (Dorn & Cutler, 1959), women's prison (Pereyra, 1961), prosti-
tutes (Rojel, 1953), patients attending venereal diseases clinics
(Greene et al, 1965) and urban women as opposed to rural women (Levin
et al, 1960). All these tend to indicate an association between the
distribution of cases of cancer of the cervix and multiplicity of
coital partners across social groups. However, Masubuchi & Nemoto
(1972) could find no difference in the number of coital mates between
their cases and controls.

Early Marriage and Cervical Cancer:

The role of marriage at early age in increased risk of cervical
cancer has been highlighted by Rotkin, (1962) and Martin, (1967).
Various workers have suggested that marriage before the age of 20
years increases the risk of carcinoma of the cervix (Stephenson &
Grace, 1954; Wynder et al, 1954; Terris & Oalmann, 1960; Stern & Dixon,
1961, Boyd & Doll, 1964; Christopherson & Parker, 1965; Figge &
Bennington, 1967).

That marriage per se is the event bearing directly upon the
initiation of carcinoma of the cervix, is probably a wrong notion.
Emphasis on early marriage, rather than on the part played by coitus
in the etiology of carcinoma of the cervix, might have arisen from the
ease with which accurate dates of marriages can be ascertained (Rotkin,

1967). Marriage remains an intersexual agreement leading to an abrupt
change in the life of the female. The pivotal act is coitus, a directly
influential factor not only during marriage, but also before and in
addition to marriage. Hence, it is the view of Rotkin, (1967) that early
marriage is less significant than age at first coitus in its relationship
to cervical cancer, provided coitus is the larger and central event in
the causation of carcinoma of the cervix.

Unstable Marriages and Cervical Cancer:

Studies have been conducted to show that there is a greater incidence
of cervical carcinoma among women who have been married two or more times
compared with those of controls who have been married only once
(Wynder et al, 1954; Terris & Oalmann, 1960; Greene et al, 1965;
Rotkin, 1967). It is known that unstable marriages are associated with
unstable sexual relationships, illegitimacy, venereal infections,
prostitution, and emotional stress, all of which appear in various
studies (Stephenson & Grace, 1954; Rotkin, 1967(b)) as correlates of
cervical cancer. More marriages indicate more contacts with separate
males as do various data for multiple control factors (Rotkin & King,
1962; Moghissi et al, 1968). Earlier age at first coitus for patients
would be related to multiple marriages and coital mates, not only
because additional time is provided, but also because early marriages
tend to be rather unstable, resulting in greater likelihood of dissolu-
tion and more attempts at remarriages. The end result is the same,
that there are more contacts with males (Rotkin, 1967(b)).

Gravidity, Parity and Cervical Cancer:

One of the factors said to contribute to increased cervical cancer risk include multiple pregnancies and deliveries. In Rotkin's studies (1967), data were provided which showed that more controls than test cases were in the class of no gravidity and no parity. However as gravidity and parity increased to the level of five to six, many more test cases were found than in controls. This was explained by the fact that carcinoma developed probably from the earlier age at first coitus for patients, affording an additional mean of some years for patients to conceive and bear more children (Wynder et al, 1954; Stocks, 1955; Rotkin & King, 1962; Boyd & Doll, 1964; Christopherson & Parker, 1965).

Circumcision and Cervical Cancer:

Epidemiologic studies of cancer of the cervix (Weiner et al, 195; Wynder et al, 1954; Pratt-Thomas et al, 1956; Kaiser & Gullian, 1958; Terris & Oalmann, 1960; Terris, 1962; Rotkin, 1967), have suggested that circumcision decreased the occurence of cervical cancer. In a study to evaluate the role of circumcision on the development of cervical cancer, Knet et al, (1963) observed that cases of carcinoma of the cervix were lower in moslem women (in Yugoslavia) whose husbands were circumcised, as compared with non-moslems whose male partners were uncircumcised. This and the known low incidence of cervical cancer among Jewish women and the almost complete absence of penile cancer in Jews, have led to the theory of the possible role of smegma as a carcinogenic agent in cervical cancer (Treusch et al, 1946; Kennaway, 1948; Haenszel & Hillhouse, 1959; Martin, 1967).

In other studies, however, higher incidences of cervical carcinoma

in moslem workers among Jewish women have been described (Versluy,1949; Hock-
man et el 1955) although this was explained by the fact that this could be
a reflection of the less complete circumcision performed in moslem
boys as late as between the fourth and tenth year of life in contrast
to Jewish boys who were circumcised immediately after birth. The sulcus
coronarius subjected to circumcision during the first day of life is
completely free and the prepuse does not exist at all in adults,
whereas in the subjects circumcised later in life, some remnants of
this persists (Kmet et al, 1963).

In a not too recent study (Rotkin, 1967(b)) whereby cancer cases
and controls were studied for exposure only to circumcised coital
mates, a difference was noticed favouring an excess of controls.
However, comparison of circumcision of first mates, though still
favouring the controls, was less significant. Patients and controls
exposed only to circumcised mates and those exposed to uncircumcised
mates were separately tested by age at onset of coitus. The signifi-
cance of differences between patients and controls for age at first
coitus appeared not to have been influenced by circumcision. It was
concluded that the strength of early first coitus as it influences
cervical cancer risk, is independent of circumcision and might be the
major event. Circumcision might be regarded only as a peripheral
supporting agent, providing increased opportunity for the storage of
carcinogenic contaminant under the prepuse.

Contraceptives and Cervical Cancer:

Contraceptives, especially the pill and intrauterine devices
(I.U.D.) have been implicated in the search for the cause or causes

of cervical cancer (McLaren, 1964; Ayre, 1965; Ishihama & Kagabu,
1965; Boyland et al, 1966; Diddle et al, 1966; Pincus & Garcia, 1967;
Richart & Barron, 1967; Melamed et al, 1969; Stern et al, 1970). In a
study designed to elucidate further the role of the contraceptives,
only three areas of difference between the test cases and controls
were noticed (Rotkin, 1967). There was a significantly greater use of
the diaphram, Jelly and the Withdrawal methods among controls than
among cancer cases, whereas more patients than controls used the
douching method. In another study, (Stern et al, 1970), a significant
association of dysplasia was found with the choice of contraceptive
pill as compared with other methods of contraception, although, the
higher prevalence of dysplasia in women chosing the pill was apparently
not attributable on demographic characteristics, since those with
dysplasia were comparable in age, race and socio-economic status to a
state of women without dysplasia.

On theoretical basis, the use of a cervical mechanical barrier
such as a diaphram or condom (Rotkin, 1967) or in the more subtle forms
as present in cervical mucus, of oral steroid users (Odelblad, 1968;
Melamed et al, 1969), should prevent entry of a possible coital
carcinogen, be it in such forms as smegma (Plaut & Kohn-Speyer 1947)
spermatozoa (Coppleson and Reid, 1967) or viral D.N.A. (Rawls et al,
1968(b)). However, it must be remembered that this coital carcinogen
probably gained entry during adolescence, as recent epidemiologic
evidence suggests (Coppleson & Reid, 1967; Moghissi et al, 1968;
Coppleson, 1969).

On the possibility of I.U.D. being a causative agent in carcinoma

of the cervix, Ayre (1965) reported on his patients using I.U.D., in two of whom dysplasia followed the insertion of a Marguile's spiral. Another three showed a pre-existing mild latent dysplasia progressing towards carcinoma in situ following insertion of I.U.D. In like manner Pincus & Garcia (1965.) showed evidence that greater women develop suspicious smears after I.U.D. as compared with the frequency before I.U.D. although the rate was not statistically significantly greater than among oral contraceptive users.

Other studies (Ishihama & Kagabu, 1965; Richart & Barron, 1967) could not report any significant difference between their test cases who had I.U.D. inserted and controls who did not. It is believed that cervical dysplasia may be associated with parity (Christopherson & Parker, 1965; Rotkin, 1967), and I.U.D. use may also be a function of parity. A difference between the incidence of dysplasia in those using I.U.D. and those that do not, may therefore be a result of differences in parity rather than occuring as a result of the use of I.U.D. (Richart & Barron, 1967).

Disagreeing with the theoretical role of contraceptives on the genesis of cervical cancer, Singer and Shearman, (1969) believe that when one looked at the proposed role of the pill on cervical cancer, it would not be surprising that the oral steroid group should have a prevalence rate of cervical dysplasia in excess of the diaphram group, as the former group would most probably be composed predominantly of high risk groups, that is, the lower socio-economic groups.

Racial and Environmental Factors and Cervical Cancer:

Many studies have stressed the part played by racial factors in

the pathogenesis of cervical cancer. These studies pointed to the
uniformly low incidence of the disease among Jewish women (Vinberg,
1906; Horwitz, 1927; Hoffman, 1933; Smith, 1941; Treusch et al, 1946;
Kennaway, 1948; Rothman et al, 1951; Winer et al, 1951; Haenszel &
Hillhouse, 1959; Martin, 1967). Similarly, the incidence of cervical
cancer has been found to be relatively low among Fijis (Handley, 1936),
and Yugoslav moslems (Kmet et al, 1963). In contrast to these however,
cervical cancer has been shown to be common among Negro populations
(Quinland & Cuff, 1940; Hynes, 1948; Robinson, 1951; Dunhan
& Bailer, 1968).

Several of these authors attributed this racial difference in the
incidence of cervical cancer to such factors as genetic immunity
(Maliphant, 1949; Stewart et al, 1966) especially among Jews, some
special hormonal factors (Clemmesen,1952), dietary deficiencies
(Horwitz, 1927; Ayre, 1947), circumcision especially among Jews and
Moslems in Yugoslavia, (Handley, 1936; Kmet et al, 1963), and the ritual
of abstenance (Smith, 1941; Kennaway, 1948; Weiner et al, 1951).

However, in a study of this topic, Wynder et al (1954), believed
that there was very little part, if any, that racial factors contribute
to the development of cervical carcinoma. Rather, the higher rate of
this disease among Negro women was attributed to their earlier ages at
first coitus and re-marriages. And the lower rate of cervical cancer
among Jewish women was found to be qualitatively consistent with their
lower exposure to uncircumcised males and later age at first coitus.

Similarly, Singer and Shearman (1969) have suggested that when one
looked at the very many proposed etiologic factors, that is, early sexual

activity, multiparity, and low socio-economic status associated with
cervical carcinoma, it would be evident that most of these factors pre-
dominate among those races where higher incidences of cervical cancer
are found. Other workers have also shown that within the low income
group, there is no significant racial difference in the prevalence of
cervical cancer (Christopherson & Parker, 1960).

Socio-economic Status and Cervical Cancer:

Kennaway (1948) suggested that there is a social gradient in the
incidence of cervical cancer. Other workers (Lombard & Potter, 1950;
Clemmesen, 1952; Stocks, 1955) have suggested that the low socio-economic
status of a woman has possible etiologic significance on cervical cancer.
In fact, Lombard & Potter (1950) believed that concomitant with low
socio-economic status are such factors as poor obstetrics care, improper
housing facilities, and poor nutrition. Smith (1941) also believes
that poor obstetric care and post partum care, and neglect of symptoms
of the lacerated and ulcerated cervix, account for the greater frequency
of cervical cancer among the poorer classes of women.

However, Clemmensen (1952) has remarked that sexual activity may
be the common denominator among these poor classes to account for the
observed difference in the incidence of carcinoma of the cervix. He
emphasized that the rural areas of Denmark had a relatively lower
incidence of cervical carcinoma, as compared with the low-income city
areas. This point was borne out even more by Vineberg (1919) when he
showed that despite the badly lacerated cervices among the Jewish women
living in the worst possible hygenic surroundings, amidst the greatest
squalor and privation such as obtained in "the lower East side of the

Metropolis", it was remarkable that so few cases of cancer of the cervix were detected among them.

Venereal Diseases and Cervical Cancer:

Several authors have reported an association between cervical cancer and syphilis (Belote, 1931; Harding, 1942; Levin et al, 1942; Rojel, 1953; Meisels, 1969), as well as Trichoniniasis (Bechtold & Reichner, 1952; Koss et al, 1959; Van Nickeik, 1963; Bertini, 1970). Most of these studies have reported twice as many syphilitic patients developing cervical cancer as in the general population.

Of particular interest, however, was the fact that those reporting a past history of syphilis and other venereal disease, also reported, as one might expect, earlier age at first coitus. In fact, the relationship between age at first coitus and prevalence of syphilis as reported, was such as to lead one to expect a two fold difference between the cervical cancer and control patients solely because of their difference in the age at first coitus (Wynder et al, 1954). This study, though fragmentary, was consistent with that of Levin et al (1942) that the statistical association between syphilis and cervical cancer could arise mainly from greater frequency of coitus among this group as compared with the controls. The latest work of Royston & Aurelian (1970) has also shown no statistical association between syphilis or trichomoniasis and cervical cancer.

In a recent epidemiologic survey of the pattern of mortality from cervical malignancy and incidence of sexually transmitted diseases, Beral (1974) postulated that a venerally transmitted factor was responsible for the causation of cervical cancer. This suggestion was

based on the fact that mortality from cervical cancer followed the trends in the incidence of sexually transmitted disease; and that the stability of the pattern observed strongly suggested that cervical cancer follows at a variable interval, exposure to genital infection in early life. He concluded that this sexually transmitted factor may not be any of the known venereal diseases, but rather, " a yet unknown factor"

Other Miscellaneous Factors and Cervical Cancer:

Various other studies have been conducted which showed many factors that have been associated with the genesis of cervical cancer. Among these are various drugs, especially hormones (Pan & Gardner, 1948; Williams et al, 1953), podophyllin (Kaminetzky, 1965), and some chemical substances (Thiery, 1960); spermicides (Boyland et al, 1966), and various immuno-suppressive drugs (Kay et al, 1970; Tallent et al, 1971). Even though most of these studies have shown proof of association of these factors with cervical cancer, there has been no proof of causation between them and the malignancy.

From all the hypothesis of the possible role of exogenous factors mentioned so far in the etiology of cervical cancer, and of the endogenous factors such as hormonal and other biologic factors, only the role of coitus has been in accordance with all available epidemiologic evidence of this disease. Only this could explain the very low incidence of cervical cancer in Nuns in comparison with the high incidence in prostitutes, the low incidence in single than in married women, the low incidence in the upper socio-economic classes where early marriages are less than in the lower socio-economic groups and the known association of the disease with venereally transmitted diseases.

2.2   <u>The search for viral etiology of carcinoma of the Cervix;</u>

Until recently, virus infections were considered responsible for
only a very small proportion of the clinical situation encountered by
Obstetricians and Gynaecologists, the world over. In fact, Cowdry(1944)
believed that while it would be foolish to deny the possibility that
man, like the lower animals, might be occasionally afflicted with cancer
viruses, there was little reason to seriously entertain the view that
viruses may be the etiologic agents of human malignant tumours. His
belief was that the proponents of the virus theory believed in "things
undemonstrable" in the form of a series of inapparent or latent viruses
which only, in exceptional cases, were supposed to reveal their presence
by cancer production. This, he stated was a considerable stretch of
the imagination; for though latent viruses do occur in animals as well
as in human beings, "they usually operate in other ways than in
carcinogenesis".

However, of recent, it has become very apparent that the spectrum
of virus-caused diseases in man may be considerably wider and more subtle
than was hitherto appreciated. Furthermore, the introduction of more
advanced techniques in virology and immunology has led to a better
understanding and definition of the role of viruses in clinical
syndromes whose etiology was previously obscure.

This trend was well illustrated by the increasing recognition of
the part viruses play in genital tract diseases (Slavins & Gavett,
1946; Varga & Browell, 1960; Frost, 1961; Kotcher et al, 1962;
Stern & Longo, 1963; Di Virgilio et al, 1965; Yen et al, 1965;
Christian et al, 1965; Nigogosyan & Mills, 1965; Josey et al, 1966;

Naib et al, 1966; Naib, 1966; Parker & Banatvala, 1967; Kleger et al,
1968). The important role maternal virus infections play in producing
diseases in the fetus and the neonate has also been highlighted (White,
1963; Mitchell & McCall, 1963; Witzleben & Driscoll, 1965; Wheeler &
Huffines, 1965; Stern & Siciliano, 1966; Sieber et al, 1966; Nahmias
et al, 1967; Nahmias et al, 1967(b); Nahmias et al, 1971).

Serologic approaches to the viral etiology of cancer have followed
several theoretical models. In one of these, it was postulated (Lewis
et al, 1965) that an acute virus infection might trigger off a cellular
alteration which subsequently led to cancer, without persistence of the
virus or of the viral antigens in the tumour. Antibody to the virus
would be no more long lasting than in a non-tumourigenic infection,
and would not be expected to be present in an excess number of persons
with the virus-induced tumour. However, antibody might still be present
during the early stages of tumouri-genesis, and epidemiologic studies
of pre-malignant lesions might reveal an increased frequency of antibody
to the initiating cause.

Cervical cancer provided a unique system for the application of
this model. There are well-defined pre-malignant lesions which could
be detected by mass screening procedures, partly because of the ready
assessibility of tissues for study, and partly because of the indications
that a sexually transmissible agent might be responsible for the production
of cervical cancer (Martin, 1967; Rotkin, 1967; Smith & Jenkins, 1969;
Beral, 1974).

The effort to rule out or establish a virus as the transmissible
agent responsible for this cancer has not been an easy task, since the

most direct approach, that of isolating the virus from malignancy, so
successful for animal tumours, was not established for humans until
late (Tokumara & Scott, 1965). Even though this has been simplified
in the past few years, it is still inaccessible to most physicians.

In this search for viral etiology of cervical cancer, it was
suggested (Lewis et al, 1965) that it would be successful if the observed
time disociation of maximal viral growth and tumour response noted in
some animals was applied. Thus, if the search was carried out prior
to the appearance of clinically obvious cervical cancer, in patients
with such lesions as dysplasia and carcinoma in-situ which are apparently
present for months or even years before the development of an invasive
lesion (Mckay et al, 1956; Te-Linde et al, 1957; Stern, 1959; Bamforth
& Gardell, 1962; Green, 1962; Kaninetzky & Swedlow, 1962; Johnson et al,
1964; Jordan et al, 1964; Richart and Barron, 1969; Reagan et al, 1969),
the search might be successful.

Hence, in the use of vaginal cytology in screening women for
evidence of malignancy, cellular changes suggestive of viral infection
had been noted by several observers (Varga & Browell, 1960; Frost, 1961;
Kotcher et al, 1962), and more recently, the correlation to a herpetic
lesion has been confirmed (Stern & Longo, 1963; Nigogosyan & Mills,
1965; Yen et al, 1965).

Extensive epidemiologic studies have indicated that a venerally
transmitted factor is responsible for the induction of squamous cell
carcinoma of the cervix (Terris et al, 1967; Rotkin, 1967; Smith &
Jenkins, 1969). Recently, a strain of Herpes simplex virus, antige-
nically distinct from the Herpes virus associated with oral lesions

(Barile et al, 1962; Dowdle et al, 1967; Figueroa & Rawls, 1967;
Ejercito et al, 1968; Nahmias & Dowdle, 1968; Rawls et al, 1968;
Plummer et al, 1970) and designated Herpes type-2 (HT-2) virus, has
been isolated from smegma samples and cervical lesion. Various studies
have now shown that HT-2 is venereally transmitted (Hutfield, 1966;
Dowdle et al, 1967; Hutfield, 1967; Parker & Banatvala, 1967; Hutfield,
1968; Nahmias et al, 1968; Rawls et al, 1968; Nahmias et al, 1969;
Rawls et al, 1971; Duenas et al, 1972; Adelusi et al, 1975(b)) and
might be an oncogenic virus transmitted venereally.

2.3   Oncogenecity of Herpes Type-2 virus in Laboratory Animals:

Early attempts to induce tumours in hamsters, using herpes virus
Hominis type 1 (Rapp & Falk, 1964; Trenton et al, 1969) were unsuccessful
due to the high mortality associated with virus infection. More recently,
tumours have been produced in animals inoculated with HT-2 virus.
Direct genital infection of mice with the virus produced cervical lesions
identical to atypia and carcinoma in-situ in women (Nahmias et al,
1971(b)) but the small number of animals used in the pilot study
frustrated any firm conclusions about the oncogenecity of the virus.

Intraperitoneal and intrathoracic inoculation of newborn hamsters
with HT-2 virus has been shown to be associated with the development
of undifferentiated sarcomas (Nahmias et al, 1970(b)). The role of
the virus in causing these tumours is uncertain. Virus could not be
recovered from these tumours and no antibodies to the virus were found
in tumour bearing animals. The C-type virus particles found in some
of the tumours posed further problems, since these viruses were known
to be associated with tumours in several vertebrate species (Kawakami
et al, 1972.

More convincing evidence of the oncogenecity of HT-2 virus has been obtained by the transformation of hamster cells in vitro by the virus (Duff & Rapp, 1971). Similarly, inoculation of HVH-transformed cells into hamsters consistently produced tumours, and antibodies to the virus were found in tumour-bearing animals, suggesting that HVH antigens were present in the tumour cells, probably on their surfaces (Poste et al, 1972).

Oncogenecity of other Herpes Viruses:

The ability to produce tumours is probably found in several herpes viruses affecting animals. Marek's disease, a lymphoproliferative disease of chickens, is caused by a herpes virus (Churchill & Biggs, 1967; 1968; Churchill & Biggs, 1968(b)) and immunisation against the virus confers protection against the development of tumours (Churchill et al, 1969). Two recently isolated viruses, Herpes virus Saniari (Melendez et al, 1969; 1971) and Herpes ateles (Melendez, 1972) induce tumours of the lymphoreticular system in a range of subhuman primates. Jaagsiekte, a neoplastic condition of sheep, characterised by primary pulmonary lesions is caused by a Herpes Virus (Smith & Mackay, 1969). An oncogenic herpes virus has also been identified as causing the Lucke renal adeno-carcinoma of frogs (Tweedell, 1967; Mizell et al, 1969; Granoff et al, 1969).

Oncogenic Potential of HT-2 in Man:

The oncogenic potential of Herpes viruses is supported in humans by follow-up studies of relating recurrent herpetic infections to an increased subsequent incidence of squamous cell Carcinoma of the lip (Wyburn-Mason, 1957) and by the consistent identification of Epstein-Barr

(EB) herpes viruses in tumour cells from patients with Burkitt Lymphomas,
and Nasopharyageal carcinomas (Epstein et al, 1964; Epstein & Achong,
1968; Grace, 1971). However, its role as a causal agent in these
conditions is still uncertain (Evans, 1971). Furthermore, the finding
of an increased incidence of cervical atypia and anaplasia before or
after herpetic genital infection (Naib et al, 1966) has led to several
experimental studies on the possible oncogenic potential of Herpes
type-2 virus (Nahmias et al, 1967(c); Nahmias et al, 1968(c)).

It was Naib et al (1966), however, who first noted the hypothesis
of a possible relationship between genital herpetic infection and cervical
carcinoma. In the study, it was noted that some of their patients, who
had cervical biopsy taken one to six weeks after the initial cytologic
diagnosis of Herpes genitalis, showed histologic evidence of acute and
chronic cervicitis, squamous atypia, dysplasia and in-situ carcinoma.
The high incidence of 15% of histologically proven squamous atypia,
dysplasia and in-situ carcinoma in cases where cytologic smears showed
herpetic cellular changes beside the ordinary pre-malignant and malignant
cells, prompted their suggestion that either a pre-existing cervical
atypia invites a secondary viral infection or else, genital herpetic
infections might have oncogenic potentiality.

Subsequent work by this group (Naib, 1966; Josey et al, 1968;
Naib et al, 1969; Nahmias & Dowdle, 1968; Nahmias et al, 1969(b);
Nahmias et al, 1970; Nahmias et al, 1971(b)) and others (Rawls et al,
1968(b); Rawls et al, 1969; Aurelian et al, 1970; Melnick & Rawls, 1970;
Centifanto et al, 1970; Plummer & Masterson, 1971; Rawls et al, 1970;
Rawls et al, 1970(b); Royston & Aurelian, 1970; Spreecher-Goldberger

et al, 1970; Skinner et al, 1971; Catalano & Johnson, 1971; Rawls et al, 1973) have substantiated this association, and raised the probability of a cause and effect relationship.

In all these studies, the method of determining the association between HT-2 virus and cervical carcinoma was by serologically demonstrating significantly higher antibody titres to the virus in women with the disease as compared with control women. However, in view of the known cross-reactivity between antibodies to Herpes type-1 (HT-1) and Herpes Type-2 (HT-2) viruses, various methods (and modification of previous methods) of measurement of antibody to Herpes viruses were devised.   These include, among others, the Complement fixation tests (Smith et al, 1967; Gerber & Rosenblum, 1968), the microneutralisation tests, (Pauls & Dowdle, 1967; Dowdle et al, 1967; Wheeler et al, 1969), neutralisation tests in which a constant number of infectious units of the virus (50 per cent Tissue Culture infective dose - $TCID_{50}$) was mixed with varying concentrations of antiserum (Wheeler et al, 1969) immunofluorescence tests, (Nahmias et al, 1969(c); Royston & Aurelian, 1970(b)), plaque reduction tests (Rawls et al, 1970; Aurelian et al, 1970), the multiplicity analysis tests (Royston & Aurelian, 1970), the micro-indirect hemagglutination tests (Fuccillo et al, 1970; Rowson et al, 1972) and inhibition passive hemagglutination tests (Bernstein & Stewart, 1971).

One fact that still needed to be clearly defined was what part racial predisposition has to play in this association between HT-2 virus and cervical malignancy. It is important to stress that the association between antibodies to the virus and cervical neoplasia has been found

mostly in Negro women from the lower socio-economic groups in the United
States (Rawls et al, 1969; Aurelian et al, 1970; Royston & Aurelian, 1970;
Nahmias et al, 1970). It has been low in Caucasian women from higher
socio-economic groups examined in the United States, Europe and New
Zealand (Melnick & Rawls, 1970; Rawls et al, 1970; Rawls et al, 1970(b);
Spreecher-Goldberger et al, 1970; Centifanto et al, 1971; Plummer &
Masterson, 1971), but not the Negro women of higher socio-economic
status. If the virus infection was a necessary pre-requisite of the
malignancy, the association between antibodies to the virus and cervical
cancer should be found in all population studies, irrespective of race.
However, most of the studies that have been presented to show the
association of HT-2 virus and cervical carcinoma have been carried out
among Negroes of lower socio-economic urban populations.

In most of these studies, a greater frequency of HT-2 virus
antibodies were found among women with cervical carcinoma than among
the controls in Uganda, the United States, New Zealand and Columbia.
This difference was statistically significant only for the groups from
Uganda and United States (Texas Negroes), while among non-Negro popula-
tions, the occurence of antibodies was considerably lower (Rawls et al,
1972). However, when the control women were selected according to age
at first intercourse, age at first marriage, age at first pregnancy,
and number of live births in addition to race, age and socio-economic
status, the differences in the occurence of HT-2 virus antibodies among
cases and controls were greatly reduced in all races (Rawls et al, 1972).

The results of Rawls et al (1972) showed that while there were
significant differences in the occurence of HT-2 virus antibodies in

different segments of the population, there was little or no racial
difference in the incidence of the virus antibodies. Furthermore, the
similar occurence of antibodies among Negro and Mestizo prostitutes
from Cali, Columbia (Duenas et al, 1972) would tend to suggest that the
racial difference noticed in most studies represent socio-cultural
differences in exposure to the virus and that both viral infection and
cervical malignancy are attributes of low socio-economic status.

Interpretation of Results of HT-2 studies:

Three susceptible interpretations have been suggested for the
association between HT-2 virus and cervical neoplasia:

(1)   The first was that the neoplastic change and the virus infection
were independent of each other and were merely joint consequences of
sexual promiscuity (Rawls et al, 1971). Indeed, cervical carcinoma has
been associated with venereal diseases such as Trichomoniasis (Koss & Wolin-
ska 1959; Meisels, 1969; Bertini, & Hornstein 1970) and Syphilis (Rojel,1953;
Meisels, 1969) and is known to be common among promiscuous women such
as prostitutes and prison inmates (Rojel, 1953; Pereyra, 1961; Greene,
1965). In most of these serologic studies, the women were matched for
age, race and socio-economic factors, but not for sexual behaviour.
The link between HT-2 virus infection and cervical cancer could there-
fore be explained by failure to consider a common factor, such as sexual
promiscuity (Rawls et al, 1971).

The cardinal importance of matching for sexual behaviour in surveys
of this type has been well illustrated in a very recent study by Adam
et al (1971). Instead of comparing the incidence of HT-2 virus antibodies
in cervical cancer patients with that in controls merely matched for

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age, race and socio-economic level, every cancer patient was compared
with a control matched for factors relevant to sexual activity; age
at first pregnancy, number of live births, number of marriages and
number of sexual partners, all of which are known to influence the
occurrence of venereally transmitted diseases.

When patients with cervical cancer were compared with these fully
matched controls, there were no significant differences in the incidence
of antibodies to HT-2 virus (Adam et al, 1971). Failure to consider
sexual factors in the analysis produced the same results as those of
previous studies on lower socio-economic groups in which a clear
association was claimed between HT-2 virus infection and cervical cancer
(Rawls et al, 1969; Aurelian et al, 1970; Royston & Aurelian, 1970;
Nahmias et al, 1970).

Furthermore, the observation in patients in whom the cytologic
herpetic changes were noticed either earlier or within a month of
detection of the cervical anaplasia (Naib et al, 1969) was said to
favour the theory of similar factors (e.g. sexual promiscuity) operating
independently to allow both infection with the venerally transmitted
herpes virus and production of cervical aplasia (Rawls et al, 1971).
Others have found that prostitutes have cervical carcinoma at a rate
of 4 to 6 times that of the normal population (Rojel, 1953; Moghissi
et al, 1968 and Pereyra, 1961).

However, evidence has been provided to suggest that the high
incidence of HT-2 virus antibodies in patients with cervical cancer
was not a simple consequence of sexual promiscuity. In a study to
elucidate this problem, Royston & Aurelian (1970) examined the relation-
ship of three sexually transmitted diseases: genital herpes, Trichomonas

and syphilis, in patients with cervical cancer, and were able to
establish a consistent association only in the case of genital herpes.
That the high incidence of HT-2 virus antibodies in patients with
cervical cancer was not related merely to sexual promiscuity was also
suggested by the finding that the incidence of antibodies in prostitutes
and patients attending a venereal diseases clinic, though higher than
that of normal population, was still lower than that in cancer of cervix
patients (Plummer et al, 1968; Rawls et al, 1969; Barron & Richart, 1971;
Skinner et al, 1971; Adelusi et al, 1975(b)).

(2)   The second possible explanation for the high incidence of HT-2 virus
antibodies in patients with cervical cancer was that the virus invades
and grows preferentially in malignant tissues (Rawls et al, 1971).   It
could be that altered cervical epithelium is more susceptible to virus
infection than normal squamous epithelium; in which case, the antibodies
in the women with invasive carcinoma may represent infection acquired
after malignant changes.   Herpes viruses have been isolated at a higher
frequency from women with abnormal cervical cytology (Naib et al, 1966).
The virus has been isolated from a case of carcinoma of the vulva
(Christian et al, 1965), and also from cervical carcinomas artificially
infected with adenoviruses (Smith et al, 1956).

In a recent study (Nahmias et al, 1970), the percentage of patients
with antibodies to HT-2 virus increased progressively with the severity
of lesions, from 56% in cervical dysplasia to 70% in carcinoma in situ
and to 83% in invasive carcinoma.   In another study (Royston & Aurelian,
1970), uniformly high percentage (90%) of patients with both pre-malignant
and malignant lesions possessed antibody to the virus.   These might tend

to suggest that opportunist invasion of virus takes place as much in pre-malignant as in malignant lesions.

These observations, however, did not distinguish between a primary herpes virus infection in the neoplastic tissue and the recrudescence of a latent virus infection acquired before the neoplastic changes occured. Indeed, Ng et al, (1970(b)) has shown one well known property of Herpes virus infection, namely, its tendency to recurrence. Thus, it was quite possible that the herpetic infections detected close to or subsequent to the diagnosis of anaplasia were recurrences of an earlier infection, first contact with the virus having occured several years previously (Naib et al, 1969). Furthermore, Naib et al, (1970) contended that the finding of HT-2 virus antibodies in the acute sera of 20 out of 21 patients with histologically confirmed cervical anaplasia would tend to show this was not their first exposure to the virus infection. These, together with the inability of various studies to demonstrate viral changes in abnormal as contrasted to normal cells when inoculated with viruses (Poste et al, 1972) showed that the theory of preferential growth of virus in malignant tissues may not be true.

(3)   The third possible explanation of the association between cervical cancer and genital herpes was that virus infection precedes the neoplastic change in the cervix, and contributes to the lesion either directly as a carcinogen, or indirectly, as a co-carcinogen (Rawls et al, 1971). If this proposed causal association between HT-2 virus and cervical neoplasia was correct, then virus infection would precede neoplastic change, and the percentage of patient with antibodies to the virus would be identical or very similar in both the pre-malignant and malignant states, as this

is a continuous process (Nahmias et al, 1970; Royston & Aurelian, 1970).
Various clinical (Fox, 1967; Hall & Walton, 1968; Richart & Barron, 1969),
epidemiologic (Johnson et al, 1964; Barron & Richart, 1971; Hulka &
Kupper, 1971) and laboratory studies (Richart, 1967) have provided
substantial evidence to support the view that cervical dysplasia,
carcinoma in-situ and invasive carcinoma represent a single spectrum of
neoplastic change. Although some evidence exists to the contrary,
(Ashley, 1966), numerous reports of clinical progression of dysplasia
and carcinoma in-situ to invasive carcinoma exist.

Investigation has suggested that carefully documented cases of
both pre-invasive and invasive neoplasia of the cervix were associated
with immunologic evidence of prior infection of these women with the
genital strain of Herpes virus, (Royston et al, 1970), whereas, no such
evidence was shown with respect to other venereally transmitted diseases
(Trichomoniasis and Syphilis), nor with respect to the prevalence of
oral strains of Herpes virus. Also, sero-epidemiologic findings of
almost 100% correlation between HT-2 virus infection and the earliest
identifiable pre-invasive stages of cervical carcinoma, suggest that the
infection is related to the induction of neoplastic lesion, rather than
the theory of preferential multiplication of the virus in neoplastic
tissue (Royston & Aurelian, 1970).

In favour of HT-2 virus being capable alone, or in conjunction with
other factors such as steroid hormones (Munoz, 1973) of transforming
normal cervical squamous cells into anaplastic ones were the age curves
which showed that the peak age of HT-2 virus detection was 10-12 years
earlier than that for detection of carcinoma in-situ (Naib et al, 1969),

and over 20 years earlier than the peak age for detection of invasive
carcinoma.

Also consistent were the observations that (a) the follow-up of a
group of women who had cervical biopsies taken 1 to 49 months after
cytologic diagnosis of genital herpes virus infection, showed that
26 (59.1%) of 44 patients had evidence of cervical dysplasia, 3 with
in-situ carcinoma (Naib et al, 1969); (b) women with cytologic evidence
of herpes genitalis infection have been shown to have a 23.7% incidence
of cervical anaplasia consisting primarily of pre-invasive cases in
contrast to a 1.6% incidence among a control group without genital herpes;
(c) herpes simplex virus is capable of producing chromosome aberations
in tissue culture (Hamper & Ellison, 1961), and karyotipic abnormalities
are characteristic features of both pre-invasive and invasive carcinoma
of the cervix (Jones et al, 1967); (d) the oncogenecity of herpes viruses
has recently been established in non-human vertebrates (Mizell et al,
1969; Churchill & Biggs, 1968; and Melendez et al, 1969).

Most evidences presented so far have lent weight to an association
between HT-2 virus and cervical neoplasia. However, in view of the
problems of comparison of control patients in most serologic surveys
completed up to date, evidence on this topic should be treated with the
greatest restraint.

2.4   Dectection of Virus or Viral Components in Neoplastic Cells:

From all the available evidences, the specific relationship between
HT-2 virus infection of the cervix and subsequent neoplastic change is
still subject to debate. At best, the association between these two is
based on circumstantial evidence (Epstein, 1971). More substantial evidence,

including the detection of the virus or viral component in neoplastic
cells, would be needed to establish proof that the virus is an etiologic
factor in cervical cancer. The basis for investigation has been the
concept that the normal cervix contains some antigens (Hollinshead et
al, 1972; Frenkel et al, 1972). The metamorphosis which occurs in
cervical tissue as it progresses from the normal state through a dysplastic
state and carcinoma in-situ to invasive carcinoma may be accompanied by
the acquisition of new antigenic determinants.

Various reports of tumour-specific or tumour-associated antigens
have been described. Abelev (1968) described the alpha-fetoprotein
association with hepatomas; Jehn et al (1970) described an antigen from
malignant melanoma; Gold and Freedman (1965) and Thompson et al (1969)
demonstrated a carcinoembryonic antigen (CEA) of the human digestive
system. Levi et al (1969) and Levi (1971) described antigenecity of ovarian
serous cystadenocarcinoma carried in tissue culture; McNeil et al (1969)
described cross reactivity between the benign ovarian mucin and extracts
of colon cancer; Hellstrom et al (1968) demonstrated antigens present
in human neuroblastoma, and Order et al (1971) described evidence of
a human-associated antigen in Hodgkin's disease. The various techniques
which have been employed to demonstrate tumour antigens include hemagglu-
tination, passive cutaneous anaphylaxis, immunodiffusion in gel,
immunoelectrophoresis, immunofluorescence, immune tolerance and colony
inhibition tests (Gall et al, 1973).

Working along this line, Royston & Aurelian (1970(b), used
immunofluorescence technique to demonstrate HT-2 virus antigens in
exfoliated cervical cells from patients with carcinoma of the cervix.

However, exfoliated cervical cells from patients without carcinoma of
the cervix did not show evidence of these antigens except those diagnosed
clinically as having herpetic cervicitis. This would tend to suggest
that the presence of herpes-related antigen in cervical carcinoma cells
is relatively specific.

Other workers (Aurelian et al, 1971; Aurelian et al, 1973; Aurelian
et al, 1973(b); Feorino & Palmer, 1973 and Gall et al, 1973) have provided
further evidences of the presence of tumour antigens in carcinoma of cervix.
While Gall et al (1973) were able to demonstrate tumour antigens present
in all their carcinoma of cervix patients but not in normal cervix,
the others were able to demonstrate antigens induced or related to HT-2
virus. I$_n$fact, Aurelian et al (1971) were able to isolate from a line
of cervical tumour cells, a virus identical to HT-2 virus with respect
to two biologic properties (plaque morphology and microtubule formation
in infected HEp-2 cells) and immunologic specificity (as determined by
immunofluorescence and neutralisation tests). More recent evidence of
the association between HT-2 virus and carcinoma of the cervix was
provided by the detection of the virus antigens in human cervical cancer
(Chang et al, 1974) by anti-complement immunofluorescence.

The finding of HT-2 virus antigens or HT-2 virus induced or associated
antigens directly in human cervical cancers, including one cancer found
earlier to possess HT-2 virus genome (Frenkel, 1972), provided further
evidence for a close relation between the virus and cervical cancer.
However, these observations did not establish conclusively a causal role
of the virus. The expression of the herpes virus in human tumour cells
might still reflect viral letency in cancer cells produced by some other

factors.

2.5 Herpes Genitalis and Cervical Cancer: Can a causal relationship be proved?

In spite of the various studies which have shown an association between HT-2 virus and cervical carcinoma, no definite cause and effect association has been provided. In the genital herpes - cervical cancer association, there was the necessity to disprove the hypothesis that certain women with increased sexual activity were pre-disposed to the development of both conditions. In order to show a cause and effect relationship between the virus and cervical cancer, Nahmias et al (1972) have suggested the adoption of five criteria of (a) Coherence, (b) Consistency, (c) Strength (d) Specificity and (e) Temporal relationship.

Coherence of Association:

This criterion requires that there must be coherence with known facts in the natural history of virus infections. The hypothesis of cause and effect association would gain coherence if it were found that the herpes virus infecting the female genital tract was different from that infecting other body sites. It has been found that the differences between genital and non-genital strains are marked (Dowdle et al, 1967; Nahmias & Dowdle, 1968; Nahmias et al, 1971(b)).

Also crucial to the coherence of the association was the need to establish that genital herpes affected the cervix, since it had generally been thought that the virus affected only the external genitalia. However, not only has it been shown that the cervix is a common site for HT-2 virus infection, it was found to be the most common site (Josey et al, 1968; Nahmias et al,1971(b)).It was also necessary to show

that the epidemiologic characteristics of the virus were similar to
those of cervical cancer. Here again, the prevalence of cervical cancer
and HT-2 virus have been shown to be the same in various studies, and
both have been shown to have the epidemiologic characteristics of a
venereal diseases (Josey et al, 1968; Nahmias et al, 1969). Results of
various other studies (McKenna et al, 1962; Watkins, 1964; Nahmias et al,
1970; Royston & Aurelian, 1970(b); Nahmias et al, 1971(b) Munoz 1973;
Nahmias et al, 1971(c); Rapp & Duff, 1971; Hollinhead, 1972) have provided
further support for the coherence of the association. However it should
be noted that even if some antigens or nucleic acids were found to be
shared by cervical cancer cells and HT-2 virus, the question of a non-
causal hypothesis, that is, that the antigens or nucleic acid originate
from viruses that were latent in the cancer cells, would still be there.

Consistency of Association:

So far, results of various retrospective studies (Rawls et al, 1969;
Nahmias et al, 1970; Royston & Aurelian, 1970; Spreecher-Goldberger
et al, 1970; Nahmias et al, 1971(b); Catalano and Johnson, 1971; Wildy, 1972)
have demonstrated consistency in the association between cervical neoplasia
and genital herpes virus. These studies have used various serologic tests
to detect HT-2 virus antibodies in women with cancer and controls.

Strength of Association:

In the retrospective and prospective studies that have shown a
consistent association, the ratio of cervical neoplasia in women with
genital herpes, as compared to those without, has varied with the presence
of dysplasia, or carcinoma in-situ or invasive carcinoma. The possibility
of a dose effect was considered, and a higher frequency of cervical cancer

was noted in the recurrent than in primary herpetic infections (Nahmias
et al, 1972). This would tend to suggest that transformation may occur
during a recurrence as well, and that it may take longer for the
development of neoplasia after a primary than a recurrent HT-2 virus
infection.

Specificity of Association:

The differences among pathologists in their histologic interpreta-
tion of what constitutes cervical displasia or in-situ carcinoma, increase
the problems involved in assessing specificity. A similar problem exists
with regard to attempts to demonstrate the specificity of the association
in connection with the serologic procedures used to detect HT-2 virus
antibodies. The reproductibility of any of the serologic tests in use
to ascertain virus antibodies, when done within the same laboratory is
no better than 80-90%, while some results obtained by some of these tests
have differed by as much as 50% when comparative serum samples were
submitted to different laboratories (Nahmias et al, 1972).

Associated causative factors might play a role in permiting cervical
neoplasia to develop after HT-2 virus infection. There appears to be an
increased incidence of cervical neoplasia when Herpes virus was detected
during pregnancy or the puerperium (Nahmias et al, 1972). In a small
series of women, for example, who developed in-situ cervical cancer prior
to 20 years of age, pregnancy in association with virus infection, appeared
to be connected with such an early development of cervical neoplasia
(Nahmias et al, 1972).

Temporal relationship of association:

The median age for genital herpes, detected by cytologic or virologic

techniques, has been found to be 5 to 30 years earlier than that for cervical dysplasia, in-situ and invasive cancer (Naib et al, 1969; Nahmias et al, 1969). Similar conclusions have also been reached by analysis of serologic data on the incidence of acquisition of HT-2 virus antibodies by age (Rawls et al, 1970)

Constitutional Hypothesis:

Increased promiscuity of women has been found to correlate with both cervical cancer and genital herpes. The question as to whether the association between the virus and cervical cancer was the consequence of such promiscuity has been raised (Rawls et al, 1971). In a study in which several factors, including those related to sexual behaviour, attendance at venereal diseases clinics and frequency of syphilis anti- bodies, were considered (Nahmias et al, 1972), it was found that control groups and patients with cervical neoplasia differed with respect only to two factors: frequency of HT-2 virus antibodies and age at first pregnancy. Similarly, Royston & Aurelian (1970) have found a greater frequency of virus antibodies in women with cancer than in controls, whereas, two indices of promiscuity: presence of Trichomonas and of syphilis antibodies, were similar to both control and cancer groups. Rawls et al (1969), in an earlier study, have also reported that the frequency of antibodies in women with invasive cervical cancer was higher than that found among prostitutes.

On the whole, the present concept is that HT-2 virus is most likely to be oncogenic, probably in association with some other factors, such as pregnancy. Coppleson (1969) suggested that benign metaplasia of the cervix, which occurs most frequently during the first pregnancy, was a

pre-requisite for the possible neoplastic transformation of cervical
cells under the influence of a venereally transmitted carcinogen, possibly
a virus. This theory might be particularly attractive, since it might
help to explain the low frequency of penile cancer among the mates of
the women with cervical cancer and Herpetic cervicitis and in the fact
of a relatively high frequency of genital herpes in males.

From all studies thus far, it is believed that, based on the five
criteria above (and as have been used to establish a causal association
between cigarette smoking and long cancer), the herpes genitalis -
cervical cancer hypothesis stands up quite well to these criteria,
enough, at least, to warrant the need for further studies.

CHAPTER 3

CLINICAL CORRELATES OF CARCINOMA OF THE CERVIX UTERI IN IBADAN

3.1 Introduction

The search for the cause or causes of cancer of the cervix uteri
has led to the implication of several non-specific factors. In various
epidemiologic studies of the disease, it has been shown that there is a
social gradient (Kennaway, 1948), the disease being commoner in married
than single girls (Martin, 1967; Rotkin, 1967); and much lower in
Jewish women (Haenszel & Hillhouse, 1959; Martin, 1967) and Yugoslav
women (Knet et al, 1963) as compared with women of other races. Such
differences have led to the suggestion that environmental and probably
genetic factors are of importance in the genesis of the disease.

The tendency to limit epidemiologic studies in any disease to a
few variables has given ground to research designs which investigate
multiple variables, and that has proved to be effective. Multivariate
methods have the advantage of determining whether a maximum number of
presumably different variables, which successfully separate patients
with cancer, for example, from controls, are independently meaningful,
or whether they are tapping different aspects of a larger domain of
influence. In cancer of the cervix uteri, patterns of sexual practice
can hardly be ignored in a programme regarding causation.

Three correlates have been shown to be of primary importance in

their relationship to cervical cancer incidence. It is known for example that the disease is uncommon in species other than man. It is very rare among Nuns (Gagnon, 1950; Taylor, 1959). It is also uncommon among virgins (Martin, 1961; Rotkin, 1967; Moghissi et al, 1968; Masubuchi & Nemoto, 1972). These and observations by other workers (Wynder et al, 1954; Terris & Oalmann, 1960; Stern & Dixon, 1961; Boyd & Doll, 1964; Christopherson & Parker, 1965; Lundin & Erickson, 1965; Harold et al, 1968) have led to the conclusion that cancer of cervix is uniquely a human cancer, and that coital experience is a pre-requisite to its causation.

Various aspects of the coital factor have been implicated in the causation of cervical cancer. Early marriage and early onset of coitus (Clemmensen, 1952; Wynder et al, 1954; Cummins, 1960; Rotkin & King, 1962; Christopherson & Parker, 1965; Figge & Bennington, 1967; Masubuchi & Nemoto, 1972) and multiparity (Wynder et al, 1954; Stocks, 1955; Rotkin & King, 1962; Boyd & Doll, 1964; Christopherson & Parker, 1965) have been implicated in the high incidence of cervical carcinoma. Intercourse at an early age, especially at puberty, was said to be significant in the occurence of cervical carcinoma (Jones et al, 1958; Christopherson & Parker, 1965; Masubuchi & Nemoto, 1972) since it was postulated (Moghissi et al, 1968) that biologically immature sexual organs are said to be highly sensitive to carcinogenic factor(s) that invades these organs through sexual intercourse.

Apart from partner heterosexual activities, an excess of multiple coital mates has been shown to be significant in cervical cancer patients as compared with controls (Kinsey et al, 1953; Rotkin, 1967(b)).

The risk of cervical cancer is said to rise with increase in marital and sexual mates (Rojel, 1953; Levin et al, 1960; Greene et al, 1965). It was postulated that sexual contact with a number of male partners would increase the chance of coming into contact with men who have carcinogenic factors (Rotkin & King, 1962; Christopherson & Parker, 1965; Moghissi et al, 1968). Others (Masubuchi & Nemoto, 1972) disagree with this view, however, as no difference in the number of coital mates could be found between cases and controls.

In the past, male circumcision was thought to decrease the occurrence of cervical cancer (Plaut & Kohn-Speyer, 1947; Kennaway, 1948; Weiner et al, 1951; Wynder et al, 1954; Pratt-Thomas et al, 1956; Kmet et al, 1963). This view has fallen into disrepute however (Jones et al, 1958; Dunn & Buell, 1959; Wynder & Licklider, 1960; Rotkin & King, 1962). Animal experiments have also shown that smegma does not give rise to cervical cancer (Fisherman et al, 1942; Reddy & Baruah, 1963). Similarly, comparison of circumcision of first mates (Rotkin, 1967(b)) did not show any significant difference in favour of control cases.

Various studies have tended to indicate a higher prevalence of cervical cancer in the lower socio-economic groups of any population (Kennaway, 1948; Lombard & Potter, 1950; Stocks, 1955). Other workers (Clemmensen, 1952) believed that sexual activity might be the common denominator among these poor classes and might account for any observable difference in the incidence of cervical cancer between them and the higher socio-economic classes

The part played by racial factor in the causation of cervical cancer

has been over-emphasised. Most of these studies (Horwitz, 1927; Treusch et al, 1946; Kennaway, 1948; Weiner et al, 1951; Haenszel & Hillhouse, 1959; Kmet et al, 1963; Martin, 1967) have shown a uniformly low incidence of cervical cancer among the Jewish and Yugoslav muslim women in comparison to the high incidence amongst Negroes (Robinson, 1951; Day, 1961; Dunham & Bailer, 1968). The low incidence of the disease amongst Jewish women was considered to be due to the custom of abstaining from sexual intercourse for 80 days after delivery and the days before and after menstruation. This protected the cervix against cancer since after delivery and menstruation, the squamo-columnar junction is very active and thus, easily affected by external stimulation especially a carcinogen (Kennaway, 1948; Weiner et al, 1951). Others (Maliphant, 1949; Stewart et al, 1966) believed that the low incidence of the disease among the Jewish women was due to hereditory resistance. However, Wynder et al (1954) concluded, from an extensive study, that there was little or no part played by racial factors in the genesis of cervical cancer, but rather, that the differences in favour of Negro women was attributable to their earlier age at first intercourse and marriage.

The various other factors implicated in the genesis of cervical carcinoma include the use or non-use of contraceptives (Pincus & Garcia 1967; Melamed et al, 1969; Stern et al, 1970), venereal diseases, especially syphilis (Rojel, 1953; Meisels, 1969) and moniliasis (Koss et al, 1959; Bertini & Hornstein, 1970) and chemical compounds (Thiery 1960 Kaminetzki & Swedlow, 1965). Opinion, however, differs on these assertions. For example, Singer & Shearman (1969) believe there is very little or

no association between cervical cancer and oral contraceptives, while Royston & Aurelian (1970) could not show any association between cervical cancer and venereal diseases such as syphilis and trichomoniasis.

From the above the only one factor that has been consistent with higher prevalence of cervical cancer is coitus. This can explain the low incidence of the disease in nuns and virgins as opposed to prostitutes, the low incidence in single women as compared with married ones, and the low incidence in upper socio-economic classes (where marriages are few and much later) as compared with lower socio-economic classes.

The clinical part of the present study was designed to determine the frequency of carcinoma of the cervix uteri among the various tribal groups of Nigerians seen at the University College Hospital (U.C.H.) Ibadan, with particular reference to the part played by their coital habits, socio-economic level and parity. In addition, the age distribution, clinical features and laboratory investigations carried out on these cases were evaluated. Findings in cancer patients were compared with those of controls.

3.2 Materials and Methods

3.2.1 <u>Study Groups</u>: The study groups were made up of the following:

(a) One hundred and fourteen women between the ages of 18 and over 65 years who were seen at the Department of Gynaecology of the University College Hospital (U.C.H.), Ibadan, during the period of October 1972 and September, 1974, with a histologic diagnosis of invasive carcinoma of the cervix uteri.

(b) Thirty six women seen in the same department and over the

same period with histologic diagnosis of cervicitis,

cervical erosion, or vaginal warts (condylomata acuminata).

(c) As controls, one hundred and six healthy women of child bearing
age attending the Family Planning Clinic of the Hospital over
the same period, were chosen. Groups (a), (b) and (c) consti-
tuted the prospective study groups.

(d) Five hundred and ninety-four (594) cases of carcinoma of the
cervix uteri, seen over the period 1962 to 1972 were included
in a retrospective study. These included patients seen and
diagnosed in U.C.H. and biopsy specimens sent into the
Hospital from outside sources.

3.2.2 Survey Method: Questionaires were prepared (specimen attached
as appendix) and patients and controls were personally interviewed
as to their age, place of origin and religion. Their socio-
economic levels were ascertained on the basis of their level
of education, occupation and the family income. Details of
their coital practice were assessed regarding their marital
status, number of marriages, age at first coitus, frequency
of coitus, number of coital partners and the circumcision or
otherwise of coital partners. The number of pregnancies as
well as the number of living children, were also ascertained.
In addition, the clinical features and the laboratory investi-
gations carried out were assessed.

3.2.3 Serum Samples: From each subject in the study groups, 10 mls.
of blood was obtained by venupuncture. After allowing this
to clot at room temperature ($25^{o}C$), serum (about 5 mls) was

separated by centrifugation at 200 rpm, for 5 minutes. The
serum samples were then stored frozen at $-20^{o}C$ until required
for use.

3.3 Results

3.3.1   Retrospective Clinical Studies

The age distribution of patients diagnosed histologically as
carcinoma of the cervix in Ibadan between 1962 and 1972, is shown in
Fig. 3.1. There were only 16 cases above the age of 65 years, and
fewer cases still below the age of 25 years. The peak age incidence was
in the 36-45 year age group and the mean age was 45.0 years (Table 3.1).
Table 3.2 shows the distribution of patients according to their places
of origin. Of the total 594 patients, 381 (64.1%) of them were from
the Yoruba speaking areas, the rest occuring in other ethnic groups.

Table 3.3 shows the marital status of the women under study. There
was no woman who was not married at one time or the other. 469 (79.0%)
were still married at the time of diagnosis while 91 (15.3%) were
widowed. The others were either separated or divorced. The obstetric
history of the women (Table 3.4) showed that a high percentage of the
patients were multiparous, with only 40 (6.7%) of them having had one
or no child, and 165 (27.8%) of them having had eight or more children.

Abnormal vaginal bleeding was the commonest symptom. This varied
from post-coital and intra-menstrual bleeding to post-menopausal bleeding
(Table 3.5). Others include foul smelling vaginal discharge, urinary
symptoms such as dysuria, frequency and incontinence. Pelvic pain and
weight loss were also common. When the results were analysed on the
basis of clinical stage of the disease, it was found that the majority

Figure 3.1
Age distribution in women with Carcinoma of

Cervix (1962-1972)

Age distribution in women with Carcinoma
of Cervix in The Retrospective Studies.
(1962 – 1972)
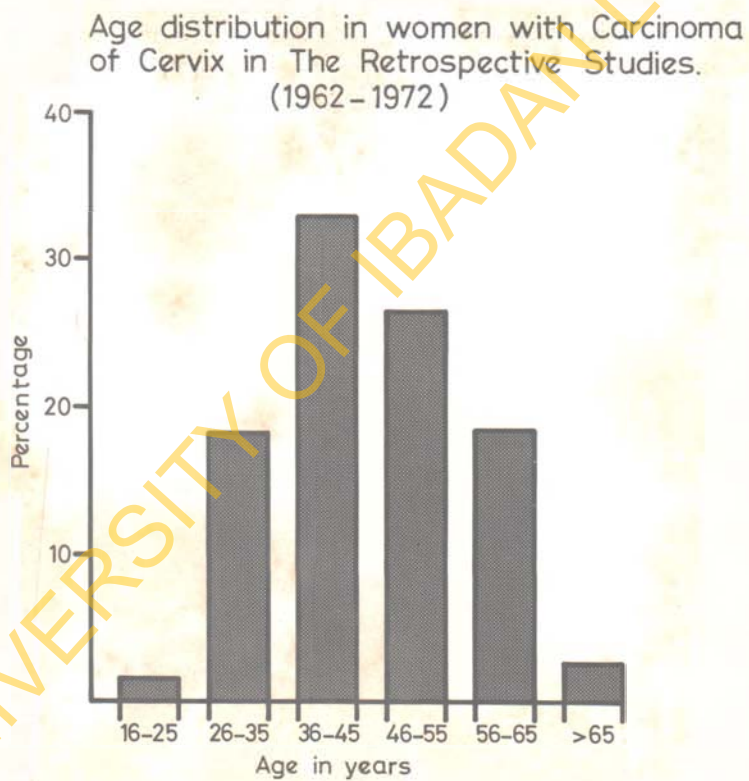
TABLE 3.1

Age incidence of women with Carcinoma of the
cervix uteri in Ibadan (1962-1972)

Age (Years)	No.	%
16 - 25	10	1.7
26 - 35	108	18.2
36 - 45	194	32.7
46 - 55	157	26.4
56 - 65	109	18.3
> 65	16	2.7
Total	594	100.0

Mean Age = 45.0 years

TABLE 3.2

Place of origin of women with Carcinoma of
the cervix uteri in Ibadan: (1962-1972)

Place of Origin	No.	%
Yoruba	381	64.1
Ibo	52	8.8
Hausa	46	7.7
Edo	43	7.2
Itsekiri	26	4.4
Efiks	20	3.4
Others	26	4.4
Total	594	100.0

TABLE 3.3

Marrital status of women with Carcinoma of
the cervix in Ibadan: (1962-1972)

Marital Status	No.	%
Single	0	0.0
Married	469	79.0
Separated	25	4.2
Divorced	9	1.5
Widowed	91	15.3
Total	594	100.0

TABLE 3.4

Obstetric history of women with Carcinoma
of the cervix uteri in Ibadan: (1962-1972)

Parity	No.	%
0 - 1	40	6.7
2 - 3	112	18.8
4 - 5	143	24.1
6 - 7	134	22.6
8+	165	27.8
Total	594	100.0

TABLE 3.5

Clinical presentation among women with Carcinoma
of cervix uteri in Ibadan: (1962-1972)

Symptoms	No.	%
Post-menopausal bleeding	276	46.5
Vaginal discharge	213	35.9
Weight loss	195	32.8
Pelvic pain	190	32.0
Inter-menstrual bleeding	133	22.4
Constipation	89	15.0
Dysuria	68	11.5
Post-coital bleeding	64	10.8
Hanaturia	45	7.6
Frequency of Micturition	40	6.7
Urinary incontinence	30	5.1
Peripheral edema	30	5.1
Diarrhea	11	1.9
Fecal incontinence	1	0.2

of the patients were seen in the latter stages of the disease (Table 3.6).
While only 62 (10.4%) of the women were seen at Stage 1 of the disease,
371 (62.5%) were seen in Stages 3 and 4.

When the clinico-pathologic Stage was analysed on the basis of
parity of the women (Table 3.7), there were many of the multiparous
women whose cervical growths had advanced to the later stages. However, the
there was no significant association (P $\geq$ 0.05) between parity and clinico-
pathologic stage. Table 3.8 shows the correlation between the clinico-
pathologic stage of disease and bladder involvement on cystoscopy. For
one reason or another, only 333 (56.1%) of the patients had cystoscopy
performed. A significant (P $<$ 0.001) majority of the advanced cases
(Stages 3 and 4) had bladder involvement. However, there was incorrect
staging of disease in some instances, where 8 cases with bladder involve-
ment were clinically staged as 1 or 2.

On histopathologic examination (Table 3.9), 491 (82.7%) of the
patients had well differentiated squamous cell carcinoma and only
27 (4.5%) had adeno-carcinoma of the cervix. In 76 (12.8%) of the
patients, the carcinoma was undifferentiated.

3.3.2 Prospective Clinical Studies:

The age incidence in cases diagnosed histologically as carcinoma
of the cervix and cervicitis, as well as the healthy controls is shown
in Fig. 3.2. The ages ranged from 16 to over 65 years for the
carcinoma group; the peak age incidence was 36-45 years and the mean
age was 46.1 years. For the healthy controls, the ages ranged from
16 to 55 years; the peak age incidence was 35-45 years and the mean
age was 36.1 years. The carcinoma of cervix cases were significantly

TABLE 3.6

Clinico-pathologic stage of disease in women with
Carcinoma of the cervix uteri in Ibadan: (1962-1972

Stage	No.	%
1(a)	0	0.0
1(b)	62	10.4
2(a)	72	12.1
2(b)	89	15.0
3	208	35.0
4	163	27.5
Total	594	100.0

TABLE 3.7

Clinico-pathologic stage of Carcinoma of cervix
uteri in Ibadan in relation to parity: (1962-1972)

Parity

Stage	0 - 1	2 - 3	4 - 5	6 - 7	8+	Total
1(a)	0	0	0	0	0	0
1(b)	5	13	13	13	18	62
2(a)	6	11	15	20	20	72
2(b)	4	15	26	19	25	89
3	11	48	48	43	58	208
4	14	25	41	39	44	163
Total	40	112	143	134	165	594

Parity Vs Clinico-pathologic stage:

$$X^2 = 9.68 \text{ on } 16 \text{ df} \quad P > 0.05$$

TABLE 3.8

Clinico-pathologic stage of Carcinoma of cervix in
Ibadan in relation to cystoscopic findings: (1962-1972)

Stage	Bladder free	Bladder involved	Total
1(b)	59	1	60
2(a)	71	1	72
2(b)	83	6	89
3	69	28	97
4	4	11	15
Total	286	47	333

Bladder involvement Vs Clinico-pathologic stage:

$$X^2 = 82.09 \text{ on } 4 \text{ df} \quad P < 0.001$$

TABLE 3.9

Histo-pathologic classification of cases of Carcinoma
of the cervix uteri in Ibadan: (1962-1972)

Histopathology	No.	%
Squamous Cell Carcinoma	491	82.7
Undifferentiated Carcinoma	76	12.8
Adeno-Carcinoma	27	4.5
Total	594	100.0

Figure 3.2

Age distribution in women with carcinoma of
Cervix, cervicitis and Healthy controls.

Age distribution in women with Carcinoma of
Cervix, Cervicitis and Healthy controls.
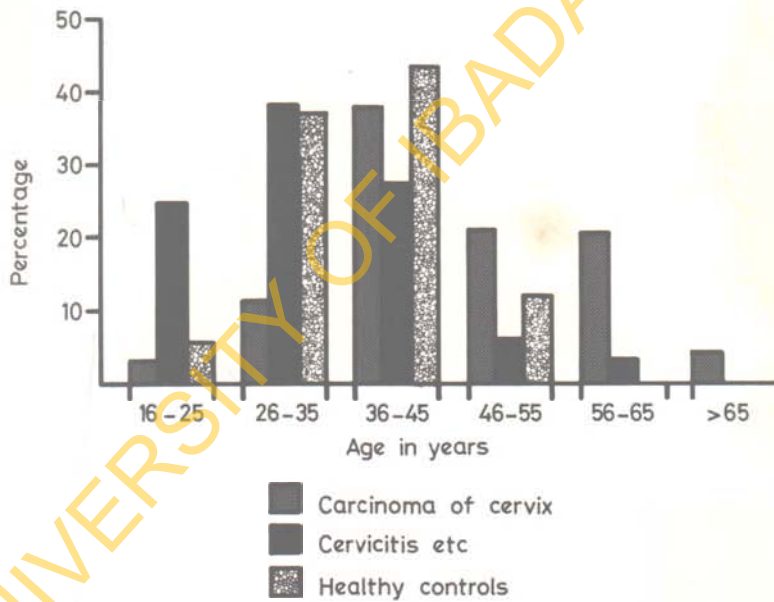
older (P<0.001) than the control cases (Table 3.10).

Table 3.11 shows places of origin of the patients with carcinoma of the cervix and cervicitis. 96 (64.0%) of all the patients were from the Yoruba speaking area of the country while the rest came from other areas. Distribution by place of origin for the carcinoma cases was not significantly (P>0.1) different from those of the cervicitis.

The religious denomination of the various groups of women under study is shown in Table 3.12. There was no association between type of cervical lesion and religion as the distribution of the women on the basis of religion showed no significant (P>0.25) difference in the two groups of women with carcinoma or cervicitis on one hand, and healthy controls.

Analysis according to the socio-economic classes of the various women under study showed that there was little difference in the distribution of the women as the majority in the three groups belong to the lower socio-economic class (Table 3.13). However, while only 3 (2.6%) of the women with carcinoma of cervix belong to the upper socio-economic class, 10 (9.4%) of the healthy control women belong to this class. On the other hand, a higher number (53.5%) of the carcinoma group belong to the lower socio-economic class compared with the healthy controls (45.3%). These differences are however not statistically significant (P>0.05).

Table 3.14 shows the marital status of the groups of women under study. There were 2 (1.9%) single women among the healthy controls, but none among the women with carcinoma of cervix. 84 (73.6%) of the carcinoma group were married at the time of diagnosis while 26 (22.8%)

TABLE 3.10

Age of women with Carcinoma of the cervix,
Cervicitis and Healthy Controls.

Age (Years)	Study groups			
	Carcinoma cervix	Cervicitis	Controls	Total
16 - 25	3	9	7	19
26 - 35	13	14	40	67
36 - 45	44	10	46	100
46 - 55	25	2	13	40
56 - 65	24	1	0	25
> 65	5	0	0	5
Total	114	36	106	256

Study Groups Vs Age (Years):

$$X^2 = 76.69 \text{ on } 10 \text{ df} \quad P < 0.001$$

Mean age (Carcinoma)   = 46.1 years

Mean age (Cervicitis) = 32.2 years

Mean age (Controls)   = 36.1 years

Overall Mean age   = 40.0 years

TABLE 3.11

Place of origin of patients with Carcinoma of the
cervix and Cervicitis in Ibadan.

| Place of Origin | Study groups | | Total | |
	Carcinoma Cervix	Cervicitis	No.	%
Yoruba	67	29	96	64.0
Ibo	12	4	16	10.7
Hausa	9	0	9	6.0
Edo	12	2	14	9.3
Itsekiri	4	1	5	3.3
Efik	3	0	3	2.0
Others	7	0	7	4.7
Total	114	36	150	100.0

Study Groups Vs Place of Origin:

$$X^2 = 8.81 \text{ on } 6 \text{ df } P > 0.1$$

TABLE 3.12

The Religious denomination of women with

Carcinoma of cervix, Cervicitis and Healthy controls

Religion	Study groups							
	Carcinoma Cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
Muslim	44	38.6	15	41.7	42	39.6	101	39.5
Protestant	40	35.1	16	44.4	46	43.4	102	39.8
Catholic	24	21.1	4	11.1	17	16.1	45	17.6
Traditional	5	4.4	1	2.8	0	0.0	6	2.3
Others	1	0.8	0	0.0	2	0.9	1	0.8
Total	114	100.0	36	100.0	106	100.0	256	100.0

Study Groups Vs Religion:

$$x^2 = 7.90 \text{ on } 8 \text{ df } P > 0.25$$

TABLE 3.13

Socio-economic classification of women with Carcinoma
of cervix, Cervicitis and Healthy Controls.

Class	Carcinoma cervix No.	Carcinoma cervix %	Cervicitis No.	Cervicitis %	Controls No.	Controls %	Total No.	Total %
			Study groups					
Upper Class	3	2.6	5	13.9	10	9.4	18	7.0
Middle Upper	19	16.7	4	11.1	14	13.2	37	14.4
Middle Lower	31	27.2	6	16.7	34	32.1	71	27.7
Lower Class	61	53.5	21	58.3	48	45.3	120	46.9
Total	114	100.0	36	100.0	106	100.0	256	100.0

Study Groups Vs Socio-economic class:

$$X^2 = 10.72 \text{ on } 6 \text{ df} \quad P > 0.05$$

TABLE 3.14

Marital status in women with Carcinoma
of cervix, Cervicitis and Healthy controls.

| Marital status | Study groups | | | | | | | |
| | Carcinoma cervix | | Cervicitis | | Controls | | Total | |
	No.	%	No.	%	No.	%	No.	%
Single	0	0.0	2	5.5	2	1.9	4	1.5
Married	84	73.6	33	91.7	100	94.4	217	84.9
Separated	2	1.8	1	2.8	1	0.9	4	1.6
Divorced	2	1.8	0	0.0	2	1.9	4	1.6
Widowed	26	22.8	0	0.0	1	0.9	27	10.5
Total	114	100.0	36	100.0	106	100.0	256	100.0

Study Groups Vs Marital Status:

$$X^2 = 39.13 \text{ on } 8 \text{ df } P < 0.001$$

were widowed. However, the percentage with husbands (at the time of study) among the carcinoma cases was significantly lower than among the other two groups (P< 0.001).

The majority of the women with carcinoma of cervix and healthy controls had been married only once (Table 3.15). While 40 (35.1%) of the women with carcinoma had had 2 or more marriages, only 20 (18.9%) of the healthy controls had been married twice or more. This difference is statistically significant (0.05 > P > 0.025). Table 3.16 shows the number of other wives of husbands of women with carcinoma of cervix, cervicitis and healthy controls. There was no significant (P > 0.05) difference between the carcinoma group and the healthy controls.

Analysis of age at first coitus among the various study groups (Table 3.17) showed that a significantly (P< 0.001) greater percentage of the carcinoma of the cervix group (31.6%) were known to have commenced heterosexual activity in early life (11-15 years) as compared with the healthy controls (10.4%). Table 3.18 shows the number of sexual partners among the various study groups. For personal reasons, it was not possible to obtain answers from 35 women. Analysis of the answers obtained showed that there was no significant (P > 0.25) difference in the distribution of numbers of sexual partners among the three groups. However, while 6 (6.1%) of the carcinoma group had 4 or more sexual partners, only 1 (1.9%) of the healthy control group had 4 or more sexual partners.

Table 3.19 shows that frequency of coitus was significantly (0.025 > P > 0.01) associated with groups in that a higher proportion of the control group had coitus less frequently than the other two groups.

TABLE 3.15

Number of marriages among women with Carcinoma of
cervix, Cervicitis and Healthy controls.

No. of marriages	Study groups							
	Carcinoma cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
1	74	64.9	26	72.2	86	81.2	186	72.7
2	33	28.9	7	19.4	19	17.9	59	23.0
3+	7	6.2	3	8.4	1	0.9	11	4.3
Total	114	100.0	36	100.0	106	100.0	256	100.0

Study Groups Vs No. of Marriages:

$$\chi^2 = 10.16 \text{ on } 4 \text{ df } \quad 0.05 > P > 0.025$$

TABLE 3.16

Number of other wives of husbands of women with
Carcinoma of cervix, Cervicitis and Controls.

No. of other wives	Study groups							
	Carcinoma Cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
None	36	31.6	5	13.9	38	35.9	79	30.9
1	41	36.0	8	22.2	42	39.6	91	35.5
2	21	18.4	15	41.7	16	15.1	52	20.3
3	15	13.2	6	16.6	10	9.4	31	12.1
4+	1	0.8	2	5.6	0	0.0	3	1.2
Total	114	100.0	36	100.0	106	100.0	256	100.0

i.   Study groups Vs No. of other wives:

   X^2 = 24.81 on 8 df   P > 0.001

ii. Ca. Cx. Vs Controls:

   X^2 = 1.52 on 3 df   P > 0.05

TABLE 3.17

Age at 1st coitus among women with Carcinoma of
cervix, Cervicitis and Healthy controls.

Age at 1st coitus (yrs).	Study groups							
	Carcinoma Cervix No.	%	Cervicitis No.	%	Controls No.	%	Total No.	%
11 - 15	36	31.6	6	16.7	11	10.4	53	20.7
16 - 20	55	48.2	17	47.2	72	67.9	144	56.3
21 - 25	22	19.3	12	33.3	18	17.0	52	20.3
> 25	1	0.9	1	2.8	5	4.7	7	2.7
Total	114	100.0	36	100.0	106	100.0	256	100.0

Study Groups Vs Age at 1st coitus:

$$X^2 = 23.25 \text{ on } 6 \text{ df } P < 0.001$$

TABLE 3.18

Number of sexual partners of women with
Carcinoma, Cervicitis and Healthy controls.

No. of Sex Partners	Study groups							
	Carcinoma Cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
1	44	44.4	18	58.1	42	46.3	104	47.1
2	32	32.3	10	37.3	32	35.2	74	33.5
3	17	17.2	2	6.4	16	17.6	35	15.8
4+	6	6.1	1	3.2	1	1.9	8	3.6
Total	99	100.0	31	100.0	91	100.0	221	100.0

Study Groups Vs No. of Sex partners:

$$X^2 = 6.34 \text{ on } 6 \text{ df } P > 0.25$$

TABLE 3.19

Frequency of coitus among women with Carcinoma
of cervix, Cervicitis and Healthy controls.

Frequency of coitus (per Wk)	Study groups							
	Carcinoma Cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
1	28	24.8	3	9.1	25	24.5	56	22.6
2	43	38.1	16	48.5	58	56.9	117	47.2
3	31	27.4	13	39.4	16	15.7	60	24.2
4	10	8.8	1	3.0	3	2.9	14	5.6
5+	1	0.9	0	0.0	0	0.0	1	0.4
Total	113	100.0	33	100.0	102	100.0	248	100.0

Study Groups Vs Frequency of coitus:

$$x^2 = 18.77 \text{ on } 8 \text{ df} \quad 0.025 > P > 0.01$$

While 42 (37.2%) of the carcinoma of the cervix cases and 14 (42.4%) of the cervicitis group had intercourse three or more times a week, only 19 (18.6%) of the healthy controls had it as frequently.

The obstetric history of the different groups of women under study is shown in Table 3.20. The majority of the women were multiparous in both the carcinoma and healthy control groups. However, while 64 (56.1%) of the carcinoma group had 6 or more pregnancies, 40 (37.7%) of the healthy controls had 6 or more pregnancies. This difference was quite significant ($P > 0.001$).

The commonest complaint in the carcinoma of the cervix group was abnormal vaginal bleeding (Table 3.21). Other complaints included foul smelling vaginal discharge, waist pain, urinary symptoms such as frequency, dysuria, hematuria, and urinary incontinence. Intestinal symptoms in the form of constipation and diarrhoea were, also common. A history of weight loss was present, while others include insomnia and peripheral edema. Pattern of symptoms, while similar in carcinoma and cervicitis groups was significantly ($P < 0.001$) different in the controls.

Among those with abnormal vaginal bleeding, post-menopausal bleeding accounted for a high percentage among the carcinoma group (Table 3.22), in contrast to the healthy controls and cervicitis groups ($P < 0.001$). While 52 (49.1%) of the carcinoma group presented with bleeding at the post-menopausal period, only 3 (11.1%) of the women with chronic cervicitis and none of the healthy controls were post-menopausal. This could however, be due to the relatively older ages among the carcinoma group.

Clinical observations on the cervix in the carcinoma patients (Table 3.23) showed that an almost equal number of women had ulcerating

TABLE 3.20

Obstetric history of women with Carcinoma of
cervix, Cervicitis and Healthy controls.

Parity	Study groups							
	Carcinoma Cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
0 - 1	6	5.3	4	11.1	4	3.8	14	5.4
2 - 3	12	10.5	12	33.3	24	22.6	48	18.8
4 - 5	32	28.1	10	27.8	38	35.8	80	31.3
6 - 7	30	26.3	6	16.7	25	23.6	61	23.8
8+	34	29.8	4	11.1	15	14.2	53	20.7
Total	114	100.0	36	100.0	106	100.0	256	100.0

Study Groups Vs Parity:

$$X^2 = 22.39 \quad on \quad 8 \quad df \quad 0.005 > P > 0.001$$

TABLE 3.21

Clinical presentation among women with Carcinoma
of cervix, Cervicitis and Healthy controls.

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Symptoms	Carcinoma Cervix	Cervicitis	Controls
Abnormal vaginal bleeding	106	32	11
Vaginal discharge	90	8	19
Pelvic pain	56	9	1
Weight loss	46	0	0
Urinary symptoms	26	2	1
Gastro-intestinal symptoms	20	8	2
Insomnia	7	0	0
Peripheral edema	6	0	0
Others	2	0	0

Study Groups Vs Clinical Presentation:

$$\chi^2 = 50.76 \text{ on } 16 \text{ df } P < 0.001$$

TABLE 3.22

Analysis of abnormal vaginal bleeding among women with
Carcinoma of cervix, Cervicitis and Healthy controls.

Type of vaginal bleeding	Study groups							
	Carcinoma Cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
Post-coital	15	14.2	17	56.6	5	65.4	37	25.2
Intermenstrual	39	36.7	10	33.3	6	56.6	55	37.4
Post menopausal	52	49.1	3	11.1	0	0.0	55	37.4
Total	106	100.0	30	100.0	11	100.0	147	100.0

Study Groups   Vs   Type of caginal bleeding:

$$X^2 = 33.72 \text{ on } 4 \text{ df} \quad P < 0.001$$

TABLE 3.23

Clinical appearance of the cervix in women
with Carcinoma of cervix, Cervicitis and Controls.

Clinical appearance of cervix	Study groups							
	Carcinoma Cervix		Cervicitis		Controls		Total	
	No.	%	No.	%	No.	%	No.	%
Normal Cervix	0	0.0	4	11.1	84	79.2	88	34.4
Cervical Erosion	0	0.0	17	47.2	16	15.1	33	13.0
Chronic Cervicitis	1	0.9	15	41.7	6	5.7	22	8.6
Ulcerating growths	56	49.1	0	0.0	0	0.0	56	21.8
Fungating growths	57	50.0	0	0.0	0	0.0	57	22.2
Total	114	100.0	36	100.0	106	100.0	256	100.0

Study Groups Vs Clinical appearance of cervix:

$$X^2 = 350.49 \text{ on } 8 \text{ df } P < 0.001$$

as had fungating growths and these two categories of women formed the
majority of the carcinoma group (50% each). In contrast, 84 (79.2%)
of the healthy controls had clinically normal cervices, and of the
rest, cervical erosion accounted for 15.1% and chronic cervicitis for
5.7%.

The clinico-pathologic staging of cervical cancer (Table 3.24)
showed that there was no case of intra-epithelial carcinoma of the
cervix. The malignancy was seen in stages 1(b) to 4 with the greater
majority (34.3%) of these in stage 3.

About half of the carcinoma of cervix cases had packed cell volumes
(PCV) of less than 30% (Table 3.25). While 48.3% of the carcinoma group
had PCV of less than 30%, only 2.9% of the cervicitis groups had PCV
below this level. Although the levels were not determined among the
healthy controls, the difference between the carcinoma group and the
cervicitis group is very significant (P < 0.001).

Hemoglobin electrophoresis patterns (Hb genotype) among the various
study groups are shown in Table 3.26. Most of the patients with carcinoma
of cervix had hemoglobin A and AS groups, while two of the hemoglobin
groups (SS and CC) were absent. Similarly, the analysis of the blood
groups in the various groups of women with carcinoma of cervix and
cervicitis are shown in Table 3.27. The majority of patients belong to
Group O, while the rest belonged to the other blood groups. The AB
blood group was the least common. No significant association (P > 0.05)
was found between any blood group or hemoglobin electrophoresis and
carcinoma of the cervix, when compared with the expected population
distribution.

TABLE 3.24

Clinico-pathologic stage of disease in women with
Carcinoma of the cervix.

Clinical stage	No.	%
1(a)	0	0.0
1(b)	7	6.1
2(a)	26	22.8
2(b)	29	25.4
3	39	34.3
4	13	11.4
Total	114	100.0

TABLE 3.25

Incidence of anemia in Carcinoma of cervix and Cervicitis as determined by the level of Packed Cell Volume (PCV).

Level of PCV(%)	Study groups					
	Carcinoma Cervix		Cervicitis		Total	
	No.	%	No.	%	No.	%
20	2	1.8	0	0.0	2	1.3
21 - 25	11	9.7	0	0.0	11	7.3
26 - 30	42	36.8	1	2.8	43	28.7
31 - 35	33	28.9	10	27.8	43	28.7
36÷	26	22.8	25	69.4	51	34.0
Total	114	100.0	36	100.0	150	100.0

Study Groups Vs Level of PCV:

$$\chi^2 = 32.70 \quad on \quad 4 \quad df \quad P < 0.001$$

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TABLE 3.26

Haemoglobin electrophoresis in women with Carcinoma of cervix and Cervicitis and the expected distribution.

Study Groups

Haemoglobin Genotype	Expected* distribution		Carcinoma Cervix		Cervicitis	
	No.	%	No.	%	No.	%
A	1980	66.0	79	69.3	23	63.9
AS	768	25.6	28	24.6	11	31.5
AC	174	5.8	4	3.5	1	2.7
SC	24	0.8	3	2.6	0	0.0
SS	51	1.7	0	0.0	0.	0.0
CC	3	0.1	0	0.0	0	0.0
Total	3000	100.0	114	100.0	35	100.0

* Esan 1970.

Study Groups Vs Hemoglobin Genotype:

$$x^2 = 9.49 \text{ on } 10 \text{ df} \quad P > 0.05$$

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TABLE 3.27

ABO Blood groups among women with Carcinoma of
cervix and Cervicitis and the Expected distribution.

Blood Group	Expected * distribution		Carcinoma Cervix		Cervicitis	
	No.	%	No.	%	No.	%
A	5544	21.3	21	18.4	4	12.9
B	6044	23.2	23	20.2	6	19.4
AB	1015	3.0	8	7.0	4	12.9
C	13404	51.5	62	54.4	17	54.8
Total	26027	100.0	114	100.0	31	100.0

* Gilles 1967

Study Groups Vs ABO blood groups:

$$x^2 = 11.57 \quad on \quad 6 \quad df \quad P > 0.05$$

Table 3.28 shows the analysis of the blood urea in women with carcinoma of cervix and cervicitis on the basis of clinico-pathologic staging of the disease. It was evident that 54 (60.7%) of the 89 women with blood urea levels below 30 mg % were in the early stages of the disease (stages 1 & 2), and only 8 (32.0%) of the 25 women with blood ureas levels above 30 mg % were in the early stages of the disease. This is quite significant ($P < 0.001$).

When the results of intravenous pyelogram (IVP) were analysed in terms of the clinico-pathologic staging of the disease (Table 3.29), 25 (31.3%) of the 80 carcinoma of the cervix patients with normal IVP had advanced carcinoma (stages 3 and 4) while 11 (78.6%) of the 14 women who had one or both ureteric obstruction on IVP had advanced disease. This association is quite significant ($P < 0.001$).

Analysis of the cystoscopic examination of the women with carcinoma of cervix is shown in Table 3.30. 59 (72.0%) of the 82 women with carcinoma of the cervix who had no bladder involvement were in the early stages of the disease (stages 1 and 2). On the other hand, only 1 (8.3%) of those who had bladder involvement were in the early stages of the disease while 11 (91.7%) of them were in the late stages (stages 3 and 4). This association is also significant ($P < 0.001$).

3.4 Discussion

One of the most rewarding aspects of the study of carcinoma of the cervix has been the comparison of the frequency with which the disease occurs in different communities in different areas and at different times. In this connection, the disease has been known to be by far

TABLE 3.28

Blood urea levels in women with Carcinoma of cervix
on the basis of clinico-pathologic stage of the disease.

Clinico-pathologic stage	Blood urea levels (mg %)				Total
	30	31-50	50-100	100	
1(a)	0	0	0	0	0
1(b)	7	0	0	0	7
2(a)	23	3	0	0	26
2(b)	24	4	1	0	29
3	29	7	0	3	39
4	6	7	0	0	13
Total	89	21	1	3	114

Blood ureak levels Vs Clinico-pathologic stage:

$$x^2 = 12.03 \text{ on } 4 \text{ df } \quad 0.025 > P > 0.01$$

TABLE 3.29

Intravenous-pyelograms in women with Carcinoma
of cervix in relation to the clinico-pathologic
stage of the disease.

Clinico-pathologic stage	Intravenous pyelogram (IVP)			Total
	Normal	Unilateral obstruction	Bilateral obstruction	
1(a)	0	0	0	0
1(b)	7	0	0	7
2(a)	23	0	0	23
2(b)	25	3	0	28
3	24	5	1	30
4	1	4	1	6
Total	80	12	2	94

IVP Vs Clinico-pathologic stage:

$$x^2 = 28.43 \text{ on } 4 \text{ df } P < 0.001$$

TABLE 3.30

Results of cystoscopy in women with Carcinoma of cervix on the basis of clinical-pathologic stage of the disease.

Clinico-pathologic stage	Cystoscopy		Total
	Bladder free	Bladder involved	
1(a)	0	0	0
1(b)	7	0	7
2(a)	24	1	25
2(b)	28	-	28
3	22	6	28
4	1	5	6
Total	82	12	94

Cystoscopic finding Vs Clinicopathologic stage:

$$X^2 = 35.56 \text{ on } 4 \text{ df } P < 0.001$$

the commonest gynaecologic   malignancy in Ibadan (Edington & Maclean,
1965; Edington & Hendrickse, 1970).   Indeed, it was shown to be the
highest malignancy in female excluding reticulo-endothelial tumours
(Edington & Hendrickse, 1972).

In the present study, in addition to this frequent occurrence, it
was found that the disease occurs at a much earlier age than elsewhere.
In most of the studies in Britain and America, the peak age-incidence
of carcinoma of the cervix has been shown to be 50-59 year age groups
(Wentz & Reagan, 1959; Wentz, 1961), whereas in the present study,
the peak age-incidence was found to be 15 years earlier (35-45 year age
group).   Similarly the mean ages in both studies differ: 46.1 years in
the present study as compared with 51.4 years (Wentz & Reagan, 1959),
and 53.1 years (Wentz, 1961).

The reasons for this high frequency as well as the early onset of
the disease has not been determined.   Various aspects of coitus which
have been implicated in the causation of carcinoma of the cervix have
been examined in the present study.   Indeed, all the women in this study
were married at one time or the other.   Also early marriage (and hence,
early initiation into sexual life) is customary in many parts of the
country (especially among the Hausa's of the North, the Ibos in the
East and the Edos in the Midwest).

In addition, a greater percentage of the carcinoma group were
known to have commenced intercourse at an early age.   While only 10.4%
of the healthy controls were found to have started heterosexual inter-
course at a very tender age (11-15 years), 31.6% of the carcinoma of
the cervix cases were known to have commenced this at the same age.

This is quite significant ($P < 0.001$) and is in keeping with the findings of others (Clemmensen, 1952; Wynder et al, 1954; Rotkin & King, 1962; Christopherson & Parker, 1965; Rotkin, 1967; Masubuchi & Nemoto, 1972), who found that more cancer of the cervix patients than controls commenced intercourse at an early age. Intercourse at an early age, especially at puberty, has been said to be significant in the occurence of cervical carcinoma (Jones et al, 1958; Christopherson & Parker, 1965; Masubuchi & Nemoto, 1972) because the immature sexual organs are highly sensitive to carcinogenic factors (Moghissi et al, 1968). It would appear that the disease is common and occurs early in this environment because of the very earlier onset of coitus.

Apart from heterosexual activities, an excess of multiple coital partners has been implicated in the causation of carcinoma of the cervix (Kinsey et al, 1953; Pereyra, 1961; Greene et al, 1965; Rotkin, 1967(b)). In the present study, it was found that the incidence of carcinoma of the cervix uteri was much higher among women who had two or more marriages. While 35.1% of the carcinoma of cervix group had been married twice or more, only 18.9% of the controls had two or more marriages, a significant finding ($P > 0.025$). Even though the difference was not significant ($P > 0.25$), a higher percentage (6.1%) of the carcinoma group had inter- course with four or more partners as compared with 1.9% among the healthy controls. It is believed that the high incidence of multiple marriages and sexual intercourse with multiple partners would enhance the chance of a woman being exposed to men having carcinogenic factors (Rotkin & King, 1962; Christopherson & Parker, 1965; Moghissi et al, 1968), although Masubuchi & Nemoto (1972) disagreed with this view.

When the frequency of intercourse per week was considered, there was no difference in the percentage of those who practise this once a week in the carcinoma and control groups. However, a significant $(P > 0.01)$ difference was noticed between the two groups when intercourse occured three or more times a week. While only 18.6% of the healthy controls practised intercourse three or more times a week, 37.2% of the carcinoma group had intercourse as many times a week. Although this is in contrast to the findings of Masters (1960) and Rotkins (1967) who found no relationship between frequency of intercourse and the incidence of carcinoma of cervix in their studies, it may be that increased frequency of coitus enhances the chances of introducing "the carcinogenic factor" into the cervix.

However in the present studies, polygamy could not be shown to have any relationship with the high incidence of carcinoma. Unlike the study of Wynder et al, (1954) where a relationship was shown between polygamy and carcinoma of cervix, there was no difference in the number of wives of the husbands of women with cervical cancer compared with the healthy control group in this study $(P > 0.05)$. The result in this study is not surprising, since the man, rather than the woman is probably the carrier (agent) of the carcinogenic factor.

In regard to the number of pregnancies and deliveries, the findings in this study were similar to those of Martin (1967), and Rotkin (1967) in that 56.1% of the carcinoma group had six or more pregnancies, and only 37.7% of the control group had this number of children. This is quite significant $(P < 0.001)$ although it may have to do with the relatively older ages of the carcinoma group. On the

other hand, it is possible that carcinoma develops because of the
earlier age at first coitus for the carcinoma patients, as opposed to
the controls (Rotkin, 1967). The early age of coitus would afford
additional years for patient to conceive and bear more children.

There is no doubt about the association of socio-economic factors
with carcinoma of the cervix in this study. The majority of all three
groups of women in the study were in the lower socio-economic status.
Even though the difference was not significant ($P > 0.05$) there were
many more women of the lower socio-economic class among the carcinoma
group than in the healthy controls. It is possible that these women
with poor education, and who do menial jobs get married to men with
similar background (Kennaway, 1948; Lombard & Porter, 1950). Hence,
they have little or no recreational facilities apart from coitus.
Sexual activity therefore becomes the common denominator among the
poorer classes (Clemmensen, 1952) to account for the observable
difference in the incidence of carcinoma of the cervix between them
and the upper socio-economic classes.

In the past, circumcision was thought to decrease the occurrence of
carcinoma of the cervix (Plaut & Kohn-Speyer, 1947; Kennaway, 1948;
Weiner et al, 1951; Wynder et al, 1954; Pratt-Thomas et al, 1956;
Terris & Oalman, 1960; Kmet et al, 1963; Rotkin, 1967). In this study,
all of Nigerian male partners of the women with carcinoma of the cervix
were circumcised. Yet carcinoma of cervix is by far the commonest female
malignancy in this part of the world. When compared with Uganda, which
has a similar incidence of carcinoma of the cervix, but where less than
10% of the males are circumcised (Dodge et al, 1963; Edington, 1970),

it is obvious that circumcision has little or no part to play in the causation of carcinoma of the cervix.

The preponderance of the Yoruba speaking women of the Western State of Nigeria in this study is largely a reflection on the location of the Hospital, as this group constituted over 80% of Hospital admissions (Medical Records). Even when the place of origin was analysed in relation to the other factors such as the clinical appearance of the cervix, the clinico-pathologic stage and histopathology of the disease, there was no significant difference in the various ethnic groups in the study. From the results in this study, it is possible that the differences observed among the races (Haenzel & Hillhouse, 1959; Kmet, 1963; and Martin, 1967) is a reflection of the coital customs of the people rather than their geographical location.

Regarding clinical symptoms, abnormal vaginal bleeding was by far the commonest symptom. In addition, contact and post-menopausal bleeding, coupled with foul-smelling vaginal discharge, pelvic pain and urinary symptoms, especially hematuria, were common. Most of these symptoms, especially pain and hematuria were probable evidence of the advanced nature of the disease by the time the patients present in hospital. The advanced nature of the malignancy is even more marked among the lower socio-economic classes where the majority presented in the late stages of the disease, a finding similar to that of Lawson et al, (1964). However, while 80% of the patients in the study of Lawson et al, (1964) and 62.5% of the patients in the retrospective study presented in the late stages of carcinoma of cervix (stages 3 and 4) only 45.7% of the patients in this study presented in stages

3 and 4. This is probably due to an increased awareness of the value of hospital care by the patients and the increase in the number of doctors in the country.

Most of the carcinoma of the cervix patients were anemic as determined by the level of packed cell volume of 30% and less. This is to be expected because of the abnormal, and sometimes very heavy and prolonged vaginal bleeding in these women. There was no significant association in the incidence of carcinoma of the cervix and hemoglobin electrophoresis (Hb Genotype) or the blood groups in this study. Increasing interest in the association between ABO blood groups, hemoglobin electrophoretic patterns and malignancies stems from the finding that hemoglobin A individuals seem more susceptible to developing Burkitts tumour (Williams, 1966) while there is a shift from blood group O in trophoblastic diseases (Llewellyn-Jones, 1965). The protection afforded against malaria by the hemoglobin S strait (Allison, 1954) on the other hand, does not appear to protect against the development of carcinoma of cervix. The high percentage of the patients in the carcinoma of the cervix group with abnormal blood ureas, abnormal intravenous pyelogram and bladder involvement on cystoscopic examination could be accounted for by the advanced stage of the disease in these women.

From the foregoing, coitus has been demonstrated as vital in the causation of carcinoma of the cervix. It is the common denominator which can explain the occurrence of the disease among women who engage in early sexual practice, have intercourse frequently, and with multiple sexual partners and have many pregnancies. It may be that a sexually

transmitted factor is important in the causation of carcinoma of the
cervix.

In support of this contention, it is known that the age of marriage
and first coitus drops with increase in standard of living in various
populations (Beral, 1974). If intercourse at an early age alone were
an important determinent of cervical carcinoma (Wynder et al, 1954;
Jones et al, 1958; Christopherson & Parker, 1965; Moghissi et al, 1968),
it was suggested that increased mortality should be expected from
carcinoma of cervix in successive generation of women. But this
has not been so (Beral, 1974). However, if cervical cancer were
caused by a sexually transmitted infection (? virus), mortality from
the malignancy might be expected to follow trends in the incidence
of sexually transmitted diseases. The mortality pattern from the cases
of carcinoma of the cervix has been found to follow trends in venereal
diseases (Beral, 1974).

It is quite possible that "a yet unknown" factor that follows the
same time pattern as sexually transmitted diseases may be the under-
lying cause of carcinoma of the cervix. Herpes Type-2 (HT-2) virus
has been shown to be venereally transmitted (Dowdle et al, 1967;
Nahmias & Dowdle, 1968; Rawls et al, 1968; Nahmias et al, 1969; Rawls
et al, 1971; Duenas et al, 1972; Adelusi et al, 1975(b)). It is
also known that the infection commences in populations around puberty
(Rawls et al, 1969; Royston & Aurelian, 1970), and reaches a peak
around the period of life when the ectocervix is known to be susceptible
to carcinogens (Coppleson & Reed, 1967). It is quite possible, therefore,
that the cervical epithelium might be susceptible to the venereally
transmitted (HT-2) virus which might help to initiate the malignant change.

CHAPTER 4

CYTOLOGIC AND HISTOPATHOLOGIC STUDIES OF
CARCINOMA OF CERVIX UTERI IN IBADAN

4.1 Introduction

There are two principal pathologic types of cervical cancer.
It would be recalled that the epithelium covering the external or
vaginal surface of the portio vaginalis of the cervix is stratified
squamous epithelium especially from the third decade of life
(Coppleson & Reed, 1967) and is continuous with the stratified squamous
epithelium of the vagina. From this type of cervical epithelium arises
squamous cell or epidermoid carcinoma. This type of the cervical
carcinoma is by far the commonest and accounts for almost 95 per cent
of all cervical carcinoma (Hull, 1973).

On the other hand, from the columnar epithelium of the cervical
canal arises the cylindric cell carcinoma which assumes a glandular
pattern in its growth and is therefore designated adenocarcinoma of
the cervix. It is alleged to develop in the columnar epithelium of
paramesonephric (mullerian) duct origin (Haggard et al, 1964). It is
less common, accounting for about 5 per cent of cervical cancers
(Hull, 1973). An adenocarcinoma of the corpus uteri infiltrating the
cervix has to be ruled out however, by uterine curretings.

Very occasionally, however, the two types of cancer may be

present in the same patient and produce a mixed cell carcinoma or adeno-squamous carcinoma of the cervix. It is thought that such tumours are very rare, and may be explicable by metaplasia of one cell type into the other. Such cancers seem to carry a more serious prognosis than either squamous cell carcinoma or adeno-carcinoma (Haggard et al, 1964). Sarcomas of the uterine cervix are found very infrequently. The exceedingly rare Sarcoma Botryoides may occasionally involve the cervix, although most cases develop primarily in the vagina.

Based on cellular morphology, a histologic classification for carcinoma of cervix, was proposed by Reagan and his associates (1957). Subsequently, Wentz and his associates (Wentz & Reagan, 1959; Wentz, 1961; Wentz & Louis, 1965) demonstrated a definite correlation between the histologic classification of carcinoma of the cervix and survival following radiation therapy. These findings were later substantiated by others (Finck & Denk, 1970). Sidhu et al (1970), using a very similar cell-type classification, analysed the results following surgical therapy and reported that survival was at least in part related to cell type. Hence, it has thus become routine to classify all cases of cervical carcinoma on a histopathologic basis, and usually by pathologists.

The sub-division of cervical carcinoma into the in-situ (intra epithelial) and invasive types is of significance, not only because of the histologic distinction between the two but also because of the clinical management. Intraepithelial carcinoma of the cervix is believed by Fluhmann (1960) and Johnson et al (1964) to originate from the transitional cells that lie at the squamo-columnar junction through "an unknown stimulus". These cells proliferate in an abnormal fashion

to form anaplastic subcylindric cells which gradually develop into
carcinoma in-situ, which in turn invades the stroma and ultimately
develops into clinically invasive carcinoma (Johnson et al, 1960; Koss
et al, 1963; Fox, 1967; Johnson et al, 1968; Richart & Barron, 1969;
Hulka. & Kupper, 1971). Opinion however differs on this hypothesis
(Ashley, 1966).

As with carcinoma elsewhere, the microscopic diagnosis of invasive
carcinoma of the cervix is based on characteristics of abnormal pattern
of architecture, and abnormalities in the constituent cells. On the
basis of this, Patten (1969) sub-divided invasive carcinoma of the cervix
into the keratinising (Group I) or the well differentiated carcinoma,
with pearl formation; the large-cell non-keratinising (Group II) carcinoma;
and the small cell (Group III) or the undifferentiated carcinoma. The
significance of distinguishing the level of malignancy of the tumour
was first proposed by Broders (1932, 1932(b)) when he graded cases of
carcinoma of the cervix into four grades on the basis of histologic
differentiation of the tumour.

The grading gave an indication of the ability of the tumour to
proliferate, invade and metastasize, and these were most likely to occur
in the more malignant grades 3 and 4. Whereas, in the normal epithelial
surface, the epithelial cells are sharply demarcated from the stroma
by the basement membrane, in cancer, the latter is broken through so
that the epithelium invades into the stroma, blood vessels and lymphatics,
at first in small buds but later in the form of long columns which grow
deep into the stroma, much as the roots of a tree grow down into the
soil (Swan & Roddick, 1973). It is this invasiveness, coupled with the

dissemination of cells through the lymphatics, which is responsible
for the characteristics traditionally associated with malignancy such
as local infiltration, metastasis and often, recurrence after incomplete
removal.

Investigating cases suspected of malignancy of the cervix has
generated a lot of interest. For example, the recent development of
an accurate cytologic method for assessing asymptomatic women with a
completely normal appearing cervix has led to the diagnosis of many
cases of early cancer long before symptomatology or overt pathologic
abnormalities are apparent.(Thomson et al, 1972). Cancer in-situ is
usually symptomless, and therefore unless vaginal and cervical smears
are taken, it is certain to be missed.

The most comprehensive investigation of the patient with cytologic
atypia has classically included cold conisation as the ultimate procedure
necessary to establish a definitive histologic diagnosis, especially
for cases of in-situ carcinoma. In some cases where there is established
clinical evidence of malignancy, however, other methods of diagnosis
have been developed, including biopsy (either punch, ring or otherwise)
especially after Schillers test. The latter is based on the fact that
cancer cell contains no glycogen, and hence does not take up iodine
like the normally glycogen-rich epithelium of the cervix. The non-iodine
taking areas are then biopsied. Where the growth is clinically obvious
however, there is no need for Schiller's test. The other methods of
diagnosis that have been developed include colposcopy (Navratil et al,
1958) and a fluorescent antibody technique (Louis, 1960). These are
relatively easy to use and eliminate the associated dangers of biopsy

especially cold conisation, and the inaccuracy of cytologic studies.

Despite all the advances made in the microscopic diagnosis of carcinoma of the cervix elsewhere, a detailed analysis of the histopathology of the disease is yet to be made in Ibadan, even though this is the commonest female malignancy seen here. This study was designed, therefore, to determine the type of cells involved in the disease, as well as the degree of invasiveness and differentiation of the cells.

4.2 Materials and Methods

4.2.1 Study Groups: Cervical biopsy specimens were obtained from
the following:

(a) One hundred and fourteen women who were seen in the Department of Gynaecology as previously described in Chapter 3. Diagnosis of carcinoma of cervix was on clinical grounds.

(b) Thirty-six women with clinically suspected carcinoma of the cervix or cervicitis, seen in the same department.

4.2.2 Cytologic Studies: All patients in the study were examined clinically, and during the pelvic examination, cervical scrapings and posterior vaginal smears were taken, using Ayre's spatula. Smears were made on clean microscope slides (size 77 x 25 mm, thickness 1.0 - 1.2 mm) from the scrapings and vaginal smears. These were fixed in cold acetone (4°C) and kept until processed for examination, usually within 7 days.

(a) Papanicolau Staining: The smears were immersed consecutively for ten seconds each in solutions of 70% and 50% alcohol, and

distilled water, and then in Harrison's Hematoxylin for 5
minutes. The fixed smears were rinsed in ordinary water for
10 seconds and then differentiated with 0.5% acid alcohol for
12 seconds. These were then rinsed quickly in tap water for
10 seconds and rendered blue in ammonia for 5 minutes. They
were again rinsed in tap water for 10 seconds, and then
consecutively for 10 seconds each in solutions of 70%, 90% and
99% alcohol.

Staining was next performed with O96 for 5 minutes, and
the smears were rinsed in 99% alcohol for 10 seconds. They were
again stained in EA 50 for 6 minutes and rinsed in 99% alcohol
for 10 seconds before being dehydrated. The smears were finally
mounted with zylene before examination.

(b) <u>Microscopic Examination</u>: The final preparation was examined
within 48 hours using a Zeiss microscope, by a trained cytologist.
Scoring of smears was based on the characteristics of the cells,
and graded according to U.C.H. scoring:

   (i)   Normal cells;

  (ii)   Slight atypia;

(iii)   Marked atipia;

  (iv)   **Dysplasia**;

   (v)   Malignant cells.

4.2.3 <u>Histopathologic Studies</u>: Biopsies (punch or cone) were taken
from each of the patients during routine pelvic examinations or
during examination under anaesthesia, and sent to the Pathology

Laboratory where these were processed and examined microscopically. Data in this study was based on reports from the Department.

The microscopic diagnosis of invasive carcinoma of the cervix was based on the two chief characteristics of abnormal architecture and abnormalities in the constituent cells.

(a) <u>Group I</u> (Keratinising Carcinoma). This was characterised by a predominance of large abnormal epithelial cells with a high degree of pleomorphism, bizzare elongate and caudate forms. The nuclei were moderatly large with a hyperchromatic coarsely granular chromatin pattern. Isolated nuclei and syncytia were relatively rare, and epithelial pearls and isolated cell keratinisation were present. A low mitotic index was commonly observed. The histologic structure was that of a <u>well differentiated squamous cell carcinoma</u> of the cervix (Fig. 4.1).

(b) <u>Group II</u> (Large cell non-keratinising Carcinoma). This was characterised by many abnormal basophilic epithelial cells varying in size. A high nuclear-cytoplasmic ratio was characteristic. The nuclei were opaque with a coarse chromatin pattern and frequent macro nucleoli. Isolated nuclei and syncytia were more common than in the other two types. There was a differentiated squamous cell carcinoma with rare isolated cell keratinisation and no epithelial pearls. There was a distinct variation in cell size and a moderately high mitotic index. The histologic structure was that of <u>poorly differentiated squamous cell carcinoma</u> of the cervix (Fig. 4.2).

(c) <u>Group III</u> (Small Cell Carcinoma) This was characterised by predominance of uniformly small basophilic cells with a high nuclear-cytoplasmic ratio. The nuclei were opaque or coarsely granular.

Figure 4.1

Well differentiated squamous cell carcinoma of the Cervix.
There are islands of malignant squamous cells with epithelial
pearls.   They are separated by bands of fibro-cellular tissue
with mono-nuclear inflammatory infiltrates.   (H & E x 175)
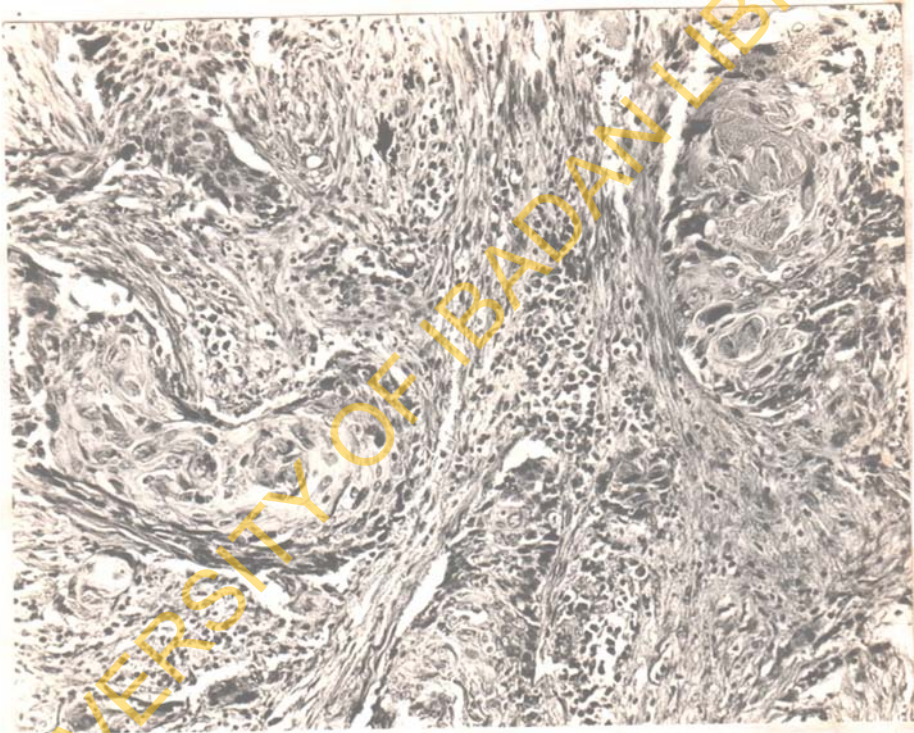
Figure 4.2

Poorly differentiated squamous cell carcinoma of the Cervix.
There are small islands some of which are interlacing
epithelial cells with foci of malignant dyskeratosis.
Epithelial nests are absent. There is a dense infiltrate
of mono-nuclear cells separating the islands of malignant
tissue. On the top right hand corner is a portion of a
dysplastic surface epithelium. (H & E x 175)
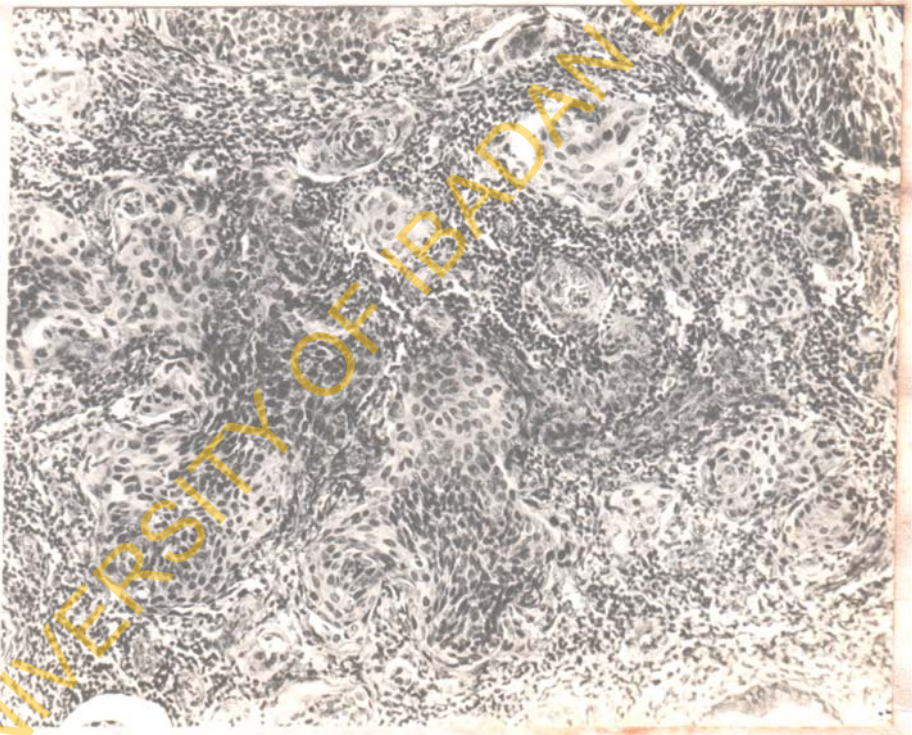
Figure 4.3

Undifferentiated Carcinoma of the Cervix. There are
sheets of epithelial cells in a syncytium. Many of the
nuclei are vesicular and several mitotic figures are present. The
cytoplasm of the cells are distinct but not separated
into individual cells. There are three small blood
vessels containing red cells and polymorphs. (H & E x 170)
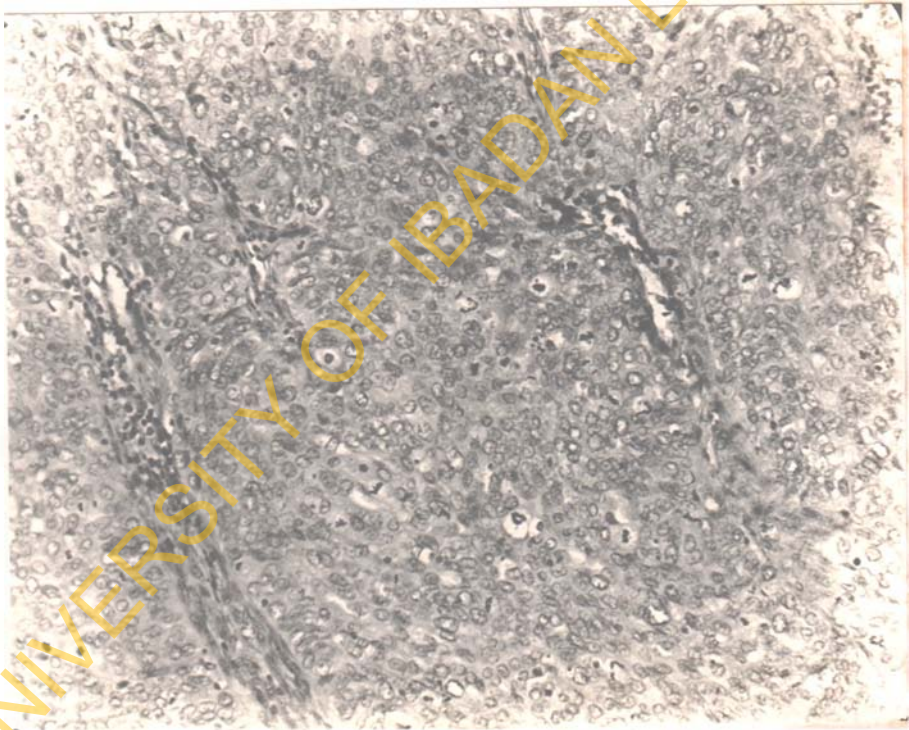
Figure 4.4

Adenocarcinoma of the Cervix. There is a superficial zone of stratified squamous epithelium with foci of vacuolar degeneration. In the fibromuscular corium are abnormally proliferating malignant endocervical glands with a papillary structure in areas. Back to Back pattern of some of the glands can be seen. There is a malignant gland abutting on the squamous epithelium. Inflammatory cellular infiltrates are sparse.

(H & E x 70)
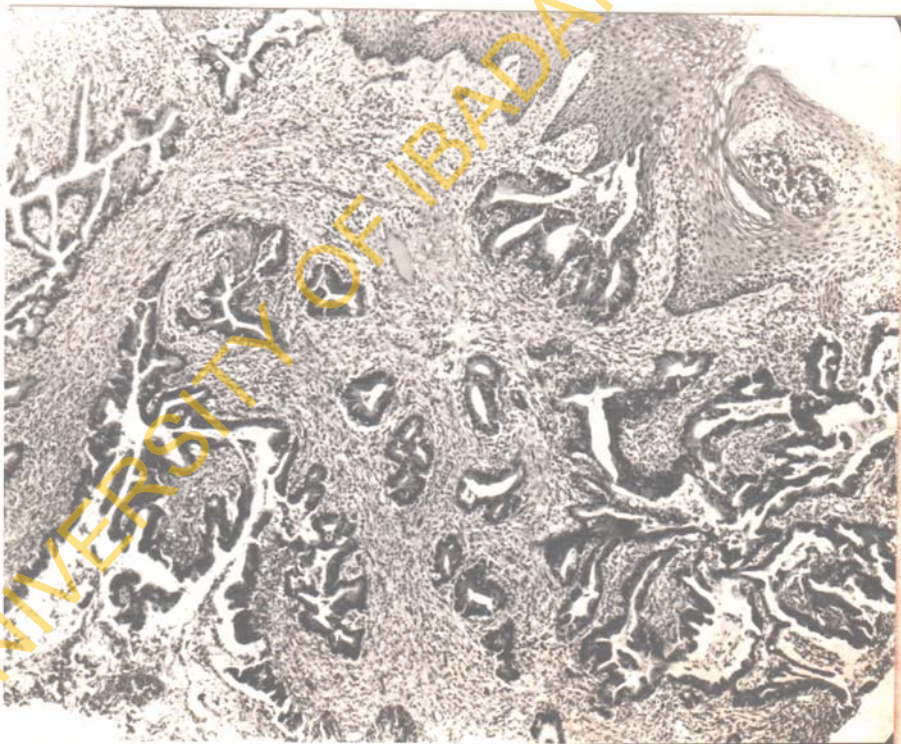
Macro nucleoli and syncytia were not as common as in Group II. The histologic architecture was remarkable for the uniformity of cell and nuclear size. Epithelial pearls and isolated keratinized cells were not observed and a high mitotic index was observed. The histologic structure was that of the undifferentiated cell carcinoma of the cervix (Fig. 4.3).

There were a number of mucin-secreting, well differentiated cylindric cells with all the features of malignancy, although these were in the minority. The histologic architecture was that of a well differentiated adenocarcinoma of the cervix (Fig. 4.4)

4.3 Results

Table 4.1 shows the cytological examination of women in the study groups. 11 (99.1%) of the women with carcinoma of the cervix showed dysplastic and malignant smears in contrast to 2 (5.6%) among the women with cervicitis and nil among the healthy controls. When the cytologic examination of smears was analysed for the carcinoma of cervix and cervicitis cases on the basis of age (Table 4.2), 25 (64.1%) of 39 women below the age of 35 years showed evidence of cellular atypia, whereas, 91.0% of the 115 women above the age of 35 years showed evidence of dysplastic and malignant smears on cytology. This difference is statistically significant ($P < 0.001$).

When the results of cytology were analysed for women with carcinoma of the cervix and cervicitis on the basis of clinical appearance of the cervix (Table 4.3), it was found that 96.5% of the 115 dysplastic and malignant smears were from women who were clinically diagnosed as carcinoma,

TABLE 4.1

Cytologic examination of smears of women with
Carcinoma of cervix, Cervicitis and Healthy controls.

Cytologic appearance	Study groups			Total
	Carcinoma Cervix	Cervicitis	Controls	
Normal	0	5	83	88
Slight atypia	0	18	17	35
Marked atypia	1	11	6	18
Dysplasia	9	2	0	11
Malignant	104	0	0	104
Total	114	36	106	256

Study Groups Vs Cytologic appearance:

$$X^2 = 326.28 \text{ on } 8 \text{ df } P < 0.001$$

TABLE 4.2

Analysis of Cervical cytology on the basis of age
in women with Carcinoma of cervix or Cervicitis.

Age (Years)	Cytologic appearance					Total
	Normal	Slight atypia	Marked atypia	Dysplasia	Malignant	
16 - 25	2	4	4	0	2	12
26 - 35	2	8	5	1	11	27
36 - 45	1	5	2	3	43	54
46 - 55	0	1	1	3	22	27
56 - 65	0	0	0	4	21	25
> 65	0	0	0	0	5	5
Total	5	18	12	11	104	150

Cytologic appearance Vs Age (Years):

$$x^2 = 60.55 \text{ on } 20 \text{ df } P < 0.001$$

TABLE 4.3

Analysis of cervical cytology on the basis of clinical
appearance of the cervix in women with Carcinoma of cervix
or Cervicitis.

Clinical appearance	Cytologic Appearance					Total
	Normal	Slight atypia	Marked atypia	Dysplasia	Maligant	
Normal	2	2	0	0	0	4
Erosion	3	10	4	0	0	17
Cervicitis	0	6	6	4	0	16
Ulcerating growths	0	0	2	5	49	56
Fungating growths	0	0	0	2	55	57
Total	5	18	12	11	104	150

Cytologic appearance Vs Clinical appearance of cervix:

$$X^2 = 171.83 \text{ on } 16 \text{ df } P < 0.001$$

a significant (P< 0.001) finding.

Table 4.4 shows the histopathologic classification of the cases of carcinoma of the cervix in the study. Of the whole, 72 (63.2%) were well differentiated (Grade I) squamous cell carcinoma, 17 (14.9%) were poorly differentiated squamous cell carcinoma (Grade II) and 18 (15.8%) undifferentiated carcinoma (Grade III). Only 7 (6.1%) of the cases were adenocarcinoma of cervix.

Table 4.5 shows the histopathologic classification in women with carcinoma of cervix in relation to the clinico-pathologic stage of the disease. There is a significant correlation (0.05 > P > 0.025) between histopathologic grading and clinico-pathologic stage of disease. The well differentiated squamous cell carcinoma group was less advanced, clinically, as compared with the undifferentiated carcinoma group. 33 (45.8%) of the well differentiated squamous cell carcinoma of the cervix, 5 (29.4%) of the poorly differentiated squamous cell carcinoma group and 3 (42.9%) of the adenocarcinoma group were in stages 3 and 4 as compared with 11 (61.1%) of the undifferentiated carcinoma group.

Invasion of the cervical tissues (stroma, blood vessels, lymphatics etc.) was noticed in the various grades of the malignancy. Most of the undifferentiated carcinoma had invasion of the stroma and fibromuscular tissues compared with the well differentiated carcinomas. On the other hand, the lymphatic involvement was more marked in the well differentiated group than in the undifferentiated group. In addition, there was a lot of inflammatory cellular infiltration of the tumours, mostly polymorphs.

4 4 Discussion

Histologic grading of carcinoma of the cervix has often been of

TABLE 4.4

Histo-pathologic classification in cases of Carcinoma
of the cervix.

Histopathology	.No.	%
*Well Diff. Sq. Cell Ca.	72	63.2
+Poorly Diff. Sq. Cell Ca.	17	14.9
Undifferentiated Carcinoma	18	15.8
Adenocarcinoma of cervix	7	6.1
Total	114	100.0

* Well differentiated squamous cell carcinoma

+ Poorly differentiated squamous cell carcinoma.

TABLE 4.5

Histopathologic classification in relation to clinoco-pathologic
stage in women with Carcinoma of the cervix.

Histopathology	Clinico-pathologic stage						
	1(a)	1(b)	2(a)	2(b)	3	4	Total
* Well diff. Sq. Cell. Ca.	0	5	15	19	29	4	72
+ Poorly diff. Sq. Cell Ca.	0	1	4	7	2	3	17
Undifferentiated Carcinoma	0	0	4	3	5	6	18
Adenocarcinoma of cervix	0	1	3	0	3	0	7
Total	0	7	26	29	39	13	114

Clinico-pathologic stage Vs Histopathology:

$$X^2 = 22.09 \text{ on } 12 \text{ df } 0.05 > P > 0.025$$

* Well differentiated squamous cell carcinoma

+ Poorly differentiated squamous cell carcinoma

special interest to physicians the world over, since the realisation
that a correlation existed between the tumour morphology and prognosis
(Reagan et al, 1957; Wentz & Reagan, 1959; Wentz, 1961; Wentz & Louis,
1965; Finck & Denk, 1970; Sidhu et al, 1970). Even Broders (1926)
whose original classification was based on the degree of differentiation
exhibited by the neoplasm, attempted to modify his classification to
support such arelationship, especially for cervical cancer. Warren (1931),
using a modification of the classification proposed by Broders, attempted
to show a relationship between tumour differentiation and metastatic
disease. Hence, it has thus become routine to grade all cases of
cervical cancer on a histopathologic basis.

On the basis of the tumour morphology, two principal pathologic
entities were identified in this study, arising from one or the other
of the two types of epithelia of the cervix. From the stratified squamous
epithelium of the cervix arose squamous cell carcinoma which accounted
for 93.9% of all the cases. And from the columnar epithelium of the
endocervix was adenocarcinoma of the cervix, which accounted for 6.1% of
all the cases. There was not a single case of the mixed adeno-squamous
form. This is in contrast to Haggard et al (1964) who frequently
demonstrated combinations of the squamous cell and adenocarcinoma in
their study, and believed that one malignant process produced both
types of carcinoma.

The question of the degree of differentiation of carcinoma (of the
cervix) is of great significance in the assessment of prognosis.
Althoug Linell & Maansson (1952), and Brack et al (1956) have shown
lack of correlation between survival and the histologic type of cancer,

Gilmour et al (1949); Miller et al, (1959); Wentz & Reagan (1959);
Wentz (1961) and Wentz & Louis (1965) have all indicated a poorer
prognosis for the most undifferentiated tumours in any given stage of
anatomical involvement. Other factors which affect the prognosis
include not only the cell type and the differentiation of the tumour
type, but also the age of the patient, the stage of the disease, the
frequency of lymph node metastasis as well as stromal and parametrial
invasion (Sidhu et al, 1970).

It was observed that most of the women with the well differentiated
squamous carcinoma of the cervix in this study were above the age of
45 years as compared with the undifferentiated carcinoma. Whereas,
47.2% of the well differentiated squamous cell carcinoma of the cervix
were below 45 years, 72.2% of the undifferentiated carcinomas were
below this age level. Even though these are not statistically
significant (P > 0.5) it is tempting to speculate that cervical cancer
manifesting itself before the age of 45 years is normally more aggresive
than that occuring in older women. The factors that may account for this
difference is not clear but these are worthy of further epidemiologic
study.

There were many more older women (above 45 years) among the patients
with adenocarcinoma of the cervix in this study. In addition, many more
(57.1%) of them had been married twice or more, as compared with the
36.1% of squamous cell carcinoma; and more (57.1%) have had eight or
more pregnancies, as compared with 22.2% of squamous cell carcinoma.
Even though these figures are not statistically significant (P > 0.5)
it may be that increasing age, more frequent marriages and parity augment

the probabilities, at least in negro populations, of a woman developing adenocarcinoma of the cervix (Haggard et al, 1964). On the other hand, the differences may be due to the ease with which it spreads to the lymph nodes (Jeffcoate, 1969), as well as its different etiology.

The clinico-pathologic stage of the disease is vital in the prognostic evaluation of cases of carcinoma of the cervix. It is generally appreciated that the five year survival in cases of carcinoma of the cervix is dependent on the stage of disease (Browne & McClure Browne, 1964; Jeffcoate, 1969). In the present study, there were 45.7% patients in stages 3 and 4, unlike in the study of Lawson et al (1964) where as many as 80% were in these late stages. Nevertheless, there were many more (34.2%) patients in stage 3 than in any other stage. This was in contrast to the studies of Wentz & Reagan (1959) and Wentz (1961) where there were many more cases in stage 2 than in any other stage. Even though the present study is an extension on that of Lawson et al (1964), there is no doubt that patients in this part of the world still report late into hospital.

Most of the undifferentiated carcinoma (61.1%) were seen at an advanced stage of the disease (Stages 3 and 4) in contrast to the well differentiated ones (45.8%). This could be explicable by the fact that undifferentiated carcinoma of the cervix is more malignant than the well differentiated types since stromal invasion is faster with the former than with the latter (Sidhu et al, 1970).

The presence of lymph-node metastasis is one of the most significant prognostic factors in carcinoma of cervix (Dobbie et al, 1962; Graham et al, 1962; Mitani et al, 1962; Masterson, 1963; Brunschwig & Barber,

1966; and Morton & Lagasse, 1967). It has been pointed out (Mitani et al, 1962) that the prognosis is poorer if more than one lymph node contains metastatic tumour. Others (Sidhu et al, 1970) have confirmed this finding. Analysis of other factors associated with lymph node metastasis, suggests that invasion of the lymphatics correlated well with the depth of invasion, and could be of prognostic signficance therefore. Compared with this on the other hand, invasion of blood vessels by the primary tumour was of limited prognostic value, contrary to earlier reports (Friedell & Parsons, 1962). This has been confirmed by other studies (Sidhu et al, 1970).

In this study, stromal invasion was more marked in the undifferentiated group than in others, while lymph-node metastasis was more marked in the well differentiated carcinoma. It is possible that one of the reasons for the relatively poor results of surgical treatment of well differentiated carcinoma of the cervix is the frequency of lymph-node metastasis which is said to be highest in this histologic grade (Botella Llusia et al, 1962; Nogales & Botella Llusia, 1965). Paradoxically, in undifferentiated (Grade III) carcinoma of the cervix, the lowest rate of lymph node metastasis was noticed. This could account for the successful surgical treatment of this grade of tumour (Dobbie et al, 1962; Blomfield et al, 1965; Walter et al, 1965; Sidhu et al, 1970).

The value of early detection, and therefore, treatment of carcinoma of the cervix has never been in doubt. It is generally agreed that carcinoma of the cervix is unique in that it is perhaps the only cancer of the female genital tract which can be detected before it becomes invasive. The recent development of routine cytologic screening of populations with a completely normal appearing cervix has led to the

diagnosis of many cases of very early cancer (in-situ) before symptoma-
tology or overt pathologic abnormalities are apparent (Thomson et al, 1972).
Furthermore, the most comprehensive investigation of a patient with
cellular atypia has included cold conisation as the ultimate procedure
necessary to establish a definitive histologic diagnosis, especially
in cases of in-situ carcinoma. Where the growth is clinically obvious,
however, biopsies (punch, ring or otherwise) have been developed.

In Ibadan, our preference has always been multiple punch biopsies
of the cervical squamo-columnar junction, if clinically obvious, or
cone biopsy where the cervix is suspect on cytologic examination.
There was no case of in-situ carcinoma seen in the present study.
However, in a wider screening programme (Ojo & Adekunle, 1976), 0.16%
of the women in their study had cone biopsies following "suspicious"
cytologic examination out of which 0.08% were found to have carcinoma
in-situ or invasive carcinoma of the cervix. Even then, this figure
is much less than in Britain (McLaren, 1963), Uganda (Trussell et al,
1968), Barbados (Vaillant et al, 1968), Japan (Kazumasa, 1969) and
Sweeden (Ringertz, 1969) where such studies have been undertaken.
This is probably due to the limited scope of the programme (screening
of Family Planning and Gynaecologic patients only) rather than a
general lack of awareness of the lesion.

Routine punch biopsy has been shown to have an accuracy of 94 to
98% (Dilts et al, 1964; Silbar & Woodruff, 1966; Krumholz & Krapp, 1972).
Cytopathology has proved to be the best screening procedure of choice,
and various authors (Griffiths et al, 1964; Anderson & Linton, 1967;
Singleton & Rutledge, 1968; Hulka, 1970) have stressed the importance

of conisation. However, many studies have demonstrated residual carcinoma,
in-situ carcinoma, or atypia in post conisation hysterectomy specimens,
suggesting that the total extent of the disease in 10-30% of the cases
was not demonstrated or eliminated by the procedure (Silbar & Woodruff,
1966; Singleton & Rutledge, 1968).

Conisation demands hospitalization and a general anaesthesia, and
is occasionally complicated by post operative bleeding and late cervical
dysfunction. If procedures less traumatic than conisation can determine
accurately the presence and extent of the neoplastic process, the more
extensive operative procedure with its associated hazzards could be greatly
reduced (Christopherson et al, 1967; Sabatelle et al, 1969; Channen &
Hollycock, 1971). Hence, the need for a search for other investigative
techniques.

Although the literature has stressed the value of colposcopy as
an important diagnostic and easy technique (Navratil et al, 1958),
there has been no emphasis on its efficacy in this country.
It is important to stress the accuracy of this as a diagnostic tool,
especially when complementary to other methods (Thomson et al, 1972).
Although the personnel trained to perform colposcopy are not available
in many centres, this is no justifiable excuse to continue to perform
a procedure that is hazzardous while the course of the disease progresses.
Such excuses were made over 25 years ago to justify the lack of
cytopathologic studies, but certainly may not be acceptable as of today.

A planned approach to the study of the patient with atypia of the
cervix on cytology would, to a major degree, eliminate the all too
common surgical conisation of the cervix which harbours invasive cancer.

The latter situation is often the result of advice from the pathologist
who directs the investigative process by suggesting "conisation" since
"the patient has a Class IV smear". The initial step in such a study
of atypia may not be conisation, but colposcopy.

CHAPTER 5

SEROEPIDEMIOLOGIC STUDIES OF HERPES TYPE 2 VIRUS
IN IBADAN

5.1 Introduction

Since the initial observation by Dodd et al (1938) on gingivosto-
matitis as a frequent manifestation of primary herpetic infection in
children, the distribution of herpes virus antibodies according to age
in various social groups has been shown by other workers (Rawls et al,
1969; Nahmias et al, 1970(c)). These studies have shown that in the
lower socio-economic populations, the incidence of herpes virus anti-
bodies rises fairly steeply up to 50-70% between the ages of 1 and 14
years, after which there is a more gradual increase approaching 100% by
late adulthood (Nahmias et al, 1970(c)). Other workers have found that
the increase in incidence after childhood is greatest during adolescent
and young adulthood (Smith et al, 1967).

Until the distinction was made between Herpes type-1 (HT-1) and
Type 2 (HT-2) viruses, it was generally assumed that the postpubertal
increase in antibodies was simply indicative of late acquisition of
the same herpetic infection found to be prevalent in children. Recent
serologic studies differentiating HT-1 and HT-2 virus antibodies
(Dowdle et al, 1967; Ejercito et al, 1968; Nahmias & Dowdle, 1968;
Rawls et al, 1968; Figueroa & Rawls, 1969; Plummer et al, 1970;

Geder & Skinner, 1971; Lowry et al, 1971; Nahmias et al, 1971(d); Josey
et al, 1972; Jeansson et al, 1972) have contributed quite significantly
to our knowledge of Herpes virus infections and the distinctive
epidemiologic features of the two types of Herpes virus Hominis, as
distinct from other human Herpes viruses (Herpes Zoster, Epstein Barr
Virus).

Herpes Type 1 (HT-1) viruses are associated primarily with most
non-genital herpetic infections such as herpes labialis, gingivosto-
matitis, kerato-conjunctivitis and encephalitis. Herpes type2 (HT-2)
viruses, on the other hand, are associated primarily with genital
infections in both males and females. However, this difference in
sites of infection is not absolute (Nahmias et al, 1968(b); Josey et
al, 1969; Nahmias et al, 1969(d); Rawls et al, 1971).

In a sociologic study of a group of 239 individuals representing
all ages in a predominantly low socio-economic population (Nahmias et al,
1970), it was found, by the application of neutralisation test for
differentiation of herpes virus antibodies, that after the newborn age
group (0-6 months) in which transplacental antibodies may be present,
HT-2 virus antibodies were present only in sera of individuals beyond
the age of 14 years. Similar results had been obtained earlier by
Rawls et al (1969) using kinetic neutralisation test. Thus, although
instances of HT-2 virus infection have been noticed in younger children,
some of whom admitted to sexual exposure (Nahmias et al, 1968(b)), it
would appear that the infection involves, primarily, individuals
beyond the age of puberty.

Since it has been shown that the genital region is the usual or

natural habitat of HT-2 virus, it is essential that the manner in which
the virus is spread be positively established. The supposition that
genital herpes might be a venereal contagion dates back to the 19th
century (Hutfield, 1966), before a viral causation of the infection
was recognised. It has been shown also that the pre-dilection of
genital herpes for the genitalis is a fundamental biologic characteristic
of micro-organisms having an essentially venereal mode of transmission
(Josey et al, 1972). It is widely accepted (Gardner & Kaufman, 1969;
Josey et al, 1969; Teokharov, 1969) that several infectious diseases
other than the classical venereal diseases are transmitted through
sexual contact. These include Trichomoniasis, Hemophilus vaginalis
vaginitis and genital mycoplasmata.

Recently, with demonstration that genital herpes virus strains
are serologically distinct from those commonly affecting other sites,
a firm micro-biologic basis was established for the concept of venereal
mode of transmission of HT-2 virus (Hutfield, 1966; Josey et al, 1968;
Hutfield, 1968; Rawls et al, 1971; Josey et al, 1972; Duenas et al,
1972; Adelusi et al, 1975(b)). The overall incidence of HT-2 virus
antibodies in lower socio-economic groups was found to be of the order of
20-30% (Nahmias et al, 1969(d)), an incidence which is three to four
times that for the higher socio-economic groups (Rawls et al, 1971).
In some segments of the lower socio-economic population with
characteristically high rates of sexual promiscuity, such as residents
of certain low income housing projects (Conger et al, 1972), the incidence
of the virus was found to approach 50%. Of further epidemiologic
significance was the finding of Rawls et al, (1969) that 54% of

prostitutes studied had serologic evidence of prior HT-2 virus infection,
whereas, Nahmias et al (1970) found that only one (2.9%) of 35 nuns had
serologic evidence of the virus exposure. The above, no doubt, is in
support of the venereal mode of transmission of HT-2 virus.

The question of the venereal mode of transmission of HT-2 virus
is of theoretical significance in relation to the potential threat to
the foetus (Naib et al, 1970; Nahmias et al, 1971; Nahmias et al, 1971(b))
and the neonate (Nahmias et al, 1967; Nahmias et al, 1969(e); Nahmias
et al, 1970(d); Zavoral et al, 1970; Nahmias et al, 1971; Nahmias et al,
(1971(b)) when this virus infects the reproductive tract during pregnancy.
It is also of interest in relation to the genital Herpes-cervical cancer
etiologic relationship hypothesis (Naib et al, 1966; Josey et al, 1968;
Naib et al, 1969; Rawls et al, 1969; Melnick & Rawls, 1970; Nahmias
et al, 1970; Spreecher-Goldberger et al, 1970; Royston & Aurelian, 1970;
Nahmias et al, 1970(b); Nahmias et al, 1971(b); Catalano & Johnson, 1971;
Adelusi et al, 1975).

In the present study, therefore, seroepidemiologic survey of the
prevalence of HT-2 virus antibodies was undertaken in different age and
social groups of the population, using the complement fixation test.
In addition, serologic evidence of the venereal mode of transmission of
the virus in Nigerians is presented as determined by the complement
fixation and immuno-fluorescence tests.

5.2 Materials and Methods

5.2.1 Study Groups: The study groups included the following:

(a)   One hundred healthy school children from a public school in
      Ibadan.

(b) One hundred and one gynaecologic patients who were seen in the out-patients department of the Hospital with complaints unrelated to cervical pathology.

(c) One hundred and two pregnant women attending Ante-natal clinic of the Hospital for routine care of normal pregnancy. Gestational ages of their pregnancies ranged from 20 to 36 weeks.

(d) Fifty-five women who were known professional prostitues, residing and earning their living by prostitution in hotels and restaurants in Ibadan.

(e) Thirty male patients who were seen in the Venereal Diseases (V.D.) clinic of the Hospital with clinical evidence of gonococcal urethritis or syphilis.

(f) As healthy controls, one hundred and one healthy sympthomless women who were seen in the Family Planning Clinic of the Hospital with problems unassociated with pelvic pathology were chosen.

Serum samples were collected from each individual in the study groups. The collection of blood samples, separation and storage of sera were as described in Chapter 3.

5.2.2 Virus Stock: Herpes type-2 (HT-2) virus (strain unspecified) was kindly supplied through Professor Fabiyi of the Virus Research Laboratory, University of Ibadan, by Mr. Vincent Monroe (Micro-biological Associates, Bethesda, Maryland, U.S.A). It has gone through several passages before being sent to Ibadan in MA-196 (Human Adult Skin). It was further passaged in the Virus Research Laboratory, Ibadan, in various cell lines and

different culture media before finally settling for monolayer
cultures of Vero (African Green Monkey Kidney) cells in Eagle's
(1955) Minimal Essential Medium (MEM) supplemented with 10%
Fetal Calf Serum. When the cell culture has become confluent, the
growth medium was replaced with maintenance medium (MEM supplemented
with 3% Fetal Calf Serum). The 50% Tissue Culture Infective Dose
($TCID_{50}$) of the virus was then determined.

Determination of $TCID_{50}$ of Virus: The determination of the 50%
Tissue Culture Infective Dose ($TCID_{50}$) of the virus was by
harvesting the HT-2 virus infected vero cells with the aid of rubber
policeman when cells have shown evidence of cytopathic effect (CPE).
The cells were sonicated to break these and release the virus
contents. Titration of the virus from 10^{-1} through 10^{-8} was done,
using Hanks BSS solution as diluent. 0.2ml of each dilution was
then innoculated into tissue culture tubes (of vero cells) containing
approximately 4×10^{6} cells, using 4 tubes per dilution. The
innoculated tubes were incubated at $37^{o}C$, and examined daily for
5 days to determine the 50% tissue culture infective dose ($TCID_{50}$)
of the virus stock, using the Reed and Muench (1938) method.

5.2.3 Complement Fixation Tests: All the serum samples were coded and
tested blind. Complement fixation (CF) tests were performed in
plastic plates, using the modified micro-titer technique of
Sever (1962), and that of the Yale Arbovirus Research Unit (YARU).

(a) Sensitisation of Sheep Red Blood Cells: Sheep red blood cells
(SRBC) were used as complement fixation (CF) indicator for the study.

The whole blood was collected in an anticoagulant (Alsever's solution) and washed twice in saline and once in Veronal Buffer (pH. 7.2). The cells were then suspended as 4% solution in veronal buffer and kept at 4°C until used. The Formula for each of the situation is as follows:-

(i) Alsever's Solution

Dextrose	20.5g.
Na Cl	4.20g.
Citric Acid	0.55g.
Sodium Citrate	8.00g.
Distilled H_2O q.s. ad	1000 ml.

Sterilised by autoclaving for 10 minutes at 10 lb. pressure.

(ii) Veronal Buffer (VB).

Oxoid CFT diluent tablet	1 tablet
Hot Distilled H_2O	100 ml.

(iii) Formula for Oxoid CFT diluent tablet

	grams per litre
Barbitone	0.575
Sodium Chloride	8.500
Magnesium Chloride	0.168
Calcium Chloride	0.028
Barbitone Soluble	0.185

(b) <u>Titration of Complement:</u> Fresh or reconstituted lyophilised guinea pig serum was the source of complement for this study. This was diluted 1:30 and 8 master tubes were set up as shown below:

Tube No.	1	2	3	4	5	6	7	8
1:30 complement	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8ml
Veronal Buffer	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2ml

Titration tubes were set up, and 0.2 ml of the mixture was transfered from each master tube into the corresponding titration tube:

	1	2	3	4	5	6	7	8
Complement	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2mls
Veronal Buffer	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1mls
Sensitised Cells	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1mls
Final Vol. of 1:30 Complement in tube	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08mls

The titration tubes were incubated at 37°C in waterbath for 30 minutes after which the titration was read. The tube showing complete or almost complete hemolysis was taken as the end point containing 1 unit of complement. Formula for calculating the correct dilution to give two units of complement is as follows:

<u>Reciprocal of original dilution of complement</u> X 0.1 (Standard
Twice amount of complement in tube   Titration Volume)

(c) <u>Titration of Hemolysin:</u> Hemolysin, an anti-sheep red blood cell serum, generally used to sensitise sheep red blood

cells was used at 4 to 6 mean hemolytic doses (MHD). The dilution of hemolysin was carried out as shown below:

Titration of Hemolysin

	1 vol. neat serum. 9 vols. diluent (1/10)		1 vol. of 1/10 9 vols. of diluent (1/100)				2 vols. of 1/100 18 vols. of diluent (1/1000)						
Vols. of Master Dilution	2	1	2	1	1	1	2	2	2	2	1	1	1
Vols. V.B. Diluent	0	4	0	1	3	7	0	1	2	3	2	3	4
Final Dilution	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1000}$	$\frac{1}{1500}$	$\frac{1}{2000}$	$\frac{1}{2500}$	$\frac{1}{3000}$	$\frac{1}{4000}$	$\frac{1}{5000}$

Titration

1 volume of Hemolysin Dilutions

1 volume 4% Sheep Cells.

From each dilution, 1 volume of hemolysin was added to 1 volume of 4% SRBC and incubated at $37^{o}C$ for 15 minutes. The series 1:10 to 1:800 were removed to another rack and left to stand at room temperature ($25^{o}C$) to observe agglutination. This should not occur in 6MHD of hemolysin, and preferably should not exceed 1:100 dilution of hemolysin.

To the series 1:1000 to 1:5000 was added 1 volume of diluent and 1 volume of 1:10 complement and incubated at $37^{o}C$ for 30 minutes. The highest dilution showing complete hemolysis was taken as 1 MHD of hemolysin.

(d) The Test: Sera were diluted 1:4 with veronal buffer (pH 7.2), inactivated at $56^{\circ}C$ for 30 minutes and further diluted in increasing 2-fold dilutions. The antigen used in the study was the undiluted HT-2 virus infected vero cells ($TCID_{50} = 10^{-5}$) which had been sonicated prior to use. The sera were distributed into appropriate wells in plates with a microtiter dropper (0.025 ml per drop). One drop of complement containing 2 units was added to each well. The antigen was then distributed into the appropriate wells. Positive and negative sera controls were set up in all the tests. Anticomplementary controls, (serum with diluent) were also included for each serum. The complement unitage was titrated in a final titration, along the test proper by adding 2 drops of complement from original master tubes to 1 drop of diluent in each well.

The plates were incubated at $37^{\circ}C$ for 1 hour; and sensitised SRBC were added, 1 drop per well. The plates were re-incubated at $37^{\circ}C$ for 30 minutes, shaking at 10 minute intervals. They were then left at $4^{\circ}C$ overnight to allow cells to settle before reading the tests. The reciprocal of serum dilution giving 4+ (no hemolysis) or 3+ (25% hemolysis) was taken as positive. Readings of 2+ (50% hemolysis) and less were taken as negative.

5.2.4 Immunofluorescence Tests: The indirect method of staining was used.

(a) Preparation of Smears: The HT-2 virus infected monolayer cultures of vero cells were harvested when more than 75% of the cells have shown CPE ($TCID_{50} = 10^{-5}$). Harvesting was by stripping the cells off the bottle with the aid of a rubber policeman. The

cells were washed three times with phosphate buffered saline
(pH 7.2) and the concentration adjusted to about 0.6×10^6 cells
per ml in the solution. Drop smears of the suspended cells were
made on clean microscope slides (size 75 x 25 mm, thickness
0.8 - 1.0 mm). The smears were air-dried, fixed in cold acetone
(4°C) for 10 minutes and stored in the cold (4°C) until used.
(usually between 1 day and 2 weeks).

(b) Immunofluorescence Staining: Sera were serially diluted with
phosphate buffered saline (PBS, pH 7.2) in 2-fold step from
1:10 dilution. The smears were washed in PBS for 10 minutes,
cleaned and then reacted with 2 drops of the diluted sera for
30 minutes in a moist chamber. After washing off excess sera,
smears were left in PBS for 10 minutes, cleaned again and
stained with 2 drops of 1:20 dilution of fluorescein isothio-
cyanate conjugated (FITC) goat anti-human globulin (Microbiolo-
gical Associates, Bethesda, Maryland, U.S.A.), for 30 minutes in
a moist chamber. After washing off excess conjugated anti-serum,
the smears were left in PBS for 30 minutes. These were cleaned,
mounted with 90% glycerine and sealed with nail vanish. Positive
and negative sera controls as well as controls for non-specific
staining by fluorescin were set up in all the tests.

(c) Microscopic Examination: The final preparation was examined
within 12 hours with a Reichart Fluorescent Microscope fitted
with an Osram HB 200 mercury vapour lamp. The presence of antibody
was recorded according to the degree of yellowish-green fluorescence
(mainly cytoplasmic) present in the cell. Scoring ranged from double

positive (++) to negative (–) when compared with negative control smears stained only with FITC goat anti human globulin. The titre of serum was taken as the reciprocal of the dilution at which fluorescence was not observed.

(d) <u>Phosphate Buffered Saline:</u> The composition of the Phosphate Buffered Saline was as follows:-

    (i) <u>Stock Solution:</u>

        1.5M sodium chloride (10 x 0.9% NaCl).

NaCl	87.675g.
Distilled H_2O qs. ad	1000 ml.

        2.0M monobasic sodium phosphate

Na $H_2PO_4H_2O$	276.02 g
Distilled H_2O qs. ad	1000 ml.

Adjusting diluents for addition of cell suspensions were prepared by combining stock solutions of 0.15 M $NaCl-0.2M Na_2 HPO_4$ and $0.15M NaCl-0.2M NaH_2PO_4$ as shown in the table of pH values.

    (ii) <u>Solution a A</u>

0.15 M	NaCl – 0.2M	Na_2HPO_4	
0.5 M	NaCl	100 ml.	
2.0 M	Na_2HPO_4	100 ml.	
Distilled Water		800 ml.	

(iii) Solution B

      0.15 M   NaCl - 0.2M NaH_2PO_4

      0.15 M   NaCl   100 ml.

      2.0 M NaH_2PO_4 100 ml.

      Distilled water   800 ml.

(iv) Phosphate Buffered Saline (PBS) pH 7.2

    72.0 ml of solution A plus 28.0 ml of solution B.

Phosphate Buffered Saline (PBS) of different pH.

Tables of Values

Final pH	Solution A 0.15M NaCl 0.2M Na_2HPO_4	Solution B 0.15M NaCl 0.2M NaH_2PO_4
5.75	3.0 ml	97.0 ml
6.00	12.5	87.5
6.20	22.0	78.0
6.40	32.0	68.0
6.60	45.0	55.0
6.80	55.0	45.0
7.00	64.0	36.0
7.20	72.0	28.0
7.40	79.0	21.0

5.2.5 Determination of "Cut-off" points:

Based on the fact that HT-2 virus antibodies neutralise HT-2 better than HT-1 viruses (Roizman et al, 1970), the determination of "cut-off" points for the presence of "significant" HT-2 virus antibodies was made at the titre where significant

differences occured between the carcinoma cases and controls,

when the cummulative distribution of antibody titres was calculated.

More often, this titre corresponded with the peak of antibody titres

in the carcinoma groups.

5.3 Results

5.3.1 Antibodies by CFT:

The distribution of HT-2 virus antibodies by titre as assayed

with complement fixation test (CFT) in the various groups studied is

shown in Figure 5.1. On the whole, titres varied from less than 1:4

to 1:64 and there was no significant $(P > 0.25)$ association in the

distribution of antibodies and study groups (Table 5.1).

When the cummulative distribution of the positive sera was

determined (Table 5.2), it was found that the prostitues and male

patients attending the venereal diseases clinic showed higher antibody

titres, especially from titre of 1:8 and above when compared with

pregnant women, patients attending the gynecology clinic, school

children and healthy controls.

Using the titre of 1:8, therefore, as a cut-off point, Table 5.3

shows the number in each group showing significant positive reaction

for HT-2 virus antibodies according to age groups. The age distribution

among the prostitues could not be determined as data on this were not

available. However, excluding the prostitutes, it was found that

antibodies were absent in all the sera from individuals below the age

of 10 years. Of the 3 positive antibody reaction in the age group

11-15 years (who were among the school children) the sera were from

two girls each 14 years old and the third was 15 years old. Then there

Figure 5.1

Distribution of Herpes Type-2 antibodies assayed
by CFT in sera of groups of patients and healthy controls.

Distribution of Herpes Type-2 antibodies in sera of
the groups of patients in the study by CFT.
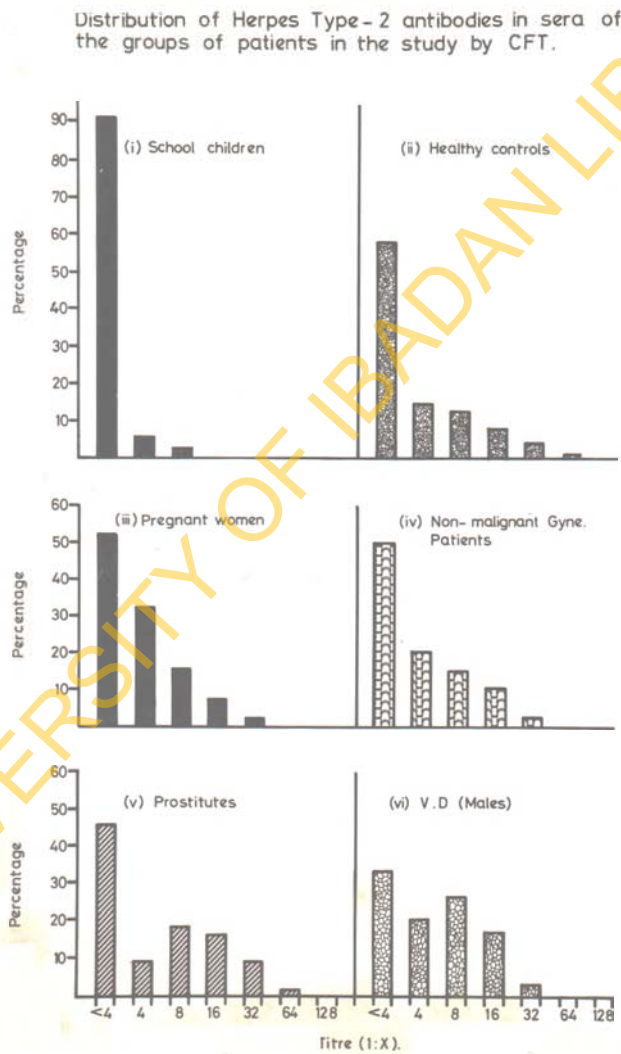
TABLE 5.1

HT-2 virus antibodies assayed by CFT in various study
groups in Ibadan.

Study groups

HT- 2 antibody Titre (1:X)	School Children	Gynec.* patients	Preg.+ women	Prosti- tutes	V.D.x (males)	Healthy controls
< 4	91	51	53	25	10	59
4	6	21	23	5	6	15
8	3	16	16	10	8	13
16	0	11	8	9	5	8
32	0	3	2	5	1	4
64 +	0	0	0	19	0	2
Total	100	102	102	55	30	101

Study Groups Vs HT-2 antibody titre:

$$x^2 = 22.41 \text{ on } 20 \text{ df } P > 0.25$$

* Gynecology Clinic patients

+ Pregnant women

x Venereal Diseases (male) patients

TABLE 5.2

Cummulative distribution of antibody titre in each group
showing positive reaction up to stated titre by C.F.T.

Study groups

HT-2 antibody Titre (1:X)	School children		Gynecology patients		Pregnant women		Prostitutes		V.D. (Males) *		Healthy controls	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
< 4	100	100.0	102	100.0	102	100.0	55	100.0	30	100.0	101	100.0
4	9	9.0	51	50.0	49	48.0	30	54.5	20	66.7	42	41.6
8	3	3.0	30	29.4	26	25.5	25	45.5	14	46.7	27	26.7
16	0	0.0	14	13.7	10	19.8	15	27.3	6	20.0	14	13.9
32	0	0.0	3	2.9	2	2.0	6	10.9	1	3.3	6	5.9
64+	0	0.0	0	0.0	0	0.0	1	1.8	0	0.0	2	2.0

*Venereal Diseases (Male) Patients

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TABLE 5.3

Age distribution in each group showing significant
levels of HT-2 virus antibodies by CFT.
(Titre 1:8 and above)

Age groups (Years)	School children		Gynecology patients		Pregnant women		Prostitutes		V.D. (Males) *		Healthy controls	
	Total	Posi-tive	Total	Posi-tive	Total	Posi-tive	Total	Posi-tive	Total	Posi-tive	Total	Posi-tive
1 - 5	15	0	0	0	0	0	?	?	0	0	0	0
6 - 10	45	0	0	0	0	0	?	?	0	0	0	0
11 - 15	40	3	0	0	0	0	?	?	0	0	0	0
16 - 25	0	0	11	3	11	4	?	?	6	2	2	0
26 - 35	0	0	32	9	41	11	?	?	17	9	32	11
36 - 45	0	0	42	13	26	5	?	?	7	3	50	13
46 - 55	0	0	14	4	24	6	?	?	0	0	17	3
>55	0	0	3	1	0	0	?	?	0	0	0	0
Total	100	3	102	30	102	26	55	25	30	14	101	27

? = Age not determined

* Venereal disease (male) patients.

was a rapid increase in the incidence of antibodies between the ages of 16 and 25 years after which there was only a slight increase with a peak in the age group 26-35 years (Table 5.4). However, on the whole, only 22.9% of the study groups showed significant HT-2 virus antibody (titre above 1:8) by CFT.

Table 5.5 shows that HT-2 virus antibodies were present above a titre of 1:8 in 25 (45.5%) of 55 prostitutes and 14 (46.7%) of 30 male patients attending the Venereal Diseases Clinic, whereas the antibodies were present in only 27 (26.7%) of 101 healthy controls, 26 (25.5%) of 102 normal pregnant women, 30 (29.4%) of 102 gynaecologic patients and 3 (3.0%) of 100 school children. The percent positive among the prostitutes and the Venereal Diseases Clinic patients is significantly (P< 0.05) higher than for the other groups.

Even when the presence of significant antibody level was determined at a higher titre of 1:16 (Table 5.6), the findings were still similar, showing that the prostitutes and V.D. (male) patients differ significantly (P< 0.001) from the healthy controls and the others by having higher HT-2 virus antibodies.

5.3.2 Antibodies by Immunofluorescence method:

Using immunofluorescence technique for the determination of the HT-2 virus antibodies in the sera of prostitutes and healthy controls, it was found that antibodies were present in all subjects up to a titre of 1:20. The distribution of HT-2 virus antibody varied from a titre of 1:40 to 1:2560 (Table 5.7) and there was a significant association between titre level and patient group (P< 0.001). There was only one peak at a titre of 1:80 among the healthy controls whereas

TABLE 5.4

Age distribution in all groups showing significant
HT-2 virus antibodies by C.F.T.
(Titre 1:8 and above)

Age groups (Years)	Total No.* Tested	No. * significant	%
1 - 5	15	0	0
6 - 10	45	0	0
11 - 15	40	3	7.5
16 - 25	30	9	30.0
26 - 35	122	40	32.9
36 - 45	125	34	27.2
46 - 55	55	13	23.6
> 55	3	1	33.3
Total	435	100	22.9%

* Excluding the Prostitutes whose ages were not
determined.

TABLE 5.5

Number of subjects in each group showing
positive reaction at titre 1:8 and above by C.F.T.

Study groups

Titre	School children	Gynae.* patients	Preg.+ women	Prostitutes	V.D.x (Males)	Healthy controls
Positive	3	30	26	25	14	27
Negative	97	72	76	30	16	74
Total	100	102	102	55	30	101
% Positive	3.0	29.4	25.5	45.5	46.7	26.7

Prostitutes + V.D.(Males) Vs Other study groups:

$$x^2 = 46.14 \quad \text{on} \quad 5 \quad df \quad P < 0.05$$

* Gynecology Clinic Patients

+ Pregnant women.

x Venereal diseases (male) patients.

TABLE 5.6

Number of subjects in each group showing positive
reactions at titre 1:16 and above by C.F.T.

	Study group					
Titre	School children	Gynae.* patients	Preg.+ women	Prostitutes	V.D. x (Males)	Healthy controls
Positive	0	14	10	15	6	14
Negative	100	88	92	40	24	87
Total	100	102	102	55	30	101
% Positive	0.0	13.7	9.8	27.3	20.0	13.9

Prostitutes + V.D. (males) Vs Other study groups:

$$x^2 = 28.60 \text{ on } 5 \text{ df} \quad P < 0.001$$

* Gynecology Clinic patients

+ Pregnant women

x Venereal Diseases (Male) Patients.

TABLE 5.7

Distribution of HT-2 antibodies assayed by Immunofluorescence
test in Prostitutes and controls.

HT-2 antibody Titre (1:X)	Study groups	
	Prostitutes	Controls *
< 40	0	0
40	6	3
80	7	19
160	4	10
320	5	8
640	10	6
1280	6	0
2560+	4	0
Total	42	45

* From the Healthy controls.

Study Groups   Vs   HT-2 antibody titre:

$$x^2 = 21.39 \text{ on } 6 \text{ df } P < 0.001$$

Figure 5.2

Distribution of Herpes Type-2 virus antibodies
assayed by Immunofluorescence Tests in sera of Prostitutes
and Healthy controls.

Distribution of Herpes Type-2 virus antibodies
in sera of Prostitutes and Healthy Controls
by The Immunofluorescence Test.
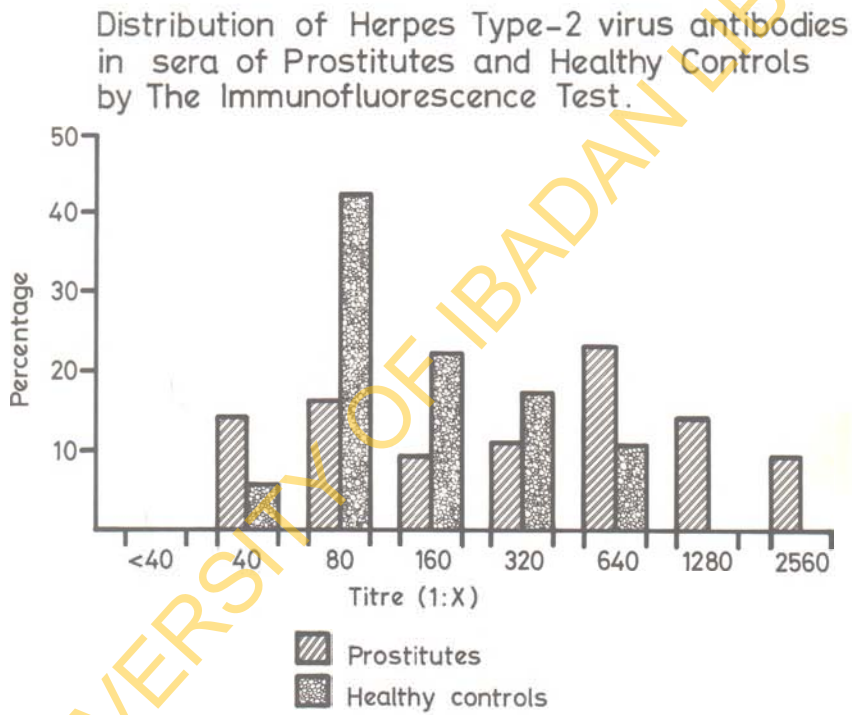
there were two peaks among the prostitutes, a lower one at a titre of 1:80 and a second (higher) peak at a titre of 1:640 (Fig. 5.2).

When the cummulative distribution of the positive titres, using the immunofluorescence technique, is determined (Table 5.8), it was found that the prostitutes showed higher antibody titres, especially from 1:320 and above as compared with controls. Using the titre of 1:640 which was the second peak in the titre of antibody among the prostitutes, as the cut-off point for significant antibody levels, it was found that HT-2 virus antibodies were present above this titre in 20 (47.6%) of 42 prostitutes whereas, these were present in only 5 (11.1%) of healthy controls (Table 5.9). The difference was statistically significant (0.005 < P < 0.001).

Even when the presence of significant HT-2 virus antibodies was determined at a lower titre of 1:320, which was the level at which difference became noticeable between the prostitutes and controls, the results obtained were still similar, (Table 5.10) showing once again the significant difference(P < 0.001) in the antibody levels among the prostitutes and healthy controls.

5.4 Discussion

Although Herpes virus Hominis (HVH) was long regarded as antige-nically homogeneous, immunologic analysis of strains isolated from a large number of patients has shown that there are two major serotypes: Type-1 (HT-1) and Type-2 (HT-2) viruses. The important observation was made by Nahmias and his co-workers (Nahmias et al, 1967(b); Dowdle et al, 1967; Nahmias & Dowdle, 1968) that HT-2 virus strains were most often associated with genital infections.

TABLE 5.8

Cummulative distribution of antibody titre
in Prostitutes and Healthy controls showing positive
reaction up to the stated titre by Immunofluorescence test.

HT-2 antibody Titre (1:X)	Study groups			
	Prostitutes		Healthy controls	
	No.	%	No.	%
< 40				
40	42	100.0	45	100.0
80	36	85.7	42	93.3
160	29	69.0	23	42.2
320	25	59.5	13	28.9
640	20	47.6	5	11.5
1280	10	23.8	0	0
2560+	4	9.5	0	0

TABLE 5.9

Number of subjects among Prostitutes and Controls
showing positive reactions at titre. of 1:640 and
above by Immunofluorescence.

Study groups

Titre	Prostitutes	Healthy Controls
Positive	20	5
Negative	22	40
Total	42	45
% Positive	47.6	11.1

Prostitutes   Vs   Healthy controls:

$$X^2 = 14.14 \text{ on } 1 \text{ df } P < 0.001$$

TABLE 5.10

Number of subjects among Prostitutes and Controls
showing positive reaction at titre of 1:320 and above
by Immunofluorescence.

Titre	Study groups	
	Prostitutes	Healthy Controls
Positive	25	13
Negative	17	32
Total	42	45
% Positive	59.5	28.9

Prostitutes Vs Healthy Controls:

$$X^2 = 8.29 \text{ on } 1 \text{ df } 0.005 > P > 0.001$$

Apart from the differences in the anatomic site of infection, the two serotypes of Herpes virus Hominis have been found to differ in their virulence for experimental animals (Plummer & Hackett, 1966; Alford et al, 1967; Nahmias & Dowdle, 1968; Plummer et al, 1968; Figueroa & Rawls, 1969), and their behaviour in embryonated eggs (Nahmias et al, 1968(d); Couch & Nahmias, 1969) and cell cultures (Rawls et al, 1968; Ejercito et al, 1968; Figueroa & Rawls, 1969; Stubbs et al, 1971). They also differ in their biochemical and biophysical properties (Goodheart et al, 1968; Schwartz & Roizman, 1969; Gerder & Skinner, 1971; Ratcliffe, 1971; Docherty et al, 1971; Thoules & Skinner, 1971). Recent studies differentiating HT-1 and HT-2 virus antibodies (Nahmias et al, 1969; Lowry et al, 1971; Nahmias et al, 1971(d); Josey et al, 1972; Jeansson et al, 1972; Sim & Watson, 1973), have contributed significantly to our knowledge of Herpes Virus infections and the distinctive epidemiologic features of the two types of Herpes Viruses.

The distribution of HT-2 virus in various age groups has been studied by several workers, using various modifications of the neutralisation and complement fixation tests (Smith et al, 1967; Rawls et al, 1969; Nahmias et al, 1970(c); Royston & Aurelian, 1970). The distribution of HT-2 virus antibodies as determined by CFT in this study was similar to that found by other workers for the lower socio-economic classes. Antibodies to the virus were not found in any group below the age of 14 years (11-15 age group). This is in consonant with the findings of Rawls et al (1969), who found antibodies in the age group 13-16 years.

However, the earliest age of detection of HT-2 virus antibodies in this study was lower than in the findings of Nahmias et al (1970) where the earliest incidence was in the age group 15-19 years, and Royston & Aurelian (1970) where the earliest incidence was in the age group 15-24 years. This differences in the age of onset of HT-2 virus infection and the percentages of those positive for HT-2 virus antibodies in each age group in this study and those of others (Rawls et al, 1969; Nahmias et al, 1970; Royston & Aurelian, 1970), could be due to the fact that the Nigerian population studied is genuinely different from the populations studied elsewhere, especially with regards to coital practice. On the other hand, the differences may be due to the different methods adopted in the tests, as well as the interpretations given to the results in each study.

The percentage in each age group with significant HT-2 virus anti-bodies was in the range of 20 to 30, with gradual rise from the 16-25 year age group to a peak at the 26-35 year age group. After this, there was a fall. The rapid increase in the proportion of persons with anti-bodies between ages 16 and 35, and the relative stable proportions of people positive thereafter is compatible with an increased susceptibility to infection during adolescence and early adulthood. The number of sera examined, however, may not be large enough to conclude statistically that the appearance of antibodies in the different age groups is as depicted in the study. However, if this represents the true occurence of antibody, the sexual habbits of the different age groups could also account for the rapid acquisition of antibody in the second decade of life.

Serologic evidence of the venereal mode of transmission of HT-2 virus:

The question of the manner in which HT-2 virus strains are spread has always been one of importance, especially when one considers the potential threat to the fetus (Naib et al, 1970; Nahmias et al, 1971; Nahmias et al, 1971(b)) and the neonate (Nahmias et al, 1967; Nahmias et al, 1969(c); Nahmias et al, 1970(d); Zavoral et al, 1970; Nahmias et al, 1971; Nahmias et al, 1971(b)) when this virus infects the reproductive tract during pregnancy. A possible etiologic role of the virus in cervical dysplasia and carcinoma has also been suggested (Naib et al, 1966; Josey et al, 1968; Naib et al, 1969; Rawls et al, 1969; Melnick & Rawls, 1970; Nahmias et al, 1970; Nahmias et al, 1971(b); Adelusi et al, 1975).

In some instances, genital herpetic infections may lead to complications such as urethral stricture, labial fusion and lymphatic suppuration (Hutfield, 1968). HT-2 virus has been found in some cases of meningitis and ascending myelitis (Nahmias, 1972). Of particular theoretical interest is the virus infection in relation to the genital herpes – cervical cancer hypothesis which supposes that both conditions are venereally acquired (Josey et al, 1968; Rawls et al, 1969).

The feeling that genital herpes might be venereally transmitted has long been suspected (Hutfield, 1966) although it was not until recently, with the demonstration that genital herpes virus strains are serologically distinct from those commonly affecting other sites (Josey et al, 1972; Jeansson et al, 1972) that a firm microbiologic basis was established for the concept (Josey et al, 1968; Rawls et al, 1971; Duenas et al, 1972; Adelusi et al, 1975(b)).

In the present study, using both complement fixation and immunofluorescence tests, it was demonstrated that higher HT-2 virus antibody levels were prevalent among prostitutes than healthy controls. Prostitutes are women regarded as promiscuous in this environment by their exposure to frequent intercourse and with multiple male partners. Higher antibody levels were also prevalent among venereal diseases clinic (male) patients who are known to patronise these women more frequently.

In a study of the United States armed forces personal in Japan, Barile et al (1962) offered evidence for the venereal mode of transmission of HT-2 virus by showing that all their patients with penile herpes admitted to recent sexual exposure, whereas, the virus could not be demonstrated in other patients who had abstained from sexual intercourse for a minimum of 30 days prior to examination. Similarly, Nahmias et al (1969) found that 7 of 8 female contacts of 7 males with penile herpes had evidence of current genital HT-2 virus infection. Other workers (Slavin & Gavett, 1946) have reported occasional instances of the genital herpes in sexual contacts of infected consorts, and experimental studies in animals (Nahmias et al, 1967(b)) have provided further evidence to support this concept of venereal mode of transmission.

In this study, there was no attempt at questioning the school children as regards sexual intercourse, most especially, those three girls around puberty who were found to have significant HT-2 virus antibodies. However, since it has been shown that the virus antibodies appear in the population usually at an age when sexual activity begins (Rawls et al, 1968(b)), this possibility cannot be ruled out here too.

However, it should be noted that HT-2 virus infections have also been known to occasionally infect children of younger age groups (Nahmias et al, 1968(b)). It would not be surprising, therefore, to find occasional individuals below the age of puberty with the HT-2 virus antibodies.

Comparing the prostitutes and V.D. (male) patients with the other groups of women, especially the non-malignant gynecologic patients and the pregnant women, who are generally of the same age groups as the prostitutes, it was found that the prevalence of HT-2 virus antibodies among the V.D.(male) patients and prostitutes was much higher than in those of the latter two. All the study groups were of comparable age groups and are mainly from the lower socio-economic sector of the population. The only major difference between them was the sexual promiscuity of the former as compared with the latter. The data presented here therefore tends to lend further weight to the concept . of venereal mode of transmission of HT-2 virus.

The incidence of other venereal diseases such as gonococcal urethritis in prostitutes in this environment was 17% as compared with 5% in the general populace (Osoba, 1970). Its incidence among the primary acceptors in the family planning clinic (Onifade & Osoba, 1972) was 7%. It therefore follows from the result of the present study that the prevalence of HT-2 virus antibodies in the prostitutes and V.D. patients follow very closely, the pattern of venereal diseases among these patients as compared with controls. This can only go further to support the concept of venereal mode of transmission of HT-2 virus infection.

Other studies (Beilby et al, 1968; Jeansson & Molin, 1971; Nahmias

et al, 1973)   have demonstrated significant association of genital
herpes with other venereal infections. In the study of Beilby et al,
(1968), 7 of 8 women with asymptomatic cervical herpes had gonorrheal
infection, 2 had trichomoniasis, one had genital warts and one had
pediculosis pubis. In a group of 80 female venereal diseases clinic
patients with bacteriologically confirmed gonorrhea studied by
Nahmias et al (1973),   5% had positive viral cultures for HT-2
virus.

Similarly, Cederqvist et al (1970), in a study of 100 Sweedish
women complaining of vaginal discharge detected HT-2 virus cytologically
in 11, with virologic confirmation in 5. The incidence of trichomoniasis
and gonorrhea in those with the virus was almost twice that of the entire
group. Amstey & Balduzzi (1970) in another study of 15 women with
cytologically conformed HT-2 virus infection, found 7 cases of gonorrhea,
one of syphilis, and one of pediculosis pubis. Rawls et al (1971), in
a study of 52 cases of genital herpes among a higher socio-economic
population and 69 cases among a lower socio-economic group in Houston,
found an association with trichomoniasis, Hemophilus vaginalis vaginitis,
gonorrhea and condylomata acuminata; and the study of Dickie (1969)
showed similar findings. All these would tend to support the concept
that HT-2 virus is a venereal contagion.

HT-2 antibody in pregnant women:

It is pertinent here to compare the finding of HT-2 virus antibody
in pregnant women in this study with those of other workers, especially
when recent reports indicate that maternal HT-2 virus infection during
early and mid-pregnancy may carry the risk of fetal death or serious fetal

malformation (South et al, 1969). In the present study, the prevalence
of HT-2 virus antibodies among the pregnant women (25.5%) were found to
be very much similar to that among the healthy controls (26.7%). This
is in contrast to the findings of the studies in the United States
(Ng et al, 1970; Nahmias et al, 1971) where genital herpes have been
shown to be about three times commoner in pregnant women than in the
remainder of the population.

It is difficult to account for this difference in the study here
and those in the United States. It was not shown, for example, that
hormonal effects predispose the genital organs to provide ideal ground
for HT-2 virus replication in pregnant women in the United States, as
this would have been equally manifested in the pregnant women here.
The question of racial difference would be difficult to postulate either
(Wynder et al, 1954; Singer & Sharman, 1969). However, the difference
in HT-2 virus antibodies between the pregnant women in this study and
in the United States may be due to differences in customs which may
influence attitude to intercourse during pregnancy (and hence, HT-2
virus transmission).

From all the foregoing therefore, it can be concluded that the
appearance of HT-2 virus antibody in the population at an age when sexual
activity has been known to commence (Parker & Banatvala, 1967), and
as has been confirmed here by the presence of virus antibodies in the
age group 11-15 years, is in support of the venereal mode of transmission
of HT-2 virus. Similarly, the demonstration of higher percentage of
prostitutes and V.D. patients with HT-2 virus antibodies in this and
other studies (Rawls et al, 1971; Duenas et al, 1972; Adelusi et al, (1975(b))
only goes further to support the concept of venereal transmission of the virus

CHAPTER 6

IMMUNOVIROLOGIC STUDIES OF CARCINOMA OF CERVIX UTERI IN IBADAN

6.1 Introduction

The search for the cause or causes of cervical cancer has led to the implication of a venereally transmissible virus. This followed the observation of cellular changes suggestive of viral infection during cytologic screening of women for evidence of malignancy (Varga & Browell, 1960; Frost, 1961; Kotcher et al, 1962; Terris et al, 1967; Smith & Jenkins, 1969). It was Slavins & Gavett, (1946); Stein & Longo, (1963); Nigogosyan & Hill, (1965) Yen et al, (1965); however, who made the correlation of these cellular changes to a herpetic lesion.

Over the past two decades, a strain of herpes simplex virus, antigenically and biologically distinct from the Herpes Simplex virus associated with oral lesions was isolated from smegma samples and cervical lesions, and designated Genital Herpes or Herpes Type-2 (HT-2) virus (Barile et al, 1962; Ejercito et al, 1968; Nahmias & Dowdle, 1968; Rawls et al, 1968; Figueroa & Rawls, 1969; Plummer et al, 1970; Geder & Skinner, 1971; Lowry et al, 1971; Nahmias et al, 1971(d); Jennsson, 1972; Josey et al, 1972). This virus has now been shown to be venereally transmitted (Hutfield, 1967: 1968; Nahmias et al, 1968; Rawls et al, 1968; Nahmias et al, 1969; Rawls et al, 1971; Duenas et al, 1972; Adelusi et al, 1975(b)).

The first suggestion of a relationship between HT-2 virus and cervical cancer was made by Naib et al, (1966), when some of their patients who had biopsies of the cervix taken 1 to 6 weeks after the initial cytologic diagnosis of Herpes genitalis showed histologic evidence of acute cervicitis, squamous atypia and in-situ carcinoma. The high incidence of atypia or in-situ carcinoma in cases where cytologic studies showed herpetic cellular changes, prompted their suggestion that either a pre-existing cervical atypia invites a secondary viral infection or that genital herpes virus infections have oncogenic potentiality for the cervix.

Subsequently, however, other studies have been conducted to substantiate this association by showing that patients with pre-invasive and invasive carcinoma of the cervix have a significantly higher prevalence of HT-2 virus antibody than matched control populations, thereby suggesting a cause and effect relationship (Josey et al, 1968; Rawls et al, 1968(b); Naib et al, 1969; Nahmias et al, 1969(b); Rawls et al, 1969; Nahmias et al, 1970; Nahmias et al, 1970(b); Aurelian et al, 1970; Melnick & Rawls, 1970; Contifanto et al, 1971; Plummer & Masterson, 1971; Rawls et al, 1970; Rawls et al, 1970(b); Royston & Aurelian, 1970; Spreecher-Goldberger et al, 1970; Nahmias et al, 1971(b); Catalano & Johnson, 1971; Skinner et al, 1971; Rawls et al, 1973; Adelusi et al, 1975).

The relationship between HT-2 virus infection and subsequent neoplastic change in the cervix has been a subject of speculation, since the association has so far been based on circumstantial evidence. There is therefore need to establish more direct evidence that HT-2 virus is an etiologic factor or co-factor in carcinoma of the cervix by detecting

the HT-2 virus or viral genome in neoplastic cells. The basis for this
type of investigation is the concept that the normal cervix contains a
definite component of antigens (Hollinshead et al, 1972; Frenkel et al,
1972), and the metamorphosis which occurs in the cervical tissue as
it progresses from the normal, through a dysplastic state and carcinoma
in situ, to invasive carcinoma might be accompanied by the acquisition
of new antigenic substances (Fox, 1967; Richart, 1967; Richart & Barron,
1969; Hall & Walton, 1968; Johnson et al, 1968; Barron & Richart, 1971;
Hulka & Kupper, 1971), which may be related to an infective agent.

On the basis of this, Royston & Aurelian (1970) demonstrated
HT-2 virus antigens by immunofluorescence in exfoliated cells from
patients with cervical carcinoma. In contrast, no similar antigens
were detected in exfoliated cells from normal patients. Recently, other
workers have further demonstrated the presence of HT-2 related, associated
or induced antigens only in patients with carcinoma of the cervix
(Aurelian et al, 1971; Aurelian et al, 1973; Aurelian et al, 1973(b);
Feorino & Palmer, 1973; Gall et al, 1973).

Indeed, Aurelian et al (1971) succeeded in isolating from a line of
cervical tumour cells, a virus identical to HT-2 with respect to two
biologic properties (plaque morphology and microtubule formation in
infected HEp-2 cells) and immunologic specificity (as determined by
immunofluorescence and neutralisation tests). More recently, Chang et al
(1974) detected HT-2 virus antigens in human cervical cancer by anti-
complement immunofluorescence studies. The finding of HT-2 virus or
HT-2 virus related or induced antigens in human cervical cancer,
including that in which Frenkel et al (1972) found cancer possessing

HT-2 virus genome, provides further evidence of the close association between the virus infection and its oncogenic potential regarding the human cervix.

Carcinoma of the cervix is very common in Ibadan. Infact, this has been found to be by far the commonest female malignancy in this environment (Edington & Maclean, 1965). The cause of the high incidence of carcinoma of the cervix in this area is not known. Oral Herpes Simplex Virus is common in Ibadan (Fabiyi, 1972). To date, there has been no studies on the prevalence of HT-2 virus antibodies in this environment, nor on its relationship, if any, to cervical carcinoma.

Since a further direct evidence of a specific virus etiology of the tumour would be strengthened if (a) the same virus is associated with the tumour in different geographic areas all over the world, and (b) the virus is unassociated with other similar tumours in the same locality, the present study has been designed to examine the prevalence of HT-2 virus antibody in Nigerians who are known to have pre-invasive and invasive cervical cancer as well as those who have squamous cell carcinoma at sites other than the cervix, and other pelvic and extra pelvic malignancies, to determine whether these patients show any difference in the prevalence and titre of HT-2 virus antibody when compared with healthy controls. The HT-2 virus antibody status of carcinoma of the cervix patients were correlated with the grade and stage of the disease, as well as the histologic type of tumour. In addition, attempts were made to demonstrate the presence of HT-2 virus antigens in exfoliated cervical cells from women with cervical neoplasia, and normal healthy controls.

6.2 Materials and Methods

6.2.1 Study Groups: The study groups were made up of the following:

I. (a) One hundred and fourteen women who were seen in the Department of Gynaecology (U.C.H.) as described in Chapter 3.

(b) Thirty-six women with clinical and histologic diagnosis of cervicitis, cervical erosion or vaginal warts (Condylomata acuminata) as described in Chapter 3.

(c) As controls, one hundred and six women attending the Family Planning Clinic of the Hospital were chosen. These have no complaints associated with pelvic pathology.

II. (a) Forty male and female patients seen in the hospital with histologic diagnosis of squamous cell carcinoma at sites other than the cervix. These included carcinoma of the Esophagus, Larynx, Bronchus, Palate, Maxilla, Intra oral tumour, Nasal tumour, Eyelid, Mons Pubis, Urinary bladder and the Skin of the back (in an albino woman) and thigh.

(b) Thirty women of child bearing age who were seen in the Department and diagnosed as malignant trophoblastic disease (MTD) on the ground of high titre of Human Chorionic Gonadotrophins (HCG), and characteristic histologic and pelvic angiographic patterns.

(c) Twenty patients seen in the hospital with diagnosis of carcinoma of the liver on the basis of histology and detection of serum alpha-feto protein.

(d) Ten women with histologically proven diagnosis of carcinoma of the breast.

(e)   Six women seen in the Department with clinical and histologic diagnosis of carcinoma of the vulva.

(f)   Twenty other patients seen in the Gynaecology Department with histologic diagnosis of invasive carcinoma of the cervix.

(g)   Ten of the 106 healthy control from the Family Planning Clinic (in Section 1) were chosen as controls for this experiment.

III.(a)   Another group of twenty-four women from the Department of Gynaecology (U.C.H.) with the clinical diagnosis of carcinoma of the cervix, from whom cervical smears were obtained for cytologic studies.

(b)   And another group of twenty-four women seen in the Family Planning Clinic of the Hospital, from whom cervical smears were also collected for cytologic studies. These were used as controls for (a) above.

The patients in group III were specially selected to buttress the finding in Sections I and II of this Chapter. Sera were collected from each individual in the study groups. The collection of blood samples separation and storage of sera were as described in Chapter 3.

6.2.2   Preparation of Rabbit Antisera: 3 Rabbits were bled before immunisation, to obtain 10 mls of blood from each rabbit from an ear vein. The animals were then immunised by intravenous injection of 1 ml of ultraviolet-light attenuated HT-2 virus infected vero cells $(TCID_{50} = 10^{-5})$ once a week for three weeks. A week after the last injection, 10 mls of blood were obtained via the ear vein from each rabbit. After clotting, sera were separated from the blood by

centrifugation, absorbed twice with uninfected vero cells (2 mls of
sera to 1 ml of cells) to make these monospecific. These were stored
at 4°C until required for testing.

6.2.3   Preparation of Cervical Smears: Exfoliated cervical cells
were obtained from both malignant and normal cervices by means of Ayre's
Spatula, and smears were made from these on clean microscope slides
(size 75 x 25 mm, thickness 0.8 - 1.0 mm). The smears were air-dried,
fixed in cold acetone (4°C) and stored at 4°C until analysed (normally
within 7 days of collection).

May Grunwald-Giemsa Staining: A smear each from the patients in Section
III was examined by staining with May Grunwald-Giemsa Stain to detect the
abundance or otherwise of malignant cells in the smears prepared.   The
slides were stained in freshly prepared 1:1 dilution of May Grunwald Stain
for 5 minutes and counter-stained in Giemsa stain for 2 minutes.   The
final preparations were rinsed in water for 5 minutes before being
mounted with Zylene for microscopic examination.

6.2.4   Test Methods:

   i.   Virus Stock: HT-2 virus was treated as described in Chapter 5.

  ii.   Complement Fixation Test: All the processes for CFT were also
      as described in Chapter 5.

 iii.   Immunofluorescence Tests: Indirect immunofluorescence tests
      were performed on two different smear preparations.

  (a)   The preparation of smears from HT-2 virus infected vero cells,
      the staining and the examination of smears were as described
      in Chapter 5.

  (b)   The smears made from exfoliative cervical cells were stained

similarly except that instead of using human sera for the test, pre-immune or immune rabbit serum was used. Examination was done with the Reichart Fluorescent Microscope as described in Chapter 5.

6.3 Results

6.3.1 Prevalence of HT-2 Antibodies:

(i) By Immunofluorescence (IM) Technique:

Table 6.1 shows that all the women in the study had HT-2 virus antibodies in their sera up to 1:40 dilution. The distribution of antibodies in the different groups of women varied from 1:40 to 1:2560. There is an association between titre and patient group, the carcinoma cases having significantly ($P < 0.001$) more cases at titre 1:640 and over than the control and the cervicitis groups. The latter two groups showed only one peak at a titre of 1:80 each while there were two peaks for the carcinoma of cervix group: a low one at a titre of 1:80 and a second (higher) peak at a titre of 1:640 (Fig. 6.1).

Table 6.2 shows the cumulative distribution of HT-2 virus antibody in each study group showing positive reactions up to the stated titre. The carcinoma of cervix group showed higher antibody titres especially from a titre of 1:640 and above, when compared with the other groups (cervicitis and healthy controls).

The prevalence of antibody titre in the carcinoma of cervix and cervicitis groups according to the clinical appearance of the cervix (Table 6.3) indicates a significant ($P < 0.001$) association between antibody titre and clinical appearance of the cervix. Most of the women

TABLE 6.1

Incidence of HT-2 virus antibody in sera of women with
Carcinoma of cervix, Cervicitis and Healthy controls.

HT-2 antibody* Titre (1:X)	Study groups			Total
	Carcinoma Cervix	Cervicitis etc.	Healthy controls	
< 40	0	0	0	0
40	0	4	16	20
80	11	12	32	55
160	6	10	25	41
320	21	6	19	46
640	38	3	10	51
1280	22	1	4	27
2560+	16	0	0	16
Total	114	36	106	256

* HT-2 virus antibody assayed
by immunofluorescence test.

Study Groups Vs Antibody titre:

$$x^2 = 98.75 \text{ on } 12 \text{ df } P < 0.001$$

Figure 6.1

Incidence of Herpes Type-2 antibodies assayed by
Immunofluorescence Tests in sera of women with
Carcinoma of cervix, Cervicitis and Healthy controls.

Incidence of Herpes Type-2 virus antibodies in sera of women
with Carcinoma of Cervix, Cervicitis and Healthy Controls by
The Immunofluorescence Test.
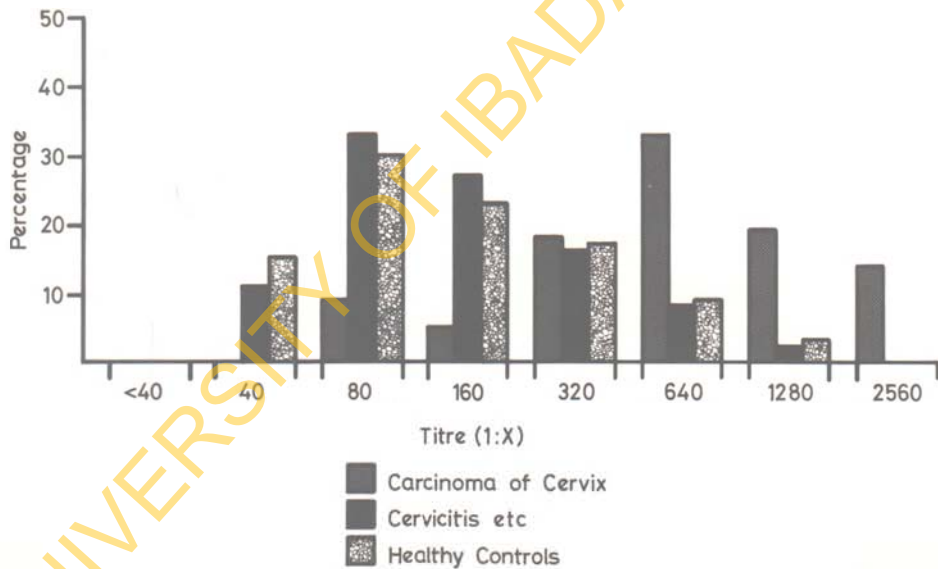
TABLE 6.2

Cummulative distribution of HT-2 virus antibody in each
study group showing positive reaction up to the stated titre.

HT-2 antibody* Titre (1:X)	Study groups					
	Carcinoma Cervix		Cervicitis etc.		Healthy controls	
	No.	%	No.	%	No.	%
< 40	0	0	0	0	0	0
40	114	100.0	36	100.0	106	100.0
80	114	100.0	32	88.9	90	84.9
160	103	90.4	20	55.6	58	54.7
320	97	85.1	10	27.8	33	31.1
640	76	66.7	4	11.1	14	13.2
1280	38	33.3	1	2.8	4	3.8
2560	16	14.0	0	0.0	0	0.0

* HT-2 virus antibody assayed by immunofluorescence
  test.

TABLE 6.3

Incidence of HT-2 virus antibody in relation to
clinical appearance of the cervix in women with Carcinoma
of cervix or Cervicitis.

HT-2 antibody* Titre (1:X)	Clinical appearance of cervix					Total
	Normal Cervix	Erosion	Cervicitis	Ulcerating cervix	Fungating cervix	
< 40	0	0	0	0	0	0
40	1	1	2	1	1	4
80	0	5	5	6	7	23
160	2	5	2	4	3	16
320	1	2	4	12	8	27
640	0	3	2	20	16	41
1280	0	1	0	9	13	23
2560+	0	0	1	5	10	16
Total	4	17	16	56	57	150

* HT-2 virus antibody assayed by immunofluorescence test.

Clinical appearance of Cervix Vs Antibody titre:

$$X^2 = 54.76 \text{ on } 24 \text{ df } P < 0.001$$

with normal cervix, cervical erosion and cervicitis had lower antibody titres. On the other hand, those with the cervical malignancy had relatively higher antibody titres.

The incidence of HT-2 virus antibody titre in relation to the cytologic appearance of smears in the three groups of women, viz: carcinoma cases, women with cervicitis and healthy controls are shown in Table 6.4 and 6.5. For the carcinoma and cervicitis cases, there was a significant association $(P < 0.001)$ between titre and cytology but there was none in the controls $(P > 0.25)$. The majority of the women in Table 6.4 showed cytologic evidence of malignancy, while only 5 of them had normal smears. Higher HT-2 virus antibody titres were present in the women whose smears showed marked atypia, dysplasia and malignancy, as compared with the antibody titres in the women with normal smears and those with mild atypia. The majority of the women in Table 6.5 showed normal cytologic appearance and low antibody titres.

Table 6.6 shows the prevalence of HT-2 virus antibody titre in the carcinoma of cervix group, based on the clinico-pathologic staging of the disease and in the group of women with cervicitis. There were 7 women in Stage 1b, 26 in Stage 2a and 29 in Stage 2b. 39 of the cases were in Stage 3 and 13 in Stage 4. The rest had cervicitis, erosion, or condylomata acuminata. There was no significant difference $(P > 0.05)$ in the level of HT-2 virus antibody and the clinico-pathologic stage of the disease, except in the group with non-malignant cervices $(P < 0.001)$ where the majority of these had a low level of antibody.

If a titre of 1:640 was taken as showing the presence of significant HT-2 virus antibodies, 66.7% of the carcinoma of cervix patients have

TABLE 6.4

Incidence of HT-2 virus antibody in relation to cervical
cytology in women with Cervicitis or Carcinoma of cervix.

HT-2 antibody* Titre (1:X)	Cytologic appearance					
	Normal	Slight atypia	Marked atypia	Dysplasia	Malignant	Total
< 40	0	0	0	0	0	0
40	0	4	0	0	0	4
80	2	7	1	2	11	23
160	1	5	2	0	8	16
320	1	1	3	3	19	27
640	1	1	3	2	34	41
1280	0	0	2	3	18	23
2560+	0	0	1	1	14	16
Total	5	18	12	11	104	150

* HT-2 virus antibody assayed by immunofluorescence test.

Cytologic appearance Vs Antibody titre:

$$\chi^2 \;=\; 62.82 \quad \text{on} \quad 24 \quad \text{df} \quad P < 0.001$$

TABLE 6.5

Incidence of HT-2 virus antibody in relation to cervical
cytology in Healthy control women.

HT-2 antibody* Titre (1:X)	Cytologic appearance					
	Normal	Slight atypia	Marked atypia	Dysplasia	Malignant	Total
< 40	0	0	0	0	0	0
40	12	4	0	0	0	16
80	25	6	1	0	0	32
160	19	2	4	0	0	25
320	14	4	1	0	0	19
640	10	0	0	0	0	10
1280	3	1	0	0	0	4
2560 +	0	0	0	0	0	0
Total	83	17	6	0	0	106

*HT-2 virus antibody assayed by immunofluorescence test.

Cytologic appearance Vs Antibody titre:

$$X^2 = 11.54 \text{ on } 10 \text{ df } P > 0.25$$

TABLE 6.6

Incidence of HT-2 virus antibody in relation to clinico-pathologic
stage in women with Cervicitis and Carcinoma of cervix.

HT-2 antibody* Titre (1:X)	Cervicitis etc.	Clinico-pathologic stage						Total
		1a	1b	2a	2b	3	4	
< 40	0	0	0	0	0	0	0	0
40	4	0	0	0	0	0	0	4
80	12	0	0	4	3	4	0	23
160	10	0	0	1	3	1	1	16
320	6	0	1	7	2	9	2	27
640	3	0	2	8	10	14	4	41
1280	1	0	3	2	5	8	4	23
2560 +	0	0	1	4	6	3	2	16
Total	36	0	7	26	29	39	13	150

* HT-2 virus antibody assayed by immunofluorescence test.

i. Carcinoma Vs Cervicitis

$$X^2 = 67.91 \text{ on } 30 \text{ df } P < 0.001$$

ii. Stages 1 - 4 Vs Antibody titre:

$$X^2 = 16.23 \text{ on } 20 \text{ df } P > 0.05$$

significant antibodies and only 4 (11.1%) of the women with cervicitis and 14 (13.2%) of the healthy control women had antibodies above this level (Table 6.7). This difference is highly significant (P< 0.001).

Table 6.8 shows the analysis of HT-2 virus antibodies in all the carcinoma of cervix group according to the histo-pathologic classification. Antibody titres were significantly higher (P< 0.001) among the squamous cell carcinoma group as compared with the other groups. Whereas 69 (77.5%) of the patients with squamous cell carcinoma of the cervix had antibodies above a titre of 1:640, only 2 (28.6%) of those with adenocarcinoma of the cervix, and 5 (27.8%) of those with undifferentiated carcinoma of the cervix had antibodies above this titre (Table 6.9). This difference is highly significant (P< 0.001). The adenocarcinoma and undifferentiated carcinoma of cervix patients showed percentages not significantly different (P> 0.05) from the healthy controls.

(ii) By Complement Fixation (CF) Test

Using the CFT, the distribution of HT-2 virus antibody titre is shown in Table 6.10. The distribution in the different groups varied from less than 1:4 to 1:256 and there was a significant (P< 0.001) association between titre and patient groups. There were many more patients at a titre of less than 1:4 in the cervicitis and healthy control groups as compared with patients in carcinoma group. For this last group, there was a peak at a titre of 1:32 (Fig. 6.2).

Table 6.11 shows the cumulative distribution of antibody titres in each study group up to the stated titre. It was noticed that the carcinoma of cervix patients showed higher antibody titres, especially from titre of 1:16 and above when compared with the cervicitis and

TABLE 6.7

Number of subjects in each group showing significant
HT-2 reaction at titres of 1:640 and above by Immunofluorescence
method.

Titre	Carcinoma Cervix	Cervicitis	Healthy controls
Significant	76	4	14
Not Significant	38	32	92
Total	114	36	106
% Significant	66.7	11.1	13.2

Carcinoma of Cervix Vs Cervicitis and Healthy controls:

$x^2 = 79.38$ on 2 df $P < 0.001$

TABLE 6.8

Analysis of HT-2 virus antibody in relation to Histopathology
in women with Carcinoma of cervix, Cervicitis and Healthy control
women.

HT-2 antibody* Titre (1:X)	Histopathologic groups					
	Sq. Cell[x] Carcinoma	Adeno. Carcinoma	Undiff.+ Carcinoma	Cervicitis	Healthy control	Total
< 40	0	0	0	0	0	0
40	0	0	0	4	16	20
80	4	2	5	12	32	55
160	2	0	4	10	25	41
320	14	3	4	6	19	46
640	31	2	5	3	10	51
1280	22	0	0	1	4	27
2560 +	16	0	0	0	0	16
Total	89	7	18	36	106	256

* HT-2 virus antibody assayed by immunofluorescence test.

x Squamous cell carcinoma

+ Undifferentiated carcinoma.

Histopathologic groups Vs Antibody titre:

$$\chi^2 = 132.32 \text{ on } 24 \text{ df } P < 0.001$$

TABLE 6.9

Number of subjects in each Histopathologic group showing
significant HT-2 antibody at titres of 1:640 and
above by Immunofluorescence method.

Titre	Histopathologic groups				
	Sq. Cell[x] Carcinoma	Adeno- Carcinoma	Undiff.[+] Carcinoma	Cervicitis	Healthy controls
Significant	69	2	5	4	14
Not Significant	20	5	13	32	92
Total	89	7	18	36	106
% Significant	77.5	28.6	27.8	11.1	13.2

x Squamous cell carcinoma

+ Undifferentiated carcinoma

i. Carcinoma of Cervix Vs Others:

$$X^2 = 99.99 \text{ on } 4 \text{ df } P < 0.001$$

ii. Adeno/Undiff. Carcinoma Vs. Controls:

$$X^2 = 3.29 \text{ on } 2 \text{ df } P > 0.05$$

TABLE 6.10

Incidence of HT-2 virus antibody in sera of women with
Carcinoma of cervix, Cervicitis and Healthy controls.

HT-2 antibody* Titre (1:X)	Carcinoma Cervix	Cervicitis	Healthy controls	Total
< 4	5	14	59	78
4	5	5	15	25
8	10	4	14	28
16	31	2	5	38
32	22	2	4	28
64	14	0	2	16
128	7	0	0	7
256+	1	0	0	1
Total	95	27	99	221

* HT-2 virus antibody assayed by CFT.

Study Groups Vs Antibody titre:

$$\chi^2 = 110.64 \text{ on } 14 \text{ df } P < 0.001$$

Figure 6.2

Distribution of Herpes Type-2 virus antibodies assayed
by CFT in sera of women with Carcinoma of cervix, Cervicitis
and Healthy Controls.

Distribution of Herpes Type-2 antibodies in Sera of women
with carcinoma of cervix, cervicitis and healthy controls by
the complement fixation test.
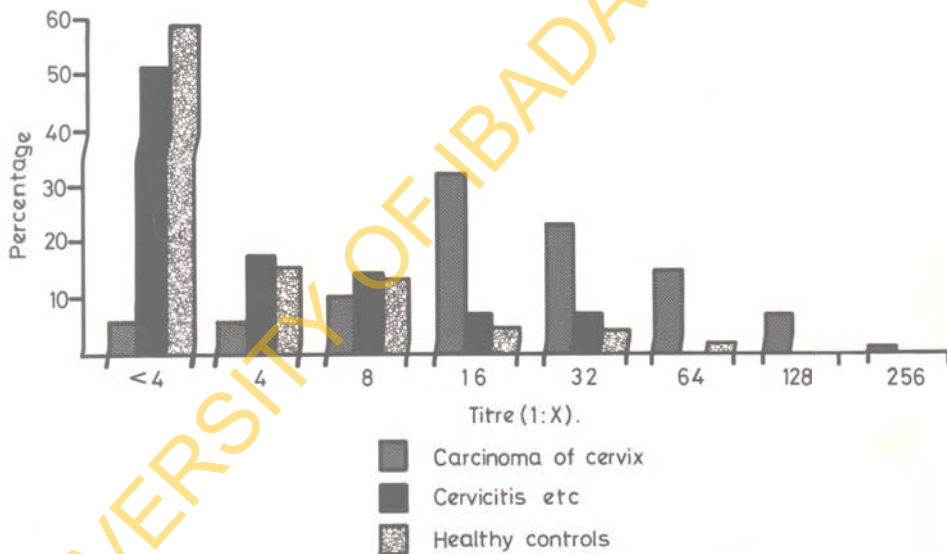
TABLE 6.11

Cumulative distribution of HT-2 virus antibody in each
study group showing positive reaction up to the stated titre.

HT-2 antibody* Titre (1:X)	Carcinoma Cervix No.	%	Cervicitis etc. No.	%	Healthy Controls No.	%
< 4	95	100.0	27	100.0	99	100.0
4	90	94.7	13	48.1	40	40.4
8	85	89.5	8	29.6	25	25.3
16	75	78.9	4	14.8	11	11.1
32	44	46.3	2	7.4	6	6.1
64	22	23.2	0	0.0	2	2.0
128	8	8.4	0	0.0	0	0.0
256	1	1.1	0	0.0	0	0.0

* HT-2 antibody assayed by CFT.

healthy control groups.

The incidence of HT-2 virus antibody in women with carcinoma of cervix or cervicitis according to the clinical appearance of the cervix is shown in Table 6.12. There was a significant ($P = 0.025$) association between the clinical appearance of the cervix and antibody titre. Most of the women with normal cervices, cervical erosion and cervicitis showed lower antibody titres as compared with the higher titres among women with cervical carcinoma. The incidence of HT-2 virus antibody in relation to the cytologic appearance of cervical smears among women with cervical carcinoma or cervicitis, and healthy control women are shown in Tables 6.13 and 6.14. While most of the women with normal cervical cells and those with mild cellular atypia had low antibody titres (Table 6.13), those with dysplasia and malignancy had significantly ($P < 0.005$) higher antibodies. But among the healthy controls, there was no significant ($P > 0.25$) correlation between the level of antibody titre and cervical cytology (Table 6.14).

Table 6.15 shows the incidence of HT-2 virus antibody in women with carcinoma of cervix based on the clinico-pathologic staging of the disease, and in the group of women with cervicitis. There were 7 cases in Stage 1b, 22 in Stage 2a, 25 in Stage 2b, 29 in Stage 3 and 12 in Stage 4. The rest consisted of the cervicitis and cervical erosion cases. There was no relationship between the clinico-pathologic stage of disease and antibody level, except between the carcinoma group on the one hand and the cervicitis groups on the other hand ($P < 0.001$).

Using a titre of 1:16 as a cut off point (Table 6.16), 75 (78.9%)

TABLE 6.12

Incidence of HT-2 virus antibody in relation to clinical appearance of the cervix in women with Carcinoma of cervix or Cervicitis.

HT-2 antibody* Titre (1:X)	Clinical appearance of cervix					Total
	Normal Cervix	Erosion	Cervicitis	Ulcerating cervix	Fungating cervix	
< 4	2	6	5	3	3	19
4	0	2	3	3	2	10
8	1	3	1	2	7	14
16	2	1	0	14	16	33
32	1	1	2	11	9	24
64	0	0	2	4	8	14
128	0	0	0	4	3	7
256+	0	0	0	0	1	1
Total	6	13	13	41	49	122

* HT-2 virus antibody assayed by CFT.

Clinical appearance of Cervix Vs Antibody titre:

$$X^2 = 44.49 \text{ on } 28 \text{ df } P = 0.025$$

TABLE 6.13

Incidence of HT-2 virus antibody in relation to Cervical cytology
in women with Carcinoma cervix or Cervicitis.

HT-2 antibody* Titre (1:X)	Cytologic appearance					Total
	Normal	Slight atypia	Marked atypia	Dysplasia	Malignant	
< 4	4	8	3	1	4	19
4	0	4	2	0	4	10
8	1	1	2	0	10	14
16	0	3	1	0	29	33
32	0	2	2	1	19	24
64	0	0	1	1	12	14
128	0	0	1	0	6	7
256	0	0	0	0	1	1
Total	5	18	12	2	85	122

* HT-2 virus antibody assayed by CFT.

Cytologic appearance Vs Antibody titre:

$$X^2 = 53.50 \text{ on } 28 \text{ df } 0.005 > P > 0.001$$

TABLE 6.14

Incidence of HT-2 virus antibody in relation to cervical
cytology in Healthy control women.

HT-2 antibody* Titre (1:X)	Cytologic appearance					
	Normal	Slight atypia	Marked atypia	Dysplasia	Malignant	Total
< 4	52	6	1	0	0	59
4	9	5	1	0	0	15
8	11	2	1	0	0	14
16	5	0	0	0	0	5
32	3	1	0	0	0	4
64	2	0	0	0	0	2
128	0	0	0	0	0	0
256+	0	0	0	0	0	0
Total	82	14	3	0	0	99

* HT-2 virus antibody assayed by CFT.

Cytologic appearance Vs Antibody titre:

$$\chi^2 = 9.50 \text{ on } 10 \text{ df } P > 0.25$$

TABLE 6.15

Incidence of HT-2 virus antibody in relation to clinico-pathologic stage in women with Cervicitis and Carcinoma of cervix.

HT-2 antibody* Titre (1:X)	Cervicitis	Clinico-pathologic stage						Total
		1a	1b	2a	2b	3	4	
< 4	14	0	0	1	2	1	1	19
4	5	0	1	1	1	2	0	10
8	4	0	1	2	4	1	2	14
16	2	0	2	5	8	12	4	33
32	2	0	2	5	4	8	3	24
64	0	0	1	5	4	3	1	14
128	0	0	0	3	2	1	1	7
256	0	0	0	0	0	1	0	1
Total	27	0	7	22	25	29	12	122

* HT-2 virus antibody assayed by CFT.

i. Carcinoma Vs Cervicitis:

$$X^2 = 101.33 \text{ on } 35 \text{ df } P < 0.001$$

ii. Stages 1 - 4 Vs Antibody titre:

$$X^2 = 14.34 \text{ on } 28 \text{ df } P > 0.05$$

TABLE 6.16

Number of subjects in each group showing significant
HT-2 reaction at titres of 1:16 and above by CFT

Titre	Carcinoma Cervix	Cervicitis	Healthy controls
Significant	75	4	11
Not Significant	20	23	88
Total	95	27	99
% Significant	78.9	14.8	11.1

Carcinoma of Cervix Vs Cervicitis and Healthy controls:

$$x^2 = 100.97 \text{ on } 2 \text{ df } P < 0.001$$

of the carcinoma group have significant antibody to HT-2 virus while only 4 (14.8%) of the cervicitis group and 11 (11.1%) of the healthy controls have antibodies above this level. This difference is highly significant (P< 0.001).

Table 6.17 shows a significant (P< 0.001) association between HT-2 virus antibody and histopathology. Antibody titres were generally high among the women with squamous cell carcinoma of the cervix as compared with the other histo-pathologic groups, the cervicitis and healthy control groups. 68 (91.9%) of the patients diagnosed as squamous cell carcinoma had antibodies above the titre of 1:16 (Table 6.18) while only 1 (25%) of those with adenocarcinoma, and 11 (35.3%) of those with undifferentiated carcinoma of the cervix showed antibodies above this titre. This is highly significant (P< 0.001). However, the latter two groups showed higher significant (P< 0.01) antibodies to HT-2 virus (at this level) when compared with the cervicitis and healthy control groups.

6.3.2 Oncogenic potential of HT-2 virus on tissues other than cervix

Table 6.19 shows the distribution of HT-2 virus antibody among the various groups of malignancies and healthy controls. Antibody was present in all sera up to a titre of 1:20 and the titres varied from 1:20 to 1:2560. While one of the study groups had more cases at a titre of 1:40 (carcinoma of the Breast) and other groups had more at a titre of 1:80 (squamous cell carcinoma of sites other than the cervix, carcinoma of the vulva, malignant trophoblastic disease, carcinoma of liver, and healthy controls), there were more cases of cervical carcinoma at a titre of 1:640 and above. There was however,

TABLE 6.17

Analysis of HT-2 virus antibody in relation to histopathology
in Carcinoma cervix, Cervicitis and Healthy control women.

| HT-2 antibody* Titre (1:X) | Histopathologic groups | | | | | |
	Sq. Cell[x] Carcinoma	Adeno-Carcinoma	Undiff.+ Carcinoma	Cervicitis	Healthy control	Total
< 4	1	0	4	14	59	78
4	1	1	3	5	15	25
8	4	2	4	4	14	28
16	26	1	4	2	5	38
32	20	0	2	2	4	28
64	14	0	0	0	2	16
128	7	0	0	0	0	7
256 +	1	0	0	0	0	1
Total	74	4	17	27	99	221

* HT-2 virus antibody assayed by CFT.

x Squamous cell carcinoma.

+ Undifferentiated carcinoma.

Histopathologic groups Vs Antibody titre:

$$x^2 = 144.90 \text{ on } 28 \text{ df } P < 0.001$$

TABLE 6.18

Number of subjects in each histopathologic group showing
significant HT-2 antibody at titres of 1:16 and above by CFT

Titres	Histopathologic groups				
	Sq. Cell[x] Carcinoma	Adeno- Carcinoma	Undiff.+ Carcinoma	Cervicitis	Healthy Controls
Significant	68	1	6	4	11
Not Significant	7	3	11	23	88
Total	74	4	17	27	99
% Significant	91.9	25.0	35.3	14.8	11.1

x Squamous cell carcinoma

+ Undifferentiated carcinoma

i. Carcinoma of cervix Vs Others:

$$X^2 = 122.43 \text{ on } 4 \text{ df } P < 0.001$$

ii. Adeno/Undiff. Carcinoma Vs. Controls:

$$X^2 = 16.25 \text{ on } 2 \text{ df } P < 0.01$$

TABLE 6.19

Distribution of HT-2 virus antibody in patients with
various malignancies and Healthy control women.

HT-2 antibody* Titre (1:X)	Study groups						
	Sq. Cell Ca.[x] (Cervix)	Sq. Cell Ca.[x] (Others)	Carcinoma Vulva	MTD[+]	Carcinoma Liver	Carcinoma Breast	Healthy Control
< 40	0	1	0	0	0	1	1
40	1	7	1	4	3	4	1
80	3	16	2	9	7	3	3
160	2	4	1	6	6	1	2
320	0	5	1	6	1	0	1
640	8	4	1	3	2	1	2
1280	4	3	0	2	1	0	0
2560 +	2	0	0	0	0	0	0
Total	20	40	6	30	20	10	10

* HT-2 virus antibody assayed by
  Immunofluorescence test.

x Squamous cell carcinoma

+ Malignant Trophoblastic Disease.

Study Groups Vs Antibody titre:

$$X^2 = 57.75 \quad \text{on} \quad 42 \quad df \quad P > 0.05$$

no statistically significant association between the groups and
antibody titres $(P > 0.05)$.

Using a titre of 1:640 as the cut off point as before (Table 6.20),
the percentage (70.0%) of the squamous carcinoma of cervix patients
showing significant HT-2 virus antibody at a titre of 1:640 and above
is significantly higher $(P < 0.001)$ than among all the other carcinoma
cases (16.0%) and healthy controls (20.0%). Even when a lower titre
of 1:320 was used as a cut off point, there was still a significant
difference $(P < 0.001)$ between the carcinoma of cervix cases and the
others. However, there was no significant difference between the
controls and the tumours of sites other than the cervix $(P > 0.05)$.

6.3.3   <u>Detection of HT-2 virus antigen in cervical smears:</u>

Using May-Grunwald-Giemsa Stain, it was possible to demonstrate
by microscopic examination, malignant cells with relatively large
nucleus and a high nucleo-cytoplasmic ratio in the smears from the
carcinoma of cervix. Fluorescence was mainly cytoplasmic in the
cells stained by the indirect immunofluorescence technique
(Fig. 6.3 & 6.4). A cell was accepted as showing positive fluorescence
if it was sufficiently bright to be clearly visible against the dark
background in the smear during examination. The proportions of
fluorescent cells observed in the different preparation varied from
patient to patient; the percentage of positive cells being very low
in some (less than 10%) and even when marked, were usually less than 50%.

Data obtained from the immunofluorescence study are analysed in
Table 6.21. Of the 24 patients previously diagnosed clinically as
having carcinoma of the cervix, smears from 22 of them showed cells with

TABLE 6.20

Number of subjects showing positive HT-2 reaction among
the test groups and Controls at titres of 1:640 and
above by Immunofluorescence technique.

Titre	Sq. Ca.x (cervix)	Sq. Ca.x (others)	Carcinoma Vulva	MTD+	Carcinoma Liver	Carcinoma Breast	Controls
Significant	14	7	1	5	3	1	2
Not Significant	6	33	5	25	17	9	8
Total	20	40	6	30	20	10	10
% Significant	70.0	17.5	16.7	16.7	15.0	10.0	20.0

x Squamous cell carcinoma

+ Malignant Trophoblastic Disease.

i. Squamous cell carcinoma of cervix Vs Others and Controls:

$$x^2 = 27.03 \text{ on } 6 \text{ df } P < 0.001$$

ii. Other malignancies Vs Controls:

$$x^2 = 0.46 \text{ on } 5 \text{ df } P > 0.05$$

Figure 6.3

Cell smears from Carcinoma of cervix showing some cells
with positive immunofluorescence in an indirect immuno-
fluorescence test with Rabbit antiserum against HT-2
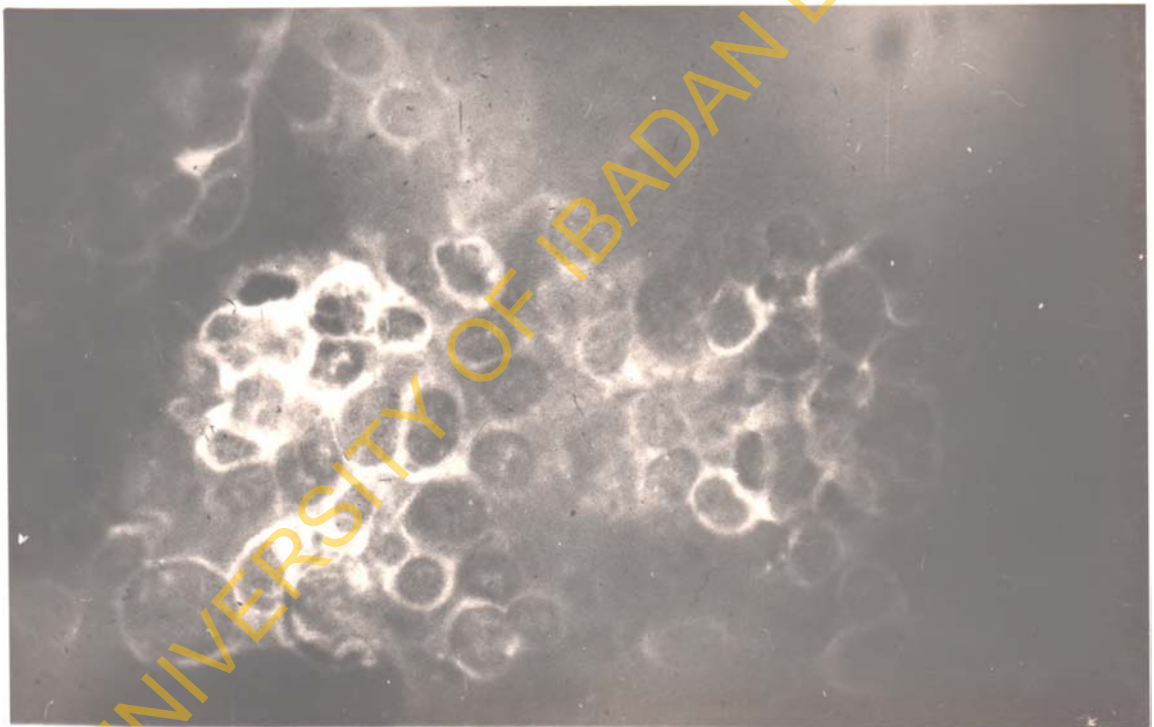virus antigen and FITC-anti Rabbit globulin. (X 1200).

Figure 6.4

Cell smears from Carcinoma of cervix showing some
cells with positive immunofluorescence in an indirect
immunofluorescence test with Rabbit antiserum against
HT-2 virus antigen and FITC-anti Rabbit globulin.
About 50% of the cells here show positive immunofluorescence.

(X 400)

positive cytoplasmic fluorescence when stained with rabbit antiserum against HT-2 virus antigen and fluorescein-conjugated anti-rabbit globulin, but not with PBS (pH 7.2) and fluorescein-conjugated anti-rabbit globulin. Smears from all the healthy control women were negative with both rabbit antiserum against HT-2 virus antigen and PBS (pH 7.2) when stained with fluorescein-conjugated anti-rabbit globulin. Similarly, smears from both malignant and normal cells did not show any fluorescence with the pre-immune (normal rabbit) serum when stained with the fluorescein-conjugated anti-rabbit globulin.

Table 6.22 shows the clinico-pathologic staging of the 24 cases of carcinoma of the cervix studied. Two cases were histologically diagnosed as chronic cervicitis. Of the remaining 22 cases, none had intra-epithelial carcinoma of cervix, 1 was in Stage 1b, 5 in Stage 2a, 7 in Stage 2b, 6 in Stage 3 and 3 in Stage 4 carcinoma of cervix. There was no relationship between the number of positive immunofluorescent cells and the clinico-pathologic staging of the disease.

6.4 Discussion

Recent results have shown an appreciation of the biologic and antigenic differences between the Herpes viruses which infect the genital and non-genital sites (Dowdle et al, 1967; Rawls et al, 1968; Nahmias & Dowdle, 1968; Figueroa & Rawls, 1969; Plummer et al, 1970; Geder & Skinner, 1971; Lowry et al, 1971; Jeansson, 1972; Josey et al, 1972. Sero-epidemiologic studies in Ibadan (Adelusi et al, 1975) and elsewhere (Naib et al, 1966; Josey et al, 1968; Naib et al, 1969; Aurelian et al, 1970; Nahmias et al, 1971(b); Catalano & Johnson, 1971; Rawls et al, 1973) have shown that there is an association between

TABLE 6.21

Number of subjects showing positive HT-2 antigen in
cervical smears by the indirect immunofluorescence method.

Study Group	No. of Patients tested	Positive Fluorescence with Rabbit anti HT-2 serum + FITC		Positive Fluorescence with PBS* + FITC		Positive Fluorescence with NRS ** + FITC x	
		No.	%	No.	%	No.	%
Patients with Carcinoma of the Cervix	22	22	100	0	0	0	0
Patients with Chronic Cervicitis	2	2	0	0	0	0	0
Healthy women (controls)	24	0	0	0	0	0	0

* Phosphate Buffered Saline

** Normal Rabbit Serum

x Fluorescein Isothiocyanate

TABLE 6.22

Clinico-pathologic stages of the 24 cases
of suspected Carcinoma of the cervix.

Staging	No. of Cases
Others*	2
1(a)	0
1(b)	1
2(a)	5
2(b)	7
3	6
4	3
Total	24

* Cervicitis.

Herpes Type 2 (HT-2) virus, the strain responsible for genital infections, and cervical cancer.

In the present study using two different methods (Immunofluorescence (IM) and complement fixation (CF)), a higher prevalence of HT-2 virus antibody titres was demonstrated in Nigerian women with carcinoma of the cervix uteri than in healthy controls. This confirms the earlier findings of an association between HT-2 virus and cervical carcinoma. These data showed that while the patients with carcinoma of the cervix possessed high HT-2 virus antibody titres, the others with chronic cervicitis, erosions as well as the healthy controls had low antibody titres. There was no difference in the level of antibody titres on the basis of cytology or clinical appearance of the cervix except that patients with malignant cells showed evidence of higher antibody titres. Similarly, the clinico-pathologic stage of the disease had no bearing on the incidence of antibody titre, as antibodies were equally distributed in all the stages of the disease. This is in keeping with the concept that once a malignant cell has been triggered off by the stimulating factor, the initiating factor need not be demonstrable, no matter the stage of the disease (Lewis et al, 1965).

However, when the analysis of the levels of HT-2 virus antibody titre was made on the basis of the histo-pathologic classification, it was shown that patients with squamous cell carcinoma of the cervix showed a marked distinction from the others. 69 (77.5%) of the patients with squamous cell carcinoma of the cervix had antibodies above a titre of 1:640 in comparison with 2 (28.6%) among patients with adeno-carcinoma of the cervix, 5 (27.8%) with undifferentiated carcinoma of

the cervix, and 4 (11.1%) with chronic cervicitis and 14 (13.2%) of healthy controls.

The association between HT-2 virus and invasive carcinoma of the cervix may not be a causal one. Three possibilities which appear very plausible have been suggested.

(i) Both the virus infection and the malignancy may be co-variables with sexual promiscuity.

(ii) The virus infection may follow neoplastic change.

(iii) The virus infection may preceed the neoplastic change and contribute to the formation of the lesion as a carcinogen or co-carcinogen.

The results of the present study is in favour of the last in which the virus infection is thought to preceed the neoplastic change in the cervix and contribute directly to the lesion as a carcinogen or indirectly as a co-carcinogen. Even though there were no pre-invasive lesions such as the intra-epithelial carcinoma of the cervix in this study, the distribution of HT-2 virus antibody titres in the different stages of the malignant disease was about equal and is similar to the finding of Nahmias et al (1970) and Royston & Aurelian (1970) where antibodies were uniformly present in the pre-malignant and malignant cervical neoplasms. This is in support of the concept that once the trigger mechanism has been initiated, it need not be present in order for the malignancy to progress. Otherwise, the antibody titres in the latter stages of the disease would have been higher than in the early stages.

The work of Royston et al (1970) has shown in carefully documented cases of pre-invasive and invasive cervical neoplasia that there is

immunologic evidence of prior infection of the women with HT-2 virus whereas there was no such evidence with respect to other venereal diseases, or with the oral strains of Herpes viruses. Similarly, sero-epidemiologic studies by Royston & Aurelian (1970) showed an almost 100% correlation between HT-2 virus infection and the earliest identifiable pre-invasive stages of carcinoma of the cervix. This suggests that the infection is related to the induction of neoplastic lesions, rather than the concept of preferential multiplication of the virus in neoplastic tissue.

In favour of this virus being capable alone or in conjunction with other factors (Munoz, 1973) of transforming normal cervical squamous cells into neoplastic ones are, the age curves of patients. The peak age incidence of HT-2 virus infection in Ibadan (Chapter 5) was 10 years earlier than the peak age incidence of carcinoma of the cervix (Chapter 3). Even though the peak age incidence in carcinoma of the cervix was much lower in this study than in others, this finding is in keeping with that of Naib et al (1969) where the peak age of incidence of HT-2 virus infection was 10-12 years earlier than that for the detection of carcinoma in-situ, and over 20 years earlier for that of invasive carcinoma of the cervix. Also consistent with the concept of an etiologic role of HT-2 virus on cervical carcinoma was the observation of a higher incidence of carcinoma of the cervix in women previously diagnosed as having HT-2 infection (Naib et al, 1969). Furthermore, the potential oncogenecity of herpes viruses has been established in non-human primates (Trentin et al, 1969; Hunt et al, 1970; Melendez et al, 1972).

The double peaks of antibody titres found in this study by the

immunofluorescence technique, one at a titre of 1:80 and a second

(higher) one at a titre of 1:640, is significant. The data suggest

that some of the carcinoma of cervix patients still behave as normal

women while others differ by producing higher levels of antibodies.

It may imply on the other hand that while the majority of cancer of the

cervix could be due to HT-2 virus infection directly or indirectly, the

rest may be due to other causes (Munoz, 1973), since cancer of the cervix

is a generic term for several types of tumours.

The oncogenesity of Herpes viruses in animals is no longer in

doubt (Tweedell, 1967; Churchill & Biggs, 1967; Biggs et al, 1968;

Churchill & Biggs, 1968; 1968(b); Mizell et al, 1968; Churchill et al,

1969; Trentin et al, 1969; Melendez et al, 1969; Smith & Mackay, 1969;

Granoff et al, 1969; Hunt et al, 1970; Melendez et al, 1971; Gravel, 1971;

Melendez et al, 1972). Similarly, the oncogenic potential of this group

of viruses in man has also been shown (Epstein et al, 1964; Naib et al,

1966; Nahmias et al, 1967(c); Epstein & Achong, 1968; Nahmias et al,

1968(c); Pope et al, 1969; Melnick & Rawls, 1970; Catalano & Johnson,

1971; Skinner et al, 1971; Rawls et al, 1973; Janda et al, 1973; Adelusi

et al, 1975). However, there is the question yet to be settled on

whether or not the HT-2 virus has a pre-dilection for squamous cells

generally. Such cells may provide the ideal ground for excessive HT-2

virus replication, and hence large production of antibodies to the virus,

in contrast to the poor growth and low antibody titre of the virus in .

other histologic types of cells. On the other hand, the virus may be

oncogenic for all types of tissues.

In the present study, where histologically diagnosed squamous cell

carcinomas of various sites other than the cervix have been used, the distribution of HT-2 virus antibodies is unlike the distribution in the squamous cell carcinoma of the cervix group. Rather, the distribution of the antibodies followed the pattern in the normal population. No similar studies of squamous cell carcinoma of sites other than the cervix was found in the literature with which to compare this result. Hence, this would require to be confirmed by other workers.

A comparable result was that by Rawls et al (1969) in which HT-2 virus antibody was determined in 22 patients with tumours of sites other than the cervix. In the study, antibodies were detected in 2 women with carcinoma of the vulva, and also in one case each of carcinoma of the ovary and testis. No antibodies were found in 5 cases of carcinoma of the prostate, 5 cases of carcinoma of the urinary bladder, 2 cases of carcinoma of the breast and one case each of carcinoma of gall bladder, tongue, stomach, tonsil and uterus.

The present study, further shows that there was no significant HT-2 virus antibody titres in patients with extra-cervical pelvic and extra-pelvic malignancies. The possibility of the virus being oncogenic for extra-cervical pelvic malignancies was not therefore borne out. 70% of the patients with carcinoma of the cervix had significant HT-2 virus antibodies, and only 16.7% of patients with malignant trophoblastic diseases, and 16.7% of cases of carcinoma of the vulva had significant levels of antibodies. It could be concluded therefore that the association already established between HT-2 virus and carcinoma of the cervix and particularly the squamous cell type (Adelusi et al, 1975) is peculiar to this growth, although other etiologic co-factors cannot be excluded

(Munoz, 1973).

The accuracy and specificity of the serologic methods employed in this study (Immunofluorescence and Complement Fixation) deserve some comments. The method of determining antibody titres by the two methods does not necessarily eliminate cross-reactivity between Herpes Type 1 (HT-1) and Type-2 (HT-2) viruses. The technique used to differentiate these two sero-types is pertinent to the interpretation of serologic data on the possible association between HT-2 virus infection and cervical malignancy.

The common techniques in current use to distinguish between HT-1 and HT-2 viruses are based on the kinetics of neutralisation of a virus isolate by antisera prepared in rabbits against both Herpes virus hominis (HVH) serotypes (Plummer, 1964; Rawls et al, 1968; Rawls et al, 1970(c)). Antibodies to sera from patients who have been exposed to HVH can be typed for specificity to HT-1 or HT-2 virus by micro-neutralisation tests which estimate the efficiency of antibodies to known strains of HT-1 and HT-2 viruses (Pauls & Dowdle, 1967; Nahmias et al, 1970(c)).

However, both the kinetic neutralisation and the micro-neutralisation tests are cumbersome and time-consuming. Furthermore, like other methods, they are not specific for either type of virus as evidenced by cross-reactivity between HT-1 and HT-2 virus antibodies. Various other studies in rabbits (Nahmias et al, 1969(f)) and new-born infants (Nahmias et al, 1969(e)) have demonstrated the problem of cross-reactivity between HT-1 and HT-2 virus antibodies by showing the presence of antibodies to antigenic determinants showed by both types 1 and 2 HVH.

Infection with the non-genital HVH strains (HT-1) is known to result

in the appearance of antibodies which neutralise HT-1 viruses more
effectively than they do HT-2 viruses. Infection of the genital tract
by HT-2 viruses causes the production of antibodies which neutralise
HT-2 viruses better than HT-1 viruses or neutralise both HT-1 and HT-2
viruses equally well (the so called intermediate antibodies) (Rawls
et al, 1970(c); Roizman et al, 1970). Hence, the use of the simpler,
yet acceptable techniques of immunofluorescence (Nahmias et al, 1969(c))
and complement fixation (Nahmias et al, 1968), since the distinction
between HT-1 and HT-2 viruses is a pre-requisite for the performance
of large sero-epidemiologic studies.

The determination of titres of antibody in the sera of patients
and controls in a blind experiment, and the use of cut-off points at
higher titres for the presence of significant antibodies in both cases
and controls, have been designed here to distinguish between two groups
of individuals with predominance of HT-1 and HT-2 virus antibodies
respectively. This concept could not be said to be completely accurate.
The fact that it is known that HT-2 virus antibodies neutralise HT-2
viruses better than HT-1 viruses (Rawls et al, 1970(c); Roizman et al,
1970), and both the cases and controls have been subjected to the same
test methods blindly, however, would make these results acceptable as
demonstrating the presence of HT-2 virus antibodies in those sera
showing high antibody titres.

In order to establish a firm evidence of cause and effect relation-
ship between HT-2 virus and carcinoma of the cervix, the presence of
either the antibody or the antigen in test cases but not in controls
need to be established. It has been shown that the cervix contains

distinct antigens (Hollinshead et al, 1972) and that the metamorphosis
which occurs in cervical tissue as it progresses from normal, through
dysplasia, to invasive carcinoma, may be accompanied by the acquisition
of new antigenic substances.

Some studies have demonstrated that exfoliated cells from the
cervix of patients with cervical carcinoma possess HT-2 virus antigen
as determined by immunofluorescence (Royston & Aurelian, 1970(b)).
Some studies have also shown evidences for the presence of tumour-specific
antigens in carcinoma of the cervix (Gall et al, 1973) while others
have demonstrated HT-2 virus induced or HT-2 virus related antigens
in the tumour (Aurelian et al, 1971; Aurelian et al,
1973; Feorino & Palmer, 1973; Aurelian et al, 1973(b)). As mentioned
earlier, Aurelian et al, (1971) succeeded in isolating from a line of
cervical tumour cells, a virus identical to HT-2 virus with respect to
two biologic properties.

In the present study, antisera raised against HT-2 virus infected
vero cells (absorbed with un-infected vero cells) reacted with exfoliated
cells from patients with histologic evidence of cervical cancer as
determined by immunofluorescence. In the normal controls, however,
none of the patients tested showed any evidence of HT-2 virus antigen
in their exfoliated cells. This result suggests that the presence of
HT-2 virus or HT-2 virus related antigens in cells is a peculiarity
of neoplastic epithelial cells of the cervix.

There was no positive control for the specificity of the HT-2
virus antiserum raised in rabbits, such as using known specific
human anti HT-2 virus serum. However, the fact that only smears from

carcinoma of the cervix patients showed positive reaction with the
immune (Rabbit anti HT-2) serum, strongly suggests that cells from
carcinoma of the cervix contain antigens which are not present in
normal cervical cells. These observations also suggest that neoplastic
cells from patients with histologically proven invasive and pre-invasive
carcinoma of the cervix contain antigens related to those present in
vero cell lines infected with HT-2 virus.

In a similar study, (Royston & Aurelian, 1970(b)), it was found
that neoplastic cells from patients with pre-invasive and invasive
carcinoma of the cervix reacted with raised rabbit anti HT-2 virus
and specific Human anti HT-2 virus sera. The cells failed to react
with pre-immune (normal rabbit) serum, raised anti-adenovirus 18,
and anti-mycoplasma orale sera. In the study, it was noticed that
reactivity could be removed by specific absorption of rabbit anti
HT-2 virus serum with HT-2 virus infected cells, but not with un-infected
cells. Also, prior reaction of neoplastic cells with antibody against
HT-2 virus infected cells blocked subsequent direct staining by
fluorescein-conjugated anti HT-2 virus serum, confirming the specificity
of the reaction.

All patients with histologically diagnosed carcinoma of the cervix
(Stages 1 to 4) in this study yielded cells which contained antigens
related to HT-2 virus as shown by immunofluorescence. These were few
in some cases, and even where many, they were generally less than 50%,
the variation showing no relationship to the clinico-pathologic
staging of the disease. This variation in the number of positive
cells (Nahmias et al, 1970) may reflect differences in the amount

of antigen present in neoplastic cells or may be due to the inability
of the test to distinguish a truely neoplastic cell from a young
metaplastic one.

The significance of the detection of HT-2 virus related antigen
in some cells and not others from cervical carcinoma in this study,
is not quite clear. It is possible that some or all of the negative
cells might be infected with the virus abortively, although, Aurelian
et al (1971) showed in their study that this was very unlikely. On the
other hand, it has been shown that some of the cells harbour only the viral
genome, and may therefore contain HT-2 virus DNA (Frenkel, 1972).

The findings in all aspects of this study thus far are in agreement
with those of others (Josey et al, 1968; Nahmias et al, 1969(b);
Rawls et al, 1970; Royston & Aurelian, 1970; Aurelian et al, 1970;
Royston & Aurelian, 1970(b); Catalano & Johnson, 1971; Rawls et al,
1973; Adelusi et al, 1975) and strengthens the evidence for an
association between HT-2 virus and carcinoma of the cervix. It cannot
yet be concluded, however, whether or not the association is an
etiologic one, although all the evidence suggests that the virus infection
is an early event, probably proceeding neoplastic changes in the cervical
epithelium. Elucidation of the precise relationship between the virus
infection and cervical neoplasm must await further investigation.

CHAPTER 7

SUMMARY AND CONCLUSION

Clinical, cytologic , histopathologic and immunovirologic parameters were employed in the study of carcinoma of the cervix uteri in Nigerians. The disease was found to occur at an earlier age in Nigerians (peak age incidence of 35 to 45 years) as compared with other studies. A greater proportion of the malignancy (78.1%) were clearly of squamous cell origin while only 6.1% were adeno-carcinoma, the rest (15.8%) being undifferentiated carcinoma. Furthermore, there was a significant correlation between the histopathology and clinico-pathologic stage of the malignancy, the undifferentiated carcinoma group being diagnosed generally at a more advanced stage than the well differentiated carcinoma group. In comparison to the study of Lawson et al (1964), many more patients were diagnosed in the present study in the earlier stages of the disease (stages 1 and 2). This was attributed to the increased awareness of the patients of hospital care, and also to the increase in number of doctors in the country.

Carcinoma of cervix appears to have a venereal origin as coitus was demonstrated as being vital in its causation. Coitus appears to be the common denominator which could explain the occurence of the disease among certain groups of women most of whom commenced sexual

intercourse in early adolescence (11-15 years). In addition, the indivi-
duals have frequent coitus usually with multiple partners and also
had many pregnancies. No association was found between the disease
and circumcision or polygamy with regard to the husbands. With the
concept that mortality from the malignancy was believed to follow
trends in the incidence of venereally transmitted disease (Beral,
1974), it was postulated that a venereally transmitted agent might be
important in the causation of carcinoma of the cervix.

The common occurrence of Herpes Type-2 (HT-2) virus infection
was demonstrated by sero-epidemiologic studies in the population
especially as from the time when heterosexual activity is known to
occur. Evidence was further provided to show that the virus was
venereally transmitted in Nigerians. It was highlighted that the virus
infection reaches a peak in the second decade of life when the
ecto-cervix is known to be susceptible to a carcinogen.

Using two different methods (Immunofluorescence and Complement
Fixation), the prevalence of Herpes Type-2 Virus antibodies was
determined in one hundred and fourteen patients with carcinoma of the
cervix uteri and one hundred and six healthy controls, to see whether
or not there was any significant association between the virus and
carcinoma of the cervix as has been postulated elsewhere. Results of
both tests showed that a significantly higher proportion of the patients
with carcinoma of the cervix had high antibodies in their sera. There
were two peaks in the distribution of virus antibody titre in the
carcinoma group, one at a lower titre of 1:80 similar to the controls,
and the other at a higher titre of 1:640. It was thought that these

peaks in incidence of virus antibodies:in the carcinoma cases might
indicate that some of these patients behave as normal women while in
others, HT-2 virus might play a part directly or indirectly, in its
causation, especially as carcinoma of the cervix is a generic term for
several types of tumours.

There was a significant association of virus antibodies with the
histopathologic cell type of the tumour, although there was no
correlation between this and clinico-pathologic stage of the disease.
No association found between the virus and squamous cell carcinoma of
sites other than the cervix, extra-cervical pelvic and extra-pelvic
malignancies. In was postulated, therefore, that HT-2 virus infection
might proceed neoplastic change in the cervix and contribute directly
to the lesion as a carcinogen or indirectly as a co-carcinogen.
Furthermore, the virus might be specific only for the epithelium of
the cervix.

In order to establish the specificity of the virus for carcinoma
of the cervix, exfoliated cells from all twenty-two women with cervical
malignancy were shown to exhibit the presence of Herpes Type-2 virus
antigens. All the findings in this study established a strong association
between the virus and cervical malignancy in Nigerians, similar to
other studies.

The findings, in this study of HT-2 virus infection being
associated with cervical cancer as in other areas, and its being
unassociated with other malignancies in this environment led to the
conclusion that a definite relationship exists between the venereally
transmitted Herpes Type-2 virus and carcinoma of the cervix uteri in

Nigerians. Even though this relationship may not necessarily be an etiologic one, it is significant enough to warrant further experimental studies in the future to determine the precise relationship of this virus to Carcinoma of the cervix. The detection, for example, of HT-2 virus or HT-2 virus-related or induced antigens in patients with carcinoma of the cervix uteri, but not in other patients with extra-cervical malignancies, and healthy controls, both here and elsewhere, would go a long way in establishing this precise relationship.

LIST OF REFERENCES

Abelev, G.I.: (1968) Production of Embryonal serum alpha
Globulin by Hepatomas: Review of Experimental and clinical
data. Cancer Res. 28: 1344-1350

Adam, E.; Levy, A.H.; Rawls, W.E. & Melnick, J.L. (1971)
Sero-epidemiologic studies of Herpes virus type 2 and
Carcinoma of the cervix. I: Case control matching.
J. Nat. Cancer Inst. 47: 941-951

_____ Kaufman, R.H.; Melnick, J.L.; Levy, A.H.; & Rawls, W.E.
(1972) Sero-epidemiologic studies of Herpes Virus
Type 2 and carcinoma of cervix. III. Houston, Texas
Am. J. Epidem. 96: 427-442.

_____ Sharma, S.D. & Zeigler, O. (1972(b)) Sero-epidemiologic studies
of Herpes virus Type 2 and carcinoma of the cervix.
II. Uganda. J. Nat. Cancer Inst. 48: 65-72

Adelusi, B.; Osunkoya, B.O. & Fabiyi, A. (1975) Antibodies to
Herpes Type-2 virus in carcinoma of the cervix uteri in
Ibadan. Am. J. Obstet. Gynec. 123:758-761

_____ Ayeni, O.; Fabiyi, A. & Osunkoya, B.O. (1975(b))
Serological evidence for the venereal transmission of Herpes
type-2 virus infection in Ibadan. Nig. Med. J. (In Press).

Alford, C.; Snider, M. & Stubbs, G. (1967) Studies on the
        virulence of Herpes Simplex isolated from different clinical
        entities. Pediat. Res. 1; 209-210

Allison, A.C. (1954). Protection afforded by sickle cell trait
        against subtertian malaria infection. Brit. Med. J. 1:290-294.

Amstey, M.S. & Balduzzi, P.C. (1970) Genital herpes virus infection:
        Diagnosis and significance. Am. J. Obstet. Gynec. 108:188-193.

Anderson, S.G. & Linton, E.G. (1967) The diagnostic accuracy of
        cervical biopsy and cervical conisation.
        Am. J. Obstet. Gynec. 99: 113-116.

Ashley, D.J. (1966) The biologic status of carcinoma in situ in
        of the uterine cervix. J. Obstet. Gynec. Brit. Comm. 73:
        372-381.

Aurelian, L.; Royston, I. & Davis, H.J. Antibody to Genital
        Herpes Simplex virus: Association with cervical atypia
        and carcinoma in-situ. J. Nat. Cancer. Inst. 45: 455-464

_____ Strandberg, J.D.; Melendez, L.V. & Johnson, L.V. (1971)
        Herpes virus type 2 isolated from cervical tumour cells
        grown in tissue Culture. Science 174: 704-707

_____ Davis, H.J. & Julian, C.G. (1973) Herpes virus type 2
        induced tumour-specific antigen in cervical cancer.
        Am. J. Epidem. 98: 1-9

_____ Schumann, B.; Marcus, R.L. & Davis, H.J. (1973(b)).
        Antibody to HSV-2 induced tumour specific antigens in serum
        from patients with cervical carcinoma. Science 181: 161-164

Ayre, J.E. (1947) Cervical Cancer: a disordered growth response
  to inflammation in the presence of estrogen excess and
  nutritional deficiency. Am. J. Obstet. Gynec. 54: 363-390

_____ (1960) Role of the halo cell in cervical carcinogenosis.
  A virus manifestation in Pre-malignancy.
  Obstet. Gynec. (N.Y.) 15: 481-491

_____ (1965) Human pre-carcinogenic cell manifestation
  associated with polythylene contraceptive device.
  Indust. Med. Surg. 34: 393-403

Bamforth, J. & Cardell, B.S. (1962) The laboratory diagnosis of
  carcinoma of the uterine cervix.
  J. Obst. Gynec. Brit. Comm. 69: 379-388

Barile, M.F.; Blumberg, J.M.; Kraul, C.W. & Vaguch, R.(1962)
  Penile lesions among U.S. forces personel in Japan.
  Acta Derm.(Chicago) 86: 273-281.

Barron, B.A. & Richart, R.M. (1971) An epidemiologic study of
  cervical neoplastic disease. Cancer 27; 978-986

Bechtold, E. & Reicher, N.B. (1952) Relationship of Trichomonas
  infestation to false diagnosis of carcinoma of the cervix.
  Cancer 5: 442-457

Beilby, J.D.W.; Cameron, C.H.; Calterall, R.D. & Davidson, D.
  (1968). Herpes virus Hominis infection of the cervix
  associated with gonorrhoea. Lancet 1: 1065-1066.

Belote, G.H. (1931) Association of cancer and Syphilis as
  determined by positive serology. Am. J. Syph. 15: 372-375

Beral, V. (1974) Cancer of the Cervix: A sexually transmitted
    Infection? Lancet 1: 1037-1040

Bernstein, M.T. & Stewart, J.A. (1971) Method for typing antisera
    to Herpes virus Hominis by indirect Hemagglutination
    Inhibition. Applied Microbiol. 21: 680-684

Bertini, B. & Hornstein, M. (1970). The Epidemiology of
    Trichomoniasis and the role of this infection in the development
    of carcinoma of the cervix. Acta Cytol. (Balt) 14: 325-332

Biggs, P.M.; Churchill, A.E.; Rootes, D.G. & Chubb, R.C. (1968)
    The etiology of Marek's disease: an oncogenic herpes type
    virus. Perspect Virol 6: 211-237

Blomfield, G.W.; Cherry, C.P. & Glucksman, A. (1965) Biologic
    factors influencing radiotherapeutic results in carcinoma of
    cervix. Brit. J. Radiol. 38: 241-254

Botella Llusia, J.; Nogales Ortiz, F.; Gimenez-Tebar, V. & Zamarriego
    Crepo, J. (1962). Effect of radiotherapy of tumour bearing
    lymph nodes in carcinoma of the cervix uteri.
    Am. J. Obstet. Gynec. 84: 508-514

Brack, C.B.; Townsend, L.; Haines, Z.W. & Dickson, R.J. (1956)
    Cervical cancer: Factors influencing prognosis in intra-epithelial
    and clinical carcinoma of cervix uteri. Obstet. Gynec. 8: 728-735

Brunschwig, A. & Barber, H.R.K. (1966) Surgical treatment of carcinoma
    of the cervix. Obstet. Gynec. 27: 21-29

Boyd, J.C.; & Doll, R.A. (1964) A study of the etiology of carcinoma
    of the cervix uteri. Brit. J. Cancer 18: 419-434

Boyland, E.; Roe, F.J.; & Mitchley, B.D. (1966) Test of certain
    constituents of Spermicides for carcinogenicity in genital tract
    of female mice. Brit. J. Cancer 20: 181-189

Broders, A.C. (1926) Carcinoma, grading and practical application.
Arch. Path. Lab. Med. 2: 376-381

_____ (1932) Carcinoma in-situ contrasted into Benign penetrating
epithelium. J.A.M.A. 99: 1670-1674.

_____ (1932(b)) Practical points on microscopic grading of carcinoma
N.Y. State J. Med. 32: 667-671.

Browne, F.J. & McClure Browne, J.C. (1964) Postgraduate Obst. &
Gynae. 3rd Ed. Butterworths. London.

Catalano, L.W. & Johnson, L.D. (1971). Herpes virus antibody and
carcinoma in-situ of the cervix. J.A.M.A. 217: 447-450.

Cederqvist, L.; Elliasson, G.; Lindell, L. & Stoemby, K. (1970)
Nuclear inclusion bodies in vaginal smears from patients
with vaginal discharge. Acta Obstet. Gynec. (Scand.) 49: 13-16

Centifanto, Y.M.; Hilderbrandt, R.J.; Held, B. & Kaufman, H.E. (1971).
Relationship of Herpes Simplex genital infection and carcinoma
of the cervix: Population studies.
Am. J. Obstet. Gynec. 110: 690-692

Chang, G.C.H.; Del Buono, I.; Tunca, C. & Nahmias, A.J. (1974)
Herpes simplex virus - 2 antigens detected in Human cervical
smears by anti-complement Immunofluorescence. (In Press)

Channen, W. & Hollycock, V.E. (1971) Colposcopy and electro-coagulation
diathemy for cervical dysplasia and carcinoma in situ.
Obstet. Gynec. 37: 623-628

Christian, R.T.; Ludovici, P.P.; Miller, N.F. & Riley, G.M. (1965)
Viral studies of the female reproductive tract.
Am. J. Obstet. Gynec. 91: 430-436

Christopherson, W.M. & Parker, J.E. (1960) A study of the relative
 frequency of carcinoma of the cervix in the Negro.
 Cancer 13: 711-713

_____ & Parker, J.E. (1965) Relations of cervical cancer to early
 marriage and childbearing. New Surg. J. Med. 273: 235-239

_____ Gray, L.A. & Parker, J.E. (1967). Role of Punch Biopsy in
 subclinical lesions of the uterine cervix.
 Obstet. Gynec. 30: 806-811

Churchill, A.E. & Biggs, P.M. (1967) Agents of Marek's Disease
 in tissue culture. Nature 215: 528-530

_____ & Biggs, P.M. (1968) Herpes type virus isolated in cell
 culture from tumours of chicken with Marek's Disease.
 I: Studies in cell culture. J.Nat. Cancer Inst. 41: 939-950

_____ & Biggs, P.M. (1968(b)) Herpes-type virus isolated in cell
 culture from tumours of chicken with Marek's disease.
 II: Studies in vivo. J. Nat. Cancer Inst. 41: 951-959

_____ Payne, L.N. & Chubb, R.C. (1969) Immunisation against
 Marek's Disease using a live attenuated vaccine.
 Nature 215: 528-530

Clemmensen, J. (1952) The Etiology of some Human Cancers.
 J. Nat. Cancer Inst. 12: 1-21

Conger, B.; Tyler, C.; Nahmias, A.J.; Naib, Z.M.; Josey, W.E. &
 Jenkins, R. (1972) Herpes Simplex Virus type 2 infection in a
 housing project. (Quoted by Josey et al 1972)

Coppleson, M. & Reid, D.L. (1967) Pre-clinical carcinoma of the
 cervix uteri. Oxford Pergamon Press.

Coppleson, M. (1969) Carcinoma of the Cervix: epidemiology and etiology. Brit. J. Hosp. Med. 2: 961-980

Couch, E.F. & Nahmias, A.J. (1969) Fillamentous structures of Type 2 Herpes virus Hominis infection of the chorio-allantoic membrane. J. Virol. 3: 228-232.

Cowdry, E.V. (1944) Factors in Cancer Production. Surg. Clin. North Am. 24: 985-993

Cross, H.E.; Kennel, E.E. & Lilienfield, A. (1968) Cancer of the cervix in an Amish Population. Cancer 21: 102-108.

Cummins, G.T.M. (1960) Carcinoma of the uterine cervix: Some epidemiological data from a Hospital in Jamaica. J. Obst. Gynec. Brit. Common. 67: 618-622

Davies, J.N.R.; Knoweldon, J. & Wilson, B.A. (1965) Incidence rates of Cancer in Kyadondo County, Uganda (1954-1960). J. Nat. Cancer Inst. 35: 789-821

_____ & Knoweldon, J. (1966) In: Cancer incidence in Five continents (Ed. by Doll, R.; Payne, P. & Waterhouse, J.) Vol. I. Spinger. New York.

Dickie, E.G. (1969) Herpes vulvitis. Obstet. Gynec. 34: 434-466

Diddle, A.W.; Watts, G.F.; Gardner, W.H. & Williamson, P.J. (1966) Oral Contraceptive Medication: A prolonged experience. Am. J. Obst. Gynec. 95: 489-495

Dilts, P.V.; Glesh, R.H. & Greene, R.R. (1964) Re-evaluation of four-quadrant punch biopsies of the cervix. Am. J. Obstet. Gynec. 90: 961-971

Di Virgilio, G.; Lavenda, N. & Siegel, D. (1965) Viruses: The

 Vaginitis-cervicitis complex and cervical cancer in man.

 Am. J. Obstet. Gynec. 93: 491-501

Dobbie, W.B.M.; Taylor, C.W. & Waterhouse, J.A.M. (1962)

 Study of Carcinoma of cervix: Series of 1030 cases of

 stages I & II seen from 1944-1953.

 J. Obstet. Gynec. Brit. Comm. 69: 543-552

Dodd, K.; Johnston, L.M. & Buddingh, G.J. (1938) Herpetic

 stomatitis. J. Pediat. 12: 95-102

Dodge, O.G.; Linsell, C.A. & Davies, J.N.R. (1963) Circumcision

 and the incidence of carcinoma of the penis and the cervix.

 E. Afri. Med. J. 40: 440-444

Doherty, J.J.; O'Neill, F.J. & Rapp, F. (1971). Differential

 susceptibility to Herpes Simplex virus of Hamster cell

 lines after exposure to chemical inactivated herpes viruses.

 J. Gen. Virol. 13: 377-384

Dorn, H.F.; & Cutler, S.J. (1959) Morbidity from Cancer in the

 U.S. P.H.S. Publ. No. 590 Washington D.C. Gort. Pig. Office.

Dougherty, C.M. (1960) Junction of Cancerous epithelium and stroma

 in the Uterine cervix: Electron microscope studies.

 Am. J. Obstet. Gynec. 79: 330-335

Dowdle, W.R.; Nahmias, A.J.; Harwell, R.W. & Pauls, F.P. (1967)

 Association of Antigenic types of Herpes virus Hominis with

 site of viral recovery. J. Immunol. 99: 974-980

Duenas, A.; Adam, E.; Melnick, J.L. & Rawls, W.E. (1972).

 Herpes virus type-2 in a Prostitute population.

 Am. J. Epidem. 95: 483-489

Duff, R. & Rapp, F. (1971) Properties of hamster embryo fibroblasts
transformed in vitro after exposure to Ultra-violet light-
irradiated Herpes Simplex virus type-2.
J. Virol. 8: 469-477.

Dunham, L.J. & Bailer, J.C. (1968) Wall maps of Cancer mortality
rates and frequency ratios. J. Nat. Cancer Inst. 41: 155-203

Dunn, J.M. & Buell, J.P. (1959) Association of cervical cancer
with circumcision of sexual partner.
J. Nat. Cancer Inst. 22: 749-764

Eagles, H. (1955) Nutritional needs of mammalian cells in tissue
culture. Science 122: 501-504

Edington, G.M. & Maclean, C.M.U. (1965) A cancer rate survey in
Ibadan, Western Nigeria, 1960-1963. Brit. J. Cancer 19: 471-481
_____ & Hendrickse, M. (1970) In Doll. R.; Muir, C. & Waterhouse, J.
(Eds.). Cancer incidence in Five continents. Vol. II
(IUCC) Spinger-Verlag N.Y.
_____ (1970) Cancer of the Uterus in the Western State of Nigeria.
Afri. J. Med. Sci. 1: 67-77
_____ & Hendrickse, M. (1972) The geographical pathology of cancer
in Africa, with special reference to the Western State of
Nigeria. Dokita 4: 1-9

Ejercito, P.M.; Kieff, Z.D. & Roizman, B. (1968) Characterisation
of Herpes simplex virus strains differing in their effect
on social behaviour of infected cells. Virol. 2: 357-364

Epstein, M.A.; Achong, B.G. & Barr, Y.M. (1964) Virus Particles
in cultured lymphoblasts from Burkitt's lymphoma.
Lancet 1: 702-703

Epstein, M.A. & Achong, B.G. (1965) Specific Immunofluorescence
    Test for the Herpes Type E.B. Virus of Burkitt Lymphoblasts
    Authenticated by Electron Microscopy.
    J. Nat. Cancer Inst. 40: 593-607

_____ (1971) The possible role of Viruses in Human Cancer.
    Lancet 1: 1344-1347

Esan, G.J.F. (1970) The Thalassemia Syndromes in Nigeria. Brit. J.
                                                    Haem. 19: 47-56

Evans, A.S. (1971) The Spectrum of infections with Epstein-Barr virus:
    A Hypothesis. J. Infect. Dis. 124: 330-342

Fabiyi, A. (1972) Personal Communications. Based on the report
    of a W.H.O. Survey of the prevalence of antibodies to
    Herpes Type-1 virus in Ibadan.

Feorino, P.M. & Palmer, E.L. (1973) Incidence of antibody to
    envelope antigen of Herpes simplex virus type 2 among
    patients with cervical carcinoma. J. Infect. Dis. 127: 732-735.

Figge, D.C. & Bennington, J.L. (1967) A study of the epidemiology
    of cervical cancer. Am. J. Obstet. Gynec. 98: 516-525

Figueroa, M. & Rawls, W.E. (1967) Biological markers for differen-
    tiation of Herpes Virus strains of oral and genital origin.
    J. Gen. Virol. 4: 259-267.

Finck, F.M. & Denk, M. (1970) Cervical Carcinoma: Relationship
    between histology and survival following radiation therapy.
    Obstet. Gynec. 35: 339-343

Fishman, M.; Shear, M.J.; Friedman, H.F. & Stewart, H.L. (1942)
    Studies in carcinogenesis, Effect of repaired application of 3,4,
    benzopyrene and of human smegma to the vagina and cervix of mice.
    J. Nat. Cancer Inst. 2: 361-367

Fluhmann, C.F. (1960) Carcinoma in-situ and the transitional zone
    of the cervix uteri. Obstet. Gynec. 16: 424-437

Frenkel, N.; Roizman, B.; Cassal, E. & Nhamis, A. (1972)
    A DNA fragment of Herpes Simplex 2 and its transcription in
    human cervical cancer tissue. Proc. Nat. Acad. Sci. (U.S.A.)
    69: 3784-3789.

Friedell, G.H. & Parsons, L. (1962) Blood vessel invasion in cancer
    of the cervix. Cancer 15: 1269-1274

Fox, C.H. (1967) Biologic behaviour of dysplasia and carcinoma
    in-situ. Am. J. Obst. Gynec. 99: 960-974

Frost, J.K. (1961) Cytology of Benign conditions.
    Clin. Obstet. Gynec. 4: 1075-1096

Fuccillo, D.A.; Moder, F.L.; Catalano, L.W.; Vincent, M.M. & Sever, J.L.
    (1970). Herpes virus Hominis types I & II: A specific micro-indirect
    hemagglutination test. Proc. Soc. Experi. Biol. Med. (N.Y.)
    133: 735-739.

Gagnon, F. (1950) Contribution to the study of the etiology and
    prevention of cancer of the cervix of the uterus.
    Am. J. Obstet. Gynec. 60: 516-522.

Gall, S.A.; Walling, J. & Pearl, J. (1973) Demonstration of
    tumour associated agents in human gynaecologic malignancies.
    Am. J. Obst. Gynec. 115: 387-393

Gardner, H.L. & Kaufman, R.H. (1969) Benigh diseases of the vulva
    and vagina. The C.V. Mosby Co. St. Louis.

Geder, L. & Skinner, G.R. (1971) Differentiation between type-1 and
      type-2 strains of Herpes Simplex virus by an indirect
      immunofluorescent technique. J. Gen. Virol. 12: 179-182.

Gerber, P. & Rosenblum, E.N. (1968) The incidence of complement
      fixing antibodies to Herpes simplex and Herpes-like viruses
      in man and Rhesus monkeys.
      Proc. Soc. Expt. Biol. Med. 128: 541-546

Gilles, H.M. (1967) Akufo. An environmental study of a Nigerian
      Village Community. Ibadan University Press.

Gilmour, M.; Glucksmann, A. & Spear, F.G. (1949) Influence of tumour
      histology, duration of symptoms and age of patient on radio-
      ariability of cervix tumours. Brit. J. Radiol. 22: 90-95

Gold, P. & Freedman, S.O. (1965) Demonstration of tumour specific
      antigens in human colonic carcinoma by immunological tolerance
      and absorption technique. J. Expt. Med. 121: 439-462.

Goodheart, C.R.; Plummer, G.; Warner, J.L. (1968) Density difference
      of DNA of Human Herpes Simplex viruses Types 1 and 2.
      Virology 35: 473-475

Grace, J.T. (1971) Studies of Epstein Barr Virus.
      Ann. N. Y. Acad. Sci. 174: 946-966

Graham, J.B.; Solto, L.S.J. & Paloucek, F.P. (1962) Carcinoma of
      the cervix. Saunders Philadelphia & Lond.

Granoff, A.; Gravell, M. & Darlington, R.W. (1969) Studies on the
      viral etiology of the renal adenocarcinoma of Rana Pipiens
      (Lucke Tumour). In: Recent results of Cancer Research.
      Springer-Verlag. New York. Pp. 275-279

Gravell, M. (1971) Viruses and renal carcinoma of rana pipiens.

    X: Comparison of Herpes Type viruses associated with Lucke

    Tumour bearing Frogs. Virology 43: 730-733

Green, G.H. (1962) Carcinoma in situ of the uterine cervix.

    Conservative management of 84 of 190 cases.

    Aust. & N.Z.J. Obstet. Gynec. 2: 49-57.

Greene, H.J.; Oppenheim, A. & Olswang, A. (1965) Results of routine

    papanicolaou Smears in two venereal Disease Clinics in New York

    City. Acta Cytol. 9: 319-321

Griffiths, C.T.; Austin, J.H. & Younge, P.A. (1964) Punch biopsy

    of the cervix. Am. J. Obstet. Gynec. 88: 695-703

Haenszel, W.; & Hillhouse, M. (1959) Uterine Cancer Morbidity in

    New York City and its relation to the pattern of Regional variation

    within the U.S. J. Nat. Cancer Inst. 22: 1157-1181

Haggard, J.L.; Cotten, N.; Dougherty, C.M. & Mickal, A. (1964).

    Primary adenocarcinoma of the cervix. Obst. Gynec. 24: 183-193

Hall, J.E. & Walton, L. (1968) Dysplasia of the cervix; a prospective

    study of 206 cases. Am. J. Obst. Gynec. 100: 662-671

Hampar, B. & Ellison, S.A. (1961) Chromosome Aberrations indiced by

    an animal virus. Nature 192: 145-147

Handley, W.S. (1936) The prevention of Cancer. Lancet 1: 987-991

Handy, V.H. & Burnette, W.S. (1966) In: Cancer incidence in Five

    continents (Ed. by Doll, R.; Payne, R. & Waterhouse, J.)

    Vol. I. Spinger, N.Y.

Harding, W.G. (1942) The influence of syphilis in cancer of the

    cervix uteri. Cancer Res. 2: 59-61.

Harold, E.A.; Elmer, E.K. & Abraham, M.L. (1968) Cancer of the
cervix in an Amish population. Cancer 21: 102-108

Hellstrom, I.; Hellstrom, K.E.; Pierce, G.E. & Bill, A.H. (1968)
Demonstration of cell-bound and humoral immunity against
neuroblastoma cells. Proc. Nat. Acad. Sci. (U.S.) 60: 1231-1238

Hendrickse, J.P. de V. (1972) Gynaecological Tumours in Ibadan.
Dokita 4: 25-30

Higginson, J. & Oettle, A.G. (1966) In Cancer incidence in Five
continents (Ed. by Doll, R.; Payne, P. & Waterhouse, J.)
Vol. I. Spinger. New York.

Hockman, A.; Ratzkowscki, E. & Schraiber, H. (1955) Incidence of
carcinoma of cervix in Jewish women in Israel.
Brit. J. Cancer 9: 358-364

Hoffman, F.L. (1933) Cancer mortality of Amstadam, Holland, by
religious sects. Am. J. Cancer 17: 142-153

Hollinshead, A.; Lee, D.B.; Mckelway, W.; Melnick, J.L. & Rawls, W.E.
(1972) Reactivity between Herpes virus type 2-related soluble cervical
tumour cell membrane antigen and matched cancer and control sera.
Proc. Soc. Expr. Biol. Med. 141, 688-693

Horwitz, A. (1927) Incidence of carcinoma of the uterus among
Jewish women. Surg. Gynec. & Obstet. 44: 355-358

Hulka, B.S. (1970) Punch Biopsy and conisation as diagnostic
procedures after abnormal cervical smears. Obstet. Gynec. 36:54-61
_____ & Kupper, L.L. (1971) An epidemiologic test of the spectrum
of disease concept in cervical neoplasia.
J. Nat. Cancer Inst. 47: 1215-1222

Hutfield, D.C. (1966) History of Herpes Genitalis.

   Brit. J. Vener. Dis. 42: 263-263

_____ (1967) Isolation studies with genital strains of Herpes

   Simplex Viruses. Acta Derm. (Stockholm) 47: 118-122

_____ (1968) Herpes Genitalis.

   Brit. J. Vener. Dis. 44: 241-150

Hull, E.C. (1973) Clear cell carcinoma of the cervix and vagina

   in young women. Am. J. Obstet. Gynec. 116: 470-481

Hunt, R.D.; Melendez, L.V.; King, N.W.; Gilmore C.E.; Daniel, M.D.;

   Williamson, M.E. & Jones, T.C. (1970) Morphology of a disease

   with features of malignant Lymphoma in marmosets and owl monkeys

   inoculated with Herpes Samiari. J. Nat. Cancer Inst. 44:447-465

Hynes, J.F. (1948) Cancer of cervix uteri: Review of 296 cases.

   Am. J. Roentgenol 60: 368-381

Ishihama, A. & Kagabu, T. (1965) Cytological studies after

   insertion of I.U.D. Am. J. Obstet. Gynec. 91: 576-578

Janda, Z.; Kanka, J.; Vonka, V. & Svoboda, B. (1973) A study of

   Herpes Simplex type 2 antibody status in groups of patients

   with cervical neoplasia in Czechoslovakia.

   Int. J. Cancer 12: 626-630

Jeansson, S. & Molin, L. (1971) Genital Herpes Simplex Infection

   Lakartindningen 68: 467-471

_____ (1972) Differentiation between herpes simplex virus type 1

   and type 2 strains by immuno-electro-osmophoresis.

   Appl. Microbiol. 24: 96-100

Jeffcoatt, J.N.A. (1969) Principles of Gynaecology 3rd Ed. Butterworths, London.

John, U.W.; Nathenson, L; Schwartz, R.S. & Skinnerm (1970) In vitro lymphocyte stimulation by soluble antigen from malignant melanoma. New Eng. J. Med. 283: 329-333

Johnson, L.D.; Hertrg, A.T.; Hinman, C.H. & Easterdy, C.L. (1960) Preinvasive cervical lesions in Obstetric patients: method of diagnosis, course and clinical management. Obstet. Gynec. 16: 133-145

_____; Easterday, C.L.; Gore, H. & Hertig, A.T. (1964) The histogenesis of carcinoma in-situ of the uterine cervix; a preliminary report of the origin of carcinoma in-situ in subcylinderical cell anaplasia. Cancer 17: 213-229

_____ Nickerson, R.J.; Easterday, C.L.; Stuart, R.S. & Hertig, A.T. (1968) Epidemiologic evidence for the spectrum of change from dysplasia through carcinoma in situ to invasive cancer. Cancer 22: 901-914

Jones, E.G.; MacDonald, I. & Breastow, L. (1958) A study of epidemiologic factors in carcinoma of the uterine cervix. Am. J. Obstet. Gynec. 76: 1-10

Jones, H.W.; Katayama, K.P.; Stafl, A. & Davis, H.J. (1967) Chromosomes of cervical atypia, carcinoma in situ and epidermoid carcinoma of the cervix. Obstet. Gynec. 30: 790-805

Jordan, M.J.; Bader, G.M. & Day, E. (1964) Carcinoma in-situ of the cervix and related lesions. An 11-year prospective study. Am. J. Obst. Gynec. 89: 160-182

Josey, W.E.; Nahmias, A.J.; Naib, Z.M.; Utley, P.M.; McKenzie, W.J.
& Coleman, M.T. (1966) Genital Herpes Simplex infection in
the female. Am. J. Obstet. Gynec. 96: 493-501

_____ Nahmias, A.J. & Naib, Z.M. (1968) Genital infection with
Type-2 Herpes virus Hominis: Present knowledge and
possible relationship to cervical cancer. Am. J. Obstet. Gynec.
101: 718-729

_____ Nahmias, A.J. & Naib, Z.M. (1969) Viral and virus-like infections
of the female genital tract. Clin. Obstet. Gynec. 12: 161-178

_____ Nahmias, A.J. & Naib, Z.M. (1972) The epidemiology of Type-2
(genital) herpes simplex virus infection.
Obstet. Gynec. Survey 27: 295-302

Kaiser, R.F. & Gullian, A.G. (1958) Some Epidemiological aspects of
Cervical cancer. Pub. Health. Rep. Wash. 73: 359-367

Kaminetzky, H.A. & Swedlow, M. (1962) Carcinoma in-situ; Factors
in its diagnosis. Obstet. Gynec. 19: 721-723

_____ & Swerdlow, M. (1965) Podophyllin and the mouse cervix:
assessment of carcinogenic potential. Am. J. Obstet. Gynec. 93:486-490

Kawakami, T.G.; Huff, S.D.; Buckley, P.M.; Dungworth, D.L. & Snyder, S.P.
(1972) C-type virus associated with gibbon lymphosarcoma.
Nature New Biol. 235: 170-171

Kay, S.; Snable, W.J. & Hume, D.M. (1970) Cervical dysplasia and
carcinoma developing in women on immunosuppression therapy for
renal transplantation. Cancer 26: 1048-1052.

Kazumasa, M. (1969) Uterine cancer control in Sweeden. Proc. World
Conf. on Ca. Uterus. New Orleans, Louisiana. Pp 41-45.

Kleger, B.; Prier, J.E.; Rostato, D.J. & McGinnis, A.E. (1968)

Herpes Simplex Infection of the Female Genital Tract.

I: Incidence of infection. Am. J. Obstet. Gynec. 102: 745-748

Kennaway, E.L. (1948) Racial and Social Incidence of Cancer of

the Uterus. Brit. J. Cancer 2: 177-212

Kinsey, A.C.; Pomeroy, W.B.; Martin, C.E. & Gebhard, P.H. (1953)

Sexual behaviours in the Human Female. Philadelphia. Saunders.

Kmet, J.; Damjanoski, L.; Stucin, M.; Borta, S. & Cakmokow, A. (1963)

Circumcision and Carcinoma Colli Uteri in Macedonia, Yugoslavia.

Results from a field study. Brit. J. Cancer 17: 391-399

Koss, L.G. & Wolinska, W.H. (1959) Trichomonas, vaginitis,

cervicitis and its relationship to cervical cancer. A

histocytological study. Cancer 12: 1171-1193

_____ Stewart, F.W.; Foote, F.W.; Jordan, M.J.; Barder, G.M. &

Day, E. (1963) Some histological aspects of behaviour of

epidemoid carcinoma in situ and related lesions of uterine

cervix: Long term prospective study. Cancer 16: 1160-1211.

Kotcher, E.; Gray, L.A.; James, Q.C.; Frick, C.A. & Botorff, D.W.

(1962) Cervical cell inclusion bodies and viral infection of

the cervix. Ann. N. Y. Acad. Sci. 97: 571-580

Krunmholz, B.A. & Knapp, R.C. (1972) Colposcopic selection of

Biopsy sites. Obstet. Gynec. 39: 22-26

Lawson, J.B.; Nwosu, S.S.O. & Olafimihan, K.A. (1964) Carcinoma of

the cervix in Nigeria.

J. Obst. Gynec. Brit. Comm. 71: 701-706

Levi, M.; Keller, S. & Mandl, I. (1969) Antigenecity of a papillary
serous cystadenocarcinoma tissue homogenate and its fractions.
Am. J. Obstet. Gynec. 105: 856-861

_____ (1971) Antigenecity of ovarian and cervical malignancies with
a view toward possible immunodiagnosis.
Am. J. Obstet. Gynec. 109: 689-698

Levin, M.L.; Kress; L.C. & Goldstain, H. (1942) Syphilis and cancer:
reported syphilis prevalence among 7,761 cancer patients.
N.Y. State J. Med. 42: 1737-1745

_____ Haenszel, W.; Carrol, E.E.; Gerhardt, P.R.; Hardy, V.H.; &
Igraham, S.C. (1960) Cancer incidence in Urban and Rural
areas of New York State. J. Nat. Cancer Inst. 24: 1243-1257

Lewis, G.C.; Rowe, W.P.; Angello, R.J.D. & Brodsky, I. (1965)
Investigation of viral etiology for cervical cancer.
Am. J. Obst. Gynec. 93: 659-668

Linell, F. & Maansson, B. (1952) Prognostic value of histologic
grading of carcinoma of the cervix uteri; study of 338 cases
treated with radium and roentgen therapy. Acta Radiol. 38: 219-238

Llewellyn-Jones, D. (1965) Trophoblastic tumours: Geographic variations
in incidence and possible etiological factors.
J. Obstet. Gynec. Brit. Comm. 72: 242-248

Lombard, H.L. & Potter, E.A. (1950) Epidemiological aspects of
cancer of cervix: Hereditary and Environmental factors.
Cancer 3: 960-968

Lowry, S.P.; Melnick, J.L. & Rawls, W.E. (1971) Investigation of
plaque formation in chick embryo cells as a biological marker
for distinguishing herpes type 2 from type 1.
J. Gen. Virol. 10: 1-10

Louis, C.J. (1960) A histochemical study by fluorescence technique
of the epithelial tumours of the cervix and uterus.
Am. J. Obstet. Gynec. 79: 336-345

Louw, J.T. (1956) Scattered thoughts on cancer of uterine cervix.
South Af. Med. J. 30: 933-937

Lundin, F.E. & Erickson, C.C. (1965) Prevalence of intraepithelial
carcinoma of the cervix in early years of marriage.
J. Nat. Cancer Inst. 35: 581-584

Maliphant, R.G. (1949) Incidence of cancer of uterine cervix.
Brit. Med. J. 2: 978-982

Martin, C.E. (1967) Marital and coital factors in cervical cancer.
Am. J. Pub. Health. 57: 803-814

Masters, W.H. (1960) The sexual response cycle of the Human Female.
West. J. Surg. 68: 57-72

Masterson, J.G. (1963) Radical surgery in early carcinoma of the
cervix. Am. J. Obstet. Gynec. 87: 601-605

Masubuchi, K. & Nemoto, H. (1972) Epidemiologic studies on uterine
cancer at Cancer Institute Hospital, Tokyo, Japan.
Cancer 30: 268-275

McLaren, H.C. (1963) The prevention of cervical cancer.
English Universities Press. London.

McLaren, H.C. (1964) The pill and Cancer. Brit. Med. J. 2: 573

McKay, D.G.; Terjanian, B.; Poschycinda, D.; Younge, P.A. & Hertig, A.T.
     (1959) Clinical and pathologic signfficance of anaplasia
     (atypical Hyperplasia) of the cervix uteri.
     Obstet. Gynec. 13: 2-21

McKenna, J.M.; Sanderson, R.P. & Blakemore, W.S. (1962) Extraction
     of distinctive antigens from neoplastic tissue.
     Science 135: 370-371

McNeil, C.; Ladle, J.N.; Helmick, W.M.; Trentelman, E. & Wentz, M.W.
     (1969) An antiserum to ovarian mucinous cyst fluid with colon
     cancer specificity. Cancer Res. 29: 1535-1540

Meisels, A. (1969) Dysplasia and carcinoma of the uterine cervix.
     IV: a correlated cytologic and histologic study with special
     emphasis on vaginal microbiology. Acta Cytol. 13: 224-231

Melendez, L.V.; Hunt, R.D.; Daniel, M.D.; Garcia, F.G.; & Fraser, C.E.O.
     (1969) Herpes virus Samiari: Experimentally indiced malignant
     lymphoma in primates. Lab. Anim. care 19: 378-386

_____ Hunt, R.D.; Daniel, M.D.; Blake, B.J. & Garcia, F.G. (1971)
     Acute Lymphocytic Leukemia in Owl Monkeys innoculated with
     Herpes virus Samiari. Science 171: 1161-1163

_____ Hunt, R.D.; King, N.W.; Barahona, H.H.; Daniel, M.D.;
     Fraser, C.E.O. & Garcia, F.G. (1972) Herpes virus ateles;
     a new lymphoma virus of monkeys. Nature New Biol. 235: 182-184

Melamed, M.R.; Ross, L.G.; Flehinger, B.J.; Kelisky, R.P. & Dubrow, H.
     (1969) Prevalence rates of uterine cervical carcinoma in situ
     for women using the diaphram or contraceptive oral steroids.
     Brit. Med. J. 3: 195-200

Melnick, J.L. & Rawls, W.E. (1970) Herpes virus type-2 and cervical
    carcinoma. Ann N.Y. Acad. Sci. 174: 993-998

Miller, N.F.; Hinerman, D.L.; Riley, G.M.; Ludovici, P.P.; Gosling,
    J.R.G.; Christian, R.T.; & Hall, D.F. (1959) The nature of
    cervix cancer. Obstet. Gynec. 14: 703-713

Mitani, J.; Fujii, J.I.; Miyamura, M.; Ishizu, S. & Matsukado, M.
    (1962) Lymphnode metastasis of carcinoma of the uterine
    cervix. Am. J. Obstet. Gynec. 84: 515-522

Mitchel, J.E. & McGall (1963) Transplacental infection by Herpes
    Simplex Virus. Am. J. Dis. Child. 106: 207-209

Mizell, M.; Stackpole, C.W. & Halpenen, S. (1969) Herpes-type virus
    recovery from "virus free" frog kidney tumours
    Proc. Soc. Expt. Biol. (N.Y.) 127: 808-814

_____ Toplin, I. & Isaacs, J. (1969) Tumour induction in developing
    frog kidney by a zonal centrifuge fraction of the frog herpes-
    type virus. Science 165: 1134-1137

Moghissi, K.S.; Mack, H.G. & Porzak, J.P. (1968) Epidemiology
    of cervical cancer. Am. J. Obstet. Gynec. 100: 607-614

Morton, D.G. & Lagasse, L.D. (1967) Pelvic lymphadenopathy as an
    adjunct to the radiation therapy of cervical carcinoma.
    Clin. Obstet. Gynec. 10: 965-973

Munoz, N (1973) Effect of Herpes virus Type-2 and Hormonal Imbalance
    on the uterine cervix of the mouse. Cancer Res. 33: 1504-1508

Naguib, S.M.; Laundin, F.E.; & Davis, H.J. (1966) Epidemiologic
    factors related to Cervical Cancer detected by a screening programme
    in Washington County, Maryland. Obstet. Gynec. 28: 451-459

Nahmias, A.J.; Josey, W.E. & Naib, Z.M. (1967) Neonatal Herpes Simplex Infection. J.A.M.A. 199: 164-168

_____ Naib, Z.M.; Highsmith, A.K. & Josey, W.E. (1967(b)) Experimental Genital Herpes Simplex infection in the mouse. Pediat. Res. 1: 209

_____ Naib, Z.M.; Josey, W.E. & Clepper, A.C. (1967(c)) Herpes Simplex Infection of the female genital tract. Virologic and cytologic studies. Obstet. Gynec. 29: 395-400

_____ Dowdle, W.R. (1968) Antigenic and biologic differences in Herpes virus Hominis. Prog. Med. Virol. 10: 110-159

_____ Dowdle, W.R.; Naib, Z.M.; Josey, W.E. & Luce, C.F. (1968(b)) Genital Infection with Herpes Virus Hominis Types 1 & 2 in Children. Pediatrics 42: 659-666

_____ Naib, Z.M. & Josey, W.E. (1968(c)) Association of Genital Herpes virus Hominis to Cervical Cancer. Proc. First Inter. Congress for Virol. Finland.

_____ Dowdle, W.R.; Naib, Z.M.; Highsmith, A.; Harwell, R.W. & Josey, W.E. (1968(d)) Relation of pock size on choiroaltantoic membrane to antigenic type of Herpes virus Hominis. Proc. Soc. Expt. Biol. Med. 127: 1022-1028

_____ Dowdle, W.R.; Naib, Z.M.; Josey, W.E.; McClone, D. & Domesick, G. (1969) Genital infection with Type 2 Herpes Virus Hominis: A commonly occuring venereal disease. Brit. J. Vener. Dis. 45: 294-298

Nahmias, A.J.; Naib, Z.M. & Josey, W.E. (1969(b)) Association of

    Genital Herpes Virus with cervical dysplasia and carcinoma.

    Inter virol. 1: 187-188

_____ Chiang, W.T.; Del Buono, I. & Duffey, A. (1969(c)). Typing

    of Herpes virus Hominis strains by a direct immunofluorescent

    technique. Proc. Soc. Expt. Biol. Med. 132: 386-390

_____ Josey, W.E. & Naib, Z.M. (1969(d)) Infection with Herpes virus

    Hominis types 1 and 2. Prog. Dermatol. 4: 7-11

_____ Dowdle, W.R.; Josey, W.E.; Naib, Z.M.; Painter, L.M. & Luce, C.F.

    (1969(e)) Newborn infection with Herpes virus Hominis types

    1-2. J. Pediat. 75: 1194-1203

_____ Dowdle, W.R.; Kramer, J.L.; Luce, C.F.; & Mansour, S.C. (1969(f))

    Antibodies to Herpes virus Hominis Types 1 and 2 in the rabbit.

    J. Immunol. 102: 956-962

_____ Naib, Z.M. & Josey, W.E.; Luce, C.F. & Guest, B.A. (1970)

    Antibodies to Herpes Virus Hominis Types 1 & 2 in Humans

    II: Women with cervical cancer.

    Am. J. Epidem. 91: 547-552

_____ Naib, Z.M. & Josey, W.E.; Murphy, F.A. & Luce, C.F. (1970(b))

    Sarcomas after innoculation of newborn hampsters with Herpes

    virus Hominis type-2 strains.

    Proc. Soc. Expt. Biol. Med. 134: 1065-1069

_____ Josey, W.E.; Naib, Z.M.; Luce, C. & Duffey, C. (1970(c))

    Antibodies to Herpes virus Hominis type 1 & 2 in humans

    1: Patients with genital Herpetic infections.

    Am. J. Epidem. 91: 539-546

Nahmias, A.J.; Alford, C.A. & Korones, S.B. (1970(d)) Infection of the new born with Herpes virus Hominis. Adv. Pediat. 17: 185-226

_____ Josey, W.E.; Naib, Z.M.; Freeman, M.G. & Fernadez, R.J. (1971) Perinatal risk associated with maternal genital Herpes Simplex virus infection. Am. J. Obstet. Gynec. 110: 825-837

_____ Naib, Z.M. & Josey, W.E. (1971(b)) Herpes virus Hominis type-2 infection - association with cervical cancer and perinatal disease. Perspect Virol. 7: 73-88

_____ London, W.; Catalano, L.; Fuccillo, D.; Sever, J. & Graham, C. (1971(c)) Genital Herpes virus Hominis type-2 infection: An experimental model in cebus monkeys. Science 171: 297-298

_____ Del Buono, I; Schneweis, K.E.; Gordon, D.S. & Their, D. (1971(d)) Type specific surface antigens of cells infected with Herpes virus (1 and 2). Proc. Soc. Expt. Biol. Med. 138: 21-27

_____ Naib, Z.M. & Josey, W.E. (1972) Genital Herpes and cervical cancer: Can a causal relation br proven? In: Biggs, P.M.; de-The, G. and Payne L.N. (Eds.) Oncogenesis and Herpes viruses I.A.R.C. Lyon. Pp 403-408

_____ (1972) Herpes virus from fish to man - a search for patho-biologic unity. Pathobiology Annual. 2: 153-182

_____ von Ryan, C.F.; Josey, W.E.; Naib, Z.M. & Hutton, R. (1973) Gonorrheal and genital herpes: Association and analogies. Brit. J. Vener. Dis. 49: 306-309

Naib, Z.M. (1966) Exfoliative Cytology of viral cervico-vaginitis Acta Cytol. (Balt) 10: 126-129

Naib, Z.M.; Nahmias, A.J. & Josey, W.E. (1966) Cytology and
histopathology of cervical Herpes simplex infection.
Cancer 19: 1026-1030

_____ Nahmias, A.J. & Josey, W.E. (1969) Genital Herpetic Infection:
Association with cervical dysplasia and carcinoma.
Cancer 23: 940-945

_____ Nahmias, A.J.; Josey, W.E. & Wheeler, J.H. (1970) Association
of maternal genital herpetic infection with spontaneous abortion.
Obstet.Gynec. 35: 260-263

Navratil, E. Burghardt, E.; Bajardi, F. & Nash, W. (1958) Simultaneous
colposcopy and cytology used in screening for carcinoma of the
cervix. Am. J. Obstet. Gynec. 75: 1292-1297

Ng, A.B.P.; Reagan, J.W. & Yen, S.S.C. (1970) Herpes Genitalis
Obstet. Gynec. 36: 645-651

_____ Reagan, J.W. & Lindner, E. (1970(b)) The cellular manifesta-
tions of Primary and Recurrent Herpes Genitalis.
Acta Cytologica 14: 124-129

Nigogosyan, G. & Mills, J. (1965) Herpes Simplex Cervicitis.
J.A.M.A. 191: 496

Nogales, F. & Botella Llusia, J. (1965) The frequency of lymph nodes
in cancer of the uterine cervix. Am. J. Obstet. Gynec. 93: 91-94

Odelblad, E. (1968) The functional structure of Human Cervical mucus.
Acta Obst. Gynec. (Scand) Suppl. 47: 57-79

Ojo, O.A. & Adekunle, J.J. (1976) The scope and value of cytologic
screening for carcinoma of the cervix in Ibadan.
West Afric. J. Surg. (In Press)

Order, S.E.; Porter, M. & Hellman, S. (1972) Hodgkin's Disease:
Evidence for a tumour-associated antigen
New Eng. J. Med. 285: 471-474

Ory, H.; Conger, B.; Richart, R. & Barron, B. (1974) Relation of
Type 2 Herpes virus antibodies to cervical neoplasia.
Barbados, West Indies 1971. Obstet Gynec. 43: 901-904

Pan, S.C. & Gardner, W.U. (1948) Carcinomas of the uterine cervix
and vagina in Estrogen and androgen treated hybird mice.
Cancer Res. 8: 337-345

Parker, J.D.J.; & Banatvala, V.E. (1967) Herpes Genitalis; Clinical
and Virological studies. Brit. J. Vener. Dis. 43: 212-216

Patten, S.R. (1969) Diagnostic pathology of the uterine cervix.
Basel S. Karger

Pauls, F.P. & Dowdle, W.R. (1967) A serologic study of Herpes virus
Hominis strains by micro neutralisation tests.
J. Immunol. 98: 941-474

Pereyra, A.J. (1961) The relationship of sexual activity to cervical
cancer. Cancer of the cervix in a prison population.
Obstet. Gynec. 17: 154-159

Pincus, G. & Garcia, C.R. (1965) Studies on vaginal, cervical and
uterine histology. Metabolism 14 (Supp.) 344-347

_____ & Garcia, C.R. (1967) Preliminary findings on Hormonal
Steroids and vaginal cervical and Endometrial histology.
Revista Inst. Nat. Cancer (Mex) 16: 452-455

Plaut, A. & Kohn-Speyer, A.C. (1947) The carcinogenic effect of
smegma. Science 105: 391-392

Plummer, G. (1964) Serological comparison of the Herpes viruses.

    Brit. J. Expt. Path. 45: 135-141

_____ & Hackett, S. (1966) Herpes Simplex Virus and paralysis in

    animals. Brit. J. Expr. Pathol. 47: 82-85

_____ Warner, J.L. & Bowling, C.P. (1968) Comparative studies type

    1 and type 2 Herpes simplex viruses.

    Brit. J. Expt. Pathol. 49: 202-208

_____ Warner, J.L.; Phuangsab, A. & Goodheart, C.R. (1970) Type 1

    and Type 2 Herpes Simplex Viruses. Serological and Biological

    differences. J. Virol. 5: 51-59

_____ & Masterson, J.G. (1971) Herpes Simplex virus and cancer of

    the cervix. Am. J. Obstet. Gynec. 111: 81-84

Pope, J.H.; Horne, M.K. & Scott, W. (1969) Transformation of fetal

    human leukocytes in-vitro by filtrates of a human leukemic

    cell line containing herpes-like virus. Int. J. Cancer 3:857-866

Poste, G.; Hawlings, D.F. & Thomlinson, J. (1972) Herpes virus

    Hominis infection of the female genital tract.

    Obst. Gynec. 40: 871-890

Pratt-Thomas, H.R.; Heins, H.C.; Latham, E.; Deins, E.J. & Ford, A.

    (1956) The carcinogenic effect of human smegma.

    Cancer 9: 671-680

Quinland, W.S. & Cuff, J.R. (1940) Primary Carcinoma in negro:

    anatomic distribution of 300 cases. Arch. Path. 30: 393-402

Rapp, F. & Falk, L.A. (1964) Study of virulence and tumorigenecity

    of variants of Herpes simplex virus.

    Proc. Soc. Expt. Biol. Med. 116: 361-365

Rapp, F. & Duff, R. (1972) Transformation of Hamster cells after
    infection by inactivated Herpes Simplex Virus type-2.
    In: Biggs, P.M.; de-The, G. & Payne, L.N. Oncogenesis and
    Herpes viruses. I.A.R.C. Lyon. Pp 447-450

Ratcliffe, H. (1971) The differentiation of Herpes simplex virus
    Type 1 and type 2 by temperature markers.
    J. Gen. virol. 13: 181-184

Rawls, W.E.; Laurel, D.; Melnick, J.L.; Glincksman, J.M.; J.M. &
    Kaufman, R.H.A. (1968) Search for viruses in Smegma,
    pre-malignant and early malignant cervical tissues.
    Am. J. Epidem. 87: 647-655

_____ Tompkings, W.A.F.; Figueroa, M. & Melnick, J.L. (1968(b))
    Herpes virus type-2: Association with carcinoma of the cervix.
    Science 161: 1255-1256

_____ Tompkings, W.A.F. & Melnick, J.L. (1969) The association of
    Herpes virus type-2 with carcinoma of the uterine cervix.
    Am. J. Epidem. 89: 547-554

_____ Gardener, H.L. & Kaufman, R.L. (1970) Antibodies to Genital
    Herpes virus in patients with carcinoma of the cervix.
    Am. J. Obst. Gynec. 107: 710-716

_____ Iwamoto, K.; Adam, E. & Melnick, J.L. (1970(b)) Herpes virus
    Type-2 antibodies and carcinoma of the cervix.
    Lancet 2: 1142-1143

_____ Iwamoto, K.; Adam, E. & Melnick, J. (1970(c)) Measurements of
    antibodies to Herpes virus types 1 & 2 in Human sera.
    J. Immunol. 104: 599-606

Rawls, W.E.; Gardner, H.L.; Flanders, R.W.; Lowry, S.P.; Kaufman, R.H. & Melnick, J.L. (1971) Genital Herpes in two ocial groups. Am. J. Obst. & Gynec. 110: 682-689

_____ Adam, E. & Melnick, J.L. (1972) Geographic variation in the association of antibodies to Herpes Virus type-2 and carcinoma of the cervix. In: Biggs, P.M.; De-The, G. and Payne, L.M. (Eds.) Oncogenesis and Herpes viruses. I.A.R.C. Pp 424-427

_____ Adam, E. & Melnick, J.L. (1973) An analysis of sero-epidemiologic studies of Herpes virus Type-2 and carcinoma of the cervix. Cancer Res. 33: 1477-1482

Reagan, J.W.; Hamonic, M.J. & Wentz, W.B. (1957) Analylitical study of the cells in cervical squamous-cell cancer. Lab. Invest. 6: 241-250

_____ Ng, A.B.P. & Wentz, W.B. (1969) Concepts of genesis and development in early cervical neoplasia. Obstet. Gynec. Survey. 24: 860-865

Reddy, D.G. & Baruah, I.K. (1963) Carcinogenic action of human smegma. Arch. Pathol. (Chicago) 75: 414-470

Reed, L.J. & Muensch, H. (1938). A simple method of estimating fifty percent end points. Am. J. Hyg. 27: 493 - 497

Richart, R.M. (1967) Natural history of cervical intra-epithelial neoplasia. Clin. Obstet. Gynec. 10: 748-784

_____ & Barron, B.A. (1967) The I.U.D. and Cervical neoplasia. A prospective study of patients with cervical dysplasia. J.A.M.A. 199: 817-819

Richart, R.M. & Barron, B.A. (1969) Follow up study of patients
with cervical dysplasia. Am. J. Obst. Gynec. 105: 386-393

Ringertz, N.O.E. (1969) Uterine cancer control in Sweeden.
Proc. World Conf. on Ca. Uterus. New Orleans, Louisiana.
Pp 41-45

Robinson, B.W. (1951) Malignancy in Negro: Statistical review.
Am. J. Roentgenol 66: 783-790

Roizman, B.; Keller, J.M. & Spear, P.G. (1970) Variability, Structural
glycoproteins and classification of Herpes simplex viruses.
Nature (Lond.) 227: 1253-1254

Rojel, J. (1953) The inter-relation between uterine cancer and
syphilis. Acta. Path et Microbiol. (Scand.) Supp. 97: 3-82

Rowson, K.E.K. & Jones, H.M. (1972) Herpes simplex type 1 and
type 2 antibody levels in patients with carcinoma of the
cervix or larynx. In: oncogenesis and Herpes viruses.
Biggs, P.M.; de-The, G. and Payne, L.N. (Eds.) I.A.R.C. Lyon
Pp 428-431

Rothman, A.: Rapoport, L.P. & Davidson, I. (1951) Carcinoma of the
cervix in Jewish women. Am. J. Obstet. Gynec. 52: 160-162

Rotkin, I.D. (1962) Relation of adolescent coitus to cervical cancer
risk. J.A.M.A. 179: 486-491

_____ (1967) Sexual characteristics of a cervical cancer population.
Am. J. Pub. Health 57: 815-829

_____ (1967(b)) Adolescent Coitus and Cervical Cancer. Associations
of related events with increased risk. Cancer Res. 27: 603-617

Rotkin, I.D. & King, R.W. (1962) Environmental variables related to cervical cancer. Am. J. Obst. Gynec. 83: 720-728

Royston, I. & Aurelian, L. (1970) The association of Genital Herpes virus with cervical atypia and carcinoma in-situ. Am. J. Epidem. 91:531-538

_____ & Aurelian, L. (1970(b)) Immunofluorescent Detection of Herpes virus antigens in exfoliated cells from Human Cervical Carcinoma. Proc. Nat. Acad. Sci. (U.S.) 67: 204-221

_____ Aurelian, L. & Davis, H.J. (1970) Genital Herpes virus finding in relation to cervical neoplasia. J. Rep. Med. 4: 109-113

Sabatelle, R.; Sedlis, A.; Sall, S. & Tchertkoff, V.(1969) Cervical biopsy versus conisation. Cancer 23: 663-666

Schneweis, K.E. (1968) Herpes Simplex Virus types 1 and 2 in various diseases. German Med. Monthly 13: 318-320

Schwartz, J. & Roizman, B. (1969) Similarities and differences in the development of laboratory strains and freshly isolated strains of Herpes simplex virus in HEp-2 cells. J. Virol. 4: 879-889

Sever, J.L. (1962) Application of a microtechnique to viral serological investigation. J. Immunol. 88: 320-329

Sidhu, A.S.; Koss, L.G. & Barber, H.R.K. (1970) Relation of Histologic factors and the response of stage 1 Epidermoid carcinoma of the cervix to surgical treatment. Obstet. Gynec. 35: 329-338

Sieber, O.F.; Fulginiti, V.A.; Brazie, J. & Umlauf, H.J. (1966) In utero infection of the foetus by Herpes Simplex Virus. J. Pediat. 63: 30-34

Silbar, E.L. & Woodruff, J.D. (1966) Evaluation of Biopsy, cone and
    Hysterectomy sequence in intra-epithelial carcinoma of the
    cervix. Obstet. Gynec. 27: 89-97

Sim, C. & Watson, D.H. (1973) The role of type specific and close-
    reacting structural antigens in the neutralisation of herpes
    simplex virus stypes 1 and 2.
    J. Gen. Virol. 19: 197-233

Singer, A. & Shearman, (1969) Contraceptives and Cervical Carcinoma.
    Brit. Med. J. 4: 108

Singleton, W.P. & Rutledge, F. (1968) To cone or not to cone:
    The Cervix. Obstet. Gynec. 31: 430-436

Skinner, G.R.B.; Thoules, M.E. & Jordan, J.A. (1971) Antibodies to
    type 1 and type 2 Herpes Virus in women with abnormal cervical
    cytology. J. Obstet. Gynec. Brit. Comm. 78: 1031-1038

Slavins, H.B. & Gavett, (1946) Primary Herpetic Vulvo-vaginitis.
    Proc. Soc. Expt Med. Biol. 63: 343-345

Smith, D.B. & Jenkins, R.S. (1969) Cervical cancer in a Housing
    Project: Report of a cluster of cases.
    Obstet. & Gynec. 104: 603-605

Smith, F.F. (1941) Nationality and carcinoma of the cervix.
    Am. J. Obstet. & Gynec. 41: 424-430

Smith, I.W.; Peutherer, J.F. & MacCullum, F.O. (1967) The incidence
    of Herpes virus Hominis antibody in the population.
    J. Hyg. (Canb). 65: 395-408

Smith, R.R.; Huclsner, R.N.; Rowe, W.P.; Schaltem, W.E. & Thomas,
L.B. (1956) Studies on the use of viruses in the treatment
of carcinoma of the cervix. Cancer 9: 1211-1218

Smith, W. & Mackay, J.M.K. (1969) Morphological observations on a
virus associated with sheep pulmonary adnomatosis.
J. Comp. Path 79: 421-424

South, M.A.; Tompkins, W.A.F.; Horris, R.C. & Rawls, W.E. (1969)
Congenital malformation of the central nervous system associated
with genital type (Type-2) Herpes virus. J. Pediat. 75: 13-18

Spreecher-Goldberger, S.; Thiry, L.; Catoor, J.P.; Hooghe, R. &
Pestian, J. (1970) Herpes Virus type-2 infection and carcinoma
of the cervix. Lancet 2: 266

Stephenson, J.J. & Grace, W.J. (1954) Life stress and Cancer of
the Cervix. Psychosom Med. 16: 287-294

Stern, E. (1959) Rate, stage and patient age in cervical cancer.
Cancer 12: 933-937

_____ & Dixon, W.J. (1961) Cancer of the Cervix: A biometric approach
to etiology. Cancer 14: 153-160

_____ & Longo, L.D. (1963) Identification of Herpes Simplex Virus
in a case showing cytological features of viral vaginitis.
Acta Cytol. 7: 295-299

_____ Clark, V.A. & Coffelt, C.E. (1970) Contraceptives and
Dysplasia: Higher rate for pill chooses. Science 169: 497-498

Stern, P.J. & Siciliano, A. (1966) Necrotising Herpes Simplex virus
infection of the cervix during pregnancy.
Am. J. Obstet. Gynec. 94: 249-252

Stewart, H.L.; Dunham, L.V.; Casper, J.; Dorn, H.F.; Thomas, L.B.; Edgcomb, J.H. & Symeonidis, A. (1966) Epidemiology of Cancers of uterine cervix and corpus, breast, and ovary in Israel and New York City. J. Nat. Cancer Inst. 37: 1-95

Stocks, P. (1955) Cancer of the uterine cervix and social conditions Brit. J. Cancer 9: 487-494

Stubbs, K.G.; Snider, M.E. & Alford, C.A. (1971) Influence of lost cell on biologic markers of type 1 and 2 Herpes virus Hominis. J. Infect. Dis. 123; 169-177

Swan, D.S. & Roddick, J.W. (1973) A clinico-pathological correlation of the cell type. Am. J. Obst. Gynec. 116: 666-670

Tallent, H.B.; Simmons, R.L. & Najarian, J.S. (1971) Primary carcinoma of cervix appearing in immunosuppressed renal transplant recepient. Am. J. Obstet. Gynec. 109; 663-669

Taylor, R.S. (1959) Mortality among women in 3 Catholic religious Orders, with special reference to cancer. Cancer 12: 1207-1223

Te, Linde, R.W.; Galvin, G.A. & Jones, H.W. (1957) Therapy of carcinoma in-situ. Implications from a study of its life history. Am. J. Obst. Gynec. 74: 729-799

Terris, M. & Oalmann, M.C. (1960) Carcinoma of the Cervix. J.A.M.A. 174: 1847-1851

_____ (1962) Epidemiology of Cervical Cancer. Ann. N.Y. Acad. Sci. 79: 808-813

_____ Wilson, F.; Smith, H.; Spring, E. & Nelson, J. (1967) The relationship of coitus to carcinoma of the cervix. Am. J. Pub. Health 57: 840-847

Teokharov, B.A. (1969) Non-gonococcal infections of the female
genitalis. Brit. J. Vener. Dis. 45: 334-340

Thiery, M. (1960) The detection of genital cancers in the female
as seen from the economic and social angle; a national summary.
Acta Gynec. Obstet. Hisp. Lusit. 8: 179-184

Thompson, D.M.P.; Krupey, V.; Freedman, S.O. & Gold, P. (1969)
The radioimmuno-assay of circulating carcino-embryonic antigen
of the human digestive system.
Proc. Nat. Acad. Sci. (U.S.) 64: 161-167

Thomson, B.H.; Woodruff, J.D.; Davis, H.J.; Julian, C.A. & Silva, F.G.
(1972) Cytopathology, histopathology and colposcopy in the
management of cervical neoplasia. Am. J. Obst. Gynec. 114: 329-338

Thoules, M.E. & Skinner, G.R.B. (1971) Differences in the properties
of thymidine kinase produced in cells infected with type 1 and
type 2 Herpes viruses. J. Gen. Virol. 12: 195-197

Tokumara, T. & Scott, T.F.M. (1965) The Herpes virus group.
In Diagnostic procedures for viral and Ricketsial Diseases.
Lennette, E. & Schmidt, N. (Eds.). Am. Publ. Health Assoc. Inc.
Pp 381-412.

Trentin, J.J.; Van Hoesier, G.L.; Ferguson, D.B. & Kitamura, I.
(1969) Oncogenic evaluation in hamsters of human picorna,
parapicorna and Herpes viruses.
Proc. Soc. Expet. Biol. Med. 132: 912-915

Treusch, J.V.; Hunt, A.B. & Rousuck, A.A. (1946) Infrequency of
 Carcinoma of the uterine cervix.among the Jewish women.
 Am. J. Obst. Gynec. 52: 162

Trussell, R.R.; Hutt, N.S.R. & Zeigler, O. (1968) Exfoliative
 cytology in the detection of cervical cancer in Uganda.
 "Cancer in Africa". (pp. 397-407) E. Afri. Pub. House., Nairobi

Tweedell, K.S. (1967) Induced oncogenesis in developing frog kidney
 cells. Cancer Res. 27: 2042-2052

Vaillant, H.W.; Cummins, C.T.M. & Richart, R.M. (1968) An Island
 wide screening programme for cervical neoplasia in Barbados.
 Am. J. Obstet. Gynec. 101: 943-946

Van Niekeik, W.A. (1963) A typical Basal cell nuclei in Cervical
 Smears, caused by Trichomonas vaginitis.
 S. Afric. Med. J. 37: 852-854

Varga, A. & Browell, B. (1960) Viral inclusion bodies in vaginal
 smears. Obstet. Gynec. 16: 441-444

Versluys, J.J. (1949) Cancer and occupation in Netherlands.
 Brit. J. Cancer 3: 161-185

Vineberg, H.N. (1906) The etiology of cancer of the Pelvic organs.
 Am. J. Obst. 53: 410-419

_____ (1919) In contributions to Medical and Biological Research
 dedicated to Sir William Osler. Hoeber, P.B. New York.

Walter, L.; Glucksman, W.A. & Cherry, C.P. (1965) Biologic and
 physical factors in radiotherapy of carcinoma of the cervix.
 J. Obstet. Gynec. Brit. Comm. 72: 575-585

Warren, S. (1931) The grading of Carcinoma of the cervix uteri as checked at autopsy. Arch. Pathol. 12: 783-786

Watkins, J.F. (1964) Adsorption of sensitised sheep erythrocytes to Hela cells infected with Herpes Simplex virus. Nature (Lond). 202: 1364-1365

Weiner, I.; Burke, L. & Goldberger, M.A. (1951) Carcinoma of the cervix in Jewish women. Am. J. Obstet. Gynec. 61: 418-422

Wentz, W.B. & Reagan, J.W. (1959) Survival in cervical cancer with respect to cell type. Cancer 12: 384-388

_____ (1961) Histologic grade and survival in cervical cancer. Obstet. Gynec. 18: 412-416

_____ & Louis, G.C. (1965) Correlation of Histologic morphology and survival in cervical cancer following radiation therapy. Obstet. Gynec. 26: 228-232

Wheeler, C.L & Huffines, W.D. (1965) Primary Disseminated Herpes Simplex of the new born. J.A.M.A. 191: 455-460

_____ Briggaman, R.A. & Henderson, R.R. (1969) Discriminating between two strains (types) of Herpes simplex virus by various modifications of the neutralisation tests. J. Immunol. 102: 1179-1192

White, J.C. (1963) Fulminating infection with herpes simplex viruses in premature and newborn infant. New Eng. J. Med. 269:455-460

Wildy, P. (1972) Herpes virus and antigens. In Biggs, P.M.; de-The, G. and Payne, L.N. Oncogenesis and Herpes viruses. I.A.R.C., Lyon Pp 409-419

Williams, A.O. (1966) Hemoglobin genotypes, ABO blood groups and Burkitts tumour. J. Med. Genetics 3: 177-179

Williams, W.L.; Paullus, K. & Erickson, C.C. (1953) Proliferative
    epithelial changes and carcinoma of cervix and vagina in mice
    with prolonged administration of alpha estradiol-sensitised
    collodion particles. Proc. Am.   Cancer Res. 1: 60-61

Witzleben, C.L. & Driscoll, S.G. (1965) Possible transplacental
    transmission of Herpes Simplex infection.
    Paediatrics 36: 192-199

Wyburn-Mason, R. (1957) Malignant change following Herpes Simplex.
    Brit. Med. J. 2: 615-616

Wynder, E.L.; Cornfield, J.; Schroff, P.D. & Doraiswani, K.R. (1954)
    Study of environmental Factors in carcinoma of the cervix.
    Am. J. Obst. Gynec. 68: 1016-1052

_____ & Licklider, S.D. (1960) The question of circumcision.
    Cancer 13: 442-445

Yen, S.S.C.; Reagan, J.W. & Rosenthal, M.S. (1965) Herpes Simplex
    infection in the Female Genital Tract. Obstet. Gynec. 25: 479-592

Zavoral, J.H.; Ray, W.L.; Kinnard, P.G. & Nahmias, A.J. (1970)
    Neonatal herpetic infection: A fatal consequence of penile
    herpes in a serviceman. J.A.M.A. 213: 1492-1493

APPENDIX

Survey of Carcinoma of the Cervix Uteri in the

University College Hospital, Ibadan.

1. Name
2. Hospital No.
3. Personal History

1	2	3	4	5	6

    (i) Age (in years)
         (Record actual age)

        (1) 0 - 20
        (2) 21 - 30
        (3) 31 - 40
        (4) 41 - 50
        (5) 51 - 60
        (6) Over 60

(7) (8)

    (ii) Place of Origin

        (1) Yoruba
        (2) Ibo
        (3) Hausa
        (4) Edo
        (5) Itsekiri
        (6) Efik
        (7) Others

9

    (iii) Religion

        (1) Muslims
        (2) Prostestant
        (3) Catholic
        (4) Traditional worshipper
        (5) Others

    (iv) Occupation

        (1) Professional/Administrator
        (2) Skilled worker
        (3) Clerks
        (4) Farmer
        (5) Housewife/Petty Trading
        (6) Others

11

    (v) Education

        (1) Nil
        (2) Primary
        (3) Secondary (including modern)
        (4) Post-secondary (other
             than University)
        (5) University

12

4. Family History:

    (i)   Husband's Occupation

             (1) Professional/Administrator
             (2) Business Executive
             (3) Skilled worker
             (4) Clerks
             (5) Labourer
             (6) Farmer/Petty Trading
             (7) Others   13

    (ii) Family Income per annum

             (1) Less than N500
             (2) N500   - N1,000
             (3) N1,000 - N2,000
             (4) N2,000 - N4,000
             (5) N4,000 and over

5. Gynaecologic History:

    (i)   Menstruation:

             (1) No record
             (2) Regular
             (3) Irregular/Heavy   15
             (4) Menopause

    (ii) Marital Status

             (1) Single
             (2) Married
             (3) Separated
             (4) Divorce   16
             (5) Widowed

    (iii) No. of marriages
          (if married)

             (1) Dont know
             (2) 1
             (3) 2   17
             (4) 3 and over

    (iv) No. of other wives

             (1) Don't know
             (2) Nil
             (3) 1
             (4) 2
             (5) 3   18
             (6) 4 and over

    (v)   Age at 1st coitus

             (1) Don't know
             (2) Under 10
             (3) 11 - 15
             (4) 16 - 20
             (5) 21 - 25   19
             (6) 25 and over

    (vi) No. of sex partners

             (1) Don't know
             (2) 1
             (3) 2   20
             (4) 3
             (5) 4 and over

(vii) Frequency of coitus weekly   (1) Don't know
(2) Once
(3) Twice
(4) Thrice
(5) 4 times
.(6) 5 times and over

21

(viii) Consorts circumcision   (1) Don't know
(2) Yes
(3) No

22

(ix) Native Vaginal Pessaries for Infertility

(1) None
(2) Herbs
(3) Chemicals
(4) Others

23

6. Previous Gynaecologic Disorders:

(1) None recorded
(2) Cervicitis
(3) Erosions
(4) Lacerations
(5) Dysplasias
(6) Others

24

7. Obstetrical History:

(i) No. of pregnancies (including abortion)

(1) Nil
(2) 1
(3) 2
(4) 3
(5) 4
(6) 5
(7) 6
(8) 7
(9) 8 and above

25

(ii) No. of living babies: (1) Nil
(2) 1
(3) 2
(4) 3
(5) 4
(6) 5
(7) 6
(8) 7
(9) 8 and above

26

(iii) Type of Contraceptive Practice:

        (1) Nil
        (2) Oral
        (3) I.U.C.D.
        (4) Others   � 27

8. Clinical Presentation:

   (i) Vaginal Bleeding:

        (1) Nil
        (2) Post coital
        (3) Intermenstrual/contact 28
        (4) Post-menopausal

   (ii) Pelvic Pain

        (1) Nil
        (2) Right sided
        (3) Left sided
        (4) Generalised   29

   (iii) Vaginal Discharge

        (1) Nil
        (2) Mild
        (3) Copius/Smelling   30

   (vi) Urinary Symptoms

        (1) Nil
        (2) Frequency
        (3) Dysuria
        (4) Hematuria   31
        (5) Incontinence

   (v) Intestinal Symptoms

        (1) Nil
        (2) Constipation
        (3) Tenesmus
        (4) Diarrhoea
        (5) Incontinence   32
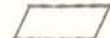
   (vi) Other Symptoms

        (1) Pedal oedema
        (2) Insomnia
        (3) Weight loss
        (4) Anemia   33
        (5) Nil

9. Examination findings:

   (i)

        (1) Normal cervix
        (2) Cervical erosion
        (3) Cervicitis
        (4) Ulcerating growth   34
        (5) Fungating growth

(ii) Clinico-pathologic staging   (1) Stage   i(a)
     (2) Stage   i(b)
     (3) Stage   ii(a)
     (4) Stage   ii(b)
     (5) Stage   iii
     (6) Stage   iv
     (7) None of above
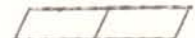

35

10. Investigations:

  (i) Cytology (Cell appearance)   (1) Not done
     (2) Normal
     (3) Slight atypia
     (4) Marked atypia
     (5) Dysplasia
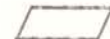   (6) Frankly malignant

36

  (ii) P.C.V. (Record actual value)   (1) Not done
     (2) Less than 10
     (3) 11 - 15
     (4) 16 - 20
     (5) 21 - 25
     (6) 26 - 30
     (7) 31 - 35
     (8) 36 and above

37   38

  (iii) W.B.C. (per cc.mm)   (1) Not done
     (2) Less than 1000
     (3) 1000 - 2000
     (4) 2001 - 3000
     (5) 3001 - 4000
     (6) 4001 - 5000
     (7) 5001 - 6000
     (8) 6001 and over

39

  (iv) Hb. Genotype   (1) Not determined
     (2) AA
     (3) AS
     (4) SS
     (5) AC
     (6) SC
     (7) CC
     (8) Others

40

  (v) Blood Group   (1) Not done
     (2) A
     (3) B
     (4) AB
     (5) O

41

(vi) Kahn Test
(1) Not done
(2) Positive
(3) Negative

42

(vii) Urine: ova of schistosima
(1) Not done
(2) Present
(3) Absent

43

(viii) I.V.P.
(1) Not done
(2) Normal
(3) Left obstructed nephropathy
(4) Right obstructed nephropathy
(5) Bilateral obstructed nephropathy

44

(ix) Blood Urea (mg%)
(1) Not done
(2) Less than 30
(3) 30 - 50
(4) 50 - 100
(5) 100 and over

45

(x) Cystoscopy
(1) Not done
(2) Bladder free from growth
(3) Bladder involved

46

(xi) Biopsy
(1) Punch
(2) Ring
(3) Cone
(4) Other

47

(xii) Histologic type
(1) Well diff. Sq. Cell Carcinoma
(2) Poorly differentiated
(3) Undifferentiated
(4) Adenocarcinoma
(5) Adeno-epidemoid car carcinoma
(6) Others

48

(xiii) Inflammatory Cellular reaction
(1) Not looked for
(2) Present
(3) Absent

49

(xiv) Fluorescent Antibody Titre
(1) Not done
(2) Less than 1:40
(3) 1:40
(4) 1:80
(5) 1:160
(6) 1:320
(7) 1:640
(8) 1:1280
(9) 1:2560+

50

(xv) Complement Fixation Test Titre

                        (1) Not done
                        (2) Less than 1:4
                        (3) 1:4
                        (4) 1:8
                        (5) 1:16
                        (6) 1:32
                        (7) 1:64
                        (8) 1:128
                        (9) 1:256+

51

11. Treatment

                        (1) None
                        (2) Cone Biopsy
                        (3) Caesium insertion
                        (4) Surgery
                        (5) Surgery + Caesium

52

12. Complications (post-therapy)

    (I) Pyrexia (of more than 24 hours) T. 39°F and above

                        (1) Nil
                        (2) Low grade
                        (3) High grade
                        (4) Swinging
                        (5) Persistent

53

    (II) Urinary Symptoms

                        (1) Nil
                        (2) Frequency
                        (3) Dysuria
                        (4) Retention
                        (5) Incontinence

54

    (III) Rectal Symptoms

                        (1) Nil
                        (2) Constipation
                        (3) Proctitis
                        (4) Diarrhoea
                        (5) Incontinence

55

    (IV) Radiation Necrosis
          (if radiotherapy)

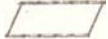   (1) Nil
                        (2) Vagina
                        (3) Bladder
                        (4) Rectum
                        (5) Other sites

56

    (V) Vaginal Stenosis

                        (1) Nil
                        (2) Mild
                        (3) Severe

57

(VI) Others

(1) Chest infection
(2) Genital sepsis
(3) Rectal fistula
(4) Vesical fistula
(5) Rectal & Vesical fistula
(6) Uterine Rupture/Perforation
(7) Vault Granulation
(8) Rectal stenosis
(9) Womb infection

58