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RECRUDESCENT HERPES SIMPLEX GENITALIS: A CLINICAL STUDY

by

Judy Smith Sanderson

B.A., University of Montana, 1975

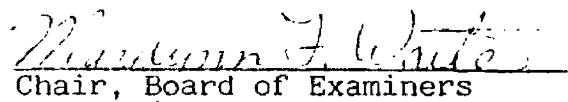
Presented in partial fulfillment of the requirements for the degree of

Master of Science

UNIVERSITY OF MONTANA

1981

Approved by:



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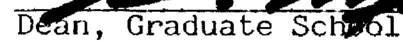

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## ABSTRACT

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Recrudescent Herpes Simplex Genitalis: A Clinical Study (83 pp.)

Director: Marilynn F. Waite

A group of 30 women with tentative diagnoses of herpes simplex genitalis was studied for six months. The women were cultured weekly for herpesvirus from their cervixes, vaginal walls and suspect lesions. Serum samples for antibody determinations were obtained from the women at various times. Each woman reported the presence of factors which previous workers had suggested might affect recurrent herpes including menstrual cycle, sexual activity and subjectively defined stresses.

The study determined that five of the women participating in the investigation probably lacked herpes simplex genitalis infections, despite their previous diagnoses. Another five women did not provide sufficient data for analysis.

The information from 20 women was statistically analyzed to determine influences on recurrent herpes genitalis. All 20 women shed herpesvirus asymptomatically during the study. The only parameter which influenced asymptomatic and recurrent herpes in this study was subjective stress. Menstrual cycle, sexual activity or birth control methods showed no correlation to the disease. Serological data cast doubt on the current concept that women with low antibody titers to herpes simplex II may be presumed not to have been infected with it.



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TABLE OF CONTENTS

Page

ABSTRACT.......... ii

ACKNOWLEDGEMNTS.......... iii

LIST OF EXHIBITS, TABLES AND FIGURES.......... vii

ABBREVIATIONS.......... ix

Chapter

1. INTRODUCTION.......... 1

Herpesvirus.......... 2

Molecular Aspects.......... 2

Relationships Between Herpes Viruses.......... 2

Viral Infection--Lytic.......... 3

Viral Infection--Latent.......... 4

Viral Infection--Transforming.......... 7

Immune Response.......... 9

Herpes Simplex.......... 10

HSV I.......... 10

HSV II.......... 10

Cervical Cancer.......... 11

Infant Disease.......... 12

Diagnosis of HSV.......... 13

Treatment.......... 14

Statement of Thesis.......... 15

2. MATERIALS AND METHODS.......... 17

Subjects.......... 17



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Chapter | Page

Initial .......... 17

Follow-up .......... 23

Culturing .......... 25

Sampling Technique .......... 25

Indicator Cell Cultures .......... 25

Virus Assay .......... 26

Freezing Cells .......... 28

Thawing Cells .......... 28

Serum Antibody Levels .......... 28

Serum Neutralization .......... 29

Virus Pools .......... 30

$TCID_{50}$ .......... 30

Virus Titration (during serum neutralization test). 30

Statistical Analyses .......... 31

3. RESULTS .......... 32

Eliminated Group .......... 32

Study Group .......... 32

Time Since Primary Infection .......... 32

Viral Isolation .......... 42

Asymptomatic Shedding .......... 42

Factors Which Might Affect Recurrences of Lesions and/or Viral Shedding .......... 45

Menstrual Cycle .......... 45



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| Chapter | Page |
| --- | --- |
| Method of Birth Control | 54 |
| Sexual Activity | 54 |
| Stress | 54 |
| Serological Data | 60 |
| 4. DISCUSSION | 64 |
| Misdiagnoses | 65 |
| Length of Infection | 66 |
| Viral Isolation from Lesions | 66 |
| Source of Virus Shedding | 67 |
| Asymptomatic Herpes Genitalis | 68 |
| Factors that Affect Recurrent Disease | 69 |
| Menstrual Cycle | 69 |
| Method of Birth Control | 70 |
| Sexual Activity | 71 |
| Stress | 71 |
| Serology | 72 |
| CONCLUSION | 74 |
| SUMMARY | 74 |
| LITERATURE CITED | 76 |
| APPENDIX | 82 |




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LIST OF EXHIBITS, TABLES AND FIGURES

Exhibit Page

A. Herpesvirus Type II: You are Infected for Life!!...... 18

B. Recurrent herpes: Patient Interview................. 19

C. Daily Calendar.................................... 21

D. Follow -up Questionaire............................ 24

Table

1. Persons eliminated from study......................... 33

2. Biographical data..................................... 34

3. Culture results and affecting factors................. 36

4. Effect of time since primary infection upon frequency of lesions, duration of lesions, frequency of viral isolation and antibody titers......................... 41

5. Isolation of virus from lesions....................... 43

6. Location of lesions................................... 43

7. Area from which positive cultures were obtained every week from each person................................ 44

8. Relationship of positive cultures to the presence (+ one week) of detectable lesions (asymptomatic positive cultures)................................... 46

9a. The relationship of menstrual bleeding to lesions and/or viral shedding.............................. 47

9b. The relationship of menstrual phase to lesion and/or viral shedding...................................... 48

10. Birth control methods................................ 55

11. Effect of birth control methods on recurrent herpes genitalis............................................ 56

12a. Effect of sexual activity on the course of recurrent herpes genitalis.................................... 57

12b. Effect of sexual activity on the course of asymptomatic viral shedding.................................... 58



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Table | Page

13. Distribution of sexual activity.......................... 59

14. Effect of stress on recurrent or asymptomatic herpes genitalis.......................................... 61

15. Serological data.......................................... 62

Figure

1. Onset of recurrent herpes in relation to menstrual bleeding:
   a. total study group
   b. women on birth control pills eliminated.......... 50

2. Effect of menstrual cycle on herpesvirus isolation:
   a. total study group
   b. women on birth control pills eliminated.......... 52




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ABBREVIATIONS

| | |
|---|---|
| BCG | bacillus Calmette-Guérin (*Mycobacterium bovis* strain) |
| C | temperature Celcius, cytosine |
| cc | cubic centimeter |
| CF | complement fixation |
| cm | centimeter |
| $CO_2$ | carbon dioxide |
| CPE | cytopathic effect |
| d | day |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| EB | Epstein-Barr (virus) |
| ELISA | enzyme-linked immunosorbent assay |
| et al. | et alii (L, and others) |
| FCS | fetal calf serum |
| g | gram |
| G | guanine |
| h | hour |
| HEp-2 | human epithelial cell line |
| HSV | herpes simplex virus |
| IHA | indirect hemagglutination |
| IUD | intrauterine d vice |
| l | liter |
| LFAV | Lucké frog adenocarcinoma virus |
| MEM | minimal essential medium |
| min | minute |



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| | |
|---|---|
| ml | milliliter |
| mo | month |
| mv | millivolt |
| nm | nanometer |
| - | negative |
| # | number |
| % | percent |
| Pd | phosphate buffered saline without calcium or magnesium |
| + | positive |
| $1^0$ | primary |
| psi | pounds per square inch |
| RML | Rocky Mountain Laboratories, NIH, NIAID |
| RNA | ribonucleic acid |
| rpm | revolutions per minute |
| s | second |
| SN | serum neutralization |
| SPSS | statistical package for the social sciences, computer program |
| $TCID_{50}$ | tissue culture infectious dose for 50% of the tissue culture cells |
| TDW | triple distilled water |
| UV | ultraviolet |
| VDRL | Venereal Disease Research Laboratories (test for syphilis) |
| VERO | African Green Monkey kidney cell line |
| vs | versus |
| v:v | volume to volume |
| $\bar{x}$ | mean |
| y | year |



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Chapter 1

# INTRODUCTION

Herpes genitalis is generally regarded as a sexually transmitted disease (36,62), but it can also be transmitted in other ways (35). It has become of national concern because of its rapidly increasing incidence (14,28,63). It presents a serious problem for several major reasons. Subsequent to primary infection the virus becomes latent in the host and often causes recurrent disease (51,66). Herpesvirus is also proposed to have a relationship to cervical cancer (44,52). Additionally, the virus may spread to the fetus before or at the time of delivery causing fetal wastage, neonatal disease or death (44). Finally, there is no effective treatment to combat the disease (5,33).

The word "herpes" was derived from the ancient Greek word ἕρπειν meaning to creep (35,76). Research on the herpesviruses may be divided into three periods (51). The first period (100 A.D. to 1920) uncovered the agents responsible for cold sores, shingles and genital blisters. From 1920 to 1960 animal models evolved to study the extending list of diseases caused by herpesviruses. The current period of research includes recognition of different biological and epidemiological variants in the herpesviruses such as herpes simplex types 1 and 2. Another important human herpesvirus is varicella-zoster virus, the causative agent of both chickenpox (varicella, primary infection) and shingles (zoster, recurrent infection).



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## Herpesvirus

### Molecular Aspects

The herpesviruses are defined on the basis of their structure. They are large DNA-containing viruses with an icosahedral nucleocapsid and a loose outer envelope. Complete viral particles have a diameter of 150 nm to 200 nm (51). Centrally located within the capsid of the complete virion is a core consisting of viral proteins and the polyamine spermine in a cylindrical structure. The linear, double-stranded DNA is wound around this core. The capsid assembles within the nucleus of the infected cells; it is composed of 162 structural subunits arranged to form an icosahedron, 100 nm in diameter. Only core-containing capsids become enveloped to form structurally complete, infectious virions. The envelope of lipids and glycoproteins is organized into a trilaminar unit membrane. It is structurally indistinguishable from the cellular nuclear membrane (54).

Variant particles have no or two membranes. Although the single-envelope particle is probably the epidemiologically important infectious unit, infectivity has been reported for the unenveloped particles (34). While there are other explanations for these results, they do raise questions about the effectiveness of the immune responses which are directed against the envelope glycoproteins. The particles with two membranes are infectious; several theories have been propounded to explain how they might arise (51), but experimental data are lacking.

### Relationships Between Herpes Viruses

Herpesvirus DNAs range from 80 to 150 million Daltons and from 32 to 74 moles percent G + C base composition. Herpes simplex I and II

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have been reported to share about 50 percent of their DNA sequences. Although usually distinguishable by cross-neutralization studies, many recombinants between herpes simplex I and II exist, leading to a 50 to 80% rate of cross-reactivity. For epidemiological reasons, it is sometimes useful to distinguish between the plethora of herpes simplex strains. This is usually done by restriction endonuclease mapping (65). The genetic relatedness between herpes simplex viruses and other human herpesviruses (varicella-zoster, cytomegalo-) is only a few percent. However, HSV shares 14 percent of its sequences with bovine mammilitis virus and 8 to 10 percent with pseudorabies virus (64).

The herpesvirus genome can code for about 100 virus-specified proteins. Of proteins recognized serologically, relatedness between the various herpesvirus groups is sparse. Consequently, serological methods have no place in the definition of the virus group (76).

Viral Infection--Lytic

Three types of infection exist: lytic, latent and transforming. Productive lytic herpesvirus infection is initiated when the viral envelope glycoprotein attaches to a pre-existing receptor site on the membrane of the host cell. There is some dispute as to whether the virus enters the cell by viropexis (pinocytosis) or by membrane fusion (34). The DNA with attendant nucleocapsid proteins enters the nucleus where approximately 50 percent to the DNA is transcribed. In the cytoplasm, virus-specific RNA enters into free and membrane-bound polyribosomes and directs the synthesis of structural and nonstructural proteins. Most of these proteins migrate to the nucleus where they aggregate with the replicated DNA to form new nucleocapsids. The

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inner nuclear membrane of the cell acquires new virus-specified proteins. Maturation occurs as the capsids bud through that modified membrane into the perinuclear space. Viral particles accumulate there and in the endoplasmic reticulum (51). Virions leave the cell by an unknown mechanism, but the majority are released after cell death and lysis.

The host cell biology is severly altered during a lytic herpes infection. Approximately 3 to 5 hours after viral entry, cellular DNA and protein synthesis cease. This metabolic shift coincides with aggregation and margination of chromatin and disaggregation of the polyribosomes. Host RNA synthesis is reduced by 70 percent and the remainder is improperly processed. Reversal of the transmembrane potential from -20 mv to +10 mv allows leakage of cellular macromolecules (51). The host plasma membrane displays antigens much like those of the viral envelope. Another labile envelope antigen which has been reported early in lytic infection is also found in cells transformed by herpesviruses. This is thought to be a "transformation antigen" similar to those found in adenovirus- and papovavirus-infected and transformed cells (51).

In lytic infection, approximately $10^5$ copies of viral DNA are made per cell; of these, only 20 percent are encapsidated; and only $10^2$ to $10^3$ viral particles are infectious (51). Whether this means that five percent or less of the nucleocapsids are functional or that the majority of the infecting particles are degraded before establishing a productive infection is not known.

## Viral Infection--Latent

Latent nonproductive herpes infection occurs when the viral

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genome is perpetuated while the cell survives. Two types of latency have been suggested, which may overlap: a dynamic state where virus replicates at low levels and a static state with little or no progeny release (22). Latency leads to recurrent disease and certain types of cancer, as will be discussed further below.

*In vitro* persistent infections of tissue culture cells have been used to study latency. These infections may or may not be valid models for *in vivo* latency. Such studies include the use of antibodies or interferon in the culture medium, or low-temperature incubation of virus-infected cells (59). Also, cell lines partially sensitive to herpesvirus infection, such as Chinese hamster cells, have been used. These cells, when infected with herpesvirus go through cycles of viral synthesis and cell dustruction, termed recrudescent infection, followed by regrowth of the cell cultures (32). During these cycles, progeny virus appears to change its level of virulence and the host cell population its susceptibility to viral infection (32). Recrudescent infection can also be seen with Earle's L cells (59).

For recurrent diseases to manifest themselves, four sources of virus may be possible: exogenous infection; endogenous infection from another site of the body; chronic, continuous low-level viral miltiplication around the site of involvement; or persistence of virus in a nonreplicating form (51).

Herpes-infected animals can be reinfected with the same strain of virus from outside their own body as has been shown by studies in Cebus monkeys (43) and observed in man, when partners with HSV reinfect each other(61). Endogenous infection from another source on the body is presumed to cause genital herpes in persons with oral HSV and no history

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of sexual intercourse. This is thought to be the cause of most genital herpes infections in celibate populations. One such group is nuns (49). Such endogenous infection could occur even in the absence of active oral lesions, since HSV has been isolated from lacrimal and oral secretions of persons between recrudescent herpes labialis episodes (23,68). The site of replication of this virus, isolated in the absence of lesions, is not known. However, because continued efforts to isolate virus from the site of lesions between recurrences have failed, chronic-continuous infection at the site of the recurrent lesions is unlikely.

For recurrences to occur, previous primary infection by herpesviruses, even if asymptomatic, must have existed and have been followed by latency. The existence of severe prodromal pain and the effect of therapeutic or traumatic severing of nerve roots lead to the hypothesis that after the primary infection, herpes simplex viruses advance to the nerve ganglia (20). This is also true in the case of varicella-zoster virus as indicated by the location of the recurrent zoster lesions. In the nerve ganglia herpesviruses appear to reside in a noncytocidal, noninfectious form. In support of this, herpes simplex antigens have been detected in at least one malignant glioma (22). Virus can be isolated from the trigeminal ganglia of human cadavers (8,10), the sacrociatic ganglia of mice after footpad injection of virus, and the trigeminal ganglia of rabbits after corneal inoculation (57,69). However, all viral isolations from ganglia must be done by nerve co-cultivation with susceptible indicator cells (10,57,69) or nerve cell pre-cultivation (8). Infectious virus has not been found in nerve

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cell homogenates (8,10,57,69). This confirms the inactivity of the infection and suggests that some step in the process of nerve tissue manipulation stimulates virus progeny production. Herpes simplex virus may also be harbored in lymphocytes (41), but the relevance of this site of infection to recurrences is unknown.

By some unknown mechanism, stimulation of virus replication in latently infected cells occurs, resulting in a recurrence of clinical disease. Precipitating factors include trauma and stress, and may include sexual activity and/or hormonal changes including those involved with menstruation. Anaphylactic and Arthus reactions in rabbits induce recrudescent herpes encephalitis (59). Such nonspecific reactivation also occurs in man. Organ transplant patients often display herpes infections of simplex, zoster or cytomegalovirus varieties (1,53). Burn patients also exhibit lesions of recurrent herpesvirus infections (51). The nature of the stimulatory effect upon the site of latent infection is unknown.

Viral Infection--Transforming

All herpesviruses share some biological and physical features. It follows that since one herpesvirus (Epstein-Barr virus) has been shown to cause cancer in man (38) and other herpesvirus cancers exist in animals (Marek's disease in chickens, Lucké renal carcinoma of frogs and lymphomas in some primates), that all herpesviruses are suspect as cancer-causing agents. In general, tumors (*in vivo*) or cell transformation (*in vitro*) appear to arise when there is interference with lytic replication of virus. In this regard, EB virus may be somewhat of an anomaly since the virus can be demonstrated in established cell

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lines derived from cases of Burkitt's lymphoma. However, the virus cannot be isolated from the tumor tissue itself (38), so this anomaly may be more apparent than real. Many unanswered questions still remain regarding the mechanism of oncogenesis of EB virus, its role in nasopharyngeal carcinoma (38), its interaction with malaria (46), and its more common role as the etiological agent of infectious mononucleosis.

The relationship between ability of the virus to cause tumors and its inability to replicate lytically has been worked out best for the naturally temperature-sensitive Lucké frog adenocarcinoma virus (LFAV). The LFAV replicates normally at cold temperatures (e.g. in the winter). Under permissive conditions virus production, cell lysis and regression of pre-existing tumors can be observed. In the summer the higher temperatures restrict the virus replication. Infected cells lack inclusion bodies and fail to produce virus, and dramatic tumor growth is found (29).

Marek's disease, an infectious lymphoma of chickens, was found, after extensive studies to be caused by a herpesvirus. At first, only a cell-associated agent could be found and no transmissable agent was isolated from the lymphoma cells. Later, non-transformed cells of the feather follicles were found to shed infectious virus (12).

Herpes simplex virus of type 1 and 2 have been shown to cause transformation of baby hamster kidney cells in culture, but only when the viruses were first inactivated with ultraviolet light (24,39). Those viruses have been implicated as causative agents of nasopharyngeal and cervical carcinomas (23). The association of HSV II with cervical carcinoma is circumstantial but persuasive, and will be discussed more

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fully below.

Another herpesvirus, herpes saimiri, appears to be involved in oncogenesis of some tissues. Isolated from healthy squirrel monkey kidneys, it will produce lymphomas in several other mammals in the absence of observable intracellular virus replication (21).

Immune Response

*In vivo*, the host reacts to a herpesvirus infection by two means, specific and nonspecific (42). The specific response includes production of anti-herpes antibody. Antibody, together with complement and activated T lymphocytes, lyse extracellular virus or virus-infected cells (which carry viral antigen on their surface) (19). A nonspecific immune response is also generated in which inflammatory cells (macrophages and lymphocytes) and several mediators (lymphotoxins, interferon, etc.) act on infected cells to prevent spread of virus to uninfected cells (42).

For a host to be susceptible to a herpesvirus infection appropriate conditions must prevail. Substantial inoculum must be introduced before disease will occur. Low viral inocula only induce infection when the host lacks sufficient antibody from previous exposure or is immunologically compromised (as with another disease or immunosuppression). Trauma is often involved with herpesvirus infections. Unlike the situation with many infectious agents, the presence of detectable anti-herpes antibodies does not indicate immunity to herpesvirus. Persons with serological evidence of exposure to herpes may exhibit recurrent infections of endogenous or exogenous origin (61).

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## Herpes Simplex

### HSV I

Herpes simplex virus type I (HSV I) is acquired by oro-respiratory transmission and is generally considered to cause above-the-waist infections (61). It is responsible for about 10 percent of genital herpes in women; in these infections there is little involvement of the cervix (16). Most people are exposed to HSV I early in life (1 to 3 years of age), with 70 to 90 percent of 5-year-olds possessing antibodies to it. This varies with socioeconomic class and geographic area and is decreasing, especially in the upper classes (61).

### HSV II

Most below-the-waist herpes infections are due to HSV II. The common HSV II diseases are contracted venereally. Consequently, the age of exposure coincides with maximum sexual activity, with 40 to 70 percent of 20- to 30-year-olds exhibiting HSV II antibody titers (61).

Males with genital herpes generally manifest sores in the penile area. The symptoms range from none (one study found that 15 percent of males harbor HSV II without a history of the disease (15)) to severe illness. Women tend to demonstrate more nonspecific symptoms of genital herpes, most frequently vaginal and/or anal burning and itching. Lesions, which can be extremely painful, commonly occur in the vagina, vulva, cervix or perineum and less frequently on the thighs, buttocks or mons pubis. Approximately 40 percent of women have only minor discomfort. Forty percent of the serologically positive women have no history of lesions (61).

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Cervical Cancer

Women with genital herpes have a higher incidence of cervical carcinoma than other women (45,49). However, the causality of the relationship is open to debate. The incidence of cervical carcinoma is strongly correlated with the onset of sexual activity at an early age and a large number of sex partners. One study shows that nuns have no incidence of genital herpes or cervical carcinoma ( 49 ). Thus, cervical carcinoma appears to be a venereal disease. The presence of cervical cancer is better correlated with the presence of anti-herpes simplex II antibodies than with any exposure to other venereal disease or with other parameters of sexual behavior (36). Antibody to the early antigen of HSV is detectable when a tumor is present, and disappears after removal. Human cervical carcinomas contain RNA transcripts complementary to 5 percent of HSV II DNA. They also contain one DNA fragment with 39 percent of the herpesvirus genome per carcinoma cell (27). In spite of these observations, HSV involvement with cervical cancer remains circumstantial. There are almost certainly other causes of cervical carcinoma. *In vitro* the cancer cells are more susceptible to viral infection than normal cell cultures. Thus it is possible that herpes simplex II virus may be a secondary infection of cells newly made cancerous by some other venereally transmitted agent.

The final proof may not occur until vaccines for either HSV II or cervical carcinoma are available, and protected vs. unprotected groups are studied. However, the risks involved with viral vaccines would appear to limit the ability to test a potential vaccine in humans. For example, inactivated vaccine would have damaged DNA which could

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itself possess transforming potential; live vaccine might possess the ability to remain latent triggering recrudescent disease; one rendered less pathogenic for a test animal such as a monkey might develop increased oncogenic potential for humans in a manner analogous to herpes saimiri; a non-replicating variant might have increased oncogenicity like the Lucké frog adenocarcinoma virus (33, 78). Also, the failure of the immune response to prevent recurrences and reinfections could prevent the development of an effective vaccine against HSV II.

## Infant Disease

Neonatal herpes is usually contracted when the fetus passes through the birth canal of an infected mother (with or without symptoms) (50). If virus is known to be present at birth, precautions such as Caesarian section decrease the chances of infection. Caesarian section is only useful if performed within 4 to 6 hours after the membranes rupture. Greater time allows migration of virus to the fetus (37). An estimated 50 percent of babies exposed to HSV at birth contract it. Of those, 50 percent die. The remainder exhibit symptoms of neonatal herpes which appear within the first four weeks of life and vary from "nonspecific failure to thrive" to severe lifelong sequelae. Recent studies indicate that infants who possess substantial maternal antibody titers to herpesvirus present less severe forms of neonatal disease than infants with little or no maternal antibody. Thus, antibody levels also influence the eventual outcome of the infection (79). Babies from mothers with high antibody titers show less severe sequelae than those from mothers with no antibodies. In the future, monitoring

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the mother's antibody level in conjunction with viral cultures may help physicians determine which women are candidates for Caesarian section (37).

Diagnoses of HSV

Herpes genitalis is a reportable disease in only two states--Arizona and Georgia. Herpes genitalis is generally diagnosed by intensive histories of patient symptoms rather than by virus identification. Some physicians and clinics choose to confirm their diagnoses through use of microbiological methods. Several available tests include direct observation, serology and culture (11).

Direct observation is done by scanning a Papanicolaou smear for giant cells with nuclear inclusions, or by observation of smears treated with dye conjugated to anti-HSV antibodies. To diagnose genital lesions the VDRL test has been used to screen out syphilis. VDRL-negative patients may then be tested for herpes antibodies (CF (11), SN (48), IHA (58) or, most recently, ELISA (74)). The state of Montana regards persons with IHA antibody titers of ≤1:8 as negative for HSV. Serology should include acute and convalescent sera to detect a rise in antibody level. The above tests are susceptible to misinterpretation because neither giant cells nor nuclear inclusions are limited to HSV. Antibody-dye techniques and other serological assays can yield false negative results. The reasons for this are unknown. Only the paired serum methods are indicative of recent infection.

To culture for HSV, a swabbed sample from the infected or suspect

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area is inoculated into a small amount of transport medium containing nutrients to stabilize the virus and antimicrobials to reduce overgrowth by bacteria or fungi. This transport medium, with or without filtration, is subsequently added to cell cultures which are used as indicators of infection (71). Herpesvirus infection induces a typical cytopathic effect in certain cells which is diagnostic (73). Further confirmation can be done with the aid of specific antiserum.

Treatment

Countless attempts have been made to find a cure for herpes genitalis. Topical ether was supposed to destroy the viral envelope, rendering the virus noninfective (5). The solvent properties of the ether actually irritated lesions so that healing was delayed (18,30), and it did not kill all the infecting virus. Another formerly popular treatment was a dye-light treatment (5). The dye was applied to denuded ulcers of herpes, followed by exposure of the area to a specific wave length of light. The heterocyclic dye intercalated into the viral DNA and broke it when exposed to the light (39). Several studies yielded conflicting results on the efficacy of this procedure (39,47), and the possibility that damaging the viral DNA could increase the risk of cancer has caused this treatment to fall into disfavor (39,47).

An attempt to treat herpes genitalis with 2-deoxy-D-glucose yielded an 89 percent cure in a two-year study of 36 women. This compound was first introduced in 1959 as a treatment for influenza. It inhibits glycosylation of glycoproteins and glycolipids so that the complex carbohydrate sidechains normally found in these molecules are not formed; precursor molecules accumulate, preventing envelope formation.

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It appears that 2-deoxy-D-glucose can prevent UV inactivated HSV from transforming primary hamster cells ( 13).

Immunological boosters have been studied for their effect on recurrent herpes genitalis. Vaccines such as smallpox, polio and BCG ( 5 ) appear to enhance cellular immunity, limiting recurrences and/or the length of attacks. The danger of encephalitis from the smallpox vaccine has limited its use. BCG still appears promising ( 6 ).

Of all the treatment attempts, nothing works as consistently for limiting the symptoms as a drying agent to speed lesions healing along with antibiotics for secondary infection of the lesions (5).

For life-threatening herpes infections, nucleotide analogues are employed. Iododeoxyuridine, cytosine arabinoside and adenine arabinoside (Vidarabine) do stop raging systemic herpes infections. Often times the virus has done considerable damage before the drug is used; and, the drugs also affect rapidly growing cells ( 5, 7). Consequently, the cure is not without dangers and an accurate diagnosis, which often requires a brain biopsy, is essential before instituting treatment ( 9). Trials with topical nucleotide analogue treatment for genital herpes have been inconclusive ( 4 ).

Statement of Thesis

This study was designed to investigate the occurrence of lesions and genital virus shedding by women with a history of recurrent herpes genitalis over an extended period of time. The University of Montana Student Health Service and local physicians regarded this study as a diagnostic aid. Based on the criteria available at the time, approximately one-sixth of the women in the study had no evidence of previous

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herpes infection. However, some evidence acquired in this study casts doubt on these criteria.

Specific aspects of the virus studies included: 1) time since primary infection; 2) success of viral cultures obtained from active lesions; 3) source of virus shedding (cervix, vagina and/or lesion); 4) frequency of symptomatic or asymptomatic virus shedding; 5) serological data; and 6) effect of factors which have been reported as related to recurrent disease or virus shedding such as a) menstrual cycle, b) sexual activity, c) method of birth control, d) emotional or physical stress.

The original goal of the study was to attempt to discover patterns of virus shedding and/or lesion occurrence either for the entire group or for the individual participants. When the data did not reveal immediately obvious trends, they were filed for 2½ years. Systematic reevaluation of the results revealed previously unperceived patterns and correlations. Computer analysis of the data, a follow-up interview and serological assays were conducted to conclude the study.

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Chapter 2

# MATERIALS AND METHODS

## Subjects

### Initial

Female volunteers were obtained with assistance from the University of Montana Student Health Service physicians and several Missoula gynecologists. Some of the original 30 patients were diagnosed as having herpes genitalis without serological or culture confirmation. Previous to 1977, our lab performed all diagnostic confirmation of herpes for free. When a charge was instituted, some physicians ordered cultures only in atypical or high risk cases. Some patients were referred to the study as a diagnostic aid by second physicians who doubted previous diagnoses. The following handout was distributed by the physicians (Exhibit A). An ad was also run in the university newspaper the Kaimin.

The potential subjects were screened in December 1977 using the following interview form: (Exhibit B).

Those persons who agreed to participate in the study were supplied with the following daily log sheets upon which specific possible contributory factors were recorded by the person. No pressure was given to provide data. (Exhibit C)

Upon completion of the study, persons keeping fewer than seven appointments and/or withholding the daily log sheets, or clearly lacking a genital herpes infection (determined serologically and/or with an in-depth discussion) were eliminated from statistical analysis (10 of 30 persons; see Table 1). For the most part, the dependable



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Exhibit A

## HERPESVIRUS TYPE II: YOU ARE INFECTED FOR LIFE!!

The incidence of Herpesvirus hominis type II (Herpes simplex) infection is increasing at an alarming rate. There are many reasons for this epidemic, but we will not discuss them at this time. Although the virus is transmitted most commonly through sexual intercourse, the disease can be contracted by other means. A particular concern is for the newborn who contracts the infection from the birth canal. Unlike the gonorrhea or syphilis microorganism, herpesvirus is much more stable, and the infection cannot be controlled by antibiotics. In fact the infection lasts a lifetime, and the individual will shed the virus at variable intervals.

Lesions (sores) do not necessarily accompany the shedding. For that matter only a small percent of those infected are aware of having contracted the virus and only because these individuals develop the sores or give birth to infants who soon show the clinical signs of the infection. In one of our studies conducted in Missoula, we found that in one large group of pregnant women without any previous history of vaginal lesions, 10% of the women were shedding the virus during the eighth month of pregnancy.

We are very interested in learning the frequency that an individual will shed the virus. By taking weekly swab samples from the vaginal wall and the cervix for several months, we will be able to prepare a profile correlating many variables (e.g., menstrual cycle, sexual frequencies, stresses of many kinds, etc.) with virus production. If you are interested in knowing your shedding frequency and wish to participate in this study, your cooperation would require weekly visits to the health service and the maintenance of an accurate record noting the factors that could trigger virus synthesis. A trained person will take the swab samples. If you are willing, 2 mls of your blood taken once a month would be used to monitor your immune response. All records will be kept confidential.

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Exhibit B

Recurrent herpes: Patient interview

Patient's name:______________________________ Date:__________

Physician:__________________________ Birthdate:______________

Interviewer:________________________

Home town:__________________________ Local address:__________

Age:______________ Sex:__________________ Race:_____________

Marital status:___________________ Year in school:___________

I. History of recurrent herpes infection:

A. type: genitalis____, labialis____, gingivo-stomatitis__________

B. First known occurrence of present condition:______________

C. Duration of $1^0$ attack:__________ treatment:______________

D. Frequency of subsequent attacks:_______ any pattern:__________

E. Average duration of $2^0$ attacks:_______ treatment:__________

F. Site of lesions:_______________ does this vary:____________

G. No. of lesions:_______________ does this vary:____________

H. Size of lesions:_______________ does this vary:____________

I. Are attacks elicited by any apparent trauma, event, food, etc.:

__________________________________________________________

how long after such trauma, etc.:_____________________________

J. History of any other herpetic type lesions (varicella-zoster):

__________________________________________________________

II. Medication history:

A. any medications taken on a regular basis:

1. oral contraceptive (brand):__________ how long:__________
2. aspirin _________________________ " " __________
3. hormones (what):__________________ " " __________
4. antihistimines (what):_____________ " " __________
5. cortisone:________________________ " " __________
6. digitalis:________________________ " " __________
7. antibiotics (what):________________ " " __________
8 other:____________________________ " " __________

B. Any changes in medication that resulted in changes in the pattern of your recurrent herpes:______________________________

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(Exhibit B continued)

III. Sexual practices:

A. Age of first sexual activity:______________________________

B. Frequency of sexual relations:______________________________

C. Type: Genital_______, Oral___________, Rectal___________

D. Total number of partners:______________________________

E. Number of partners with whom you are presently having sexual relations:______________________________

F. Have any of your partners suffered genital and/or oral herpetic type lesions either before or after contact with you:___________ at the time of sexual contact:______________________________

G. present contraception method employed:______________________ for how long:______________________________

H. Do you see any relationship between any changes in contraception methods and your recurrent infection:______________________

I. Have you ever had any other venereal disease:________________ when:______________________ Rx:______________________

J. Have you ever been pregnant:_____________ when:_______________

K. Outcome of pregnancy:______________________________

L. Any change in the pattern of your recurrent infection during pregnancy:______________________________

IV. Notes and Comments:

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Exhibit C

DAILY CALENDAR

Record several criteria which could influence the herpes shedding (some of which are listed below). Please turn in the previous month's record on your first visit to the health service each month.

Calendar information:

e.g. WEDNESDAY

| 1 | I | II | III | IV |
|---|---|---|---|---|
| | | | V | VI |

I menstrual cycle--record day 1 as the first day of flow

II birth control pills (what kind?)--start with 1 for each month

III sexual activity--indicate different partners if applicable

IV lesions--record a + during each day noticed, also note location and number

V stress

physical

F--fatigue

I--general infection, especially with a fever

V--vaginal infection (other than herpes)

°--(fever--record)

environmental

H--extreme heat or rigorous exercise

E--exam

O--some other tension causing event

VI culture--vaginal and cervical (we will add)

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(Exhibit C continued)

MAY

| SUNDAY | MONDAY | TUESDAY | WEDNESDAY | THURSDAY | FRIDAY | SATURDAY |
|---|---|---|---|---|---|---|
| I II III IV V VI | 1 I II III IV V VI | 2 I II III IV V VI | 3 I II III IV V VI | 4 I II III IV V VI | 5 I II III IV V VI | 6 I II III IV V VI |
| 7 I II III IV V VI | 8 I II III IV V VI | 9 I II III IV V VI | 10 I II III IV V VI | 11 I II III IV V VI | 12 I II III IV V VI | 13 I II III IV V VI |
| 14 I II III IV V VI | 15 I II III IV V VI | 16 I II III IV V VI | 17 I II III IV V VI | 18 I II III IV V VI | 19 I II III IV V VI | 20 I II III IV V VI |
| 21 I II III IV V VI | 22 I II III IV V VI | 23 I II III IV V VI | 24 I II III IV V VI | 25 I II III IV V VI | 26 I II III IV V VI | 27 I II III IV V VI |
| 28 I II III IV V VI | 29 I II III IV V VI | 30 I II III IV V VI | 31 I II III IV V VI | I II III IV V VI | I II III IV V VI | I II III IV V VI |

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participants in the ordeal (7 to 17 appointments) were extremely helpful and enthusiastic about their role in contributing to the future knowledge of herpes genitalis.

Follow-up

In April 1981 a three-year follow-up study of the 1978 genital herpes group was initiated. The following letter was sent to 13 persons whose addresses were found:

> Dear ______:
>
> I am carrying out a follow-up on the 1978 study at the University of Montana Student Health Service of which you were a part. If you are willing to participate in a follow-up interview, please contact me at the above address or phone number. I appreciate any further assistance that you can give me. Thank you.

None of the letters were returned by the Postal Service, suggesting that they all reached their destinations. Seven of the 13 persons responded affirmatively. They were sent the following form: (Exhibit D). Three persons were sent an additional note:

> Dear ______:
>
> Since I work in the medical field, I overlooked the possibility that having blood drawn could cost a sum of money. Please excuse this thoughtlessness. Feel free to send me the bill; or, call me collect--I may be able to make arrangements by phone for someone to draw the sample for nothing. Thanks again for your help. I hope to hear from you soon.

One reply included "... It only cost a small donation and a bruised and sore vein.....No need to reimburse me--I am glad to be a part of the study..."

Due to circumstances beyond control, the serological study included in the follow-up was not completed for this manuscript.

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Exhibit D

Dear______:

Please answer the following questions as completely as possible and return this questionaire with the enclosed self-addressed mailer. Thank you, again.

1) Did the study change your attitude about your herpesvirus infection?

2) Did it make you more aware of (your) herpesvirus?

3) How many outbreaks have you had since June 1978? (approximate if you don't know the exact number)

4) How frequent are the recurrences now?

5) Do you have a pattern of recurrent infections? (can they be predicted?)

6) Has the pattern changed? (elaborate)

I have included a 5cc tube and mailer. I am extremely interested in a follow-up serological study of the persons in the 1978 study. If you could get a friend or clinic to draw a small amount of blood for serum, and remove the serum into the tube provided here, and return it with the questionaire I would be most grateful. I will alert you to any publications that arise from the study.

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## Culturing

### Sampling Technique

Medium disposable plastic specula (Sani-Spec, Burnett Inst. Co., Lawrence, Kansas) were reused for each participant in the study (numbered upon first use). One subject required a smaller size; a small stainless steel instrument was borrowed from the Student Health Service for each visit. These specula were steam sterilized between uses and stored in clean drawers used only for that purpose at the health service.

Each visit conducted at the health service consisted of an external exam for lesions or other infections, followed by insertion of the speculum. (Several of the first-week exams were performed by the health service nurse practitioner for demonstration purposes.) Separate samples were obtained from the cervix, the vaginal walls and lesions if present, using sterile wooden-shafted cotton swabs. Each swab was placed in a separate transport tube containing medium (see appendix), and was stored at 4°C for 5 h or less before inoculation onto cell cultures.

### Indicator Cell Cultures

African Green Monkey Kidney (VERO) cells (initially obtained from Donald Lodmell, Ph.D., RML) were utilized for virus growth studies. The cells (in 75 $cm^2$ Corning tissue culture flasks) were passaged weekly (split ration 1 to 3) using a standard trypsinization procedure. Medium was removed by aspiration and the cell layer washed

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with Pd(appendix) containing 1% glucose. Trypsin (DifCo) solution (0.15% plus versene) was added (1.0 ml per flask). Cells were incubated with trypsin solution at 37°C for up to 15 min. Following detachment of the cells, 5 to 10 mls of medium with 10% FCS were added to the flasks to terminate the enzyme action. Vigorous pipetting further dispersed the cells. Suspended cells were removed to a 15 ml conical centrifuge tube and centrifuged at 700 to 1000 rpm for 5 to 10 min. The supernatant fluid was aspirated, and the cell pellet was resuspended in the desired volume of medium by forceful trituration.

One day before testing samples for herpesvirus, glass tubes (GIBCo) were seeded with 1 ml of MEM + 5% FCS containing 1 to $5X10^5$ VERO cells. A sterile Cornwall syringe was employed to dispense the cell suspension. The cultures were incubated at 37°C for 18 to 24 h. Other cell lines used in tandem with VERO cultures included HEp-2 (weeks 5,6 and 7) and primary rabbit kidney (week 11), both obtained from Rocky Mountain Laboratory. In no case did either of these cell types exhibit a herpes CPE when the VERO cells did not; in fact, the VERO cells were more sensitive to herpes than either HEp-2 or rabbit kidney cells, and the use of the alternate cells was abandoned.

Virus Assay

Patient samples were inoculated onto cells within five hours after they were obtained. The first four weeks, one VERO cell tube was used per sample. Subsequently, each sample was cultured in duplicate either in one VERO cell culture and one other cell type

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or in duplicate VERO tubes. In only 40 cases (6% of the total number of cultures) was virus growth obtained in one tube but not in the other. After inoculation the volume of medium in each tube was brought to approximately 2 ml. The tubes were incubated at 37°C in a roller drum so as to avoid possible loss of a culture due to a skewed tube. Each tube was observed daily on a tube holder (GIBCo) with an inverted microscope (American Optical) for cytopathic effect of the cell layer due to herpesvirus infection. The results were observed as 1+ for 25% involvement of the monolayer to 4+ for 100% affected cells. Cytopathology consisted of rounded, balooning cells which originated in "foci" in the monlayer and spread to involve the entire culture. (Since all 1+ cultures eventually became 4+, results are recorded as + and - only.)

One isolate from each patient was centrifuged at 1000 rpm for 10 min and the supernatant fluid passed to a new indicator tube. All such isolates showed CPE on serial passage, indicating that they were herpes simplex infections. Varicella-zoster, the herpesvirus which causes chickenpox and shingles would not have caused cytopathology upon subcultivation since it is a cell-associated virus (20). The isolates could not have been cytomegalovirus because those CPE do not appear for 10 to 21 days (73) and not in the cell lines used in this study. Neither could they be Epstein-Barr virus because EB virus cannot be grown in normal cells (38).

To further confirm that the CPE was that of herpes simplex virus, isolates from three patients (F, I and R) were tested by serum neutralization using known antisera prepared in guinea pigs. All three

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isolates were neutralized by a 1:64 titered anti-herpes simplex II serum, confirming that the isolates were herpes simplex virus.

Freezing Cells

Seed cultures were stored at -70°C (REVCO) and -192°C (liquid nitrogen refrigerator--Linde) in 1 ml quantities at a concentration of $5X10^6$ cells/ml. Dimethyl sulfoxide (DMSO-- Mallinckrodt) was added to the medium as a cryoprotective agent to a concentration of 10% (v:v). The cells were slow-frozen by placing the vials on aluminum canes (Minnesota Valley Engineering) and submerging the canes in a 95% ethyl alcohol-filled cylinder. The cylinder was placed at -70°C for 2 to 4 h after which some canes were transferred immediately to the liquid nitrogen storage container. Other vials were removed from the canes and boxed in the REVCO.

Thawing Procedure

To revitalize the frozen cells, a vial either from -70°C or -192°C was quick-thawed in a 37°C water bath. The cells were centrifuged from the DMSO-containing medium and resuspended in 20 ml of medium containing 10 to 20% FCS in a 75 $cm^2$ flask. The flask was gassed for 10 s with a 5% $CO_2$-95% air mixture, closed and incubated at 37°C.

Serum Antibody Levels

Serum samples were drawn by the Student Health Service lab personnel, once a month, or as often as the subjects were willing (at least once during the study). They were stored at -20°C and heat inactivated at 56°C for 1 h just prior to the assay.

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Serum Neutralization

Flat-bottomed, sterile microtiter plates (#3040--Falcon, with appropriate lid) consisting of 8 rows of 12 wells each were used (total of 96 wells/plate). With sterile, disposable, calibrated pipettes (Cooke Laboratory Products), 0.05 ml of MEM with 10% FCS was added to wells 2 through 11 of each row, and 0.1 ml to wells 1 and 12. Then 0.05 ml of the test serum sample(s) was added to wells 1 and 2 in every row. Using 0.05 ml microdiluters (Cooke; sterilized with 70% alcohol), serial two-fold dilutions of the test sera were made from well 2 through 10. The drop remaining in the microdiluted was discarded. Well 1 of each row was the serum control to determine if the patient's serum was cytotoxic to the cells. Duplicate plates of the samples were set up to test for antibodies to HSV I and HSV II.

The virus pools previously titered by the $TCID_{50}$ method (described later) were diluted to a stock concentration of 100 $TCID_{50}$/0.05 ml. Then 0.05 ml of the appropriate virus was added to wells 2 through 10. Virus titration (described later) was carried out in the wells in column 11. Well 12 of each row received neither virus nor serum and served as the control to illustrate how normal cells should appear. The plates were covered and incubated at room temperature ($22^0$) for 1 h.

During this hour, VERO (indicator) cells were prepared. A 75 $cm^2$ flask of cells was trypisinized and the cells were suspended at a concentration of $2X10^5$ cells/ml. Fifty microliters of that suspension were added to each well on all plates ($1X10^4$ cells/well). The plates

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were incubated at 37°C in a sealed box flushed with 5% $CO_2$-95% air. The plates usually were read in two days (see virus titration).

To determine the neutralization index, the highest dilution that inhibited the cytopathic effect was considered the neutralizing titer. The wells were read + or - for viral CPE.

## Viral Pools

Flasks (75 $cm^2$) of VERO cells were infected with HSV I (KOS strain) or HSV II (strain 196; both obtained from Donald Lodmell, Ph.D., RML). When 80 to 100% of the cells were rounded the flasks were freeze-thawed twice to release maximum virus from the cells. The suspension was centrifuged at 2500 rpm for 15 min to remove the cellular debris. The pool was frozen in one-use vials in the REVCO (-70°C) for later use.

## $TCID_{50}$

Determination of the virus titers was performed as follows. A tube from each virus pool was thawed. Ten-fold serial dilutions were made of each stock virus (10 for HSV I, 7 for HSV II). A 3040 plate previously seeded with VERO cells was used for each virus. A 0.05 ml aliquot of the undiluted stock virus or the appropriate virus dilution was added to the first 11 wells of each row. Row 12 received only diluent and remained as uninfected cell control. The plates were incubated at 37°C in the $CO_2$ box previously described. They were read (+ or -) at 48 h. The 50% endpoint was calculated by the method of Reed and Muench (40).

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Virus Titration (during serum neutralization test)

To confirm that approximately 100 $TCID_{50}$ were used per well in the serum neutralization test, a virus titration was performed in parallel with each test. Three serial 10-fold dilutions were made and inoculated into a like number of wells in column 11. The actual titer of the inoculum was calculated by the method of Reed and Muench and was 50 to 200 $TCID_{50}$ (considered accurate to within one dilution).

Statistical Analyses

The data from Table 3 of the Results section were entered into a DEC-20 computer system at the University of Montana in SPSS program format by Daryl Paulson, M.S. The data were not normally distributed, therefore non-parametric techniques were used. Among the techniques he applied to this study are: multiple regression analysis with up to 13 variables with partial, multiple partial and residual analyses; analysis of variance; analysis of covariance; other non-parametric statistical procedures; and procedures involving binomial and Poisson distributions (25,55,57 ). Most of the tests revealed no statistically significant differences between groups and no correlations between lesions, positive cultures and occurences postulated by other workers to be precipitating factors. The statistical analyses upheld the conclusions drawn from inspection of the data. Thus most of the analyses were not discussed.

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## Chapter 3

## RESULTS

### Eliminated Group

From the onset of the study, the diagnosis of herpes genitalis in several patients was in doubt because it was made without viral culture or serological confirmation. Ten of the original 30 candidates for the study were eliminated; half of those apparently did not have herpes simplex genital infection. Thus the study allowed five women who thought they had genital herpes to be reassured that this was not the case. Although four of the remaining five clearly had genital herpes simplex infections, they did not participate in the study long enough to allow the accumulation of adequate data. (Table 1)

### Study Group

Biographical information about the remaining women who participated in this study are found in Table 2. A compilation of culture results and specific factors affecting herpes recurrences or asymptomatic shedding can be seen in Table 3. Various parameters will be dealt with separately below.

#### Time Since Primary Infection

Table 4 shows the study group in ascending order for length of time since primary herpes genitalis infection. One-half of the group had been infected for less than one year. The other half ranged from 17 mo to over 5 y. The four participants who manifested lesions most frequently during the study were in the "short infection" group (S, C, L and G). However, three of four participants who had no lesions



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Table 1

Persons eliminated from study.

| group | | age (1978) | marital status (1978) | date herpes diagnosed | SN titer HSVI/HSVII | outcome of cultures | # of exams | probable diagnosis |
|---|---|---|---|---|---|---|---|---|
| 1 not herpes | a | 25 | single | 11/77 | 4/0,2/2 | negative | 9 | condyloma acuminata |
| | b | 22 | single | 11/77 | <4/4 | no virus yeast contamination | 8 | Candidiasis |
| | c | 24 | single | 5/77 | 0/0,2/<2 | 1+ from cervix, not tested for zoster | 5 | genital zoster |
| | d | 18 | single | 11/77 | 8/4 | negative | 4 | nongenital zoster |
| | e | 28 | married | α | - | negative | 1 | no infection |
| 2 insufficient data | f | 23 | single | 1/76 | 64/>128 | 1+ from lesion | 5 | genital HSV |
| | g | 22 | single | β | 16/64 | negative | 5 | insufficient participation |
| | h | 25 | single | 11/76 | 64/128 | 2+ isolates | 4 | genital HSV |
| | i[γ] | 30 | married | 4/77 | >64/8 | 1+ from lesion | 2 | genital HSV I |
| | j | 21 | single | 1/77 | 32/128[δ] | 1+ from vagina | 1 | genital HSV |

α--never diagnosed; husband affected
β--never diagnosed; exposed twice
γ--initial diagnosis by virus culture
δ--virus isolated at same time

Table 2:

| patient | age (1978) | marital status (1978) | number of pregnancies | when |
|---|---|---|---|---|
| A | 27 | single | 1 | 1971[i] |
| B | 25 | married | 1 | 1977[i] |
| C | 23 | single | 1 | 1978[i] |
| D | 20 | single | 0 | - |
| E | 25 | married | 1 | 1975[l] |
| F | 20 | single | 1 | 1976[i] |
| G | 24 | single | 1 | 1974[m] |
| H | 26 | single | 0 | - |
| I | 26 | single | 0 | - |
| J | 28 | married | 0 | - |
| K | 28 | single | 0 | - |
| L | 28 | single | 0 | - |
| M | 21 | single | 0 | - |
| N | 29 | single | 0 | - |
| O | 25 | married | 2 | 1969[l]<br>1971[l] |
| P | 25 | single | 1 | 1970[l] |
| Q | 31 | single | 0 | - |
| R | 22 | single | 0 | - |
| S | 18 | single | 0 | - |
| T | 22 | single | 0 | - |

i=induced abortion

l=live birth

m=miscarriage

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Biographical Data

| age of first intercourse | # of sex partners | | time since HSV onset | other diseases |
|---|---|---|---|---|
| | total | 1978 | | |
| 21 | 10 | 1 | 1 mo | none |
| 19 | 10 | 1 | 24 | none |
| 16 | >1 | 1 | 5 | none |
| 19 | 1 | 1 | 17 | none |
| 16 | ? | 1 | 52 | none |
| 15 | 15 | 1 | 37 | none |
| 21 | 1 | 1 | 9 | none |
| 16 | ? | 1 | 64 | yeast |
| 21 | 6 | 1 | 40 | none |
| 17 | <10 | 1 | 28 | none |
| 20 | 23 | varies | 2 | none |
| 19 | 1 | 1 | 6 | none |
| 17 | 1 | 1 | 4 | none |
| 20 | 5 | 2 | 30 | none |
| 15 | 2 | 1 | 6 | none |
| 17 | 5 | 1 | 3 | none |
| 19 | unknown | 1 | 35 | gonorrhea 2X |
| 22 | 1 | 1 | 1 | yeast & Haemophilis |
| 16 | 4 | 1 | $1^{0}$ | yeast & warts |
| 16 | >2 | 0 | 40 | gonorrhea |

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Table 3

Culture Results and Affecting Factors
(Legend)

(patient)
1 pregnant
2 no sexual activity
3 BCG
  a after study
  b before study
4 primary infection

(culture results)
o negative culture
c positive isolate from cervix
v positive isolate from vagina
l positive isolate from lesion
e positive isolate from eye
a absent
b break (Spring)

(affecting factors)
m menstrual bleeding
s sexual activity
t t1 illness
  t2 trauma
  t3 emotional stress
  t4 exam
  t5 exercise, overheated, etc.

@ serum drawn for antibody titers
(see Table 15)

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| patient | week 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | s ss | b<br>ss ssss |
| B | a<br>mmmmm<br>s<br>ℓ ℓ | o | cv<br>s s s<br>ℓℓℓℓ | o<br>s<br>ℓ | o<br>mmmmmm | o<br>s sss | o<br>111111<br>ℓ ℓ | @<br>cvl<br>1111111 | o<br>1111<br>mmmmm<br>ℓ | b<br>s |
| C[1] | o<br>mmmmmm<br>ℓℓℓℓℓ | a<br>ss s<br>ℓℓℓℓ | @<br>o<br>s sss | o<br>sss | o<br>1111 | cv<br>s s s | v | cv | @<br>o<br>11111<br>s<br>ℓ | b<br>1111111<br>s |
| D | o<br>mmmmmm<br>sss<br>ℓ | @<br>o<br>s | o<br>ss | o<br>s ss<br>t t | a<br>s sssss<br>ℓℓℓ | c<br>mmm<br>ss ss | o<br>ℓℓ | @<br>cv<br>ℓℓ | o<br>ss | b<br>1111<br>mmmmmm<br>ssssss |
| E[2,3] | o | @<br>o | o<br>mmmmm | v | o | o | a | @<br>cv<br>1<br>mmmm | cv | b |
| F | o | o<br>ss s<br>ℓ ℓ | @<br>o<br>ss sss | o<br>s sss<br>ℓ | c<br>mmmmm<br>sss ss<br>ℓ | o<br>ss sss<br>ℓ | o<br>s | @<br>cv | cv | |
| G | o<br>111<br>mmmmm<br>ℓℓℓ | o<br>s | a | @<br>o<br>111<br>mmm | o<br>11<br>mm<br>s<br>ℓ | c | o | cv<br>mmmmm | @<br>v | b<br>ℓ |
| H[3] | o<br>s | @<br>o | o<br>mmmm<br>ss s | c<br>s | o<br>s | c<br>s s<br>ℓℓ | @<br>cvl<br>1111<br>mmmm | cv | o | |
| I | o<br>s s<br>ℓℓℓℓℓ | o<br>mmmmmmm<br>s ss<br>ℓℓℓℓℓℓ | o<br>sss s<br>ℓ | c<br>s s ss | o<br>s sss | o<br>mmmmmmm<br>s s | cv<br>s s | @<br>c<br>s s | a<br>ss | b<br>mmmmmm<br>s sss |
| J[2] | | | | | a<br>mmmm | a | @<br>cv<br>ℓℓℓℓℓ | o<br>ℓ | o<br>mmmm<br>ℓ | b |

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| 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|
| @<br>o<br>s ssss | o<br>ss ssss | o<br>ssss s | o<br>mmmmm<br>sss ss | v<br>ssss s | a<br>ss | o<br>ss s | o<br>ssss s | o<br>1<br>s s<br>ƚƚƚƚƚƚƚ | 1 |
| a<br>s ss | o<br>s s | v<br>mmmmmm<br>s s | o | o<br>s | o<br>s | o<br>mmmmmm<br>ƚƚ | a<br>s | o | |
| a<br>ƚ | a<br>s | a<br>s | a<br>ƚ3 | a | @<br>o<br>s | o<br>111 | cv<br>mmmmm<br>s | o<br>s | v<br>11 |
| cv<br>11111 | o<br>mmmmm<br>ss | o<br>s | o<br>ss | o | o | o<br>mmmmmm<br>ss | a<br>ssss | cv<br>sss<br>ƚƚƚ | o<br>ss |
| a<br>mmmm | o | cv | o<br>mm | a<br>mmm | a | o<br>m | o<br>mmm | @<br>o | c |
| | | | | | | | | | |
| v<br>mmm | o<br>mm<br>ƚ | v<br>ƚ | o | c<br>mmmmm | a<br>ƚƚ ƚƚ | o<br>ƚƚ | o<br>ƚ | c<br>mmmmm | o |
| | | | | | | | | | |
| o<br>ssss ss | o<br>s sss | c<br>s ss<br>ƚ | o<br>s<br>mmm | @<br>o<br>11111<br>mm<br>s | o<br>s ss<br>ƚƚƚ | @<br>o<br>s s<br>ƚƚƚƚƚ | a<br>mmmm<br>ssss | a<br>1111<br>m<br>s s s | c<br>1 |
| cv<br>ƚ | c<br>1 | cv1<br>111<br>mmm<br>ƚ | cv<br>ƚ | o<br>ƚ | o<br>mmmm<br>ƚ | @<br>o<br>ƚ ƚ | cv<br>ƚ ƚ | cv<br>ƚ ƚ | o |

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|---|---|---|---|---|---|---|---|---|---|---|
| K | o<br>mmmm<br>t t | @<br>o<br>t | o | o<br>mm | @<br>c<br>mm | c<br>t t t t t | c<br>t t t | @<br>o<br>mmmmm<br>s s s s s s<br>t t t | c<br>11<br>t t | b |
| L | | a<br>s s<br>t | o<br>11<br>s<br>t t | v<br>11<br>mmm<br>s | o<br>s | v<br>s | c<br>1<br>s | o<br>1 1<br>mmmm | @<br>o<br>s s<br>t t t | b<br>1111<br>s s<br>t |
| M | | | | @<br>cv | o<br>mmmm | o | a | cv | a<br>mmmm | b |
| N | | | o<br>s s<br>t t t t | c<br>mmmmm<br>s s s<br>t t t t | @<br>o<br>111<br>s s<br>t | cv<br>s s<br>t | v | cv<br>mmmmmm<br>s<br>t t | c<br>s<br>t t t t t | b |
| O | | | | | @<br>o<br>s s<br>t t | o<br>s s s<br>t t | o<br>mmmm<br>s s s<br>t t t | cv<br>s s s<br>t | cv<br>s s s<br>t t t t | b<br>s s<br>t t |
| P[2] | | | o | o | @<br>o<br>mmmm<br>t | c | o | a<br>mmm | cv | b |
| Q | | | o | c<br>s s<br>t t t t | @<br>o<br>t | o | a<br>mmmm<br>s<br>t | cvl<br>1111<br>s<br>t | cv<br>s<br>t | b<br>s<br>t |
| R | a<br>mmm<br>s<br>t | a<br>s<br>t | @<br>o<br>s | o<br>s<br>t t | o<br>mmmmmm<br>t | o<br>t | cv<br>s s s<br>t | v<br>t | @<br>o<br>t t t t | b<br>mmmmm |
| S | | | a<br>1111111<br>mmmmmm | @<br>1<br>1111111<br>t t t t t t | o<br>1111111<br>t t t | o<br>t t t | cv<br>mmmmm<br>t | @<br>cv<br>t | o<br>t t | b<br>s s s |
| T | | | | @<br>v | o<br>mmmm | v<br>t t t t t t t | cv | v | @<br>o<br>mmmmmm<br>s | b<br>11<br>t t t t t |

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| cv<br>1111<br>mmmmm<br>ℓℓℓ | o | v | c<br>ℓℓℓ | o<br>11<br>mmm<br>ℓℓℓℓℓ | o<br>ℓℓℓℓ ℓ | o | o<br>mmmm | o | o<br>1<br>ℓℓ |
| cv | cv | o | a | cv<br>mmmmm | @<br>cv | | | | |
| cv<br>mmmmmmm<br>s ℓ | o<br>s s ℓ | @<br>c<br>s s ℓ | c<br>s s s s ℓℓ | o<br>mmm<br>ss ss ℓ | c<br>s sss ℓℓℓℓ | o<br>s s s ℓ | c<br>s ss ℓ | v<br>s s s s ℓ ℓ | o<br>1<br>mmm<br>sss ℓℓ |
| o<br>mmm<br>s ssss ℓ | o<br>sss s ℓℓ | cv<br>ss ℓℓℓ | o<br>ss s ℓ | v<br>mmm<br>sss s | @<br>cv<br>s ss ℓℓℓ | o<br>111111<br>s s s | v<br>mmmm<br>s ℓℓℓℓ | c<br>s s ss | a<br>ss ℓ |
| v<br>mmmmm | a | o | o | o | a<br>mmmmm | a | a | o | o |
| cv<br>s sss ℓ | a | a<br>mmmmm | a<br>ss s ℓ | a<br>s s | a<br>mmmm<br>s ℓ | a<br>s s s ℓ | a<br>s ℓ ℓ | a<br>sss s | a<br>ss ℓℓ |
| o<br>ℓ ℓ | o<br>ℓ ℓ ℓℓℓ | o<br>ℓ | cv<br>mmmmm | v<br>ℓ | o<br>ℓ | o<br>ℓ ℓ | a<br>ℓℓ | o<br>mmmmm<br>ℓℓℓℓ | o |
| o<br>1<br>ℓ | o<br>mmmmmm | cv<br>ℓℓℓ | o<br>ℓℓℓ ℓℓ | @<br>c<br>ℓℓ | cv<br>s ℓ | o<br>111<br>mmmmmm<br>ℓℓ | cv | v<br>ℓ ℓ | o |
| cv | cve | o<br>mmmmm | o | @<br>o | o<br>mmmm | o | o | c | c<br>1<br>ℓℓℓℓ |

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Table 4

Effect of time since primary infection upon frequency of lesions, duration of lesions, frequency of viral isolation and antibody titers.

| patient | age | number of months since 1° infection | # of lesions / days in study | sum lesion length / number of lesions | # isolates / attempts | high SN |
|---|---|---|---|---|---|---|
| S | 18 | * | 3/126 | 26†/3 | 8/16 | 64/32 |
| A | 27 | 1 | 1/70 | 2/1 | 1/8 | 32/16 |
| R | 22 | 1 | 0/140 | - | 4/16 | 16/64 |
| K | 28 | 2 | 1/63 | 2/1 | 4/9 | 4/4 |
| P | 25 | 3 | 0/126 | - | 3/12 | 32/16 |
| M | 21 | 4 | 0/112 | - | 6/9 | <8/16 |
| C | 23 | 5 | 4/140 | 21/4 | 5/13 | 32/16 |
| L | 28 | 6 | 8/133 | 18/8 | 6/17 | 16/2 |
| O | 25 | 6 | 1/112 | 6/1 | 7/14 | 32/16 |
| G | 24 | 9 | 3/140 | 8/3 | 7/17 | 8/8 |
| D | 20 | 17 | 2/140 | 9/2 | 4/17 | 16/32 |
| B | 25 | 24 | 1/133 | 17/1 | 3/15 | 8/16 |
| J | 28 | 28 | 1/140 | 4/1 | 8/16 | 16/128 |
| N | 29 | 30 | 2/119 | 4/2 | 11/17 | 64/<4 |
| Q | 31 | 35 | 1/126 | 4/1 | 4/7 | 16/64 |
| F | 20 | 37 | 0/63 | - | 3/9 | 512/256 |
| I | 26 | 40 | 2/140 | 10/2 | 5/16 | 16/32 |
| T | 22 | 40 | 2/119 | 3/2 | 8/16 | 64/128 |
| E | 25 | 52 | 1/133 | 1/1 | 5/15 | 256/128 |
| H | 26 | 64 | 1/63 | 4/1 | 4/9 | 32/8 |

* entered study during 1° attack

† 1° lasted 22 days

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during the study were also in that group (R, P and M). No difference was seen between the length of lesions or the number of positive cultures for those persons recently infected and those infected for a considerable length of time ($\bar{x}$ length of lesion 4.1 d vs. 4.3 d, $\bar{x}$ percent positive cultures 39 vs. 41).

Viral Isolation

Whether lesions were present or not, attempts were made to culture virus from the cervix and from the vagina each time the patient was examined. Whenever a lesion was apparent, it was swabbed directly and an attempt was made to culture virus. Successful culture of virus directly from lesions was possible 5 of 22 times (22.7% ; see Table 5). Lesions were found at a variety of locations listed in Table 6. One patient (T) experienced optic neuralgia. A culture from her lacrimal secretions yielded herpesvirus. (See Table 7 for source of positive cultures.) Only 5, or 3.1% of the positive cultures came from lesions. The cervical isolates (84 or 52.2% of the total) were slightly more numerous than vaginal isolates (72 or 44.7% of the total). Two participants had positive viral isolates from the cervix more than twice as often as from the vagina (I and K). They did not have cervical lesions during the study.

Asymptomatic Shedding

Of 268 culture sets (cervical, vaginal, plus any suspect lesion), 106 cultures were positive for herpesvirus (39.6%). The 39.6% were further divided into those that could be related to lesions ($\pm$1 wk) and those that involved asymptomatic shedding. In this study, 74.5%

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Table 5

Isolation of virus from lesions

| patient with lesion on culture day | culture negative (failure) | culture positive (success) |
|---|---|---|
| B | 2/3 | 1/3 |
| C | 4/4 | 0/4 |
| D | 1/1 | 0/1 |
| E | 1/1 | 0/1 |
| G | 1/1 | 0/1 |
| H | 0/1 | 1/1 |
| I | 1/1 | 0/1 |
| J | 0/1 | 1/1 |
| K | 1/1 | 0/1 |
| L | 3/3 | 0/3 |
| N | 1/1 | 0/1 |
| Q | 0/1 | 1/1 |
| S* | 2/3 | 1/3 |
| total | 17/22=77.3% | 5/22=22.7% |

* $1^{0}$ lesion included

Table 6

Location of lesions

| | |
|---|---|
| A | slightly forward of clitoris[α] |
| B | left side of vulva and anal |
| C | left side of vaginal opening |
| D | vaginal |
| E | perineum |
| G | (did not specify) |
| H | left side of labia |
| I | perineum |
| J | left labia |
| K | inside base of vulva |
| L | vaginal |
| N | lower outer vulva |
| O | pubic area |
| Q | right lip of vulva[β] |
| S | around anus |
| T | inside vaginal opening |

α unable to culture

β one attack may have been exogenous reinfection (manifest at a new site which was not specified)

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Table 7

Area from which positive cultures were obtained every week from each person (week 10 was Spring Break--no examinations).

| patient | week 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | total c | v | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | o | o | o | o | v | a | o | o | o | | 1 | 0 | 0 |
| B | a | o | cv | o | o | o | o | cvl | o | a | o | v | o | o | o | o | a | o | | 2 | 3 | 1 |
| C | o | a | o | o | o | cv | v | cv | o | a | a | a | a | a | o | o | cv | o | v | 3 | 5 | 0 |
| D | o | o | o | o | a | c | o | cv | o | cv | o | o | o | o | o | o | a | cv | o | 4 | 3 | 0 |
| E | o | o | o | v | o | o | a | cv | cv | a | o | cv | o | a | a | o | o | o | c | 4 | 4 | 0 |
| F | o | o | o | o | c | o | o | cv | cv | | | | | | | | | | | 3 | 2 | 0 |
| G | o | o | a | o | o | c | o | cv | v | v | o | v | c | | a | o | o | c | o | 4 | 4 | 0 |
| H | o | o | o | c | o | c | cvl | cv | o | | | | | | | | | | | 4 | 2 | 1 |
| I | o | o | o | c | o | o | cv | c | a | o | o | c | o | o | o | o | a | a | c | 5 | 1 | 0 |
| J | o | o | a | c | a | a | cv | o | o | cv | c | cvl | cv | o | o | o | cv | cv | o | 8 | 6 | 1 |
| K | o | o | o | o | c | c | c | o | c | | | | | | | | | | | 4 | 0 | 0 |
| L | | a | o | v | o | v | c | o | o | cv | o | v | c | o | o | o | o | o | o | 3 | 4 | 0 |
| M | | | | cv | o | o | a | cv | a | cv | cv | o | a | cv | cv | | | | | 6 | 6 | 0 |
| N | | | o | c | o | cv | v | cv | c | cv | o | c | c | o | c | o | c | v | o | 9 | 5 | 0 |
| O | | | | | o | o | o | cv | cv | o | o | cv | o | v | cv | o | v | c | a | 5 | 6 | 0 |
| P | | | o | o | o | c | o | a | cv | v | a | o | o | o | a | a | a | o | o | 2 | 2 | 0 |
| Q | | | o | c | o | o | a | cvl | cv | cv | a | a | a | a | a | a | a | a | a | 4 | 3 | 1 |
| R | a | a | o | o | o | o | cv | v | o | o | o | o | cv | v | o | o | a | o | o | 2 | 4 | 0 |
| S | | | a | l | o | o | cv | cv | o | o | o | cv | o | c | cv | o | cv | v | o | 6 | 6 | 1 |
| T | | | | v | o | v | cv | v | o | cv | cve | o | o | o | o | o | o | c | c | 5 | 6 | 0 |
| | | | | | | | | | | | | | | | | | | | | 84 | 72 | 5 |
| | | | | | | | | | | | | | | | | | | | | 52.2% | 44.7% | 3.1% |

v=vaginal c=cervical l=lesion e=eye o=negative culture a=absent
blank=not in study at that time

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of the positive isolates had no relationship to noticeable lesions. In all but two cases (C and L) the majority of positive cultures were obtained in the absence of lesion. Also note that all participants shed virus asymptomatically. (See Table 8.)

Although only 25.5% of the viral isolates were obtained during recurrences of lesions, only 10.8% of the attempts to isolate viruses were made during these recurrences. The remaining 89.2% of the attempts, made in the absence of lesions, led to 74.5% of the virus isolations. Thus it would appear that the likelihood of isolating virus during a recurrence is more than 2.8 times greater than it would be from an asymptomatic patient. The McNemar related sample test (non-parametric) gave a confidence limit between .85 and .90, which may be significant considering the small sample size.

Factors Which Might Affect Recurrences of Lesions and/or Viral Shedding

Menstrual Cycle

For purposes of this study, menstruation was analyzed in two ways. Menstrual bleeding or "menses" began on day 1 of bleeding and included all days of bleeding. Of the time period covered by this study, 15.8% of it occurred during menses, with 20% of the lesions and 13.9% of the positive viral cultures. (See Table 9a.)

The menstrual phase was defined as two days before bleeding and all days of menses, to include the post-secretory phase. (This is 2 days longer than denoted by "m" on Table 3.) The menstrual phase included 22.6% of the study period, 34.6% of lesions and only 17.8% of positive cultures. Because of scatter of the sample group, these

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Table 8

Relationship of positive cultures
to the presence (± one week) of detectable lesions
(asymptomatic positive cultures)

| patient | positive* cultures / times cultured | positive cultures in absence of lesions (± 1 wk) | (% of positive cultures) |
|---|---|---|---|
| A | 1/8 | 1 | (100) |
| B | 3/15 | 2 | (66.7) |
| C | 5/13 | 2 | (40) |
| D | 4/17 | 3 | (75) |
| E | 5/15 | 4 | (80) |
| F | 3/9 | 3 | (100) |
| G | 7/17 | 6 | (83.3) |
| H | 4/9 | 2 | (50) |
| I | 5/16 | 4 | (80) |
| J | 8/16 | 6 | (75) |
| K | 4/9 | 3 | (75) |
| L | 6/17 | 1 | (16.7) |
| M | 6/9 | 6 | (100) |
| N | 11/17 | 10 | (90.9) |
| O | 7/14 | 6 | (83.3) |
| P | 3/12 | 3 | (100) |
| Q | 4/7 | 2 | (50) |
| R | 4/16 | 4 | (100) |
| S | 8/16 | 6 | (75) |
| T | 8/16 | 5 | (62.5) |
| total | 106/268=39.6% | 79 | (74.5) |

*positive culture from any site tested

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Table 9a

The relationship of menstrual bleeding to lesions and/or viral shedding.

| patient | days of bleeding / total d in study | lesions in menses / total lesions | + cultures in menses / total positive cultures | lesions and + cultures in menses[d] / total lesions and positive cultures |
|---|---|---|---|---|
| A | 5/70 | 0/1 | 0/1 | 0/2 |
| B | 28/133 | * | 1/3 | 1/3 |
| C[a] | (11/140) | (0/4) | (1/5) | (1/8) |
| D | 26/140 | 1/2 | 1/4 | 2/6 |
| E | 22/133 | 0/1 | 0/5 | 0/5 |
| F | 5/63 | - | 1/3 | 1/3 |
| G | 30/140 | 2/3 | 3/7 | 5/10 |
| H | 8/63 | * | 1/4 | 1/4 |
| I | 30/140 | 0/2 | 0/5 | 0/7 |
| J | 15/140 | * | 1/8 | 1/8 |
| K | 13/63 | 0/1 | 0/4 | 0/4 |
| L | 19/133 | *1/7 | 0/6 | 1/9 |
| M | 13/112 | - | 1/6 | 1/6 |
| N | 24/119 | 0/2 | 3/11 | 3/13 |
| O | 14/112 | 0/1 | 0/7 | 0/7 |
| P | 17/126 | - | 0/3 | 0/3 |
| Q | 13/126 | 0/1 | 0/4 | 0/4 |
| R | 24/140 | - | 1/4 | 1/4 |
| S | 23/126 | †*1/2 | 1/8 | 2/10 |
| T | 19/119 | 0/2 | 0/8 | 0/9 |
| totals | 348/2198=15.8% | 5/25=20% | 14/101=13.9% | 19/117=16.2% |

[a]this person became pregnant and was deleted from the statistics

*lesions which began outside menses and continued into it were omitted

†$1^0$ lesion

d when there were simultaneous lesions and positive cultures, they were counted as one

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Table 9b

The relationship of mestrual phase to lesions and/or viral shedding.

| patient | days of menstrual phase / total days in study | lesions in phase / total lesions | + cultures in phase / total positive cultures | lesions and + cultures in phase / total lesions and positive cultures [d] |
|---|---|---|---|---|
| A | 7/70 | 0/1 | 0/1 | 0/2 |
| B | 38/133 | * | 1/3 | 1/3 |
| C[a] | (15/140) | (0/4) | (1/5) | (1/8) |
| D | 36/140 | 1/2 | 1/4 | 2/6 |
| E | 32/133 | 0/1 | 0/5 | 0/5 |
| F | 7/63 | - | 1/3 | 1/3 |
| G | 42/140 | 2/3 | 4/7 | 6/10 |
| H | 12/63 | * | 1/4 | 1/4 |
| I | 40/140 | 0/2 | 0/5 | 0/7 |
| J | 23/140 | 1/1 | 1/8 | 1/8 |
| K | 19/63 | 0/1 | 0/4 | 0/4 |
| L | 29/133 | *3/7 | 2/6 | 5/9 |
| M | 19/112 | - | 1/6 | 1/6 |
| N | 34/119 | 0/2 | 3/11 | 3/13 |
| O | 22/112 | 0/1 | 1/7 | 1/7 |
| P | 25/126 | - | 0/3 | 0/3 |
| Q | 19/126 | 0/1 | 0/4 | 0/4 |
| R | 34/140 | - | 1/4 | 1/4 |
| S | 31/126 | †*2/2 | 1/8 | 3/10 |
| T | 27/119 | 0/2 | 0/8 | 0/9 |
| totals | 496/2198=22.6% | 9/26=34.6% | 18/101=17.8% | 26/117=22.1% |

[a] this person became pregnant and was deleted from the statistics

*lesions which began outside menstrual phase and continued into it were omitted

†1° lesion

d when there were simultaneous lesions and positive cultures, they were counted as one

differences are not significant. Patients G and L had greater than 50% of their lesions and/or positive cultures during the menstrual phase. Only participant G had 50% of positive cultures during "menses". (See Table 9b.)

Figure 1 illustrates the reported onset of lesions plotted in relation to the first day of menstrual bleeding. Because the menstrual cycles of the women in the study ranged from 16 to 40 days the data were plotted ± 15 days of the first day of bleeding to minimize possible effects of variable cycle length. Panel A illustrates onset of lesions for the entire study group. Because the hormonal levels of those women on birth control pills is different from those women not on birth control pills, they were removed from a portion of this analysis. Panel B shows the incidence of lesions of women not on the "pill". Although the highest incidence of reported lesion onsets occurred on the first day of bleeding, computer analysis for linear trends showed that for these test groups no day of the menstrual cycle was more likely to yield lesions than another. However, two individuals appeared to have lesions grouped in one portion of the menstrual cycle. Patient G had all three episodes of recurrent disease in the first 8 days of the cycle, and patient I had both episodes one week after the first day of bleeding or a little over 3 weeks before that first day of bleeding.

In the same manner, Figure 2 is demonstrative of trend for isolation of virus relative to menstruation. Again, computer analysis for linear trend indicated that no day of the cycle was more or less likely to yield positive cultures than another.

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Figure 1

Onset of recurrent herpes in relation to menstrual bleeding.

(Day 1 is the first day of menstrual bleeding. Negative numbers are days prior to the onset of bleeding. Positive numbers are days after the onset of bleeding.)

a. total study group

b. women on birth control pills eliminated

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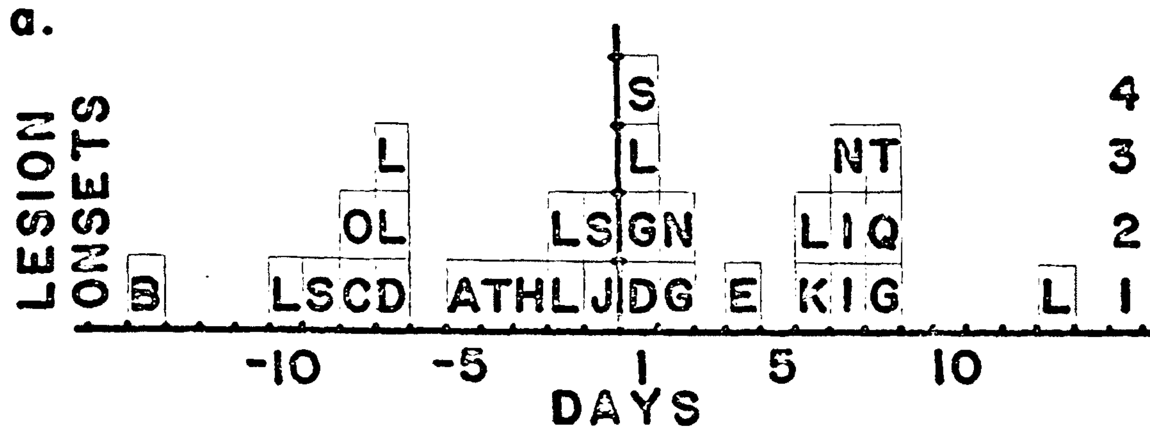
a.




b.

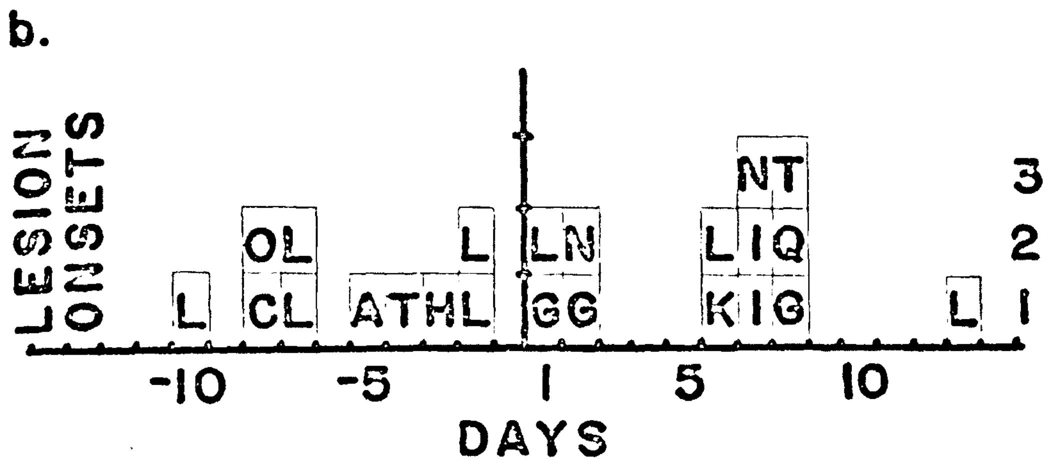



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Figure 2

Effect of menstrual cycle on herpesvirus isolation.

(Day 1 is the first day of menstrual bleeding. Negative numbers are days prior to the onset of bleeding. Positive numbers are days after the onset of bleeding.)

a. total study group

b. women on birth control pills eliminated

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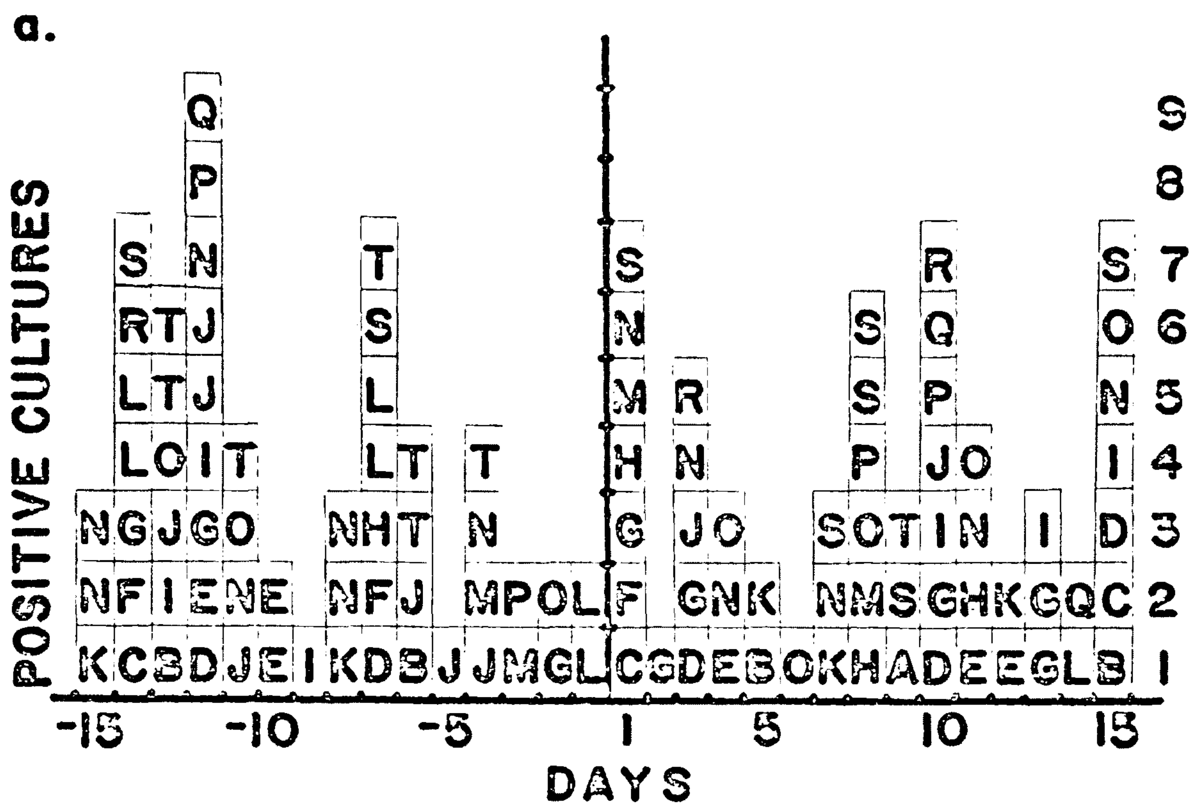


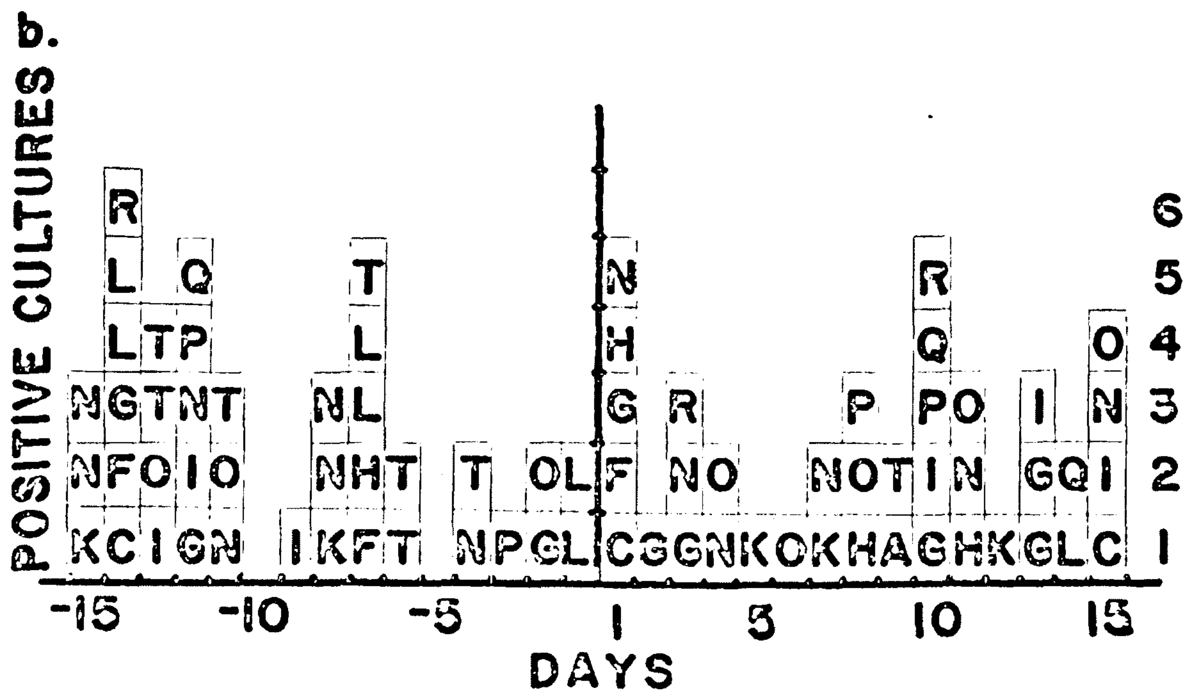


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Method of Birth Control

Birth control methods (and success) are recorded in Table 10. Table 11 shows the effect of birth control methods on lesions and positive cultures. The number of women in the groups using birth control pills and IUD's were equal. To facilitate statistical analysis, the groups should have been of equal size. Two persons were eliminated from the group using other methods (none or chemical/barrier), the person who became pregnant and the lesbian. A three factor, one-way analysis of variance indicates that there is no difference in the groups. However, the length of the lesions appeared to be somewhat longer for the group on birth control pills ($\bar{x}$ lesion length of 5.0 d vs. 3.0 d and 3.2 d for the other groups).

Sexual Activity

Tables 12a and b illustrate that in this study, the probability that sexual activity induced lesions or positive cultures was greater than 30% (not significant). (Patients M and P were deleted from this portion due to lack of response about sexual activity.) Table 13 shows how the participants were divided to give the groups shown in Table 12.

Stress

For this study, stress included other illnesses, trauma, emotional stress, exams and excessive excerise. Patients E and M did not report stress, therefore they were eliminated from the stress analysis. As a group the remaining participants reported experiencing stress during 14.5% of the study period. Thirty-six percent of lesions were within

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Table 10

Birth control methods used during study.

| group | | patient | type | how long | comments |
|---|---|---|---|---|---|
| 1 birth control pill | | B | Orthonovum | 6y | induced abortion (6 mo before study) |
| | | D | Orthonovum | 6mo | |
| | | E | Ovcon 50 | 2y | live birth (when off pill) |
| | | J | Nornal | 8y | |
| | | M | Ovral | 1y | |
| | | S | Orthonovum | 1y | |
| 2 IUD | | A | | 3y | |
| | | F | | 9mo | induced abortion (when on pill previously) |
| | | G | | 6mo | |
| | | I | | 2y | |
| | | N | | 3½y | |
| | | P | | 6y | |
| 3 other | a | C | diaphragm | 1½y | pregnant--induced abortion (during study) |
| | | H | diaphragm | 3y | |
| | | K | diaphragm | 2y | had Dalkon shield for 5 y (previously) |
| | | R | Encore oval | 1mo | |
| | b | L | | | lesbian |
| | | O | | | husband had vasectomy |
| | | Q | sterilized | | tubal ligation |
| | | T | | | not sexually active |

a chemical-barrier methods
b no birth control used

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Table 11

Effect of birth control methods on recurrent herpes genitalis.

| group | patient | # of lesions | total days with lesions / number of recurrences | positive cultures / total # of cultures |
|---|---|---|---|---|
| 1 birth control pills | B | 1 | 17/1 | 3/15 |
| | D | 2 | 9/2 | 4/17 |
| | E | 1 | 1/1 | 5/15 |
| | J | 1 | 4/1 | 8/16 |
| | M | 0 | - | 6/9 |
| | S | 3 | *4/2 | 8/16 |
| totals | | 8 | 35/7=5.0 d $\bar{x}$ | 34/88=38.6% of total |
| 2 IUD | A | 1 | 2/1 | 1/8 |
| | F | 0 | - | 3/9 |
| | G | 3 | 8/3 | 7/17 |
| | I | 2 | 10/2 | 5/16 |
| | N | 2 | 4/2 | 11/17 |
| | P | 0 | - | 3/12 |
| totals | | 8 | 24/8=3.0 d $\bar{x}$ | 37/96=38.5% of total |
| 3 other | H | 1 | 4/1 | 4/9 |
| | K | 1 | 2/1 | 4/9 |
| | R | 0 | - | 4/16 |
| | O | 1 | 6/1 | 7/14 |
| | Q | 1 | 4/1 | 4/7 |
| | T | 2 | 3/2 | 8/16 |
| totals | | 6 | 19/6=3.2 d $\bar{x}$ | 31/71=44.7% of total |

*1° lesion 26 d--omitted

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Table 12a

Effect of sexual activity on the course of recurrent herpes genitalis.

| group | patient | d with sex / # days in study | lesions within 4d after sex | lesions not within 4d after sex | sex followed by lesions in no more than 4d | sex with no lesions within 4d |
|---|---|---|---|---|---|---|
| 1 | A | 47/70 | 1 | 0 | 1 | 46 |
| active | D | 43/140 | 2 | 0 | 3 | 40 |
| | F | 24/63 | 0 | 0 | 0 | 24 |
| | I | 54/140 | 2 | 0 | 2 | 52 |
| | N | 41/119 | 2 | 0 | 5 | 36 |
| | O | 47/112 | 1 | 0 | 2 | 43 |
| totals | | | 8 | 0 | 13 | |
| 2 | B | 20/133 | 1 | 0 | 3 | 17 |
| moderate | C | 20/140 | 2 | 2 | 4 | 16 |
| | H | 8/63 | 0 | 1 | 0 | 8 |
| | K | 6/63 | 1 | 0 | 3 | 3 |
| | L | 11/133 | 3 | 5 | 4 | 7 |
| | Q | 26/112 | 1 | 0 | 1 | 25 |
| totals | | | 8 | 8 | 15 | |
| 3 | E | 0/133 | 0 | 1 | 0 | 0 |
| in- | G | 2/140 | 1 | 2 | 1 | 1 |
| active | J | 0/140 | 0 | 1 | 0 | 0 |
| | R | 7/140 | 0 | 0 | 0 | 7 |
| | S* | 4/126 | 3 | 0 | 2 | 2 |
| | T | 1/119 | 1 | 1 | 1 | 0 |
| totals | | | 5 | 5 | 4 | |

* sexual encounter before $1^0$ lesion not on Table 3

Table 12b

Effect of sexual activity on the course of asymptomatic viral shedding.

| group | patient | d with sex / # days in study | Cultures: + within 4d of sex | + not within 4d of sex | - within 4d of sex | - not within 4d of sex | # isolates / attempts |
|---|---|---|---|---|---|---|---|
| 1 | A | 47/70 | 1 | 0 | 7 | 0 | 1/8 |
| active | D | 43/140 | 2 | 2 | 6 | 7 | 4/17 |
| | F | 24/63 | 1 | 2 | 4 | 2 | 3/9 |
| | I | 54/140 | 5 | 0 | 11 | 0 | 5/16 |
| | N | 41/119 | 8 | 3 | 5 | 1 | 11/17 |
| | O | 47/112 | 7 | 0 | 6 | 1 | 7/14 |
| totals | | | 24 | 7 | 39 | 11 | 31/81=0.38 |
| 2 | B | 20/133 | 2 | 1 | 6 | 6 | 3/15 |
| moderate | C | 20/140 | 3 | 2 | 4 | 4 | 5/13 |
| | H | 7/63 | 3 | 1 | 2 | 3 | 4/9 |
| | K | 6/63 | 1 | 3 | 1 | 4 | 4/9 |
| | L | 11/133 | 1 | 5 | 3 | 8 | 6/17 |
| | Q | 26/112 | 4 | 0 | 1 | 2 | 4/7 |
| totals | | | 14 | 12 | 17 | 27 | 26/70=0.37 |
| 3 | E | 0/133 | 0 | 5 | 0 | 10 | 5/15 |
| in- | G | 2/140 | 0 | 7 | 0 | 10 | 7/17 |
| active | J | 0/140 | 0 | 8 | 0 | 8 | 8/16 |
| | R | 7/140 | 2 | 2 | 2 | 10 | 4/16 |
| | S | 4/126 | 0 | 8 | 2 | 6 | 8/16 |
| | T | 1/119 | 0 | 8 | 0 | 8 | 8/16 |
| totals | | | 2 | 38 | 4 | 52 | 40/96=.42 |



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Table 13

Distribution of sexual activity

| patient | # of wks in study | $\bar{x}$ # of X/wk | group |
|---|---|---|---|
| A | 10 | 4.7 | 1 |
| B | 19 | 1.1 | 2 |
| C | 20 | 1.0 | 2 |
| D | 20 | 2.2 | 1 |
| E | 19 | 0 | 3 |
| F | 9 | 2.7 | 1 |
| G | 20 | 0.1 | 3 |
| H | 9 | 0.9 | 2 |
| I | 20 | 2.7 | 1 |
| J | 20 | 0 | 3 |
| K | 9 | 0.7 | 2 |
| L | 19 | 0.6 | 2 |
| N | 17 | 2.4 | 1 |
| O | 16 | 2.9 | 1 |
| Q | 18 | 1.4 | 2 |
| R | 20 | 0.4 | 3 |
| S | 18 | 0.2 | 3 |
| T | 17 | $<0.1$ | 3 |
| | | $\bar{x}=1.2$ times/week | |

1. sexually active
2. average sexual activity
3. below average sexual activity

| 3 | 2 | 1 |
|---|---|---|
| $\leq 0.4$ | 0.6 - 1.4 | $\geq 2.2$ |

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four days after stress and 53.5% of all positive cultures were within four days after stressful events. See Table 14. Friedman's multiple correlation coefficient analysis revealed a correlation coefficient of .774 between lesions and stress and .855 between positive cultures and stress (eitherbefore or after). These statistics were the most significant of the study. Factor analysis suggested that stress was dominant over lesions. Therefore, more lesions may have been caused by stress than stress caused by lesions. There is an interrelationship between these variables so they can not be totally separated. Stress contributes to lesions and lesions to stress.

Serological Data

Table 15 displays the serological data for this study. Only one individual had dominant type 1 or type 2 antibodies (four-fold difference in titers) more than once during the study. That participant (H) had dominant type 1 antibodies both times her serum was assayed even when virus was isolated.

The average neutralizing antibody titer for the study was approximately 32 for HSV I and 32 for HSV II in the absence of lesions or positive viral cultures. When positive cultures were present concominantly, the average serological titers were approximately 64 for HSV I and 32 for HSV II. This held true even if lesions were present. When lesions alone were present, in the absence of positive cultures, the serum neutralization antibody titers were approximately 16 for each virus. A possible reason for this is suggested in the discussion.

Serological data is also presented in Table 4, where the highest value for neutralizing antibody titers for each participant is shown

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Table 14

Effect of stress on recurrent or asymptomatic herpes genitalis.

| patient | # d with stress / reported # of days | total lesions | lesions related to stress[a] | + cultures related to stress[b] / cultures related to stress[c] |
|---|---|---|---|---|
| A | 7/70 | 1 | 1 (100%) | 0/1 |
| B | 12/133 | 1 | 0 (0) | 2/3 |
| C | 12/140 | 4 | 0 (0) | 0/0 |
| D | 13/140 | 2 | 0 (0) | 2/3 |
| F | 5/63 | 0 | 0 - | 0/1 |
| G | 14/140 | 3 | 2* (67) | 4/6 |
| H | 2/63 | 1 | 1 (100) | 1/1 |
| I | 21/140 | 2 | 0 (0) | 0/3 |
| J | 18/140 | 1 | 0 (0) | 3/3 |
| K | 16/63 | 1 | 1* (100) | 3/5 |
| L | 25/133 | 8 | 4 (50) | 2/6 |
| N | 33/119 | 2 | 1* (50) | 6/8 |
| O | 29/112 | 1 | 0 (0) | 5/9 |
| P | 1/126 | 0 | 0 - | 0/1 |
| Q | 17/105 | 1 | 0 (0) | 1/2 |
| R | 30/140 | 0 | 0 - | 1/7 |
| S | 32/126 | 3 | 1 (33) | 5/9 |
| T | 16/119 | 2 | 1 (50) | 3/3 |
| totals | 303/2093 (14.5%) | 33 | 12 (36%) | 38/71 (53.5%) |

[a] lesions occurring within 4 d after subjectively perceived stress

[b] + cultures occurring within 4 d after subjectively perceived stress

[c] cultures taken within 4 d after subjectively perceived stress

* exam on first day of lesion suggested as stress prior to lesion (studying)

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Table 15

Serological Data

| patient | SN antibody titer HSV I/HSV II |
| --- | --- |
| A | 32/16 |
| B | 8/16[α,γ] |
| C | 32/16, 8/8[β], 8/8 |
| D | 16/16, 16/32[α] |
| E | 256/128, <2/<2[α,β], 128/64 |
| F[ε] | <4/16, 512/256[α,†] |
| G | <4/16, 8/8[α] |
| H | 32/8, 16/2[α,δ] |
| I | 16/32[α], 8/2[β], 4/8 |
| J | 16/128[α], 32/32 |
| K | 4/4, 4/2[α], 4/2 |
| L | 16/2 |
| M | <8/16[α], 8/8[α] |
| N | 8/16[β], 64/<4[α] |
| O | 8/16, 32/16[α] |
| P | 32/16 |
| Q | 16/64 |
| R | 16/32, 16/64 |
| S | 64/32[*,γ], 8/2[α], 8/64[α] |
| T | 64/128[α], 4/64, 32/16 |

α + isolate same day
β first day of lesion
γ lesion present for >1 wk
δ fourth day of lesion
ε only one outbreak in 1974
† one + isolate 2 wks previously
* $1^0$ lesion

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along with the length of time each had been infected at the start of the study. The participants were divided into two groups of ten. Those who had been infected 9 mo of less had a mean high neutralizing antibody titer of 30, while those who had been infected 17 mo or more had a mean high neutralizing antibody titer of 126. There is overlap between the groups, but there are also differences. Four of the people in the "long infection" group had neutralizing antibody titers higher than that found in any of the "short infection" group and two of the "short infection" group had high serum neutralizing antibody titers lower than any of the "long infection" group. The difference between the two groups in this study is highly significant ($p<0.01$).

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## Chapter 4

## DISCUSSION

Many of the participants in this study were extremely eager to take part. Several of them were unaware of the permanence of herpes genitalis or its consequences until they read Exhibit A. The physicians had not taken time to explain the details to their patients. Numerous persons were eager to discuss herpes genitalis with someone possessing a little knowledge, time and patience (even persons outside the study). One male eagerly volunteered to participate. When he was rejected, he coerced his girlfriend (R) into volunteering for the study. Although she never had a lesion, she was culture positive during the study.

The follow-up study was also accepted eagerly by the participants. Recent newspaper and television coverage of herpesvirus, its vast number of victims and research for cures had increased the interest of many of the people. Some unusual reactions were revealed in the follow-up questionnaire. One person had decided to try acupuncture treatments for the disease. Another woman suggested that her disconnection of emotion from sex might cause herpes recurrences to persist. Each follow-up participant had obviously thought about the problem extensively.

This study consisted of approximately 750 cultures (358 culture sets, cervical and vaginal, plus 34 lesions) and 52 antibody determinations. In 1978 the charge for a herpes culture was $15 and for a serum neutralization assay $7.50. Thus, the total cost for the study would have been $11,640 in 1978 dollars, if anyone had been paying for it.



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The high cost of this study may explain the paucity of comparable data.

Misdiagnoses

In the group which was eliminated from the statistical analyses of herpes genitalis parameters, 50% probably did not have herpes simplex. That group represents 16.7% (1/6) of all those persons studied. Even if the two participants with presumptive herpes zoster infections were considered to have "herpes genitalis", 10% of the study group were still misdiagnosed. If that percentage is representative of the number of persons who are informed that they have genital herpes without actually having it, many persons are given undue fears by that burden. One participant (T) practically gave up sex because of her herpes infection. Participant S, age 18, commented that she would become a nun if it were not already too late for that. One person (a) who was eliminated from the statistics because she lacked herpesvirus had given up sex. She also had become extremely emotionally disturbed. Each of her 9 visits included lengthy discussions about the disease and the lack of counseling by the physician.

Another concern for misdiagnosis of herpes genitalis is elicited by the use of serological methods (without culture) to eliminate herpes as a cause of infection. Culture alone has drawbacks (to be discussed more extensively below). During an active recrudescence of herpes infection, antibodies may be depleted by neutralizing the infecting virus (to be discussed below). Thus, false negative results may lead to diagnoses such as "nonspecific vaginitis", which would be very misleading for patients and their doctors in the future.

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Length of Infection

No correlation was found between the length of time an individual had been infected with herpesvirus (0 to 64 mo at the start of the study) and either the number of recurrences, length of lesions or number of positive herpes isolates. Yet it is generally believed that herpes genitalis becomes less severe for the victim with time (61). Perhaps this study did not demonstrate a lessening of recurrent disease because all but 2 of the women in the group were within the peak age range for herpes genitalis of 20 to 29 (>50% of women with the disease (61)). Another alternative is that lessening is not apparent until after 3 y of disease have passed, and three-fourths of the women in the study had been infected 37 mo or less. However, the four participants who entered the study at 40 to 64 mo after their primary attack did not differ significantly from those who had been infected for 9 mo or less. In the follow-up study, while one individual (N), at 32 years old after 6 y of genital herpes, had been 2 y without a lesion, another participant (I) at 30 years old after 7 y with genital herpes, had disease with greatly increased severity (from 1 to 2 recurrences per year to 1 every month or 2--a 6-fold increase). Individual deviations such as these cannot be explained; the participants themselves, by now well informed about genital herpes, were unable to offer even tentative explanations.

Viral Isolation from Lesions

Successful virus isolation directly from swabs of genital herpetic lesions was low in this study (23%) probably because few cultures were attempted during optimal conditions. Chang, et al. (17) reported

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an 82% rate of isolation from fluid obtained by aspiration from intact vesicles. No attempt was made to aspirate intact vesicles in this study. Of studies of recurrent oral lesion, one by lesion puncture yeilded an 89% isolation rate (68); and another performed in a manner similar to this study yielded a 24% isolation rate (23). Sumaya et al. (70), reported a 68.4% rate of virus isolation from lesions, but only cultured 8% of the lesions that they saw and did not report how the 8% were selected. These data suggest a cause for concern about accurate diagnoses of the etiological agents of lesions even by culturing. Clinicians are seldom presented with prime conditions for viral cultures because the victims, either through procrastintion until the disease is unbearable or through difficulty in getting an appointment, rarely see a physician during the first few days of lesion formation, prior to vesicle rupture. The reasons for failure to isolate virus from open lesions are not clear.

Source of Virus Shedding

Of the 161 positive cultures 3.1% (5) were from lesions, 52.2% (84) from cervixes and 44.7% (72) from vaginal walls. Seventy-five percent of the positive cultures were obtained in the absence of lesions. Even when lesions were present, there was only a 50 % viral isolation rate from all sources. Those isolates were 36.4 % cervical, 41.0% vaginal and 22.7% from lesions. These data indicate that cervical and/or vaginal cultures were superior indicators of viral presence even when lesions were present. Patients I and K had virus isolated from the cervix more often than from vaginal walls. It was noted that few other studies reported attempts to isolate virus from areas other

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than the lesion when lesions were present. Guinan, et al. (31) cultured the cervix and reported a 33% viral isolation rate. No cervical lesions were seen in their study or this one.

According to this study, one is only 2.8 times more likely to isolate virus when a lesion is present than in the absence of lesions. Others have had much less success in isolating virus from asymptomatic patients. A possible explanation comes from the results of Guinan, et al. (31) who found virus titers much higher during lesions than asymptomatic isolation ($10^3$ vs $10^1$ plaque-forming units).

Asymptomatic Herpes Genitalis

The epidemiological importance of asymptomatic genital shedding of herpes simplex virus by people known to be infected has received limited attention. Most studies have focused on recurrences of lesions or the association of herpesvirus type 2 infection with neoplasia of the cervix (36,44,49). Several studies of asymptomatic virus shedding have generally been carried out in women with no history of active herpes lesions. A venereal disease clinic in London found .89% asymptomatic shedding (77), and a study of hospitality women in Thailand revealed an 8.9% rate of asymptomatic virus shedding (72). It has usually been assumed that people who have recurrent herpetic lesions are infectious only when lesions are present (16). It is also widely believed that only a few women shed virus asymptomatically. Other studies have reported 2 of 6 (60), 2 of 10 (26), 1 of 27 (31), 4 of 49 (2), and 14 of 157 (72). Adam, et al. (2) noted that women with asymptomatic viral isolates were unaware of overt genital herpes

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in their recent sex partners before the viral isolations; thus the isolated virus probably was produced by the women themselves and not by their sex partners. All 20 women with herpes simplex genitalis who completed this study shed virus asymptomatically at least once during the study. And 75% of positive cultures in this study were obtained in the absence of lesions. Most of the isolations could not be a consequence of a recent sexual encounter with an infected partner. The data presented in this study suggest that virus shedding occurs during a large portion of the lives of most victims of genital herpes.

In this study, unlike those reported above, the virus was not frozen prior to culture which may have accounted for the higher frequency of virus isolation in the absence of lesions. Guinan, et al. (31) reported a low titer of infectious virus in their study. These data do not provide information about the potential infectivity of asymptomatically shed virus. However, one participant (R), who has never (up to 1981) had a lesion, reported that she apparently infected her husband.

## Factors that Affect Recurrent Disease

### Menstrual Cycle

Neither the menstrual bleeding nor stage in the menstrual cycle of persons in this study appeared to affect the course of recurrent or asymptomatic herpes genitalis. The study group varied so much that scatter resulted in no correlation for the group. However, the recurrences of patients G and L did appear to be affect by the menstrual cycle (>50% of lesions or positive cultures occurred during the

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post-secretory phase). Also, patient G incurred 4 of 7 positive cultures during menstrual bleeding.

Rattray, et al. (60) reported that no discernable relationship between menstrual period and onset of recurrent genital infection was present. This study confirmed their finding and extended it to include no correlation between menses and viral isolation. And yet, Nahmias and Roizman (51) in a recent review indicated that the menstrual cycle, or at least hormones, affected recurrent disease. A biochemical study may be necessary to determine if hormones actually affect the disease. It may be that stress precipitated by the emotional changes accompanying menstruation rather than the hormone levels themselves lead to reactivation of virus. (This question of the effects of hormone levels is possibly relevant to the next two sections as well.)

Method of Birth Control

It has been suggested that birth control pills may influence the course of recurrent herpes genitalis (3,44,77). This study revealed that birth control methods (pill vs. IUD vs. other) had no influence on the number of recurrent lesions or viral isolations of the women. It did appear that the pill prolonged the duration of lesions, but because of variation within the groups, the difference was not statistically significant. Birth control methods may not influence herpes (28,44); the use of reliable contraceptives such as birth control pills or IUD's may lead to the increased sexual activity, and hence a greater incidence of herpes genitalis among the users of birth control methods.

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Sexual Activity

The amount of sexual activity of a person with recurrent herpes genitalis has also been postulated to affect the recurrence of the disease (62). This study showed no correlation between sexual activity and lesions or positive cultures ($p > .30$). This is in agreement with Rattray, et al. (60) who found no association ($p > 0.05$) between the number of days since sexual activity and recurrences or viral isolation. The lack of correlation between sexual activity and positive virus cultures, as well as the presence of detectable virus in people with no recent sexual experiences suggests that the "asymptomatic virus shedding" detected in this study was actually virus shedding and not detection of virus transmitted by a sexual encounter.

Stress

Stresses of all types have been suggested as reactivators of recurrent herpes genitalis (3,51). However, since stress is largely subjective, adequate study of such a parameter is difficult. For purposes of this study, several types of stressful situations including exams, terminations of important relationships, deaths in the family, excessive physical exercise and illness were grouped together (the previously discussed sexual activity was not included). Statistical analysis of the data revealed that subjectively perceived stress had a significant influence on both recurrent and asymptomatic herpes genitalis. In fact, stress was the only variable that was found to substantially influence the incidence of disease or virus shedding.

In the follow-up study, the one participant (R) who herself had never experienced overt lesions noted that she apparently transmitted

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herpes to her husband..."during a particularly stressful month for me"... Four of the seven who participated in the follow-up reported stress as the only notable event related to their recurrences.

Another interesting finding from this portion of the study was that stress and herpes genitalis were statistically correlated in two ways: in addition to finding that stress induced herpes, it also appeared that herpes induced stress. That herpes attacks induced stress seems reasonable. Contraction of or recurrence of an incurable sexually transmitted disease would seem likely to cause emotional stress (56). It would appear that when a patient is disturbed by an undesirable reminder of this, she finds other situations more stressful than she would if she were in a "normal" state of mind.

Serology

Except in one case, the serological data were not adequate to reveal whether the participants were infected by herpes simplex I or II. Participant H probably had HSV I because both sera had $\geq$4-fold higher HSV I antibodies than HSV II antibodies.

Participant K demonstrated such low antibody titers that if diagnosis of the disease had been by this serological method, she probably would have been found negative. Participant F also had very low serum antibody titers and had had only one lesion, a primary, in 1974. She exhibited asymptomatic virus shedding during this study, and the serum sample taken two weeks later revealed the highest antibody titer in the study. It is unfortunate that she was lost to follow-up. It would have been interesting to know whether her very high antibody response would have put her infection back into a

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quiescent phase and again allowed her antibody titer to drop. Participants F and K provide warning that low titers of neutralizing antibody for herpes simplex do not indicate lack of infection or preclude recurrences.

Another type of "false negative" antibody titer is exhibited especially well by participant E. Near the beginning of the study her antibody titers were 256 for HSV I and 128 for HSV II. In mid-study, when her only lesion was present, neutralizing antibody titers dropped to undetectable levels. Later in the study the titers were resotred to 128 for HSV I and 64 for HSV II. This suggests that the neutralizing antibodies were utilized to inactivate the virus when the lesion occurred. This is a possible reason for the difficulty in isolating virus from lesions after the first days of a recurrence. It also provides a warning for those who would use antibody titers to rule out the presence of herpes infections: they may be reduced during times of active lesions or asymptomatic viral shedding. Participants I, N, S and T also demonstrated significant reductions in antibody titers when lesions were present or virus was isolated. Further, low antibody levels in some people do not preclude recurrences, therefore, the diagnostic value of low serum antibody levels, especially early after infection, is negligible. More rigorous study of serology with virus isolation is necessary to establish the virus' influence on antibodies.

Although serology has been used extensively to diagnose herpes cases (11,17,48,75), and has been the prime method used to correlate herpes simplex II with cervical carcinoma (49), the reliability of

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most methods have been questioned. Prakash and Seth reported that indirect hemagglutination did not differentiate between HSV I and II (58). Douglas, et al. (23) found no correlation between serum neutralizing antibodies and viral presence for herpes labialis. In this study 11 out of 41 serum samples from patients with active (virus-producing) genital herpes simplex infections were borderline (neither HSV I nor HSV II titers over 8) or negative (titers 4 or less). These data suggest that serological methods should not be used without concimitant cultures. However, it should be borne in mind that negative culture data does not always mean lack of infection.

## CONCLUSION

Although diagnosis of herpesvirus infections is becoming more common, the accuracy of those diagnoses may be in question. Herpes genitalis is an extremely complicated disease which may be affected by a large array of biological and environmental factors. Extensive study of individuals or even partners with recurrent herpes genitalis is greatly needed to help understand the disease, its gamut of complications, and its effects upon the minds and bodies of the victims of this wide-spread sexually transmitted disease.

## SUMMARY

In all, this study of genital herpes simplex infections included 30 women. Ten were excluded from the final analysis. Five, all of whom apparently had genital herpes, did not participate sufficiently (less than six cultures). Five others apparently did not have genital herpes simplex infections, a misdiagnosis rate of 16.7%.

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Twenty women with genital herpes infections were followed for 7 to 20 weeks. The major contribution of this study is the observation that all twenty of these women shed virus asymptomatically. Among the five eliminated for insufficient participation, two shed virus in the absence of symptoms. Virus was isolated from asymptomatic women 29% of the time, and from symptomatic women 50% of the time. In these symptomatic women, 23% of the isolations were from swabs of the lesion and 77% were from the cervix or vaginal walls. The amount of virus from each source was not determined. A second major observation was that some women who had recurrent herpes lesions or viral shedding also had such low serum antibody titers that they might have been presumed to be uninfected. In some cases the low titer persisted over a prolonged period. Another group of women had significangly decreased antibody titers only when lesions or postive cultures were present, as if the antibody was bound up in attempts to neutralize the virus. A third major observation of this study is that neutralizing antibody titers increased with length of time since primary infection.

A number of parameters were analyzed to determine their possible affect upon lesions or positive cultures. Subjectively defined stress was the only variable which exhibited a significant positive correlation to the disease. Sexual activity, method of birth control, menstrual cycle, and length of infection did not correlate with recurrent lesions or positive cultures of herpes simplex genitalis.

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## APPENDIX

### Media Formulations

Cell Culture Medium

MEM . . . . . . . . . . . . . 9.6 g

TDW . . . . . . . . . . . . . 1.0 l

FCS . . . . . . . . . . . . . 100 ml

$NaHCO_3$. . . . . . . . . . . . 1.0 g

L-Glutamine . . . . . . . . 0.1 g

antibiotic stock* . . . . 1.0 ml

Cell Maintenance Medium--same as Culture Medium with only 10 ml FCS.

Transport Medium--same as Culture Medium with added nystatin 100 u/ml

1. Thoroughly dissolve all ingredients except $NaHCO_3$. Add $NaHCO_3$ and dissolve. Adjust pH to 7.2 to 7.4 and filter sterilize through a 2μ pore-size filter.
2. Add nystatin after filtration.
3. Dispense media into sterile 100 ml, 500 ml or 1 l containers ( 2 ml tubes for transport media).

*Antibiotic Stock (100X)

Penicillin G . . . . . . . . . 0.627 g

Streptomycin . . . . . . . . . 1.0 g

TDW . . . . . . . . . . . . . . . 10.0 ml

1. Dissolve pen-strep in TDW. Filter sterilize.
2. Dilute 1.0 ml (100X) in 1 l of medium.



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