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Concerning antigenic properties of human spermatozoa as carcinogen in cervical carcinoma

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CONCERNING ANTIGENIC PROPERTIES OF HUMAN SPERMATOZOA
AS CARCINOGEN IN CERVICAL CARCINOMA

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TABLE OF CONTENTS

	Page
I. Introduction.	1
II. Literature Review of Etiology of Cervical Carcinoma.	3
(a) Marital Status	4
(b) Number of Marriages.	4
(c) Age at First Marriage and First Coitus	4
(d) Circumcision	5
(e) Socioeconomic Status	7
(f) Nutrition.	7
(g) Number of Pregnancies.	7
(h) Abortions.	8
(i) Syphilis	8
(j) Douching	8
(k) Contraception.	8
(l) Heredity	9
(m) Hormonal Factors	9
(n) Summary.	10
III. Inflammatory Reaction and Carcinoma	11
IV. Antigen-Antibody Reaction and Inflammation.	15
V. Production of Antibodies Against Mammalian Spermatozoa	17
VI. Antibody Titers in Patients with Carcinoma of the Cervix	22
(a) Methods.	23
(b) Results.	25

VII. Summary	27
VIII. Conclusion	29
IX. Bibliography	

INTRODUCTION

The purpose of this study is to demonstrate whether or not human spermatozoa can act as a carcinogen through the medium of an antigen-antibody reaction in the production of cervical carcinoma. It is a well-known fact that various mechanical and chemical factors, antigen-antibody reactions, and living organisms produce inflammation and chronic irritation in body tissues. The role of chronic inflammation in the production of carcinoma will be reviewed.

To date no definite cause of carcinoma of the cervix has been established, although exhaustive studies have been undertaken in an attempt to link numerous etiological factors with the disease. Effects of agents ranging from religion to hormone imbalance have been implicated. A review of the literature concerning these various factors is presented in an attempt to point out the results and their meaning in terms of the production of carcinoma.

The basis for this study has been drawn from well-documented evidence that human spermatozoa not only carries but also possesses antigenic properties in itself and that antibody responses are found present in the serum and cervical mucous of subjects exposed. Laboratory methods were employed in this study in an attempt to confirm the presence of these antigens and antibodies and to determine whether the resultant reactions were present or more prevalent in patients and controls. It is felt that the demonstration of consistently high antibody titers against spermatozoa in patients with cervical

carcinoma as opposed to low or absent titers in controls would establish considerable evidence in favor of the reaction as significant in the etiology of carcinoma of the cervix.

LITERATURE REVIEW OF ETIOLOGY OF CERVICAL CARCINOMA

As early as 1842 the effects of marital status were noted to be significant in the incidence of cervical carcinoma. Rigoni-Stern examined the death registers in the city of Verona, Italy, from 1769-1839 and found that the death ratio from cancer of the breast and uterus varied from 1:4 in married, 3:1 for single women other than Catholic Sisters, to 9:1 in Sisters.⁴⁹ Since that time many other investigators have discovered the marked infrequency of cervical carcinoma in nuns. In 1950 Gagnon investigated Catholic Sisters in Canada with the following results: (1) No CA of the cervix in 13,000 Sisters over a 20 year period; (2) Of 140,000 pathology reports of malignant uterine tumors in Montreal and Eastern Quebec hospitals only 3 confirmed reports of CA of the cervix were found among nuns; (3) Records of four religious orders with an average yearly population of 3,280 showed 130 cancers from 1929-1949 with no cervical carcinoma. Later figures (1949-1953) brought the total number of cancers to 222, still without a case of cervical CA; (4) A review of six cancer centers in Ontario and Quebec from 1952-1953 revealed no cervical carcinomas among nuns. Study of death records in Weirzberg, Germany, from 1900-1952 by Schomig demonstrated one case of CA among 1,025 Sisters. In this country Taylor reviewed the records of 3 orders of Catholic nuns, two in Massachusetts, one in New York with the finding of a "great deficiency of CA of the cervix".⁴⁹

When nuns are excluded from statistics the infrequency of CA of

the cervix among single women still seems to hold true. In an extremely detailed study of Wynder et. al. the liability of cancer of the cervix was found to be greater at every age among married and widowed women than among single women, especially between the ages of 45-65 when it was seven times as great.⁵⁶ Similar findings were present in a London study by Harnett. These facts were confirmed by other investigators who found consistently smaller numbers of single women with cervical carcinoma.^{37, 16}

The significance of these reports implies the importance of sexual intercourse in the production of cancer, since it is quite unlikely that the act of marriage itself can be of any significance. Jones, Rotkin and Terris in separate studies have attempted to determine the importance of the age at first marriage and first coitus. Jones found a significant excess of carcinoma among women with their first pregnancy before 20 and the birth of the last child before 25, as well as first marriage before 20.¹⁶ Rotkin likewise found the mean onset of coitus in patients with CA to be between 15 and 17 years of age and between 18 and 23 in controls.^{37, 38, 39} Terris confirmed these reports.⁵¹ Wynder found that 13% of white and 32% of negro patients were married before the age of 16, whereas only 5% and 13% respectively of controls had married by this age. He estimates that women married once with the onset of coitus at or before age 16 have twice the risk of developing carcinoma (both white and negro), those with coitus after 25 have 30% less chance,

as compared with those between 20 and 24. It was also discovered that the risks doubled for those married twice or more.

The significance of the number of marriages is disputed. In contrast to Wynder, Jones and Rotkin found no difference in the number of sexual partners, frequency of coitus, or the number of years of sexual relationships.^{16, 37} In a detailed study at the California Institute of Women from 1957-1958, Peyeyre studied a population of prison women with one mass social characteristic, the diversity of their sexual activity.³⁰ PID, cervicitis, trichinosis, and cervical lacerations were common. The incidence of carcinoma of the cervix was 15 per thousand as compared with an average rate of 5-6 per one thousand in other population groups. All admitted to diverse and frequent sexual activity; 50% were prostitutes.

Stern and Dixon have performed a statistical assay using multiple regression analysis on 6,058 controls and 403 cases of carcinoma at the Cancer Detection Center, Cancer Prevention Society, Los Angeles, California.⁴⁵ They found that "the variable, marital events, was shown to be the most important variable, excluding the fact that the person is Jewish."

The question of circumcision has long been debated since the recognition of the very low cancer rate in Jewish females. It is interesting that Stern and Dixon also concluded in their multiple regression analysis that "such factors as race, religion, nationality, and circumcision, thought to be important when considered singly,

because of their obvious association with special groups of women in whom cervix cancer rates were exceptionally high or low, were found to be of secondary significance. Omission of Jewish women (from the study) seemed to have no effect on the relationship of the other variables to the cancer status. In particular the status of circumcision remains of secondary importance."

In the study of Jones, McDonald, et. al. of 429 cases and 429 controls at Los Angeles County Hospital and 155 private cases and controls, it was found that circumcision was present equally in cancer and control groups.¹⁶ Rotkin found more circumcision in a control group with a Chi^2 of 0.176 indicating a greater than 50% probability that the difference is due to chance.³⁷ Wynder also recognized a low incidence of cervical carcinoma in Jews, Fijis and Moslems, all of whom practice circumcision.^{56, 57} He demonstrated by studies in both India and the U. S. A. that there were more circumcised husbands in control groups than in carcinoma groups. It was further found that the risk of CA increased slightly when married twice, even if both partners were circumcised. A more meaningful test was the comparison by Wynder of the cancer ratio in Jewish women married to circumcised mates. Although the study was limited to a small number of patients, no significant difference was detected.

Dr. Weiner, in a review of the status of carcinoma among Jewish women, cites Kennaway, Vineberg, and Smith who attribute the low incidence of CA to the custom of 14 days abstinence from sexual activity after

the beginning of the menses.⁵⁴ His study presents data to confirm the view that the rising incidence of Jewish carcinoma is due to laxity in following this custom today.

Experimental evidence on the carcinogenic properties of smegma has been intensively investigated by Pratt-Thomas.^{32, 33} In his investigation human smegma was introduced into the vaginal canals of female mice with the almost exclusive result of cervical carcinoma after varying periods of time. From his work it cannot be denied that smegma is a carcinogen, at least in mice.

There appears to be an inverse relationship between the incidence of carcinoma and socioeconomic factors as indicated by education, occupation of father, and crowding in houses.¹⁶ However, the number of marriages, age at first marriage and coitus, hygiene, and cervicitis also is inversely proportional to the socioeconomic status so that a conclusion as to what aspects of the lower privileged group are of significance cannot be stated. Studies on nutrition have not indicated any difference among cancer cases and controls.¹⁶

Rotkin has reported from three separate studies of environmental factors and cervical carcinoma that there are an equal number of deliveries and pregnancies in cases and controls.^{37, 38, 39} Wynder has found that when the effect of the number of pregnancies is held constant, patients and control groups differ in the age at first marriage, however "it is far from clear, that when the age at first marriage is held constant, that any difference varies in the

frequency of pregnancies in the two groups."⁵⁶ These studies indicate that the number of pregnancies provides no evidence of an association with the development of cervical carcinoma.

Studies from the same groups support the generalization that when the cause is not taken into consideration, there is no difference in the percentage of abortions among patients and controls. However, Rotkin found an increased number of induced abortions among cancer patients (33% of total pregnancies of patients compared with 13% among controls.)

Many references are present in the literature citing the prevalence of syphilis among patients with carcinoma of the cervix.^{1,13,16,22,36} Most statistics indicate this prevalence to be twice as great as among the general population. When breaking these figures down into racial groups. Wynder found this ratio to be true in the white population, but not in the Negro.⁵⁶ These studies probably reflect the greater frequency of coitus and diversity of sexual partners.

Douching materials have been investigated from the standpoint of carcinogenic agents. Lombard and Potter have found suggestive evidence that coal-tar douches have been significant, however, others have not been able to substantiate this fact.^{37, 56}

Reliable statistics on the use of contraceptives, like the data on circumcision, are very difficult to obtain. It would seem that comparison of Roman Catholics with a population of people who practice contraception regularly would be most helpful in establishing

information as to the significance of circumcision, smegma, spermatozoa, and general hygiene in the role of carcinogens. However, there are few people who practice contraception with any one type of contraceptive or with any degree of regularity. Both population groups have to be screened very carefully and in general indicate that there is little uniformity in contraceptive practice. Wynder has the most recent and largest group of subjects in whom he found no difference among cancer patients and the control group in the use of contraceptives without reference to the types used.⁵⁶ Frequency was not obtained. This study coincides with that of Rotkin in that patients used slightly fewer condoms than controls, but the difference was far from significant.³⁷

A thorough review of the current literature was made in 1961 by Rotkin, in addition to his study of patients from the Kaiser Foundation and the Permanente Medical Group at Oakland and Vallejo, California, to determine hereditary factors in carcinoma of the cervix.³⁹ It was concluded that "genetic transmission of cervical cancer was not demonstrated by a sample of families of cervical cancer patients when compared with families of matching controls despite exceptionally complete and accurate data."

Hormone imbalance came to the forefront in the study of carcinoma of the cervix when Gardner produced CA in inbred strains of mice with large doses of estrogen. Ayre then found that vaginal cytologic studies showed an unusually high estrogen level in women with carcinoma. Subsequently Jones determined 24-hour urine estrogen levels in patients

with carcinoma and found no difference when compared with normal. Rotkin found nonspecific hormone imbalance was significant to a Chi² of 3.018 with a probability of between 5-10 per cent of this being a chance variable.³⁷ To date, however, no proof is available to substantiate a definite relationship.

In summary numerous etiological factors have been studied, but none have been linked directly as the cause of cervical carcinoma. However, certain facts stand out as being significant. Marital status, age at first coitus, the number of marriages, and the circumcision status of the male appear consistently to be prominent in the natural history of carcinoma patients. It appears that we can deduce from this that there is some factor in the sexual act itself which is influencing the development of carcinoma.

INFLAMMATORY REACTION AND CARCINOMA

Histologic studies on cervical tissue during the actual development of carcinoma are paramount in determining the pathogenesis of the disease. Such studies have been undertaken with great detail after introduction of various known carcinogens into the vaginas of experimental animals. All experimentors have found evidence of inflammatory reaction.

Emmerich von Hamm induced cervical carcinoma in mice by introduction of a carcinogen.⁵² He found that dysplasia preceded CA, but also that the dysplasia was preceded by a period of inflammatory reaction which began a few days after administration of the carcinogen. The inflammatory reaction had reached its peak and had begun to subside by the time the cellular dysplasia became present. This evidence explains Reagan's findings in a similar experiment where he found no more marked exudate in areas of cancer cells than in other areas of the cervix.³⁵ However, all cases produced infiltration of neutrophils and lymphocytes into the stroma at an earlier period. A large percentage of the mice did develop carcinoma after introduction of the carcinogen.

Pratt-Thomas found that chronic irritation and infection is a pathogenic mechanism for carcinoma of the cervix in his experiments with smegma.³³ However, he was unable to isolate a specific carcinogen.

J. Herald found that there is a perivascular cervicitis and an atypical epithelium as the initial cancerization of the cervix in his

study of women with positive Papanicolaou smears.¹⁴ He believes these changes to be induced by a virus which causes cervicitis by nature of its fulfillment of metabolic needs.

Taylor correlated a proliferation of the basal cells of the stratified epithelium of the cervix in women with ectropion, erosion, inflammation and pregnancy.⁴⁸ He did not compare the incidence of cervical carcinoma in these patients with that in the general population. Sirtoni found similar changes in nonspecific cervicitis.⁴³ In addition to hyperplasia of the basal cells, these changes developed into a glandular epidermization or Keratinization of the ectocervical squamous epithelium.

Wolfgang Karte developed a schematic classification for the causal pathogenesis of cervicitis following years of experimental work.¹⁸ He found an unquestionable relationship between cervicitis and carcinoma of the cervix. He believes that "metaplastic cervical inflammation can follow from every chronic inflammatory irritation, which is associated with a permanent or changing acidification of the alkaline milieu of the cervix. In the course of many cell generations there occurs in the cervix itself a transformation of cervical epithelium into lower degrees of epithelial differentiation. Here a chronic intraepithelial inflammation is always demonstrable."

Gagnon believed this fact to be extremely important clinically and followed 4,000 patients of his own which were cured of chronic cervicitis.⁹ Among all of these cases there was not one instance of

of cervical carcinoma. Cashman also found that if cervicitis is treated, there is less likely a chance of developing CA.⁷ Crossen and Crossen cite Craig in their text book of Operative Gynecology who did not observe a single case of cervical carcinoma in a 10 year period in 2,895 treated and cured cases of cervicitis.⁸ Pemberton and Smith found no carcinoma in 1,408 women treated and cured of cervicitis.⁵⁰

This evidence confirms the opinion that there is an undeniable relation between the development of carcinoma and a preceding hyperplastic process of cervicitis. It appears that years of regeneration of cells from certain specific inflammatory stimuli will eventually cause some change in the mitosis of the cells. This metaplasia is seen in many cases of cervical carcinoma as is substantiated by wide success of Papanicolaou smears in detecting early lesions.

Berenblum summarizes the case for cervicitis and hyperplasia well.³ "Every carcinogen that produces a tumor at the site of application or injection is an irritant in the sense that it induces a continued state of reproductive hyperplasia. Furthermore, in all cases where sufficiently accurate observations can be made, it is seen that the primary tumor is preceded by hyperplasia. From these facts it is concluded that hyperplasia is an essential precursor of neoplasia. But it is now certain that only some, not all, irritants are carcinogenic. Therefore, hyperplasia must be a specific type, biologically (and it is claimed even morphologically) distinct from

ANTIGEN-ANTIBODY REACTION AND INFLAMMATION

Antigen-antibody reactions are considered along with physical and chemical agents and living organisms as the basic mechanisms responsible for the production of inflammation. These hypersensitivities are considered to cause allergic inflammation. Some foreign substances, especially proteins, cause no injury or inflammation the first time they enter tissues, but on subsequent exposure injury and inflammation may be intense. The tissue cells have been sensitized by the first exposure, and thereafter they are hypersensitive. Various types of responses are seen clinically and are described as various syndromes. Atopy, infectious allergy, drug allergy, and precipitin allergy have been described.

The precipitin allergies are artificially induced syndromes in man and animals, and constitute the allergic disease with which we are concerned here. The antigen is usually serum or some other soluble protein but may be an antigenic polysaccharide or synthetic antigen. Precipitins are usually present in the serum in some phase of the development of the condition. The antibodies are characterized by giving an observable precipitate in the test tube, being relatively thermostable, passing the placental barrier, and being passively transferred with serum to other animals of the same or related species.

The original allergic reaction in the rabbit reported by Arthus was a local reaction. It consisted of injection of horse serum at appropriate intervals subcutaneously into rabbits. Progressively

more severe reactions occurred at the site of each succeeding injection. The mechanism is considered to be the formation of precipitating antibodies in response to the introduction of a foreign protein (horse serum). When antigen later enters the body, it unites with antibody present in the tissue fluid. This union causes the release of substances that cause the muscle in the arterioles to constrict and the vascular endothelium and leukocytes to become sticky. An inflammatory reaction and tissue necrosis results. The Arthus phenomenon is described in man who is less reactive than the rabbits in his ability to produce precipitins and form the classic reaction. Red edematous areas are not at all unusual following subcutaneous injection of horse serum in patients who have had a previous injection of serum some days or weeks previously.

It is proposed here that mild or subclinical reactions may be produced in the tissues of the human cervix by introduction of small amounts of protein antigen over long periods of time. This protein antigen is spermatozoa. If this hypothesis is true, continuous hyperplasia and eventually metaplasia leading to carcinoma may result.

PRODUCTION OF ANTIBODIES AGAINST MAMMALIAN SPERMATOZOA

Landsteiner first introduced the concept of the antigenic properties of human spermatozoa in 1926 when he demonstrated that blood group antigens were found present on sperm.²¹ Using agglutination-inhibition techniques he was able to demonstrate that A and B antigens were carried by sperm. In the same year Yamakami found blood group antigens in semen, saliva and vaginal secretions. Intensive investigation of these properties began in the study of infertility. It was believed that in blood-group incompatible marriages, sperm was agglutinated by antibodies present in the cervical mucous of mates. Blood-group incompatible matings are those in which the female carries antibodies in her serum against the blood group antigens of her husband. That is, women with blood type B are incompatible with A or AB husbands, A women are incompatible with B or AB husbands, and the serum of O women postulated as capable of agglutinating sperm from A, B, or AB husbands. Gershowitz and Behrman found in a study of 102 infertile couples that 87.3% were heterospecific with respect to blood groups.⁴ They believe that the antibodies in the cervical mucous immobilize sperm and render them incapable of fertilization. In earlier work they detected hemagglutinins in type A, B, and O women, but none in AB women. In 1957 Gullbring found that only single blood group antigens are carried on sperm.¹² In his serologic studies he found that A sperm carried only the A antigen, B sperm only the B antigen, and that AB sperm carried one or

the other but never both. Naturally sperm from O males carries neither. Shahani et. al. confirmed this work in 1962 feeling that the inability of AB males to carry both antigens was undoubtedly due to the meiotic division of the reproductive cells.⁴²

Strangely enough the antigenic properties of spermatozoa itself also came to light in the study of infertility. Pioneer work was done in 1928 by Pommerenke who introduced semen and testicular extracts into the vaginas of female rabbits and injected these substances into their blood stream.³¹ He observed the following facts: 1. The serum and vaginal secretions of a female rabbit injected with rabbit spermatozoa or a fresh testicular extract became toxic for rabbit spermatozoa. 2. The longevity of spermatozoa normally deposited in the genital tract of the female rabbit is greatly decreased by the previous IV injection of rabbit spermatozoa or testicular extract. 3. Infertility for 2 to 25 weeks follows repeated injections of sperm or testicular extract. Controls used were salivary gland extract and semen. 4. Rabbit-sensitized serum (with rabbit sperm) is also toxic for rat sperm and visa versa. 5. Repeated intravaginal injections of rabbit spermatozoa by natural and artificial means, in the female rabbit may produce antigenic effects in the blood and vaginal secretions of the rabbit. There was inconclusive evidence on whether this caused sterility.

These facts readily demonstrate the antigenicity of spermatozoa and confirm the principle that the antibody properties are present at the tissue level in the cervical secretions. Further work was

done by Parson in 1940 when he produced serum antibody titers against homologous and heterologous sperm introduced intravaginally into the rabbit and guinea pig.²⁹ Karte likewise found antibodies present in the serum and uterine secretions of rabbits following the local application of homologous sperm or testicular extract.¹⁸ Anti-sperm agglutinins were found in the cervical secretions of human females shortly after ovulation and in the second trimester of pregnancy by Lindahl et. al. in 1956.²³ Baskin presented a series of 20 cases of sterilization employing immunization techniques. He injected sperm into females producing spermicidal cervical mucous.⁴⁰

Straus attempted to determine the quality and quantity of antibodies in the blood and secretions following local and parenteral introduction of antigen.⁴⁷ All of his work was done with humans. He speculated that the antibody response in vaginal secretions following parenteral active immunization is quicker and shorter acting than the serum antibody and that the response was probably not cumulative in contrast to serum antibody. In his experiment he used conventional typhoid bacillus vaccine as the parenteral agent and a soluble typhoid bacillus for local application in the vagina. The method consisted of subcutaneous injection of 0.5 ml of typhoid vaccine which was repeated when the vaginal mucous agglutinin was no longer detectable, although the serum agglutinin persisted. Local antigen was applied with a junior-sized tampax. He found that vaginal antibody was produced in response to either parenteral or locally applied antigens,

but the response to the latter was superior (1:200 as compared with 1:2000). Serum agglutinin responses were higher in parenteral administrations as would be expected. The vaginal agglutinin appeared earlier than the serum antibody, reached a peak sooner, and disappeared in 7 to 8 weeks although the serum antibody persisted, often in a relatively high titer. Vaginal antibody response to primary and secondary inoculations appeared to be nearly the same and was not effected by menstruation or pregnancy. On the other hand the secondary serum response was greater than the primary as is normally found.

These findings suggest local antibody formation to locally applied antigen. This is supported by the stronger response of vaginal antibody to the local application.

Further evidence to support intravaginal absorption of antigen with concomitant production of circulatory and local antibodies was obtained by Kibrick et. al. and McCartney.^{19, 25}

Electrophoretic studies were done on mucous proteins by Moghissi in an attempt to determine the composition of this substance especially with reference to antibodies.²⁷ The presence of antibodies naturally implies the presence of gamma globulins. Proteins are present in the following order of decreasing concentration: albumin, gamma, beta, and alpha-2 globulins. The gamma globulins are significantly decreased during the midphase of the menstrual cycle. This supports the evidence of Lindahl who discovered an antiagglutin factor in the cervical

secretions of humans which occurs during and just before ovulation and in the middle part of pregnancy.²³ This factor is stimulated by estrogens and decreased by progesterone and may be necessary for fertilization by virtue of its antisperm agglutinating ability.

Moghissi feels that the cervical mucous factor probably originates primarily from the blood serum. He bases this premise on the evidence of von Kaulla who found that when I¹³¹ labeled human serum albumin is injected IV into women it appears shortly in the cervical mucous.⁵³ This, of course, does not reflect the nature of gamma globulin in these secretions.

In summary there is little doubt that spermatozoa introduced intravaginally can produce an antibody response readily demonstrable in cervical mucous and serum of sensitized females. The frequency with which this actually occurs is unknown, however, those investigators who postulate spermicidal cervical mucous in sterile females feel that this mechanism may be encountered quite frequently.

ANTIBODY TITERS IN PATIENTS WITH CARCINOMA OF THE CERVIX

The experiments undertaken in this study have attempted to establish the antigenicity of human spermatozoa and the antibody response present in the serum of cervical mucus. The initial work consisted of determining whether the blood group antigens carried on sperm might induce an inflammatory hypersensitivity. This work was done by the methods used by Gershowitz and Gullbring, employing standard antiserum preparations.^{10, 12} Sperm was selected from six husbands of patients treated for cervical carcinoma. Two of these patients demonstrated blood group antigens on their sperm. These two and one other were secretors and contained the antigen in their semen. It was felt that these results indicated no consistency in the presence of these antigens in sexual partners of carcinoma patients.

Work was then done using patients with proven but untreated carcinoma of the cervix. Cervical mucus was drawn from these patients using a tight-fitting polyethylene catheter on the end of a 14 gauge needle. The mucus was then examined for the presence of blood group antibodies using fresh one per-cent red cell suspensions as the antigen. The method was similar to that used above. Of the four patients selected only one demonstrated antibodies. This was from a blood-group O female who contained both A and B antibodies in her cervical mucus.

The two initial experiments resulted in the conclusion that

blood group antibodies were not the agent responsible as the carcinogen in the production of cervical carcinoma. Neither the antigen nor the antibody response were found consistently present.

An attempt was then made to observe macro- and microscopic agglutination of human spermatozoa using sperm and mucous obtained from the above experiments. It was found that the cervical mucous was too viscid for determination of an agglutination process. The sperm was simply suspended in a very thick, adhesive fluid.

Work was then begun on the premise of Straus, McCartney, Moghissi, Pommerenke, Dragstdt, Freund, and others that antibodies of the precipitin type will be present in the serum of all patients sensitized by intracutaneous methods. There is little doubt that sperm does penetrate the cervical epithelium as indicated by Kohlbrugge as early as 1912.³¹ Investigation of circulating antibodies includes the detection of antigenic response to spermatozoa itself, and does not depend on blood group responses.

The methods used in this experiment are identical with those used by Audrey Smith who perfected a technique for the identification of serum antibodies developed in response to spermatozoa.⁴⁴ The test used here is the one recommended by her after trial with several agglutination and precipitin reactions.

Antiserum dilutions were made 1 in 10, 1 in 20, 1 in 40, and so on down to 1 in 1280. Two-hundredths milliliter of each dilution was transferred to an agglutination tube (3 in. x 0.3 in.) and 0.2 ml.

of spermatozoa suspension mixed with it. The final concentration of the sperm was thus halved. The sperm was diluted with 0.85% saline to 1:20 to give a density of between 1×10^4 and 2.5×10^4 sperm per cubic millimeter. Human spermatozoa was used, in five cases that of the husband. A control tube containing 0.2 ml. of spermatozoal suspension mixed with an equal volume of 0.85% saline was included. Tubes were left to stand one hour at room temperature and then shaken gently and examined microscopically. The following degrees of agglutination were recognized:

- + Fine floccules just perceptible to the naked eye.
- ++ Medium-sized floccules suspended in opalescent or turbid fluid.
- +++ A single pellet or large flocculent masses suspended in a clear surrounding fluid.

In each sample a drop was examined microscopically for agglutination and its type. Only tail agglutination was considered specific as is also recommended by Henle. Head agglutination is considered nonspecific and is sometimes found in normal sera.

Kibrick describes a very similar method pointing out that the morphology and motility of the sperm are important factors in the sensitivity of the test.¹¹ All samples were fresh and were examined microscopically before being used.

Sera was obtained from patients with proven carcinoma, ranging from Stage 0-11. Ten cases were selected. In each instance there was no demonstrable macroscopic agglutination. When examined

microscopically, two samples contained small amounts of nonspecific head agglutination, but no tail agglutination was observed.

The last experiment precludes the possibility that the antigenic properties of spermatozoa were significant in the production of carcinoma of the cervix in the patients selected with two possible exceptions. Most of the patients had been treated for cervical carcinoma several years before and it may be that the antibody response had disappeared. However, three of the patients were those from whom sera had been drawn before treatment and who were receiving antigenic stimulus until the time of the study. The other possibility is that the antibody response may be present only at the tissue level and that circulating antibodies may not be produced.

These results leave two very interesting areas of investigation yet to be explored. It is possible to select quantities of cervical mucous from carcinoma patients and render this material less viscous. This may be done by collecting the material at the time of ovulation or after sexual stimulation when the material is thinner or by using a tissue enzyme such as hyaluronidase to reduce the viscosity of the mucous. When this is done it may be possible to observe specific agglutination of sperm microscopically without interference of the nature of the suspending medium.

Another area open to investigation is the study of a tissue extract for the presence of antibodies. This could be obtained from cervical tissue acquired at the time of definitive surgery. This

material would surely contain any antibodies resulting from antigenic spermatozoa.

In conclusion it can be said that much work remains to be done in the field of antigen-antibody induced hyperplasia as a carcinogen in carcinoma of the cervix. This paper has presented evidence questioning the significance of blood group antigens as a factor in the production of such a reaction. We have also attempted to demonstrate antisperm antibodies in the serum of carcinoma patients and found no correlation between the two. A vast area of study in the investigation of tissue antibodies, however, is left undone, and experiments such as those cited above remain.

SUMMARY

A review of the current literature on the etiological factors of carcinoma of the cervix reveals that no definitive cause can be established even though extensive studies have been done in this area. However, certain factors stand out as being associated with carcinoma. These are marital status, age at first coitus, the number of marriages, and the circumcision status of the partner. All of these facts imply that something in the sexual act itself is related to the etiology of cervical carcinoma. Spermatozoa could obviously be one of these factors.

It is a well-known fact that antigen-antibody reactions occurring at the tissue level in humans are a cause of inflammation. The full-blown picture is present as the Arthus reaction.

Antibodies have been demonstrated in the cervical mucous and serum of humans in response to introduction of spermatozoa by both intravaginal and intravenous methods. If hypersensitivity is obtained from repetitive exposure to sperm, precipitin antibodies may be demonstrable in the antiserum by conventional methods.

It is postulated that continual hyperplasia and inflammation induced by certain agents may lead to the dysplasia seen characteristically as a precursor to carcinoma. It was further postulated in this thesis that local tissue reactions induced by an antigen-antibody reaction may be the agent responsible for this dysplasia. However, upon examination of the serum from ten patients with proven

carcinoma, it was found that there were no circulating antibodies present. It is therefore considered that the postulate of antigen-antibody induced inflammation is unlikely, although the possibility of only a local response has not yet been eliminated.

It would also be meaningful to determine whether sperm in itself could be the agent inducing a chronic hyperplasia process in female patients and whether the chronic hyperplasia was prone to develop into the dysplasia precursor to carcinoma. A study of this type would involve methods similar to those used by Reagan,³⁵ Pratt-Thomas,³³ Berenblum,³ von Hamm,⁵² and others who introduced various carcinogens in the vaginas of experimental animals and observed the changes histologically. By this method various constituents of semen could be tested to determine whether they possess carcinogenic properties.

CONCLUSION

1. Etiological factors considered significant in the etiology of carcinoma of the cervix have been found to be marital status, age at first coitus, the number of marriages and the circumcision status of the partner, employing the sexual act as the common denominator.
2. Chronic hyperplasia and inflammation induced by certain specific agents have been demonstrated consistently to precede the development of carcinoma of the cervix in experimental animals through an intermediate stage of metaplasia. There is much clinical evidence to indicate that this is also true in humans.
3. Antigen-antibody reactions have been well established in various hypersensitivity states to induce an inflammatory reaction, frequently referred to as allergic inflammation.
4. Antibodies can be demonstrated in the cervical secretions and serum of patients in response to intravaginal introduction of spermatozoa.
5. There is no correlation between the level of circulating antisperm antibodies and the patients with carcinoma of the cervix used in this experiment.
6. There is a great deal of work remaining to be done to determine whether a local antibody response is present in cervical carcinoma.

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