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Received September 20, 1963. P.S.E.B.M., 1963, v114.

Inhibition of Viruses by Secretions from the Female Genital Tract.* (28789)

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For several years an extensive search has been made in this laboratory for viruses in human vaginal and cervical secretions. This work was directed toward establishing a baseline of the viral flora of the normal vagina and cervix as a prelude to studies on the possible role of these agents in the etiology of cervical cancer. A total of 229 washings and swabbings taken from adult women all proved negative for viruses as determined in tissue culture. These findings could be interpreted either as meaning that viruses are not normally present in the female genital tract or that if present they are masked or restrained by some type of inhibitor. The present report relates the demonstration of such an inhibitor.

Materials and methods. Volunteers providing secretions. The volunteers were principally patients at the Jackson Memorial Hospital in Miami, Fla. and at University Hospital, University of the West Indies in Jamaica. Economically and socially they cut across most strata of the population, but the highest number were women of the lower social and economic status.

Cervicovaginal secretions were obtained by using Tassettes,† which are rubber cups normally used to collect menstrual secretions. The Tassette was kept in the vagina for 6–24 hours. The secretions obtained were very small in quantity (0.5–1 ml), usually ranged in color from gray to light green and had a pH of 3–4. They were highly tenacious and required dilution approximately 5-fold with Hanks' balanced salt solution to facilitate removal from the Tassettes. The Hanks' solution contained 1,000 units penicillin, 0.5 mg streptomycin and 0.025 mg fungizone per ml. The secretions were frozen at –20°C until used. In the initial experiments, and in all experiments with Rous sarcoma virus, the individual secretions were tested separately. However, no single specimen sufficed for more than one test. Two or 3 secretions were therefore pooled in several instances to obtain a workable quantity of material. All secretions were brought to a pH of 7.0 to 7.6 at time of the test.

Tissue culture. HeLa or KB cell lines cultivated and maintained in Eagle's medium supplemented with calf serum (10% for growth; 5% for maintenance) were used. After inoculation, cultures were examined daily for cytopathogenic effect (CPE) and experiments were terminated after 7 days of observation.

Viruses. Rous sarcoma virus was supplied by Dr. W. Ray Bryan, NIH. Herpes simplex strain GCA3 was received from Dr. T. F. McNair Scott, Children's Hospital, Philadelphia. Poliovirus type I, Mahoney strain, was obtained from The Connaught Laboratories. Serial passages in tissue culture of both herpes and poliovirus were made in this laboratory.

Attenuated trivalent oral polio vaccine was provided by Dr. Herald Cox, Lederle Laboratories. The initial titer of this vaccine was greater than $10^{6.0}$ $TCID_{50}$ per ml, but after

* This work was supported in part by NIH grant and in part by NIH Training Grant.

† Obtained from Tassette, Inc., Stamford, Conn.

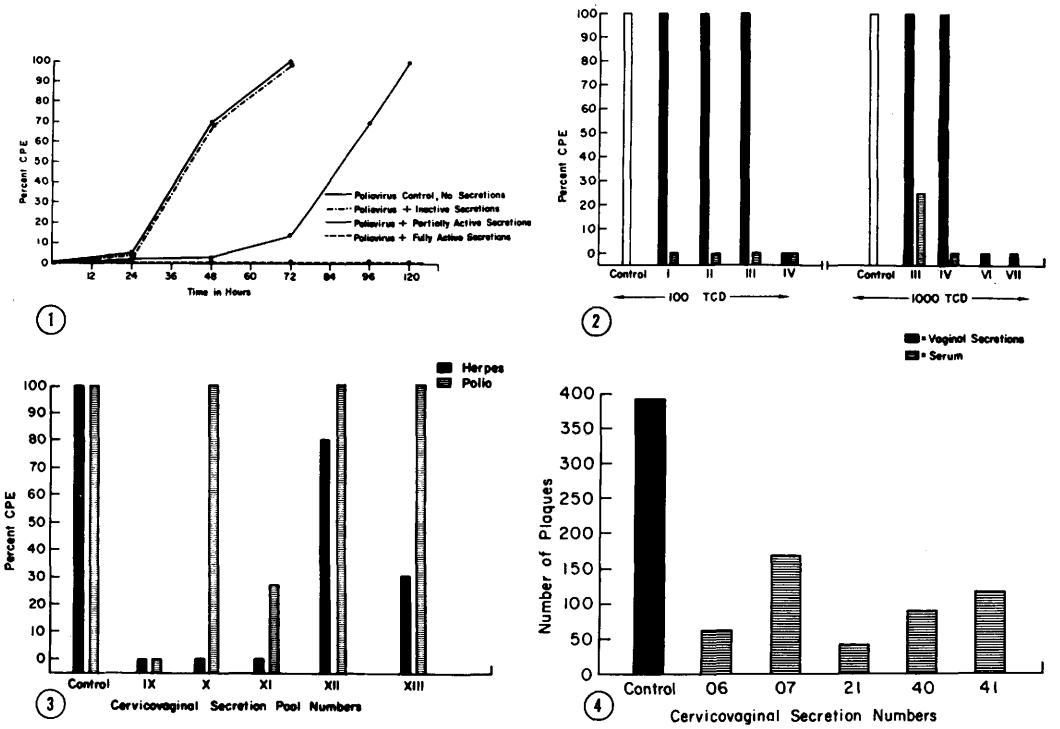

FIG. 1. Inhibition of poliovirus Type I by vaginal secretions.

FIG. 2. Comparative inhibition of poliovirus Type I by vaginal secretions and serum pools. Numbers I-VII denote pool compositions as follows:

Pool No.	Vaginal secretions	Sera	Pool No.	Vaginal secretions	Sera
I	1, 3, 5	1, 3	IV	51, 52, 53, 54	51, 52, 54
II	10, 11, 13	10, 11	VI	11, 17, 19	None
III	6, 7, 8	6, 8	VII	13, 16, 18	"

FIG. 3. Comparative inhibition of poliovirus Type I and herpes simplex virus by vaginal secretions. Numbers IX-XIII denote pool compositions as follows:

Pool No.	Vaginal secretions	Pool. No.	Vaginal secretions
IX	10, 21	XII	16, 17
X	40, 41	XIII	23, 24
XI	43, 45		

FIG. 4. Inhibition of Rous sarcoma virus by individual vaginal secretions.

transshipment to Jamaica the potency was found to be lowered and the first tissue culture assay of viral infectivity revealed an endpoint of $10^{-4.5}$.

Experimental results. Several secretions were toxic to tissue culture. In most cases toxicity could be removed by centrifugation of the secretions at 2500 RPM for 30 minutes and discarding the sediment.

Inhibition of poliovirus: In vitro *experiments.* Each of 21 cervicovaginal secretions (CVS) was mixed with an equal volume of poliovirus type I diluted to give a final concentration of 1000 $TCID_{50}$ per ml. All mixtures were incubated at 37°C for 30 minutes and 0.1 ml aliquots (representing 100 $TCID_{50}$ of virus) were inoculated into tissue cultures. The inhibition of poliovirus by the secretions is shown in Fig. 1. Three secretions were decisively inhibitory (fully active), 9 were partially inhibitory and 5 had no inhibitory effect. In addition, 4 specimens were contaminated despite the high concentration of antibiotics used.

Following these observations, several CVS pools were tested for their activity. For a

TABLE I. Search for Polioviruses in Vaginal Washings and Sera Following Introduction of Attenuated Polio Vaccine into Vagina.

Patient	Washing 1	2	3	4	5	6	7	Evidence of viremia*	Rectal swabs†
A	—	+	—	—	—	—	Cont.	—	—
B	—	+	—	—	—	Cont.	N.D.	—	—
C	—	+	—	—	—	—	—	—	—
D	—	+	—	—	—	Cont.	N.D.	—	—
E	—	+	+	—	—	—	—	—	—

Washings were taken at the following intervals after introduction of virus into vagina: (1) Before starting experiment. (2) 2 hr 40 min. (3) 5 hr 30 min. (4) 30 hr. (5) 55 hr. (6) 79 hr. (7) 270 hr.

N.D. = Not done. Cont. = Bacterial contamination.

* Blood samples taken at each of above time intervals were tested.

† Taken 55 hr after introduction of poliovirus.

number of the individual secretions comprising the pools, corresponding serums obtained from the volunteers at the time of CVS collection, were available. The inhibition of poliovirus by appropriate pools of these serums was compared with that by the respective CVS pools. Results are shown in Fig. 2. Inhibition by CVS pools has no definite correlation with that by serum pools. All serum pools caused inhibition, whereas only 3 out of 6 CVS pools manifested this effect. The fact that the CVS pools contained 3–4 samples and the serum pools consisted of 2–3 corresponding specimens cannot explain this discrepancy. Two CVS pools showed complete inhibition of 1000 $TCID_{50}$ of poliovirus type I, but unfortunately the corresponding sera were not available.

In vivo *experiments*. Poliovirus in the form of trivalent attenuated vaccine was instilled in the vaginal canal of 5 volunteers. Two ml of material were introduced by means of a pipette. At intervals (Table I) vaginal washings and blood specimens were collected. No virus was isolated from the washings obtained before instillation of the vaccine. Poliovirus was isolated from the vaginal washings of all 5 individuals 3 hours after introduction, but from only one individual at 5½ hours. No virus was recovered at any time thereafter. No effort was made to determine the type of the recovered virus. Virus could not be isolated from the blood of these individuals at any interval. Neutralization tests made on sera obtained before introduction of virus and 12 days later showed no significant change in antibody titer.

Inhibition of herpes simplex virus. CVS pools were divided in 2 parts. One aliquot of each pool was mixed with herpes virus and the mixture incubated at 4°C for 20 minutes. The other was mixed with poliovirus and incubated as already described. Tissue cultures were inoculated with 0.1 ml volumes (representing 100 $TCID_{50}$ herpes or poliovirus) of the various mixtures. The results (Fig. 3) show that there was no correlation of inhibition between these 2 viruses. CVS pools 1 and 3 suppressed both polio and herpes infection to about the same extent, while pools 2 and 5 had a selective activity against herpes virus.

Inhibition of Rous sarcoma (RSV). A number of individual CVS were tested for inhibitory effect against RSV, utilizing the chick embryo chorioallantoic membrane for plaque formation. These secretions were mixed with equal amounts of virus diluted 1:50,000. To the mixtures was added hyaluronidase in a final concentration of 30 USP units/ml. The mixtures were incubated for one hour at 4°C and 0.05 ml amounts were inoculated onto the chorioallantoic membrane of 9-day-old embryos, 10 eggs being used for each mixture. All secretion pools caused inhibition of Rous plaques (Fig. 4).

Discussion. Secretions from the female genital tract were found capable of inhibiting polio, herpes and Rous sarcoma viruses *in vitro* and poliovirus *in vivo*. In the latter case, the inhibitory effect may have been

associated with a low pH of the vagina, but this effect cannot be ascribed to the inhibition *in vitro* where the secretions were brought to pH 7 prior to testing. The nature of the inhibitory substance has not been determined. It may represent specific antiviral antibody. Polio antibody has been reported to be present in urine of normal human subjects(1). The work of Strauss(2) may be interpreted as meaning that antibody to the typhoid bacillus may not only be found in the mucus of the vagina, but that such antibodies can also be locally formed. The presence of anti-polio and anti-herpes virus substances in the secretions can be explained on the basis of antibody derived from circulation, or in terms of local production of antibody in view of the known occurrence of herpes infection in the vagina and of the close proximity of the vagina to the rectum which may furnish polio antigen from time to time. Yet the lack of correlation between the neutralizing capacity of cervical secretions and corresponding sera coupled with the presence of inhibitory action against RSV (which inhibitor has rarely been found in the human sera) suggests that the substances in question were of a nonspecific or non-antibody nature. It is possible that more than one substance of an inhibitory nature, both antibody-like and nonspecific inhibitor-like, may be present in the secretions.

Summary. A viral inhibitor(s) effective against polio, herpes simplex and Rous sarcoma viruses has been demonstrated in the female genital tract. This may explain why it was not possible to isolate any virus(es) from the vaginal washings and swabbings of 229 volunteers. The nature of this inhibitor has not been determined. Some of the effects described, especially the activity against RSV, suggest that at least part of the inhibitory activity is not due to antibody.

The authors gratefully acknowledge the invaluable help in collection of specimens by Drs. T. E. Been and C. P. Douglas, Univ. of the West Indies, Jamaica, and Dr. Denis Cavanagh, School of Med., Univ. of Miami, Coral Gables, Fla. Much of the initial work aimed at the detection of viruses in vaginal secretions was carried out by Dr. T. Burnstein. Thanks are also due Prof. David B. Stewart and Dr. Louis S. Grant, Univ. of the West Indies, for their interest and support of this work.

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Received September 20, 1963. P.S.E.B.M., 1963, v114.

Production of Fatty Liver in the Rat by Cortisone.* (28790)

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Among other effects of cortisone, there are clearly effects on lipid metabolism. Marked changes in fat distribution occur in humans subjected to excessive doses of cortisone, giving rise to a characteristic body habitus. In addition, some inconstant changes in serum lipids have been reported(1), and it appears that fatty liver may result in some patients (2). Among experimental animals, the rabbit develops gross lipemia and fatty liver when cortisone is administered(3), but this animal is abnormally prone to develop these changes under a variety of relatively minor stimuli. Other animals have not been carefully studied in this regard, although a few undocumented references to the phenomenon exist(4,5). The present communication reports the development of hyperlipemia and lipid deposition in the livers of rats subjected to cortisone administration.

Materials and methods. Animals. Female rats weighing approximately 200 g were obtained from Holtzman Co. After a short period of acclimatization, the rats were di-

* This work was supported in part by USPHS grant, Pathology of the Cell.