20. Winget, C. M., Smith, A. H., *Am. J. Physiol.*, 1959, v196, 371.
21. ———, ———, *Poultry Sci.*, 1958, v37, 509.
22. Riddle O., Honeywell, H. E., *Am. J. Physiol.*, 1924, v67, 316,333,337.

Received July 31, 1961 P.S.E.B.M., 1961, v108

Occurrence and Titer of Isohemagglutinins in Secretions of the Human Uterine Cervix.*† (27022)

G. I. SOLISH**, H. GERSHOWITZ AND S. J. BEHRMAN (Introduced by J. V. Neel)
Departments of Human Genetics and Obstetrics and Gynecology, University of Michigan Medical School, Ann Arbor

Gershowitz, Behrman, and Neel(1) have demonstrated the presence of anti-A and anti-B agglutinins in the cervical secretions of normal women and suggested that this might play a role in the deficiency of type A offspring in incompatible marriages of blood type A fathers to type O mothers. An incompatible marriage is here defined as one in which the male possesses an ABO blood group antigen for which the female partner has the corresponding antibody. Behrman, et al.(2) found an excess of type O women as well as a greater than expected number of incompatible matings in a series of 102 childless couples studied, for whom no apparent cause for infertility could be found "by the most exhaustive gynecological and urological examinations possible," and who had been attempting to conceive for at least 5 years. Matsunaga(3) observed both a reduction in fertility and an almost 2-fold increase in the frequency of childless couples in an incompatibly-mated group compared with a compatibly-mated control group. These observations, together with the finding of antigenic dimorphism in human spermatozoa by Gullbring(4) and the demonstration of the presence of antibodies to sperm antigens in the serum of some women by Rao and Sadri (5), lend support to the hypothesis of an immunological selection mechanism operating at a preconception level.

The present study represents an exploration into the quantitative levels of ABO agglutinins in the cervical secretions of women representing the different ABO blood groups, the relationship of these titers to the day of the menstrual cycle, and to the level of agglutinins in the serum.

Materials and Methods. A total of 428 samples of cervical mucus was collected from 182 women. Three of these were postmenopausal while the remaining 179 were in the reproductive age group. Blood and saliva specimens were also obtained in most cases. These subjects were selected from several sources including the Outpatient Gynecology Clinic of University Hospital (largely patients with infertility problems), inpatients from the Ypsilanti State Psychiatric Hospital, volunteers from medical students' wives groups, student nurses, and patients attending the Ann Arbor Planned Parenthood Clinic. The representativeness of the first source may be questioned but in fact these individuals, who comprise about 60% of Group I, contribute about 50% of the positive cervical specimens in this group and thus do not appear to bias the sample.

The total of 182 women was divided into 2 groups—Group I, comprising 141 subjects, from whom only a single specimen of cervical mucus was obtained, and Group II, 41 subjects, from whom 2 or more specimens were taken at different times of the menstrual cycle.

* Supported in part by grants from U. S. Atomic Energy Commission and from Association for Aid of Crippled Children.

† We are grateful to Dr. James V. Neel for many helpful suggestions during this study and for his critical review of the manuscript.

** On USPHS Training Grant from Dept. of Obstetrics and Gynecology, State Univ. of New York Downstate Medical Center, Brooklyn, New York.

Essentially 2 methods of collection were employed. In each of a small number of volunteers a menstrual cup (Tassette) was inserted into the vagina at times other than during the menstrual flow, and left in place for about 4-6 hours while the subject continued her usual activities. After removal, the sample was obtained by aspiration of the mucoid material at the bottom or adhering to the sides of the cup. The second and most frequently used method of collection was the direct aspiration of cervical mucus from the cervical os by means of a specially devised polyethylene pipette attached to a 10 or 20 cc syringe. Storage time until analysis was variable.

Determination of antibody titer was by means of a standard doubling dilution titration technique in saline, using 2% washed red blood cells; the end point (the last tube showing agglutination) was expressed as $-1\times \log_2$. The tests were incubated at room temperature for one hour and read macroscopically before and after centrifugation. All doubtful tests were reexamined microscopically.

Since viscosity of the mucus constituted a major technical problem, a neuraminidase-containing crude enzyme extract prepared from *Clostridium perfringens*‡ was added in very small quantities (0.01 to 0.02 ml to total volume of specimen). In a series of preliminary observations, this enzyme preparation seemed to have no effect on serum and saliva hemagglutinin titers even after more than 10 days of incubation at 4°C. Sufficient decrease in the mucilaginous consistency of the cervical secretions occurred within 24-48 hours at 4°C to permit performance of the test. Prevention of hemolysis of erythrocytes by the crude enzyme solution was accomplished by addition of 0.01 to 0.02 ml of a 1% solution of ethylenediamine tetraacetic acid (Versene) as a metal-chelating agent, but was not necessary when purified neuraminidase‡ was used.

The serum α and β isoagglutinin titers were determined in a similar way, testing against 2% suspensions of A_1, B, and O red cells, the O cells serving as a control. Determination of the secretor status was performed by an inhibition hemagglutination technique using boiled saliva diluted 1:20 with normal saline.

Results. The distribution of Group I and Group II subjects possessing isoagglutinins in their cervical mucus tabulated with respect to ABO blood types is shown in Table I. Agglutinins were present in 23.7% of Group I women, who were selected only because of their availability for this study. This ratio compares favorably with the 22.1% frequency of positives found in the earlier study by Gershowitz, Behrman, and Neel(1), using diluted specimens of cervical mucus. However, when multiple specimens were obtained from the same individual, viz. Group II, the proportion of individuals with at least one specimen positive for agglutinins rose to 63.4%. The number of samples obtained from women in Group II ranged from 2 to 15 with the median at 6 specimens.

The frequency of positive samples among all the women was found to be significantly greater in type O than in the combined sample of type A and type B women (X^2 with 1 d.f. = 11.56, $p<0.001$). Among those specimens with detectable antibody present, the mean anti-B titer *in the cervical secretions* was significantly higher in type O women than in type A women ($t' = 2.34$, $0.02<p<0.05$) and, correspondingly, the anti-A titer was significantly greater in type O women than in type B women ($t' = 2.61$. $0.01<p<0.02$).‡‡ However, such differences in mean antibody titer were not encountered when *serum* anti-B titers of types A and O women were compared, nor when *serum* anti-A titers of types B and O women were compared. This lack of difference held for both the cervical hemagglutinin positive group (for anti-B $t=1.75$, 46 d.f., $0.05<p<0.10$; for anti-A $t=1.39$, 37 d.f., $0.10<p<0.20$) and the entire series of 141 cases for which serum antibody titers were available (for anti-B $t=0.56$, 127 d.f., $0.40<p<0.60$; for anti-A $t=1.98$, 58 d.f., $0.05<p<0.10$).

The presence of anti-A and the presence of anti-B in the cervical secretions of type O women in Group I do not appear to be independent events ($X^2=17.68$, 2 d.f., $p<0.001$)

‡ We are greatly indebted to Drs. Saul Roseman and George W. Jourdian of the Rackham Arthritis Research Unit, Univ. of Michigan, for supplying these materials.

‡‡ Cochran's approximation of the Behrens-Fisher test was used because the variances were distinctly different, $F(25,15)=4.87$ for anti-B and $F(27,6)=7.28$ for anti-A.

TABLE I. Cervical secretion hemagglutinins and blood type.

	Group I (single specimen)			Group II (multiple specimens)		
Blood type	Total No. tested	No. positive for cerv. hemaggl.	Mean titer ($\bar{x} \pm$ S.E.) —†	Total No. tested	No. with at least one specimen positive for cerv. hemaggl.	Mean titer ($\bar{x} \pm$ S.E.) —†
A	63	7	$2.0 \pm .436$	19	9	$1.46 \pm .175$
B	9	4	$2.0 \pm .408$	6	3	$1.0 \pm .00$
AB	9	0	—	0	—	—
O	54	anti-A only 8 anti-A $= 2.47 \pm .585$ anti-B only 6 anti-B $= 2.31 \pm .645$ both 7 Total 21		16	Both anti-A and anti-B = 7 anti-A only = 1 anti-B only = 0 **Both and anti-A only = 5 **Both and anti-B only = 1 Total 14	anti-A $= 2.84 \pm .227$ anti-B $= 2.36 \pm .263$
Totals	135*	32		41	26	

* Blood type not available in 6 additional cases.
†— Endpoint of doubling dilution method expressed as $-1 \times \log_2$.
** In these 2 categories some specimens had both anti-A and anti-B while other specimens taken from the same subjects at other times had only anti-A or anti-B.

and in this respect these antibodies resemble the serum hemagglutinins. However, in contrast to the finding of both anti-A and anti-B in the sera of type O individuals, these antibodies are found either singly or together in the *cervical secretions* of type O women (see Table I).

As in the earlier study, no antibodies were demonstrable in the cervical secretions of the 9 AB women in this series. However, in 3 instances, all involving type A women, the cervical secretions appeared to contain agglutinins not present in the subject's serum and not consistent with her ABO blood group. Two of these subjects were A_2 and one A_1.

The secretor type was determined in 163 women in this study by testing samples of boiled saliva for presence of soluble A, B, and H specific substances. No difference in ratio of secretor to non-secretor types in the positive cervical antibody and the negative cervical antibody groups was found (see Table II).

Among 17 patients from whom 6 or more consecutive specimens of cervical mucus were obtained at various points in the menstrual cycle, no consistent pattern of fluctuation in titers was detectable.

Among women positive for cervical agglutinins, there is at most a weak correlation between serum and cervical secretion titers. Thus, the regression coefficients of cervical secretion antibody titer on serum antibody titer were -0.068 for anti-B agglutinin in type A women, 0.149 for anti-A agglutinin in type B women, 0.108 for anti-B agglutinin in type O women, and 0.430 for anti-A agglutinin in type O women. None of these regressions is significant, but the numbers involved are small.

TABLE II. Relationship of ability to secrete ABO substances in saliva to presence of hemagglutinins in cervical secretions.

	No. of secretors	No. of non-secretors	Total
No. of cervical hemagglutinin: Negative women	83	27	110
No. of cervical hemagglutinin: Positive women	41	12	53
Total	124	39	163

$x^2 = .0053$, 1 d.f.

Discussion. Two explanations for the occurrence of hemagglutinins in the cervical secretions are suggested by the results of recent work which are pertinent to the present study.

The first arises from the studies of the transplacental passage of immune anti-A and anti-B from mother to fetus. Rosenfield and Ohno (6) observed that in 90% of cord sera of group O infants of group O mothers both anti-A and anti-B agglutinins were present, but when the mother was type A or type B, only 35% of compatible infants demonstrated the presence of the appropriate antibody in their cord bloods. More recently, Freda and Carter (7) have demonstrated a selective transmission of isogglutinins across the placenta dependent

upon the blood type of the mother, type O mothers transmitting larger amounts of antibody to the fetus than A_2 mothers and the placentas in A_2 mothers permitting a freer exchange than placentas of A_1 mothers.

These findings in addition to the preponderance of group O women found among mothers of infants with ABO hemolytic disease, provide strong clinical evidence that the isohemagglutinins produced by group O individuals differ from those produced by group A and group B persons. Biochemical evidence in support of this observation has recently been supplied by Rawson and Abelson(8), who found that type O individuals produce isoagglutinin composed largely of γ_2 globulins, of relatively low molecular weight, separating in the ultracentrifuge with the 7S component, and readily crossing the placenta. Non-O subjects, on the other hand, tend to produce increased amounts of 19S antibody located in the γ_1 fraction of globulins which are rarely found to cross the placental barrier. If these observations are confirmed, it would not be amiss to propose that a similar mechanism may be responsible for the selective appearance of antibodies of given molecular size in other body tissues and fluids such as those of the uterine cervix, if such antibodies are in fact derived from the blood serum.

The second type of explanation arises from the work of Straus(9) who has been able to demonstrate the occurrence of specific antibody in human vaginal mucus as well as serum in response to the local application of soluble typhoid antigen. If the existence of such a local antibody-forming mechanism can be substantiated for other antibodies, then a similar scheme might be envisioned for production of hemagglutinins in response to specific antigen, perhaps of bacterial origin, within the vagina. These same antigens may also be effective in distorting the measurable agglutinin titers in the cervical mucus by inhibiting their response both *in vitro* and, more importantly *in vivo* against sperm antigens. Whatever may be the origin of the cervical hemagglutinins, be it through passive transfer from the serum, local production, or both, they appear from the present studies to be related in a quantitative as well as qualitative way to the ABO blood group of the individual.

The foregoing serves to emphasize the possible complexity of the basis for the deficiency of type A and B children in ABO incompatible matings, especially of O women with A or B men, the evidence for which has recently been reviewed by Levene and Rosenfield(10). In view of the well-known occurrence of ABO hemolytic disease, most of the attention has centered on the action of maternal antibodies on the fetus as the basis for the deficiency (3,11,12). The present data strengthens our earlier suggestion of an alternative mechanism, involving selection at a gametic level. The relative role of each of these two mechanisms remains to be clarified.

Summary. An analysis of the cervical mucus from 182 women randomly selected, revealed that 23.7% contained isohemagglutinins when a single specimen was examined but when more than one specimen was obtained the percentage rose to 63.4%. Type O women had agglutinins in their cervical secretions more frequently than either type A or type B women, and, in general, the titers were higher. There was no significant correlation between cervical secretion titers and serum hemagglutinin levels. The occurrence of antibodies in the cervical mucus seemed to be unaffected by the secretor type. Observations on multiple samples obtained from 41 women failed to suggest any correlation between antibody titer and phase of the menstrual cycle. These findings extend the data which earlier suggested the hypothesis of ABO selection at the gametic level as a factor in explaining the deficiency of certain types of offspring in ABO incompatible matings.

1. Gershowitz, H., Behrman, S. J., Neel, J. V., *Science*, 1958, v128, 719.

2. Behrman, S. J., Buettner-Janusch, J., Hegler, R., Gershowitz, H., Tew, W. L., *Am. J. Obst. & Gynec.*, 1960, v79, 847.

3. Matsunaga, E. *Am. J. Hum. Genet.*, 1959, v11, 405.

4. Gullbring, B., *Acta med. Scand.*, 1957, v159, 169.

5. Rao, S. S., Sadri, K. K., *VIth International Conference on Planned Parenthood,* (Vigyan Bhavan, New Delhi), 1959, 313.

6. Rosenfield, R. E., Ohno, G., *Rev. d'Hémat.*, 1955, v10, 231.

7. Freda, V. J., Carter, B. A., *Obst. & Gynec.*, 1961, v17, 597.

8. Rawson, A. J., Abelson, N. M., *J. Immunol.*, 1960, v85, 640.

9. Straus, E. K., PROC. SOC. EXP. BIOL. & MED., 1961, v106, 617.

10. Levene, H., Rosenfield, R. E., *Progress in Medical Genetics, I*, 1961, Grune & Stratton, New York, p. 120.

11. Matsunaga, E., Itoh, S., *Ann. Hum. Genet.*, 1958, v22, 111.

12. Chung, C. S., Morton, N. E., *Am. J. Hum. Genet.*, 1961, v13, 9.

Received August 3, 1961 P.S.E.B.M., 1961, v108

Ineffectiveness of Pitocin on the Sheep Gravid Uterus: The Role of Pitocinase.* (27023)

S. R. M. REYNOLDS AND JOHN D. MACKIE

Department of Anatomy, University of Illinois, College of Medicine, Chicago

Continuous slow infusion of a low concentration of oxytocin is highly effective in stimulating rhythmic contractility of the human uterus(1,2). With faster infusion, the uterus remains contracted. The effect of intravenous infusion on the uterus of other animals has not been tested although Assali *et al.*(3) have done so in a study on uterine blood flow in sheep. The present study was undertaken to compare the response of the myometrium of the ewe with that of the human to obtain a controlled means of stressing the fetus if the uteri of the 2 species behave similarly.

In pregnant women, rhythmic contractions of the gravid uterus can be induced in an otherwise virtually quiescent uterus. Strength and frequency vary progressively, within limits, with amount of pitocin infused(2). Sensitivity of the uterus increases as pregnancy advances. To obtain comparable outputs of contractility *(force, in mm Hg, X frequency of contraction per unit of time)*, Caldeyro-Barcia and Poseiro (2) found that Syntocinon dosage requirement decreased with increase in time of pregnancy.

We decided to investigate the sensitivity of the gravid sheep uterus in an exactly similar manner. Six pregnant ewes in the last half of gestation (circa 150 days) were used. Four ewes were lightly anesthetized with diallylethylbarbituric acid and urethane (Dial), 0.2 ml per kg of body weight, given intravenously. Two ewes were prepared under local anesthesia (Procaine) only.

* Supported by USPHS Grant

The ewe was placed on her side and part of the uterus delivered through a midline abdominal incision. Intrauterine pressure was recorded by a polyethylene catheter admitted to the amniotic sac and connected to a pressure transducer. At the same time, umbilical artery and vein pressures were recorded by small catheters admitted through branches of these vessels, as the membranes were maintained intact and the uterus closed after the operation. The fetus was *in utero*, in its normal habitat (4). In addition, maternal respiration was recorded from an intrapleural trocar and carotid arterial pressure by a catheter in that artery; the trocar and catheter were connected to Statham pressure transducers. Records were obtained on an ink-writing Offner recorder.

Syntocinon, synthetic pitocin, was infused into the jugular vein of the ewe by a continuous infusion pump (Harvard). Dilutions and rates of infusion were carefully controlled. After an initial 20-30 minute recording period, infusion was commenced, always starting at the lowest infusion rate. The starting rate was either 0.76 or 1.9 mμ Syntocinon per min. After 20 min the rate of infusion was doubled. This was repeated successively until 191-1928 mμ per min was being infused.

In no case was rhythmic uterine contractility observed prior to, or during, infusion of pitocin. An increase in tonus (intrauterine pressure) occurred. It will be seen that the onset of increased tonus commences with dose levels of 0.76 and 19.1 mμ of Syntocin per min (Fig. 1). The lowest thresholds (0.76 and 1.9 mμ per