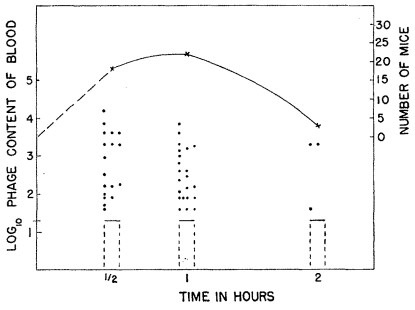

Fig. 1. Passage of *B. megatherium* bacteriophage through the skin of white mice. Numbers of active phage particles recovered from the total blood circulation of 96 mice. Thirty-two mice were tested at each time interval.

the skin of the abdomen, the same techniques were followed with the exception that the Pentathal sodium was administered by the subcutaneous route into the interscapular area of the mice instead of intraperitoneally. Two-tenths of a milliliter of the high-titer phage suspension was applied to the fur of the abdomen and gently spread with the tip of the pipette to effect penetration through the fur and onto the skin. The phage-treated area was equal in size to that of a 2.0 cm circle. At indicated time intervals after the administration of the bacteriophage suspension, the chest area was thoroughly sterilized with iodine and alcohol, and 0.1-ml samples of blood were taken by cardiac puncture with a heparinized 1-ml syringe. Following this procedure, the mice were sacrificed. Full-strength and serial two-fold dilutions of such blood samples were then assayed for the presence of virus particles. Control experiments on the accidental contamination of blood samples with phage were performed by placing 0.2 ml of the high-titer phage suspension on the chest area. After the routine sterilization procedure, blood samples were taken by cardiac puncture. From these as well as from other normal control animals no phage particles were recovered.

The recovery of active virus particles from the circulation of mice thus treated was quite irregular in both the number of positive recoveries and in the amount recoverable from the circulation of each mouse. There was essentially no difference in the rate and quantities of recovery by the two experimental methods. Figure 1 cites the cumulative results of one series of such combined experiments. The quantity of phage particles per 0.1 ml of sample has been converted by a factor of 20 and is cited for an average total blood volume of such mice of 2 ml. Thirty-two mice were sacrificed at each time interval. As can be seen, the number of mice yielding recoverable virus particles decreases with time, for only three mice yielded particles at 2 hours. These results suggest that this effect is possibly due to the immune mechanisms of the experimental animals. In this respect it is worth while to note that Van Vunakis *et al.* (*6*) have reported in vitro inactivation of *Escherichia coli* phage by normal mouse serum. The factor responsible was shown to be the properdin system previously described by Pillemer (*7*). Sulkin and his associates (*8*) reported evidence for the in vivo inactivation of *Staphylococcus* phage by the properdin system in rabbits.

In this study great variations in the recovery of active phage particles from the blood circulation were noted after intraperitoneal inoculations into different strains of normal mice. It may be possible that these variances were due to differences in titer of natural antibodies. Since current work is showing that the rate of arterial disappearance of this bacterial virus from the circulation of dogs is very rapid (*9*), the actual number of particles which were able to pass the skin barrier is possibly of a larger magnitude.

The size of the bacteriophage particles used in this study has been determined (*10*) to be 49 mμ for the width of the head and 330 by 15 mμ for the length and width of the tail. On the basis of the size of the head only, this particle then would be in the size range of the small animal viruses.

It is hoped that this report will stimulate reinvestigations of the possibility that virus infections may occur by penetrations through the intact skin and of the effects that this rather exotic mode of transmission may have in the epidemiology and pathogenicity of virus infections of man (*11*).

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Hemagglutinins in Uterine Secretions

Two kinds of findings have led to the suggestion that the ABO blood group phenotypes may be subject to the action of natural selection. One line of investigation has purported to show that there is a relative deficiency of living children of blood type A among the offspring derived from the "incompatible" mating, mother type O, father A (see, for example, *1, 2*). For a number of reasons considerable uncertainty exists concerning the existence and/or magnitude of this deficiency (*3*). The other line of investigation has suggested that in blood-group incompatible matings, the frequency of abortions is higher and the mean number of living children is lower than in compatible matings (*4*). (An incompatible mating is defined as one in which the male possesses an antigen which is lacking in the female; in the ABO system, the male possesses an antigen for which his spouse has the corresponding antibody.)

There are two possible mechanisms whereby selection due to the ABO blood groups may alter the number of children born with a given blood type. Many have assumed that immune antibodies produced by the mother could damage the fetus and result in its loss (for example, *1*). Another possibility, which formed the starting point for this investigation (*5*), is that selection may be exercised during the preconception period on the spermatozoa themselves. These two mechanisms are, of course, not mutually exclusive.

The postulate of spermatozoal selection involves one or more basic assumptions. One is that human sperm possess the specific blood group antigens of the donor. Previous findings on this point (for example, *6*) need to be reevaluated in light of the possible contamination of blood group substances in the seminal fluid. (Sperm from a nonsecreter donor would obviate such a possibility.) A second is that two antigenically different kinds of sperm are produced by a heterozygous AO male—that is, sperm bearing and sperm lacking A antigen. This antigenic dimorphism in the sperm population of heterozygous males has not, to our knowledge, been demonstrated. A third assumption, aside from the cellular antigens of the sperm, is that the presence of soluble blood group substances in the seminal fluid may itself be the (or one of the) determining agent(s) in spermatozoal selection.

If any of these assumptions is correct, then it becomes conceivable that there may be specific selection of sperm in the female reproductive tract, either by selective impedance of motility, complete inactivation, neutralization of a point of

attachment, or by some other, as yet unknown, mechanism. Such inactivation might also serve as a possible cause for infertility among otherwise physiologically normal couples.

The object of this study has been to determine whether or not the secretions of the uterine cervix contain hemagglutinins, as an initial step in an effort to work out the possible physiological basis for ABO selection. The impetus for the study stemmed from the feeling that the known facts regarding the consequences to the fetus of ABO isoimmunization (see 7) were not sufficient to account for the magnitude of selection effects postulated for ABO incompatible matings. The detection, some 30 years ago, of hemagglutinin in the cervical fluid was apparently limited to the analysis of a *single* sample (*8*).

All the women investigated in the course of this study were patients in the Out-Patient Gynecology Clinic of the University Hospital. Their reasons for the clinic visit were varied; only five were seen because of failure to conceive and in whom the cause of infertility was still unknown after careful study of the marital partners. The collection of cervical secretion depended only on whether or not a sample could be obtained from any particular woman. Blood for typing was obtained at the same time. All secretions were frozen soon after collection and all were tested within a period of 5 days.

The method of testing was as follows. To each tube containing the sample, which was usually quite small, was added 0.15 ml of saline. Because the quantity of secretion obtained varied from subject to subject, the dilution factor is variable. The contents of the tube were then thoroughly agitated with an applicator, the tube was centrifuged, and the clear supernatant fluid was tested with fresh A_1, B, and O red blood cells. All tests were performed in microtubes (6 by 50 mm) to which 0.01 ml of a 2 percent washed red blood cell suspension was added to 0.01 ml of the diluted secretion. The tests were allowed to stand for 1 hour at room temperature and were read macroscopically both before and after centrifugation.

The results are given in Table 1. Only distinctly positive reactions are tabulated, although the inclusion of weak, ambiguous reactions would not have substantially changed the magnitude of the difference presented in the table. Fifteen of the 35 diluted secretions (42.8 percent) of blood type O women contained antibody of the ABO system. Of 30 type A and 5 type B women, two cervical secretions (5.7 percent) had detectable antibody. Hemagglutinins were not found in the secretions of seven type AB women, which is additional evidence for the ABO group specificity of these antibodies. Only one of the women with cervical hemagglutinins had been seen because of infertility. A comparison of the findings in type O women with those in types A and B by the exact treatment of a 2×2 table yielded the two-tailed probability of 0.0053 that they were drawn from the same population. The fact that the over-all percentage of O, A, and B women exhibiting detectable hemagglutinins was only 24.3 may be due in part to the degree of dilution necessary to bring the volume up to 0.15 ml for scanty specimens, but this suggestion leaves unexplained the differences between A or B and O women. To some extent the latter differences may be due to the greater opportunity to detect agglutinins in O women, who have *two* agglutinins. Studies on undiluted secretions are in progress.

These findings would appear to support the possibility of gamete selection as a means of accounting for the distortions of the expected ratios in marriages of A fathers and O mothers, a possibility which, after the completion of this study, was discovered to have been proposed by Matsunaga in 1953 (*2*). Although several further lines of investigation readily present themselves, really critical data on this point may be difficult to obtain. The observed departures from the Mendelian ratios could be produced by a relatively slight impairment of sperm motility within women of the appropriate genotype, an impairment difficult to demonstrate with certainty. Inasmuch as the increased frequency of abortions in incompatible marriages seems securely established (*2, 9*), the present findings may indicate that the departure from Mendelian ratios in the children of A male × O female marriages has a complex background.

Note added in proof: It has recently come to our attention that the demonstration of antigenic dimorphism in the sperm of an AB male has been made by Gullbring (*10*). This finding would seem to support the possibility of preconception selection by blood group antibody in the uterine secretions.

Table 1. Number of diluted uterine cervical secretions containing hemagglutinin, tabulated by blood type of the donors.

Blood type	No. tested	No. containing only anti-A	No. containing only anti-B	No. containing anti-A and anti-B	Total no. with hemagglutinins
A	30	0	2	0	2
B	5	0	0	0	0
AB	7	0	0	0	0
O	35	4	2	9	15
Totals	77	4	4	9	17

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Concentration of Albumin in Renal Papilla

Recent investigations (*1*) have focused attention on the renal papilla for its role in concentrating urine. Freezing-point determinations of papillary tissue have demonstrated a tissue osmolarity up to three times that of the blood or renal cortex, because of a high concentration of sodium, chloride, and urea. Completely unsuspected, however, is the observation reported in this note of the high albumin concentration in the mammalian papilla. This may well bear an important relation to water transport in the papilla as part of the mechanism of concentrating urine.

Fourteen normally hydrated, nonanemic dogs were anesthetized with pentobarbital sodium. About 500 μc of I^{131}-labeled human serum albumin was injected systemically. The kidneys were exposed through an abdominal incision, and a loose tie was placed around the pedicle. In four dogs one kidney was ligated 3 minutes, the other kidney 60 minutes, after the injection of the radioactive material. In the remaining ten dogs both kidneys were ligated simultaneously after about 1 hour. The kidneys were removed and frozen in Dry Ice. Sections were cut on a band saw, and