ACTIVE AND PASSIVE SENSITIZATION OF THE UTERUS OF THE COW *IN VIVO* AGAINST *TRICHOMONAS FOETUS* ANTIGEN AND THE EVIDENCE FOR THE LOCAL PRODUCTION OF ANTIBODY IN THAT SITE

By W. R. KERR and MURIEL ROBERTSON*

The Ministry of Agriculture, Stormont, Belfast, and the Lister Institute, London, S.W.1

In the cow the flagellate *Trichomonas foetus* is a uterine parasite. The infection is transmitted by the bull at coitus, and the organism is very closely adapted to the conditions in the uterus at the inception of pregnancy. The parasite does not pass into the blood stream at any time (Kerr & Robertson, 1943).

In the infected animal, antibody is found in the circulating blood, and it has been shown that this is induced by the antigen absorbed from the uterus. Filtered solutions of laked *Trichomonas* and also suspensions of freeze-dried antigen when instilled into the uterus produce good titres against *T. foetus* in the blood (Kerr & Robertson, 1943).

In the course of the work it was found that the uterus sometimes became sensitized, and local, and sometimes general, reactions of an acute allergic type were produced. In addition, during attempts to immunize animals against infection and during the study of the experimental re-infection of animals which had had the disease in an acute form, it was discovered that the uterus, on occasions, contained free antibody (Kerr & Robertson, 1947).

The present paper deals with:

(1) The presence of antibody in the uterus.
(2) The active sensitization of the uterus.
(3) The evidence for the local production of antibody in the uterus.
(4) The passive sensitization of the uterus *in vivo*.

MATERIALS AND METHODS

Trichomonas foetus is cultivated in a medium devised by one of us (W. R. K.). It consists of inspissated slopes of horse serum covered with 10 ml. of tryptic digest broth with 2% of glucose. The fluid is then covered with about half an inch of sterile liquid paraffin and steamed three times. The broth is adjusted to pH 8 before adding the glucose, and should be at 7·2–7·3 when inoculated.

The inoculum used is about 0·2 ml., and the organisms are subcultured after 48 hr. when the number is about 5 million per ml. Growth in bulk is obtained as follows.

A serum broth is used (Kerr & Robertson, 1947). The glucose broth is put in 16 oz. medicine flats in 300 ml. and in Roux bottles in 900 ml. quantities. Sterile

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horse or cattle serum is added so as to produce a final concentration of 7 % serum. The pH is 7·2–7·4 when ready for use. 5–7 ml. of a 2-day growth of *Trichomonas* are seeded into each flat. The flats are incubated at 35–37° C. for 44–48 hr., by which time there should be a heavy growth of 5–7 million organisms per ml. The duration and temperature of the incubation must be carefully watched, because when the peak growth is reached the culture dies off extremely rapidly. 25–50 ml. are transferred from the flats to the litre bottles and these are in turn incubated for 44–48 hr. The *Trichomonas* are then spun down in the centrifuge or in an Alfa-Laval separator. The deposited organisms, which should be very motile, are washed in saline at least three times and should still be motile when the next step is taken. The antigens are all prepared from living washed *Trichomonas* in saline. Growing *T. foetus* in bulk requires some experience, but can be carried out as a routine with yields of about 0·5 g. of dried organisms per litre.

Antigens

Freeze-dried (F.D.). The most useful antigen was freeze-dried *Trichomonas* bodies.

Acetone-dried antigen. Another good antigen was made by precipitating the organisms from the saline suspensions with a large volume of acetone, washing them three or four times in the centrifuge in acetone, drying them overnight in the incubator at 37° C., and desiccating for 48 hr. *in vacuo* over P_2O_5.

Blendor antigen. Blendor antigen was made by blending the living suspension in a Waring Blendor for about 2 min. The resulting milky fluid was dialysed in distilled water at 1° C. to get rid of the salt and then freeze-dried. This is a more soluble antigen but was not much used for big animals. It is very useful as an antigen in precipitation tests with rabbit sera.

Three fluid antigens were used in our earlier work.

Fluid antigen 1 (*Fl.* 1). *Trichomonas* suspensions were washed with saline, laked in distilled water and Seitz filtered. The salt content was adjusted to 0·85 % with sodium chloride. Fl. 1 was sometimes precipitated with trichloracetic acid.

Fluid antigen 2 (*Fl.* 2). The filtrate from laked organisms was concentrated by distillation at 28° C. under reduced pressure. Doses were measured in terms of the numbers of organisms in the original suspension before laking.

Fluid antigen 3 (*Fl.* 3). Fl. 3 was a saline extract. Washed organisms were heated to 75° C. for 1 min., which killed and fixed them, and extracted for some days in the cold in the saline in which they had been heated. The organisms were spun off and the supernatant was used. This antigen did not keep well.

Material used for skin testing

The fluid for skin tests was a glycol extract of freeze-dried or acetone-dried bodies prepared by the method of Feinberg & Morgan (1953). This is not antigenic and apparently causes no disturbance of the immunological state of the animal. The glycol extract contains the substance that determines the serological properties of the variety from which it is made.

Serological tests

The sera, after heating to 56° C. for 20 min., were tested for agglutinins by the method of Kerr & Robertson (1941) and Pierce (1947*b*), and uterine samples were tested for antibody by Pierce's (1947*a*) agar-plate method, the dilutions running from 1/10 to 1/80.

Uterine samples were withdrawn as described by Kerr & Robertson (1941).

Definitions

Free antibody in the uterus is defined as antibody which can be withdrawn from the uterus without trauma, by irrigating the organ with saline.

The local uterine reaction of the sensitized uterus evoked by the instillation of antigen consists of an increase in size, the development of tenseness and, in severe cases, in the outpouring of a clear mucous fluid, with maximum effect after about 2 hr., often disappearing at 4 hr. It is considered to be an allergic reaction of the anaphylactic type, and the words 'local anaphylactic reaction' are used in this sense throughout the paper.

The uterus was examined *per rectum* for the local reaction usually 2 and 4 hr. after the instillation of antigen.

Unless otherwise stated, 'titre' refers to *T. foetus* agglutinins.

RESULTS

(1) *Presence of the antibody in the uterus*

The uterine samples were tested by the agar-plate method (Pierce, 1947*a*). Owing to the difficulty in getting the samples by irrigation with saline the antibody is diluted to an unknown extent and no great weight can be placed upon the exact titre of the material withdrawn. The positive findings are of great importance but the negatives may be less reliable.

In all, six animals (D 4, D 14, K 2, K 9, K 7 and D 13) gave positive samples. They were without exception animals in which antigen, either in the form of living organisms or derived from organisms grown *in vitro*, had been present in the uterus at some time. Intramuscular injection alone did not induce free antibody in the uterus.

A larger number of animals with free antibody in the uterus might have been found if samples had been taken from all the suitable animals under experiment. In six animals sampled the blood contained antibody, and usually the skin reaction was positive. The blood titre, as has been shown in earlier work, rises during active infection with *Trichomonas* and after the introduction of antigen into the uterus. Some of the animals also showed sensitization of the uterus, but we were unable to find any close correlation between the two sets of observations, possibly because of the experimental difficulties. They are therefore treated separately for the sake of clarity.

An animal with free antibody in the uterus after infection with the living organism

D 4. This animal was described by Kerr & Robertson (1947). Its history is recapitulated here because the presence of antibody in the uterus was the sequel

to infection with the organism and not to the instillation of prepared antigen. It had received intramuscular injections of living *Trichomonas* antigen, to which it had responded well. Fifty-six days after the birth of the second calf, D4 was infected with *T. foetus* at insemination, oestrus reappearing in 31 days. The animal was immediately inseminated and exposed to infection. *Trichomonas* appeared in large numbers in 16 days. The parasites were thus present in the uterus of this animal for at least 47 days. They were not seen again by direct examination, though they may have been present for some time longer. Sixty-three days after the last observation of living *Trichomonas* and 13 days after the last oestrus, a uterine sample had a relatively high agglutinin titre (+ + 1:80). Shortly afterwards the animal was successfully inseminated and produced a healthy calf at full term.

The important features about D4 are: (*a*) that the uterine antigen consisted of live *Trichomonas* harboured for at least 47 days: (*b*) that the positive sample occurred 13 days after one oestrus and 10 days before the next; and (*c*) that the recent presence of antibody of relatively high titre did not prevent conception and a normal pregnancy.

Animals with free antibody in the uterus after uterine instillation of antigen

*D*14 was a normal calved cow (see Kerr & Robertson, 1947). Three injections of antigen were given intramuscularly, producing a good blood titre. Fifty-nine days later uterine instillations were begun with freeze-dried *Trichomonas* antigen. Nine days after the fourth instillation the first uterine sample with a titre of + + 1:80 was obtained. Among many negative samples, positive samples were obtained at various intervals after the last instillations of antigen. During the 3 months when the animal was receiving uterine instillations at fairly regular intervals antibody could be demonstrated in good titre on five occasions. Antibody persisted in the uterus certainly for 7 weeks after the cessation of the instillations but could not be demonstrated in samples taken later. D14 showed sensitization of the uterus during the course of the instillation of antigen (see below, p. 410).

*K*2 and *K*9. Free antibody was found in K2 on three occasions and in K9 on one. Neither of these animals was sensitized.

*K*7 and *D*13. In these animals the uterus was sensitized by the instillations and good local reactions were produced. K7 showed free antibody in the uterus on one occasion (+ 1:40); and D13 a titre of + 1:80, 9 days after an instillation. A sample taken 6 days later was negative. These examples demonstrate the presence of free antibody in the uterus; it was obtained only when antigen had been present in the uterus a relatively short time before.

(2) *Sensitization of the uterus*

Sensitization of the uterus to *T. foetus* antigen was first observed during experiments to find whether antigen instilled into the uterus would induce the formation of antibodies in the blood stream.

Eight non-gravid cows of unknown history, cast out of herds for various reasons, were used (Kerr & Robertson, 1943). Two produced no circulating antibody after instillation and six did so. Of these six animals, five had generalized acute allergic

reactions, some of them severe. This early work was carried out with the antigen Fl. 1. Reproducible local anaphylactic uterine reactions, sometimes very acute, were produced with freeze-dried organisms. The fluid antigens, Fl. 1, Fl. 2 and Fl. 3 tended to deteriorate on keeping. Unless stated to the contrary, freeze-dried *Trichomonas* bodies were used as antigen.

Of fifteen animals instilled with antigen, eleven became locally sensitive and one of them (D 13, see below) was sensitized twice in experiments separated by $2\frac{1}{2}$ years. Some of the animals were injected intramuscularly with freeze-dried antigen or with live organisms before the first instillation.

Animals which had received no antigen intramuscularly

D 12. Calved cow of unknown history.

0 day. First uterine instillation (0·5 g.). No local reaction.

18th day. Second instillation (0·5 g.). Definite local reaction at 1 hr. which persisted at 2 hr. At 4 hr. the reaction was declining and at 5 hr. condition was almost normal.

63rd day. Third instillation (0·5 g.). No local uterine reaction.

D 16. Calved cow.

0 day. Seven weeks after calving. First instillation (0·5 g.). No local reaction.

14th day. Second instillation (0·5 g.). Moderate local reaction.

80th day. Third instillation (Fl. 2 = 50 million organisms). No reaction.

93rd day. Fourth instillation (Fl. 2 = 750 million organisms). Very marked local reaction.

109th day. Fifth instillation (Fl. 2 = 1510 million organisms). Moderate local reaction.

D 18.

0 day. Three months after calving, first instillation (0·1 g.). No local reaction.

13th day. Second instillation (0·3 g.). Marked local reaction.

66th day. Third instillation (Fl. 3 = 2097 million organisms). No reaction.

73rd day. Fourth instillation (Fl. 3 = 2097 million organisms). Slight local reaction.

81st day. Fifth instillation (Fl. 2 = 375 million organisms). Marked local reaction.

These animals showed early sensitization from the first uterine instillation, the second instillation setting off the local anaphylactic reaction. Resensitization of the uterus was evoked in D 16 and D 18 by further instillation.

E 11 was not sensitized with the first four instillations. After a pause of 70 days instillations were started again and there was a moderate local reaction at the third instillation.

E 1 was sensitized with four uterine instillations and reacted at the fifth.

D 13 was sensitized with the first uterine instillation of antigen and reacted markedly at the second instillation.

E 4 was not sensitized by five instillations of freeze-dried antigen given at intervals of 3 weeks. No great weight is attached to this animal as it was found to be heavily infected with liver fluke.

Up to this point evidence has been produced:

(1) That antibody is found in the uterus of those animals only which have had antigen in some form introduced into the uterus.

(2) That in normal animals the uterus can be sensitized by the repeated introduction of antigen.

(3) That uterine instillations of antigen produce circulating antibody in the blood stream (Kerr & Robertson, 1943).

We have not produced evidence in the foregoing experiments as to whether the antibody in the uterus was produced locally or reached that site from the circulation since at the time of the finding of the free antibody or of the manifestation of the sensitivity there was always some circulating antibody in the blood.

In the following experiments the matter was approached from the opposite direction. If the uterus could be sensitized from the blood stream in animals highly immunized by intramuscular injection alone, it should be possible to set off the local anaphylactic reaction in the uterus by the first instillation of antigen. This we were never able to do.

(3) *Evidence for the local production of antibody in the uterus*

Animals injected intramuscularly with antigen before the instillation of antigen into the uterus

All of ten animals described in this section had high blood titres for *T. foetus* 1/384 to 1/1576, at the time of the first uterine instillation. Where *Brucellus abortus* dead vaccine was injected the *Br. abortus* titres ranged from 1/640 to 1/1280. None of the ten animals reacted to the first uterine instillation of antigen.

Six animals (D 10, D 14, K 7, K 10, D 13 (1952) and P 6) did not react to the first instillation of antigen but reacted to subsequent instillation.

D 10.

0 day. Three months after calving. First instillation of antigen (Fl. 3 = 500 million organisms). There was no local reaction.

18th day. Second instillation (Fl. 3 = 1500 million organisms). Definite local reaction.

52nd day. Third instillation (Fl. 3 = 1500 million organisms). No local reaction.

66th day. Inseminated. Pregnancy carried through to full term with birth of a healthy calf.

This animal, in spite of a high blood titre at the time of the first instillation, did not react. Like the animals not injected intramuscularly, such as D 12, D 16 and D 18, it was sensitized by the first instillation and the second set off the reaction.

It should be noted that in D 10 the instillation of antigen as recently as 14 days before insemination did not prevent conception.

D 14. Free antibody was obtained from this animal on a number of occasions (see above, p. 408).

0 day. First uterine instillation of antigen (0·25 g.). No local reaction.

7th day. Second instillation (0·25 g.). No reaction.

14th day. Third instillation (0·25 g.). No reaction.

28th day. Fourth instillation (0·5 g.). Severe local reaction.
37th day. Uterine agglutinins + + 1:80.
44th day. Fifth instillation (Fl. 1 = 1000 million organisms). Severe local reaction.
45th day. Uterine agglutinins + 1:20.
50th day. Uterine sample negative.
74th day. Sixth instillation (0·5 g. trichloracetic precipitated Fl. 1 antigen). Moderate local reaction.
80th day. Uterine agglutinins + + 1:80.
105th day. Uterine agglutinins + 1:80.
121st day. Uterine sample negative.
127th day. Uterine agglutinins + 1:80.

After this the uterine samples were negative. This animal was clearly sensitized by the first three instillations of antigen, and it would appear that the 0·5 g. of the fourth instillation not only combined with the antibody presumably fixed in the cells of some part of the uterus to set off the local anaphylactic reaction, but, in addition, stimulated the production of free antibody found in the sample taken 9 days later.

K 7 was given injections of *Br. abortus* dead vaccine so as to have a high titre against both organisms at the time of the uterine injections. The abundant antibody in the blood had not sensitized the uterus which did not react to the first instillation. However, this instillation sensitized the uterus, and a fortnight later the second instillation produced a marked local reaction. The third and fifth instillations did not, but there was a very severe reaction after the fourth. Uterine samples tested throughout the experiments contained neither *Trichomonas* nor *Br. abortus* agglutinins, except that taken 13 days after the fifth instillation which had a low titre (+ 1:10 for *T. foetus*).

K 10 had a high titre against *T. foetus* maintained by repeated intramuscular courses of antigen for 17 months and had also a recent titre against *Br. abortus* (dead vaccine). Instillations of *T. foetus* antigen were begun 2 months after calving. There was no local reaction to the first four instillations. The uterus reacted markedly to the fifth instillation. Uterine samples were negative for both organisms.

D 13. 1952 series. There was a high titre against *T. foetus* and *Br. abortus* (dead vaccine) when these experiments were begun. The animal did not react to the first four instillations but did to the fifth. The uterine samples were negative for both organisms.

P 6 had a high blood titre against *T. foetus* maintained by intramuscular injections of antigen for 1 year and 5 months before the beginning of the instillations. It did not react to the first four uterine instillations of antigen but reacted severely to the fifth instillation and moderately to the sixth. P 6, like the animals described above, failed to react to the first instillations, showing that the uterus had not been sensitized by the antibody circulating even for a prolonged period in the blood stream. Three animals (K 2, K 9 and P 5) of the ten in this series did not become sensitive upon repeated instillation of antigen.

K 2. A high blood titre and a very good skin sensitivity (8·5 mm.) were induced in this animal by intramuscular injection of antigen. It did not respond to the first instillation nor to six subsequent ones, although free antibody was found on two occasions.

K 9. Had a high blood titre against *T. foetus* for more than a year and before these experiments had also had *Br. abortus* dead vaccine injected subcutaneously. None of five uterine instillations of *T. foetus* antigen produced a local reaction. Uterine samples tested at intervals throughout the experiments were negative for both organisms, with the exception of that taken 9 days after the fifth instillation which had a *T. foetus* titre of + 1:40 but contained no agglutinins for *Br. abortus*.

P 5 had a high antibody titre against *T. foetus* for 15 months before the beginning of the instillations. There was not reaction to five instillations. The uterus was, however, sensitized later (see below, p. 413) so that it is possible that these three animals might have been sensitized if the instillations had been resumed after a pause, but the purpose of the experiments at this time was to see if the uterus could be sensitized by intramuscular injection alone (Table 1).

Table 1. *Active sensitization of the uterus of the cow by the local instillation of* Trichomonas foetus *antigen*

State of blood at time of first instillation of antigen	Animals sensitized per group	Instillation at which 1st local anaphylactic reaction took place					
		1st	2nd	3rd	4th	5th	6th
No antibody	6/7	0	4	0	0	1	1
Antibody present	6/9	0	2	0	1	3	0

K 5. The last animal of the ten was treated differently in that live organisms were used. The blood titre 3 weeks after the third intramuscular injection was 1:784. About 10,000 million orgnisms (equal to approximately 0·8 g. dried weight) were instilled. There was no local reaction and the animal was not further treated. K 5 does not appear in Table 1, although it is included in the ten animals with circulating antibody considered above.

It is interesting to note that this instillation of live organisms did not produce any infection. At no time has the uterine instillation of live organisms ever resulted in an infection in our experiments except when the parasites were introduced into an animal at oestrus and accompanied by semen, or after service by the bull. The attempt to produce infection even at oestrus without semen has been made in this work and has never succeeded. The successful establishment of the uterine infection seems to be determed by conditions prevailing at the beginning of pregnancy. Live organisms were used for K 5 as it was thought at the time that they might possibly be a better antigen.

(4) *Passive sensitization of the uterus*

It seemed relevant to the work described above to see if the uterus of the cow could be sensitized passively *in vivo* by the instillation of serum with a high antibody titre against *T. foetus*.

Sixty ml. of such antiserum were instilled into the uterus of a normal cow (L 2) about 4 months after it had calved. There was no reaction to the serum. Antigen 0·5 g. was instilled into the uterus 9 days later, and there followed a characteristic local anaphylactic reaction. A second instillation of antigen 7 days later also produced a local reaction. This second response may have been due either to the residual passive sensitization or to the active sensitization of the uterus by the antigen. It should be noted that passively sensitized animals showed a clear rise in the *T. foetus* blood titre 7 days after the instillation of antigen, so that the antigen not only set off the anaphylactic reaction but was also to some extent absorbed. No change in the titre of the circulating serum could be detected after the instillation into the uterus of the anti-trichomonas serum.

Two animals, K 9 and P 5, which were not actively sensitized by five instillations of antigen were used for passive sensitization experiments.

K 9, after a rest of 3 months, was successfully sensitized by instillation of anti-trichomonas serum (E 7*c*), and gave a characteristic uterine reaction when 0·5 g. of antigen was introduced 6 days later.

P 5, after a rest of $4\frac{1}{2}$ months, had 60 ml. of anti-trichomonas serum instilled into the uterus. Seven days later 0·5 g. of antigen was instilled but produced no uterine reaction. A second instillation a week later produced a marked reaction as did an instillation 7 days later. Forty days later the uterus was still sensitive. Here the serum with a titre of 1:768 did not sensitize the uterus passively but the instillation of the antigen did.

P 10 as a control was given, 6 weeks after calving, a uterine instillation of 60 ml. of the pooled serum of two normal young heifers. The instillation of 0·5 g. of antigen produced no local reaction. Fifteen days later a second instillation of 0·5 g. was followed by the characteristic reaction. A third instillation of antigen 7 days later was also followed by a definite local reaction, the uterus becoming large and tense. In this animal the normal serum, as expected, had not sensitized the uterus, but the presence of antigen produced an active sensitization as shown by the reaction to the second and third instillation of antigen. P 5 and P 10 can be considered as animals which were actively sensitized by the instillation of antigen. They are not included in Table 1. No further work was done on the passive sensitization of the uterus *in vivo*.

It is of interest to record that *Trichomonas* antigen (Blendor) can be used to produce the classic anaphylactic reactions in guinea-pigs. Mr A. E. Pierce has kindly permitted us to quote some of his unpublished results on sensitization in the guinea-pig. He was able to sensitize the uterus of guinea-pigs passively *in vivo* by intravenous injection of rabbit anti-trichomonas serum and to produce the anaphylactic reaction in the Dale bath. He was also able to sensitize the guinea-pig passively *in vivo* by intravenous injections of anti-trichomonas rabbit serum

and bring about fatal anaphylactic shock by intravenous injection of *Trichomonas* antigen (Blendor). Mr Pierce was not able to sensitize the uterus of the guinea-pig either *in vivo* or *in vitro* by the use of bovine anti-trichomonas serum.

This is in agreement with the results recorded by Hartley (1951) that antitoxin prepared in the serum of rabbits, man and guinea-pig would sensitize guinea-pigs, while that made in horse, sheep, ox, goat and pig would not.

DISCUSSION AND CONCLUSIONS

Our results establish:

(1) The presence in the uterus of antibody accessible to withdrawal by irrigation. This antibody has always resulted from the presence of antigen in the lumen of the uterus.

(2) That neither free antibody in the uterus nor the sensitization of the uterus are produced by intramuscular injection of antigen, even when antibody has circulated in the blood stream for periods from a few weeks to many months.

(3) An active sensitization of the uterus follows only upon repeated introduction of antigen into the uterus.

We conclude that antibody can be produced locally by some of the cells in the uterus when antigen is present in the lumen. Local antibody production is no longer an unexpected phenomenon. McMaster & Hudack (1935) showed that agglutinins are formed within the draining lymph nodes of mice following intradermal injection of killed cultures of micro-organisms, and more recently, Oakley, Batty & Warrack (1951) have shown that antibody can be produced in the skin, fat or voluntary muscles of guinea-pigs and rabbits by a single secondary injection of alum-precipitated toxoid into these tissues.

We have evidence that the uterus will show an anaphylactic reaction with antigen as much as 2 months after the last instillation of antigen, but that appears to be about the limit of the duration of the sensitivity in our experimental work. The longest period after the instillation following which we have still found free antibody was 7 weeks, but that may not be the actual limit. There is one example (D 4) where free antibody was found 63 days after the last appearance of living *Trichomonas* in the uterus.

Free antibody could be obtained and the anaphylactic reaction could occur at all periods during the 3-week cycle of the uterus of the cow. Both these findings appear to be independent of oestrus itself. We have not been able to get detailed evidence of how exactly oestrus is related to the processes described.

The relatively short duration of the uterine sensitivity is a feature that may seem unusual when compared with the persistence of other types of allergic sensitivity. We would suggest rather tentatively that the disappearance of the uterine sensitivity with the passage of time in the absence of further antigen would appear to be related to the special circumstances of the site. It might be that the cells in which the antibody is formed belong to the deciduous cells of the organ, and that they are gradually shed in the cyclical process of preparing the uterus for the reception of the ovum.

With the limitations of experimental work of this kind *in vivo* with large animals this can only be a speculation, but it does agree with the facts.

SUMMARY

1. Antibody occurs in the uterus and can be withdrawn by irrigation. Intrauterine antibody results from the introduction of antibody into the lumen of the uterus.

2. Neither free antibody nor the sensitization of the uterus has been produced by intramuscular injection of antigen, even when antibody has circulated in the bloodstream for periods from a few weeks to many months.

3. Active sensitization of the uterus follows upon the repeated introduction of antigen into the uterus.

4. It is concluded that antibody can be produced locally by some of the cells in the uterus when *T. foetus* antigen is present.

5. The uterus of the cow can be sensitized passively *in vivo* by the instillation of bovine anti-trichomonas serum so that the instillation 6–10 days later of *Trichomonas* antigen will produce the characteristic local anaphylactic reaction.

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