THE RELATIONSHIP OF POLYMORPHONUCLEAR LEUKOCYTES TO INFERTILITY IN UTERI CONTAINING FOREIGN BODIES*

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The intrauterine contraceptive device is a potentially important factor in the world population problem, primarily because it provides an effective method of contraception that does not require continuous motivation on the part of the user. In addition, its cost is trivial, its insertion requires only a few minutes, and normal fertility returns quickly after its removal. There is also some reason (1, 2) to hope that the difficulties in its use, such as expulsions and rare pregnancies, can be overcome, since we still have much to learn about it. Even the basis of its contraceptive action remains unknown.

Previous observations in the rat and other species indicated that a foreign body in the uterus had no systemic effect, but only a local effect in the uterus. This local effect in the uterus appeared to be a direct toxicity for blastocysts or spermatozoa, rather than an inhibition of implantation. For example, a foreign body in the rat uterus (3, 4) had no effect on fertilization or tubal transport of ova, but a few hours after blastocysts entered the horn containing a foreign body they could no longer be recovered; blastocysts could be recovered from control horns for about 24 hr. Thus, in the rat horn containing a foreign body, blastocysts were no longer present at the time of implantation. In the mouse (5), similar observations were made, and in addition it was noted that spermatozoa were killed. In the sheep (6), spermatozoa were killed in the uterus and fertilization was prevented. And in the rabbit (7), pregnancy occurred throughout the uterus except for the portion in direct contact with the foreign body. Observations such as these have led to the notion that a foreign body creates a hostile environment in the uterus (8). The experiments reported here clarify the role of inflammation and of infection in the infertility of uteri containing a foreign body.

Materials and Methods

Text-fig. 1 is a diagram of the rodent uterus, detailing the regions of special interest in these studies. The region FB (foreign body) was the segment of the uterine horn which contained

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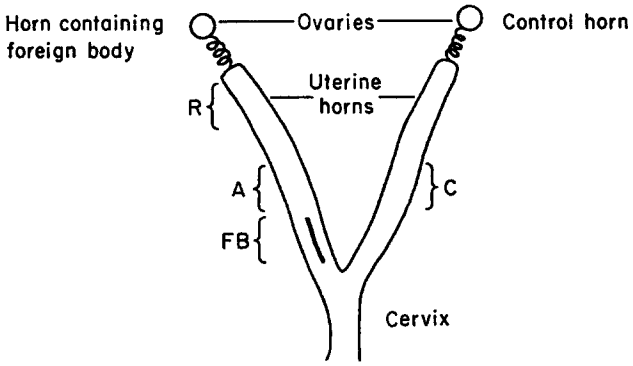
the foreign body in the lumen. The segment A (adjacent) was a few millimeters removed from contact with the foreign body, and R (remote) was far removed from the foriegn body. The midportion of the control horn was segment C (control). In each of the species used in this study (rat, rabbit, mouse) the foreign body occupied roughly 20% of the length of the uterine horn. In most cases the foreign body was located in the cervical end of the uterine horn, but in some cases it occupied the ovarian end or the center.



TEXT-FIG. 1.

Rats.—Holtzmann rats were used in these studies. With the animal under ether anesthesia, a uterine horn was exteriorized and approximately 10 mm of 3-0 surgical silk suture was passed along the lumen with the aid of a straight atraumatic needle. A knot at one end of the suture was drawn up against the serosal surface of the horn; the other end of the suture lay free in the peritoneal cavity. Fig. 6 shows such a foreign body in the uterus. Although the sutures were either autoclaved or soaked in benzalkonium chloride (Zephiran), the conditions of foreign body insertion cannot be considered aseptic.

For bacteriological studies, diestrus animals whose foreign bodies had been in place for 30–60 days were killed by a blow on the head. Whole uterine horns were removed under sterile conditions by transecting at the uterotubal and uterocervical junctions and by trimming away most of the mesometrium. The horns were then homogenized in sterile tissue grinders; foreign bodies were left in place. The bacteriological study included animals into which foreign bodies were inserted on different days, and which were housed in separate cages.

For measurements of lysozyme in the uterine lumen, whole horns were obtained from animals in diestrus and on day 5 of pregnancy. Day 1 of pregnancy refers to the day spermatozoa were found in the vaginal smear; implantation of blastocysts normally occurs on day 5 of pregnancy. Tissues for histological study were taken from regions FB, R, and C in all stages of the estrus cycle and pregnancy; smears (or touch preparations) were also made from freshly cut ends as the uterine horns were transected in these regions.

Rabbits.—Female rabbits weighing 2.0–4.5 kg were anesthetized by intravenous injection of 25 mg/kg of pentobarbital, followed by ether. A 20 mm length of polyethylene catheter tubing (Clay Adams Inc., N. Y., PE 200) was inserted into each uterine horn through a 2 mm incision. A short length of silk suture was attached to the catheter tubing and passed out through the incision. The incision in the uterus was closed with another suture, and the catheter tubing was secured by joining its suture to the one closing the incision. Catheter tubes, with their sutures attached, were soaked in benzalkonium chloride for at least 1 wk before use.

The rabbits were mated after allowing at least 14 days for recovery from the surgery; the

day of mating was designated day 1 of pregnancy. Animals were killed on day 14 of pregnancy for bacteriological, histological, and fertility studies, and animals were killed on day 5 of pregnancy for histological studies and lysozyme measurements. Tissues for bacteriological studies were removed from region FB and homogenized in sterile tissue grinders. Tissues for histological studies were removed from regions FB and R, and from regions adjacent to implantation sites. For measurements of lysozyme in the uterine lumen, each horn was transected in situ 5 mm beyond the end of the foreign body. The resulting four segments, two containing foreign bodies and two not, were then removed from the animal and lysozyme was measured in each.

Mice.—Albino mice were anesthetized with ether and a short length of 3-0 silk suture was inserted into one uterine horn, as described for rats. After 15 days the mice were killed without regard to the stage of the estrus cycle. Tissues for histological examination were obtained from regions FB, R, and C; smears were also made from the freshly cut ends as uterine horns were transected in these regions.

Germfree Rats.—Male and female germfree rats were purchased from Carworth Farms, New City, N. Y., and Charles River Laboratories, North Wilmington, Mass., and maintained in our laboratory in plastic isolators (Isolab from Bioquest, Hackensack, N. J.). Packaged food and bedding, sterilized by irradiation, were also obtained from Bioquest. A 4% peracetic acid spray (FMC Corp., N. Y.) was used for sterilization; other procedures were standard in germfree work (9). Silk or stainless steel sutures (Ethicon Inc., Somerville, N. J. 3-0) were inserted as described for conventional rats, except that pentobarbital was used for anesthesia and operations had to be performed in the isolators through plastic gloves. When the foreign body had been in situ for the desired length of time, the female was placed with a male and vaginal smears were made each morning. The smears were placed on glass microscope slides that remained inside the isolator until an entry of food or water into the isolator was required, at which time the slides were removed and the smears examined; spermatozoa were easily recognized. The day on which spermatozoa were present in the smear was termed day 1 of pregnancy; the animals were killed with ether (to avoid rupturing the cecum) on day 8 of pregnancy.

Some of the females did not mate, although they spent as long as 30 days with a fertile male. These females cycled irregularly or not at all and they were killed in diestrus. Although our experience was confined to a small number of animals, it was clear that the reproductive performance of Carworth animals was superior to that of Charles River animals, under identical conditions in our laboratory.

A fresh pellet of feces was collected for culture inside the isolator immediately prior to sacrificing an animal. The cervix and vagina, and occasionally a small piece of the uterus with suture, were then removed under sterile conditions and prepared for culturing. The rats were considered germfree if the cecum was distended and no bacteria could be cultured from the tissues or feces.

Tissues for histological studies were obtained from regions FB, A, and C. Lysozyme in the uterine lumen of pregnant females was measured only in region FB. For lysozyme in diestrus females, the whole horn containing a foreign body was either used intact, or was transected between FB and A and each of the two regions was treated separately. The whole control horns of the diestrus females were used for lysozyme measurements.

Preparation of Tissues for Culturing.—Tissues were homogenized in glass tissue grinders containing 2.0 ml of sterile, charcoal-filtered water. Pellets of feces were suspended by shaking in broth medium. Aliquots of 0.5 ml of the tissue homogenate or feces suspension were transferred to broth media and 10-fold dilutions were made to 10^{-8}. Loopsful of homogenate or suspension were plated on agar media.

Culture Media.—The base medium of Schaedler et al. (10) was used as a broth for aerobic

and anaerobic incubations, and base medium plus 1.5% agar agar was used for plates and slants. The base medium agar plates were incubated anaerobically and in a candle jar. The base medium slants were covered with beef serum and incubated anaerobically. Trypticase soy (Baltimore Biological Laboratories, Baltimore, Md.) broth and agar plates were incubated aerobically. All incubations were at 37°C. Details of anaerobic incubation have been described by Schaedler et al. (10).

Histological Procedure.—After fixation in Bouin's solution, the tissues were dehydrated in graded alcohols, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissue smears were made by touching the freshly cut end of a uterine horn to a glass slide, flash drying in a jet of air, and staining with Wright's blood stain.

Lysozyme Measurements.—Lysozyme served as an index of inflammation, since it was present in polymorphonuclear leukocytes but was not detectable in washings of the normal uterine lumen. Washings were collected from the rat and rabbit uterine lumen by flushing 0.5 ml and 1.0 ml respectively of Ringer's solution through the lumen. Fluid remaining in the lumen was gently squeezed out with forceps. This technique almost certainly did not result in recovery of all the lysozyme, since histological observations showed that some leukocytes remained in the lumens of horns that had been so flushed.

Immediately after collection, the suspension was centrifuged at 1000 *g* for 10 min to deposit the cells, and the supernatant was assayed for lysozyme activity. The cell pellet was disrupted by repeated freezing and thawing, suspended in 0.5 ml or 1.0 ml (rabbits) of 0.05 M phosphate buffer at pH 6.8, centrifuged at 1000 *g* for 10 min, and the supernatant was assayed for lysozyme. 0.1 ml of the solution to be measured was added to 0.9 ml of a suspension of *Micrococcus lysodeikticus* in 0.05 M phosphate buffer at pH 6.8, and an optical density of about 0.3/cm. The measurements were carried out in a Zeiss spectrophotometer with sensitivity and stability sufficient to detect a Δ OD of -0.001/min. The lysozyme activities in the supernatants and cell extracts were compared to the activities of standard solutions of 3 × crystallized lysozyme (Pentex Corp., Kankakee, Ill.) and converted to total amounts of lysozyme present.

RESULTS

Conventional Rats.—As seen in Table I, polymorphonuclear leukocytes were found along the entire length of the conventional rat uterine horn containing a silk suture at all stages of the estrus cycle and pregnancy (Fig. 1). The leukocytes were present in the lumen and occasionally in glands, between the epithelial cells lining the lumen, and especially in the endometrial stroma just beneath the epithelial basement membrane. In contrast, no polymorphonuclear leukocytes were found in control uterine horns. The presence of leukocytes in the rat uterine horn containing a silk or stainless steel suture has also been reported by Greenwald (11). Many studies (3, 8, 11–13) have shown that the entire rat horn containing a silk suture is infertile, whereas the control horn remains normally fertile. The leukocyte infiltration was thus associated with the infertile region.

At least 95% of the leukocytes that entered the uterine lumen were polymorphonuclear cells. This was observed as early as 2 days after insertion of the foreign body, and remained unchanged at least as long as 100 days. The inflammatory response was thus unusual in that the acute, or polymorphonuclear, phase persisted indefinitely.

Rat polymorphonuclear leukocytes were found to contain lysozyme, which served as a convenient marker for these cells. Lysozyme was always recovered from the lumens of horns containing foreign bodies but never from control horns, thus corresponding to the distribution of polymorphonuclear leukocytes (Table II). Furthermore, the lysozyme that was recovered from the uterine lumen was partly contained in polymorphonuclear leukocytes and partly free in solution. The concentration of free lysozyme in solution in the uterine lumen, assuming a fluid volume there of 0.01 ml, would be of the order of 100 μg/ml. It is probable that lysozyme and other components of polymorphonuclear leukocytes are released as a consequence of the death of the cells. In support

TABLE I

Histological Studies of Uterine Tissue

Number of specimens showing infiltration of polymorphonuclear leukocytes.

Animal	Horn containing foreign body				Control horn	
	Segment in contact with foreign body		Segment not in contact with foreign body*			
	Polymorphs		Polymorphs		Polymorphs	
	Present	Absent	Present	Absent	Present	Absent
Conventional rat	100	0	100	0	0	100
Germfree rat	11	0	1‡	9	0	6
Mouse	15	0	15	0	8	7

* In the germfree rat this tissue was obtained at a distance of 2–4 mm from the foreign body.

‡ This is the infertile horn of column 3, Table V.

of this concept, we observed that many of the polymorphonuclear leukocytes recovered from the lumen of the horn containing the foreign body were unable to exclude a protein-bound dye.

Bacteria were cultured from uteri containing foreign bodies but none were found in uteri of unoperated rats (Table III). In all but one of the animals, at least 10^8 bacteria were present in horns containing foreign bodies, and 10^1–10^4 bacteria were found in control horns. Two species of bacteria were recovered in anaerobic cultures from almost every animal, and at least a dozen other species were isolated from one or more animals. The species present were quite diverse in morphology and growth requirements. Although none of them was identified, the most common one was clearly a vibrio and almost all were gram-negative. The species present in the control horn were usually identical with those recovered from the horn containing the foreign body. An attempt was made in several animals to recover bacteria from the horn containing a foreign body

by flushing the lumen with sterile water; this was not successful. The bacteria appear to be embedded either in the endometrium or in a relatively insoluble mucous layer.

TABLE II

Lysozyme in the Uterine Lumen

Infertile Region			Fertile Region	
Rats in diestrus and on day 5 of pregnancy				
Horn containing foreign body			Control horn	
Supernatant	Cell extract		Supernatant	Cell extract
μg	μg		μg	μg
0.66	0.80	Diestrus	0.00	0.00
1.8	0.96		0.00	0.00
0.96	1.0		0.00	0.00
0.66	1.3	Day 5	0.00	0.00
0.52	0.56		0.00	0.00
0.81	0.81		0.00	0.00
0.55	0.69		0.00	0.00
0.81	0.61		0.00	0.00
Rabbits on day 5 of pregnancy				
Segment containing foreign body			Remainder of horn*	
Supernatant	Cell extract		Supernatant	Cell extract
μg	μg		μg	μg
0.62	1.4		0.10	0.15
0.90	2.8		0.08	0.10
0.17	1.4		0.00	0.10
0.22	0.75		0.00	0.00
0.31	0.25		0.12	0.00
0.23	0.48		0.00	0.00
0.63	1.0		0.03	0.12
0.63	1.5		0.00	0.03
0.12	0.12		0.00	0.00
0.08	0.12		0.00	0.00

* Transected 5 mm from the end of the foreign body.

The administration of antibiotics: streptomycin-penicillin (Strep-Combiotic®, Charles Pfizer & Co., New York), 15 mg per day, or oxytetracycline (Terramycin®, Pfizer), 5 mg per day, or a combination of both, for seven days, did not eliminate the infection; in fact, if anything, larger numbers of bacteria

were recovered from horns containing foreign bodies. The horn containing the foreign body in rats given antibiotics remained infertile and the control horn remained fertile.

Mice.—The results of histological examinations of mouse uteri are presented in Table I. The pattern of polymorphonuclear leukocyte infiltration was different from that in the rat in one respect. Whereas these leukocytes were never observed in control horns of rats, they were found in 8 of the 15 mouse control horns examined. In the horn containing the foreign body, polymorphonuclear leukocytes occurred along the entire length of the horn (Fig. 2). Doyle and Margolis (5) found that the entire mouse horn containing a foreign body was infertile, and that the fertility of the control horn was also markedly reduced. Thus the pattern of infertility in the mouse also corresponded to the pattern

TABLE III

Bacterial Flora of Uteri From Conventional Rats

Animal	Horn containing foreign body		Control horn	
	No. of bacteria	Species of bacteria	No. of bacteria	Species of bacteria
1	10^8	5	10^3	5
2	10^8	3	10^3	3
3	10^8	4	10	1
4	10^8	6	10^2	5
5	10^8	3	10	1
6	0	0	0	0
7	10^8	3	10	1
8	10^8	3	10^4	3

of polymorphonuclear leukocyte infiltration. The intensity of the inflammation in horns containing foreign bodies and the extension of some inflammation to control horns suggest that bacteria were present, but no cultures were made from these uteri.

Rabbits.—Previous studies had established that a foreign body in the rabbit uterus was rather ineffective in preventing pregnancy when compared to the rat or mouse. In the present study, the number and positions of embryos in 10 rabbit uterine horns containing 20 mm long pieces of polyethylene catheter tubing were observed on day 14 of pregnancy. It was concluded from these observations that the entire rabbit uterus, except for the segment in direct contact with the foreign body, was fertile, as evidenced by the frequent occurrence of normal embryos immediately adjacent to the foreign body (Fig. 3). No embryo was ever observed in the segment with the foreign body, but 42 embryos were present elsewhere in the 10 horns. Embryos were found on the cervical side of the foreign body as well as on the ovarian side.

The histological examination of the rabbit horn containing a foreign body revealed only a few leukocytes per transverse section, and did not demonstrate markedly more leukocytes in the segment that contained the foreign body than in the remainder of the horn. Measurements of lysozyme in the two segments did reveal differences, however (Table II), indicating a slight inflammatory response to the presence of the foreign body. The 2 cm segment in contact with the foreign body always contained lysozyme, whereas the remaining 8 cm had only a trace. We also observed that the number of polymorphonuclear leukocytes in the tissue and the amount of lysozyme recovered from the uterine lumen depended on the handling of the foreign body before its insertion. In early experiments in which the polyethylene tubing was held with bare hands during the attachment of its suture, then sterilized in benzalkonium chloride for only 20 min., 10–20 μg of lysozyme was recovered from the lumen, a dense suspension of leukocytes accumulated in the lumen of the tube, and extensive inflammation was observed histologically in region FB (Fig. 4). In later experiments, in which the catheter tubing was handled throughout with sterile technique and was not placed in benzalkonium chloride, slightly less lysozyme was recovered than the amounts in Table II and few leukocytes accumulated in the tube. The restriction of the inflammation to the segment containing the foreign body, demonstrated by the measurements of lysozyme, was consistent with the fertility pattern observed in this species.

The bacteriological studies on the rabbit uterus were entirely negative. No growth was ever obtained from the segment in contact with the foreign body, in any medium. This observation, and the absence of extensive inflammation, indicate that the foreign body does not usually lead to infection in the rabbit uterus.

Germfree Rats.—Our studies on the conventional rat uterus containing a silk suture indicated that bacterial infection regularly occurred. This infection might be responsible for all or part of the inflammation, and might also exert an antifertility effect. Studies were therefore undertaken in the germfree rat to attempt to separate the effects of inflammation from those of infection. A foreign body in the uterus of germfree rats caused an infiltration of polymorphonuclear leukocytes into the uterus (Fig. 5). However, in the germfree uterus these polymorphonuclear leukocytes were present only in the tissue and lumen immediately adjacent to the foreign body, whereas in the conventional rat uterine horn containing a foreign body the polymorphonuclear leukocytes were distributed through the entire length of the horn. Since the foreign body occupied only about 20% of the uterine horn, the remaining 80% of the germfree horn was free of inflammation. Even at a distance of only 2 mm from the foreign body there were no polymorphonuclear leukocytes. As shown in Table I, there was only one exception to this pattern in the 10 germfree animals examined.

The measurements of lysozyme in uterine horns of germfree rats are presented in Table IV. In horns containing a foreign body, the 1 cm segment in contact with the foreign body always contained lysozyme, whereas the remaining 4 cm segment had essentially none. The lysozyme measurements thus were in accord with the histological observations on the distribution of leukocytes.

There was no consistent difference in the histological appearance of germfree uteri containing a stainless steel, as compared to a silk, suture. Similarly, the amounts of lysozyme recovered from uteri with the two kinds of foreign bodies did not appear to be different. Three animals were kept in germfree conditions

TABLE IV

*Lysozyme in Uterine Lumens of Germfree Rats**

Horn containing foreign body				Control Horn	
Segment in contact with foreign body		Segment not in contact with foreign body‡			
Supernatant	Cell extract	Supernatant	Cell extract	Supernatant	Cell extract
μg	μg	μg	μg	μg	μg
1.4	0.69	0.06	0.00	0.00	0.00
0.70	0.20	0.00	0.00	0.00	0.00
0.65	0.07	0.00	0.00	0.00	0.00
0.40	0.16				
0.35	0.30				
0.26	0.17				

* Foreign bodies were in place 25–55 days. Animals were killed in diestrus or on day 8 of pregnancy.

‡ Transected 2 mm from end of foreign body.

for 50 days with their foreign bodies in place, and the inflammation at the end of this period was still a polymorphonuclear response of typical intensity.

It was of interest to study the fertility of uterine horns containing foreign bodies in the germfree rat, since a large portion of such horns was free of inflammation. The results of these studies are presented in Table V. When the foreign body was in the ovarian end of the uterine horn, blastocysts entered the inflamed region and probably spent about 1 hr passing through it (14). This apparently resulted in the death of all blastocysts entering that horn, for no embryos subsequently grew in the noninflamed lower uterine segment. When the foreign body was in the cervical end, blastocysts entered a noninflamed region. Only those few blastocysts that would normally migrate to the cervical end for implantation were exposed to the inflammation there. As Table V shows, most blastocysts survived and implanted when the foreign body was in the cervical end; the implantation sites were examined histologically and were

found to contain normal embryos. Fig. 6 shows the appearance of the uterus from a germfree rat on day 8 of gestation.

One germfree animal, having five embryos above a cervical end foreign body and eight embryos in the control horn, was released to conventional environment on day 8 of pregnancy. At term she delivered 10 normal pups, and was then mated in the postpartum estrus to a conventional male. On the 10th day of gestation there were eight embryos in the control horn again and none in the horn with the foreign body. This horn now harbored 10^8 bacteria of three species, and inflammation was present throughout its length. These results showed that a uterus containing a silk suture could acquire a bacterial flora even when the conditions of foreign body insertion were absolutely aseptic, and that inflammation and infertility of the entire horn developed in associ-

TABLE V

Influence of the Position of the Foreign Body on Fertility in the Germfree Rat Uterus: Number of Embryos per Horn

Foreign body in ovarian end	Control horn	Foreign body in cervical end	Control horn
0	7	5	6
0	7	3	6
0	6	5	8
		2	4
		0*	4

* Inflammation throughout this horn; reason unknown.

ation with this bacterial infection. Bacterial cultures were made from the uteri of two other germfree animals that received foreign bodies under germfree conditions and were later released to the conventional environment. Neither of these females was mated. After 21 days in the conventional environment, one of the animals had acquired a bacterial flora in the uterus while the other apparently had not.

DISCUSSION

In rats, rabbits, and mice, there was a chronic infiltration of polymorphonuclear leukocytes into the uterine lumen in the infertile regions of uteri containing foreign bodies. In rats, and probably in mice, the presence of the foreign body was associated with a bacterial infection that spread the inflammatory response throughout the horn containing the foreign body, and probably into the mouse control horn as well. In rabbits, the presence of the foreign body did not seem to lead to infection. Polymorphonuclear leukocyte contents, as judged by the behavior of lysozyme, were liberated into solution in the uterine lumen. Since the uterine lumen contains little fluid when blastocysts

arrive, the concentration, along the epithelial surface, of substances derived from polymorphonuclear leukocytes could be very high.

Consistent with the correlation between inflammation and infertility in the three species, the noninflamed region of the germfree rat uterus with a foreign body in its cervical end was fertile; and if the foreign body was in the ovarian end of the horn so that all ova were exposed to the inflamed region the entire horn was infertile. Thus in a single species a change from the generalized inflammation of the conventional rat horn to the localized inflammation of the germfree rat horn resulted in a change from complete infertility to local infertility. On the assumption that the uterine physiology of conventional and germfree rats is identical except with regard to the bacteria and inflammation, it appears that inflammation is largely responsible for the contraceptive action of a silk or stainless steel foreign body in the uterus. Bacteria may make some contribution to the toxicity of the rat uterine lumen, but the results obtained in germfree rats prove that bacteria are not essential for contraception.

In addition to the species studied here, inflammation has been noted in association with a foreign body in the uteri of cows (15, 16) and sheep (17). Studies of biopsy specimens from human uteri containing foreign bodies showed that leukocytes appeared in only about 20% of the specimens (1, 18–22). The failure to find leukocytes in every uterus by this technique has been interpreted to mean that inflammation is not a significant part of the response to a foreign body in the human uterus (23). Recent studies, however, which utilized whole human uteri, found that leukocytes were always present in the tissue that was in contact with the foreign body (24–26). This suggests that inflammation is limited to the region of the foreign body in the human uterus, and that the absence of leukocytes in many biopsy specimens is a consequence of random sampling. Consistent with this interpretation is the fact that no bacteria could be cultured from endometrial scrapings of human uteri containing plastic foreign bodies (27). The human uterus thus seems to fall into the pattern of rabbit and germfree rat uteri.

In the rabbit uterus, a clean polyethylene tube caused only a slight inflammatory response, and was only minimally effective in preventing pregnancy. The fact that polyethylene was chosen as a material for the intrauterine device in humans largely because it does not provoke a significant tissue reaction should therefore be considered in relation to the possibility that inflammation is the basis of the contraceptive action of intrauterine devices. Such consideration should caution that a polyethylene foreign body in the human uterus may not provide an optimum contraceptive effect.

Although in this paper we do not wish to discuss in detail the other effects of uterine foreign bodies and their relation to the inflammatory reaction, a few comments with regard to spermatozoa are pertinent. Substances in the inflammatory exudate may be toxic for spermatozoa as well as blastocysts. A study of

the effects of a silk suture in the mouse uterus (13) showed a high percentage of unfertilized ova in the oviducts after mating, suggesting an effect of the foreign body on spermatozoa. A foreign body in the cow uterus also prevented fertilization of ova (15). Similarly, a foreign body in the sheep uterus prevented fertilization (6), and spermatozoa recovered from the uterus were found to be broken at the midpiece.[1] Spermatozoa may disappear more quickly than normal from the human uterus containing a foreign body (28). The early disappearance could be caused by phagocytic activity of viable polymorphonuclear leukocytes in the uterine lumen, or toxicity against human spermatozoa in the inflammatory exudate. In any event, a reduction in the number of viable spermatozoa might result in a reduced rate of fertilization in women using the intrauterine device for contraception. A reduced rate of fertilization would explain the observed reduction in the number of ectopic pregnancies occurring in women using the intrauterine device (29). This interpretation is as consistent with the available data as the interpretation proposed by Tietze (29); namely, that the foreign body must influence the oviducts in order to reduce the number of ectopic pregnancies. Furthermore, if human spermatozoa are indeed susceptible to the inflammatory exudate, there exists the possibility that a foreign body less inert than polyethylene might elicit sufficient inflammation to prevent fertilization altogether in women. In the rat (8), and perhaps other species, fertilization is not impeded. Whether or not spermatozoa of a particular species are affected may depend on differences in the speed with which they traverse the uterus or differences in the susceptibility of the spermatozoa to the toxic effects of an inflammation.

The great species variation in the effects of a foreign body in the uterus results from differences in the inflammatory response, determined largely by the presence or absence of bacterial infection, and from differences in the susceptibility of the spermatozoa to the inflammation. In each species it appears that inflammation can account for the effects of a uterine foreign body. Since the inflammatory cells that reach the uterine lumen are almost all polymorphonuclear leukocytes, some component of these cells is apparently toxic for blastocysts and/or spermatozoa. We are currently seeking a factor derived from polymorphonuclear leukocytes which, in meaningful concentrations, exhibits toxicity towards blastocysts.

SUMMARY

A chronic infiltration of polymorphonuclear leukocytes was invariably found in the infertile regions of uteri containing foreign bodies in conventional rats, germfree rats, mice, and rabbits. Polymorphonuclear leukocytes were never found in the fertile regions of these uteri.

[1] Hawk, H. W. 1967. Personal communication.

A foreign body in the uterus of the rat, and probably also the mouse, was associated with a bacterial infection which spread the inflammatory response throughout the horn containing the foreign body, and in the mouse occasionally into the control horn as well. No bacteria could be cultured from the rabbit uterine horn containing a foreign body.

In the germfree rat, both the infiltration of polymorphonuclear leukocytes into the uterus and fertility were significantly different from that observed in the conventional rat. Whereas in the conventional rat the inflammation and infertility extended along the entire length of the uterine horn containing a small foreign body, in the germfree rat the inflammation and infertility were closely correlated to the position of the foreign body.

As judged by measurements of lysozyme in the uterine lumens of rats and rabbits, polymorphonuclear leukocytes released their contents into solution in the uterine lumen. It is concluded that some substance derived from polymorphonuclear leukocytes may exert toxic effects on fertilized ova or on spermatozoa and thus be responsible for the infertility of uteri containing foreign bodies.

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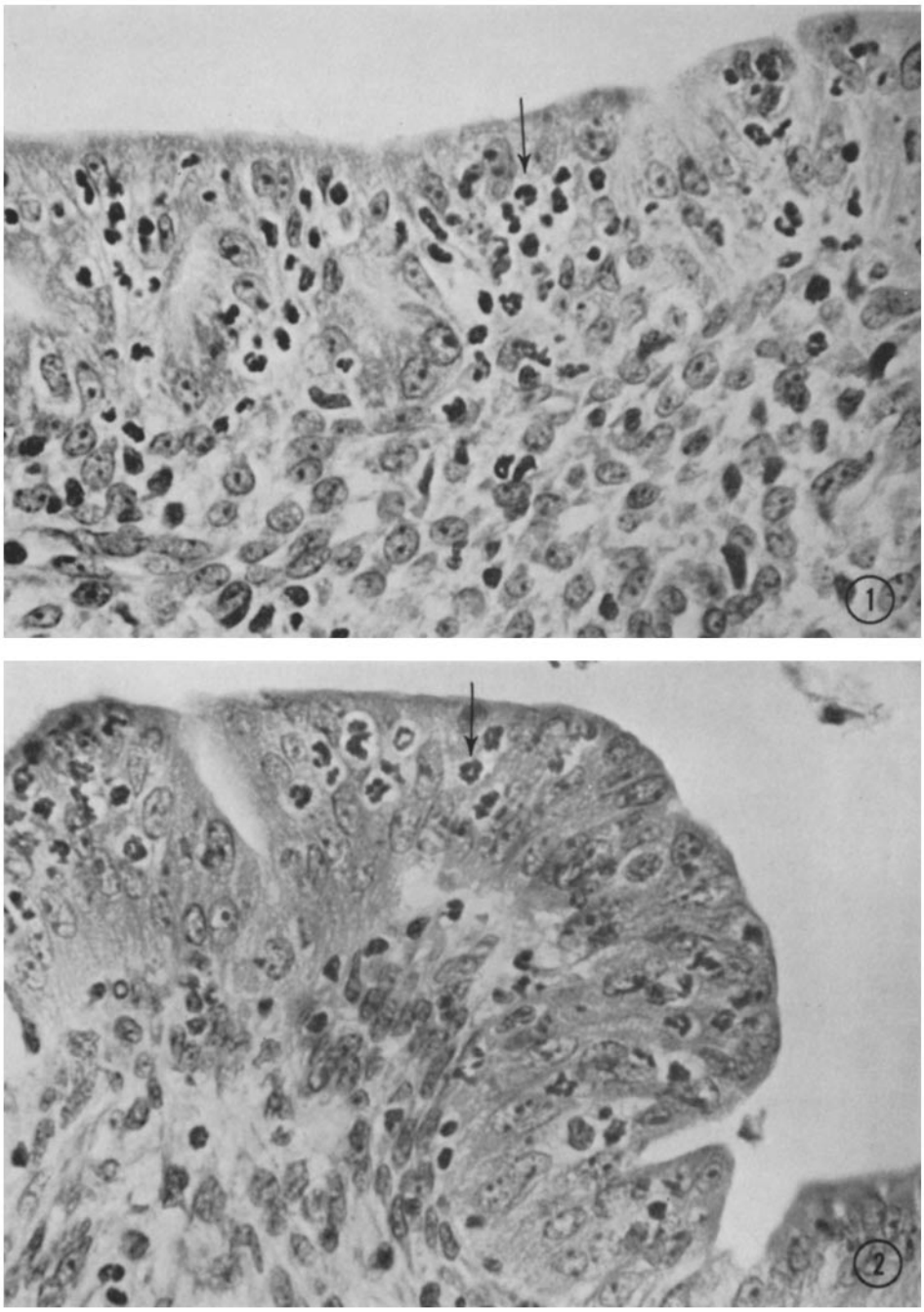
EXPLANATION OF PLATES

PLATE 38

FIG. 1. Section of rat uterine horn containing a foreign body, showing numerous polymorphonuclear leukocytes in the epithelium and subjacent stroma. The leukocytes can be distinguished by their nuclear morphology (see arrow). × 720.

FIG. 2. Section of mouse uterine horn containing a foreign body, showing numerous polymorphonuclear leukocytes in the epithelium and subjacent stroma. The leukocytes can be distinguished by their nuclear morphology (see arrow). × 720.

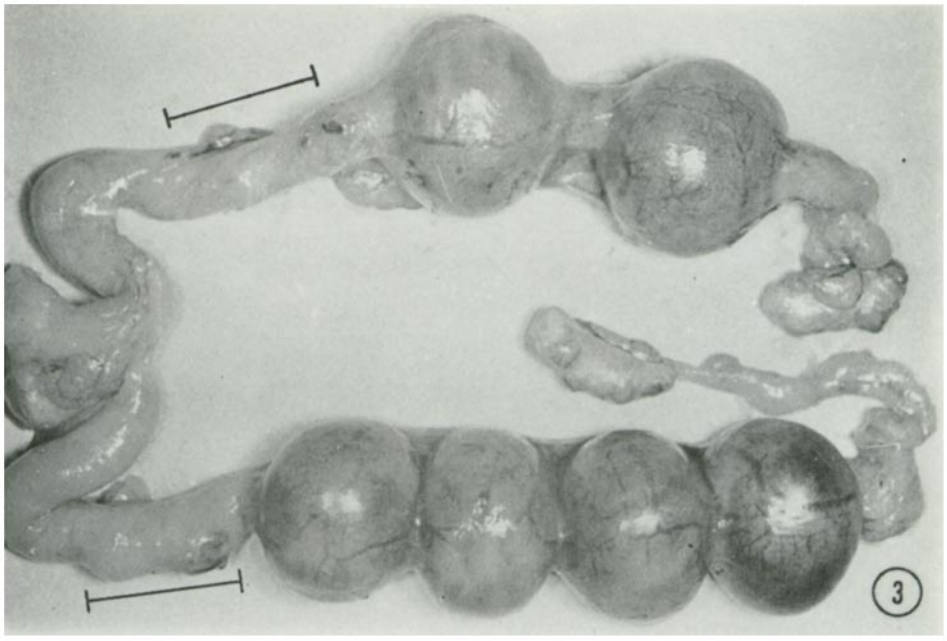


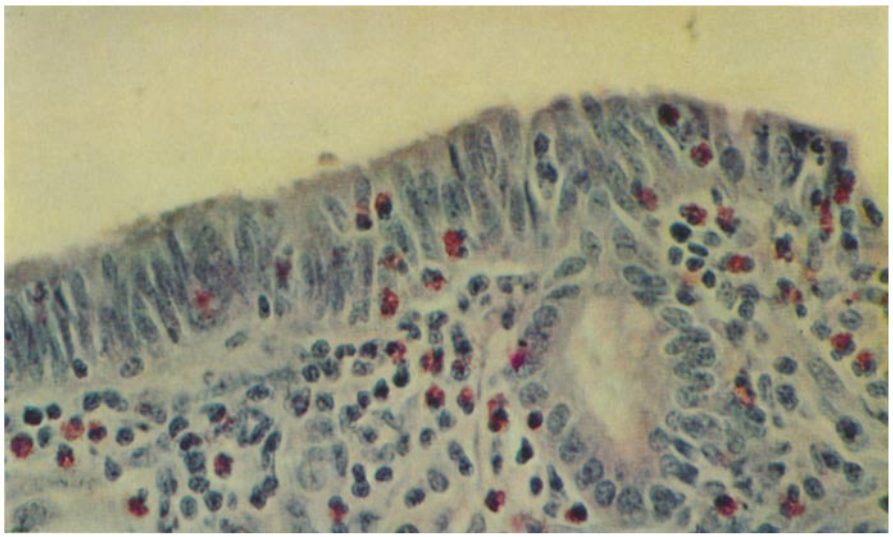
(Parr et al.: Uterine foreign bodies and inflammation)

PLATE 39

FIG. 3. Photograph of rabbit uterus on day 14 of pregnancy. A 20 mm polyethylene tube is present in each horn, as indicated by the brackets. Normal rabbit embryos have developed in the portion not in contact with the foreign bodies. × 1.

FIG. 4. Section of the segment of a rabbit uterine horn that contained a foreign body, showing numerous polymorphonuclear leukocytes in the epithelium and subjacent stroma. The eosinophilic granules of rabbit polymorphonuclear leukocytes distinguish these cells from stromal cells and the color plate thus illustrates the extent of the polymorph infiltration. At higher magnification these cells were clearly identified as heterophils, not true eosinophils. × 640.





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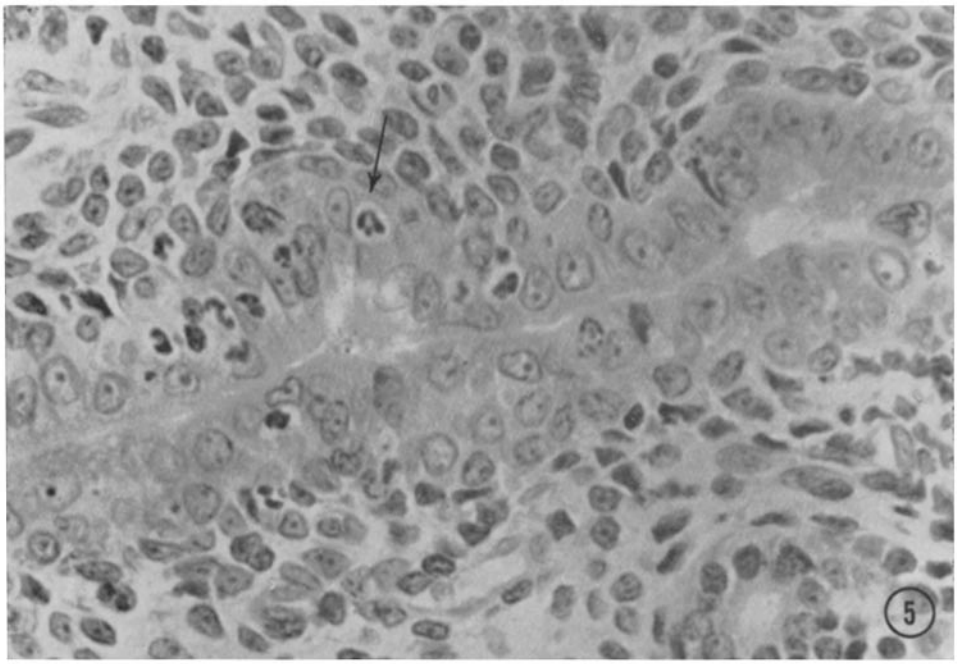
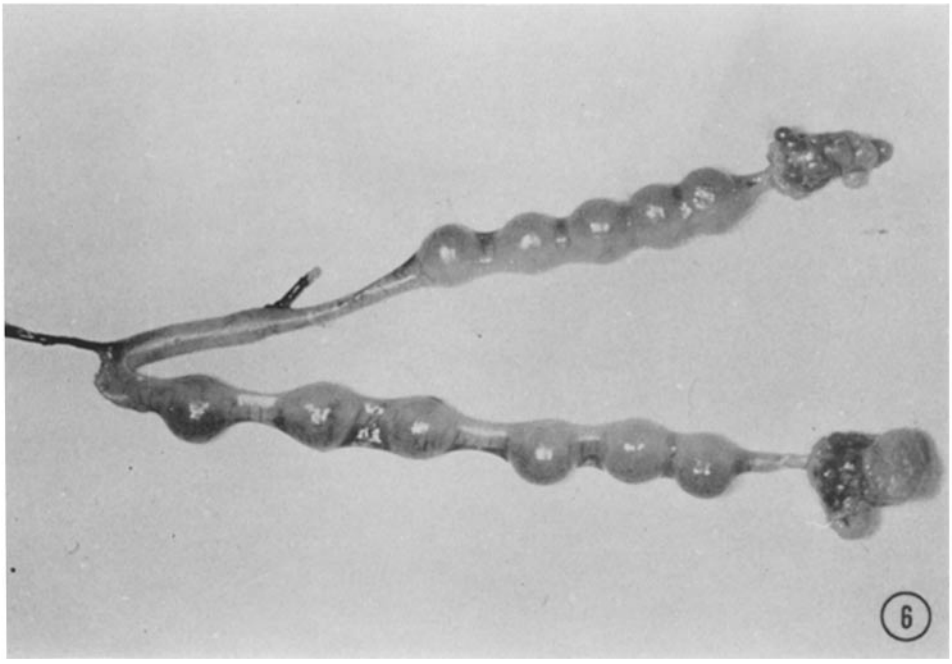
(Parr et al.: Uterine foreign bodies and inflammation)

PLATE 40

FIG. 5. Section of the segment of a germfree rat uterine horn that contained a foreign body, showing polymorphonuclear leukocytes in the epithelium and subjacent stroma. The leukocytes can be distinguished by their nuclear morphology (see arrow). × 720.

FIG. 6. Photograph of germfree rat uterus on day 8 of pregnancy. A silk suture is present in the cervical end of the upper horn. Normal rat embryos have developed in the portion not in contact with the foreign body. × 1.





(Pari et al.: Uterine foreign bodies and inflammation)