# THE SELECTIVE LOCALIZATION OF MURINE PLEURO-PNEUMONIA-LIKE ORGANISMS IN THE FEMALE GENITAL TRACT ON INTRAPERITONEAL INJECTION IN MICE

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PLATE 40

(Received for publication, May 18, 1954)

The application of mass production to the rearing of mice, a requirement imposed by the demand, has been followed by an increase in the incidence of infectious catarrh. Otitis media continues to be the most common manifestation of the disease (1). Recent examinations, made in this laboratory, of mice from both breeding and holding colonies have never failed to reveal middle ear involvement in some individuals. Infectious catarrh is readily communicable and the accepted transmission of the causal pleuropneumonia-like organisms (PPLO)[1] or coccobacilliform bodies is by way of the nasal passages (2). From their point of entry, the organisms apparently migrate through the Eustachian tube to the middle ear where they provoke a vigorous counter inflammation.

It was of interest to inquire into other possible routes by which PPLO might be carried to the middle ear. As an experimental approach, migration from the abdominal cavity was first considered. In the initial experiments intraperitoneal injection was indeed attended by otitis media. Unexpectedly, however, the results also suggested a selective preference of PPLO for the female genital tract. These observations were extended and the findings reported in the present paper.

Relatively few observations have been reported on the behavior of PPLO in the abdominal cavity of mice. Klieneberger (1938) noted that her $L_3$ strain tended to provoke abscesses at the site of injection (3). The organism was recoverable from the purulent contents and also from the spleen. Sabin (1939) observed only arthritis with cultures of his A and B types (4). The organisms disappeared from the peritoneal cavity shortly after injection. Sullivan and Dienes (1939) failed to produce any pathologic change with a culture of PPLO, serologically identical with Sabin's type A (5). Edward (1940) observed small areas of consolidation in the lungs and occasionally splenic enlargement (6). Histologic changes also occurred in the liver but PPLO were recovered only from the lung. Pearson (1942) using lung suspensions from infected

[1] PPLO stands for pleuropneumonia-like organisms.

mice reported the survival of coccobacilliform bodies for several weeks in the mesentery, spleen, and occasionally in the lung (7). His findings were suggestive of some protection against PPLO injected intranasally. Mooser (1951) commented on the enhancement of growth by ectromelia virus. PPLO alone produced at most a scant peritoneal exudate with few bacteria in films (8). In the presence of virus their number was greatly increased and both agents were demonstrable in the blood. Examinations of the genital tract and the middle ears were not reported in any of these studies.

## *Source of PPLO Strains and Outline of Methods*

Four strains of the catarrhal type (designated J, C, P, and F) and one strain of the conjunctival type were used in the following experiments. The cultural and morphological characteristics of these strains were sufficiently distinctive to differentiate them.

The catarrhal types were originally obtained from natural cases of infectious catarrh, as follows: the J strain from a colony of Japanese mice, C from a colony maintained at the former "metallurgical" laboratory in Chicago, Illinois, P from a colony in Princeton, New Jersey, and F from a commercial colony in New York. Strains J, C, and P have been carried for a period of years either by the nasal passage of middle ear exudate or by direct contact. Strain F is of recent isolation and has been maintained by intraperitoneal passage. The conjunctival type was isolated some years ago from a colony in Princeton and has been continued solely by direct contact (9). Most of the passages were made in female weanlings of the Princeton strain (12 to 15 gm. in weight). Although this colony is not completely free from infectious catarrh the disease incidence in young mice is so low that the risk of contamination is negligible. Swiss weanlings of the original, unselected, Rockefeller Institute strain were also used.

Nasal instillation was conducted in mice, lightly anesthetized with ether, by the inhalation of middle ear exudate in saline, ovarian suspensions in saline, or undiluted 20 per cent horse serum–bouillon cultures (48 hours' growth of the 2nd or 3rd transfer). About 0.03 ml. was delivered dropwise on the nares from a 24 gauge needle. The intraperitoneal injections were made midway over the abdomen, using 0.1 ml. of inoculum.

Tissues and fluids removed at autopsy were cultured on 20 per cent horse serum–heart infusion agar (pH 8) plates containing 2500 units of penicillin. The inoculated plates were sealed with scotch tape and incubated at 37°C. for 5 to 7 days. The plates were examined several times at a magnification of 100. Block transfers were made to serum-bouillon. Morphological verification was chiefly by phase microscopy but Gram, Giemsa, and Wayson stains were also used. With the above noted concentration of penicillin the rate of contamination was exceedingly low. No difficulty was experienced with L type variants from other bacterial species. In the many hundreds of cultures from animal sources made in this laboratory L type variants have been observed only on plates from rats infected with *Streptobacillus moniliformis*. Suspensions from spleens, ovaries, testes, and lungs were made in a concentration of approximately 10 per cent in saline with a glass tissue grinder. Uterine material was removed to saline by aspiration with a fine capillary pipette. Middle ear, nasal, conjunctival, and seminal suspensions were similarly made by aspiration. For the peritoneal washings repeated pipettings were made with about 1.0 ml. of saline. Serum-agar plates were inoculated by means of a 3.0 mm. loop. Blood was removed from the heart, immediately after killing with ether, by aspiration with a pipette. The drawn blood was added in nearly equal volume to 1.0 per cent sodium citrate in saline. Cultures were made directly on serum-agar and again after a preliminary enrichment in serum-bouillon.

## *The Intraperitoneal Injection of Catarrhal Type PPLO in Mice*

*The J Strain.*—Chattering was observed in one male from the group of mice injected with middle ear exudate. The other mice of this series showed no

outward sign of disease and gained weight normally during the period of observation. As summarized in Table I, the autopsy findings indicated, however, a localized multiplication of PPLO in the female genital tract with a resulting inflammation of the ovaries and Fallopian tubes. The primary lesion was commonly followed by a secondary localization in the middle ears. Male mice showed no involvement of the genital tract and only an occasional invasion of the middle ear.

At autopsy there were no signs of a generalized peritonitis in the mice of either sex. The spleens, livers, and lungs were macroscopically normal. The male genital organs were also

TABLE I

*The Examination of Mice Injected Intraperitoneally with the J Strain of PPLO*

| No. of experiment | Inoculum | Sex | Autopsy findings with each group of 5 mice | | | | Presence of PPLO in cultures |
|---|---|---|---|---|---|---|---|
| | | | Peritoneal cavity | Genital organs | Middle ears | Nasal passages | |
| 1 | Middle ear exudate | Male | 5− | 5− | 5− | 5− | 5 from spleen − |
| 2 | " " | " | 5− | 5− | 1+ 4− | 1+ 4− | 1 each from middle ears and nasal passages +<br>5 from seminal fluid − |
| 3 | " " | " | 5− | 5− | 1+ 4− | 5− | 1 from middle ears +<br>5 " testes − |
| 4 | " " | Female | | | | | 4 from ovaries +<br>5 " middle ears +<br>1 " nasal pass. + |
| 5 | " " | " | 5− | 5+ | 5+ | 5− | 5 from uterus +<br>5 " middle ears + |
| 6 | " " | " | 5− | 5+ | 5+ | 5− | 5 from ovary +<br>5 " middle ears + |
| 7 | Culture | Female | 5− | 5+ | 3+ 2− | 5− | 5 from ovary +<br>3 " middle ears − |

normal and bacteriologically sterile. The one mouse that chattered showed both otitis media and rhinitis. PPLO were demonstrable in cultures from both loci. Otitis media was also observed in one additional male but in the absence of rhinitis.

Fourteen of the 15 females showed well defined inflammatory changes in the genital tract, together with positive cultures, and all of them otitis media with involvement of one or both middle ears. The nasal passages of the 15 females were normal. The response in the 5 females injected with a pure culture of PPLO essentially duplicated these findings.

Additional experiments were carried out in female mice to determine the distribution of PPLO with varying time intervals. The organisms were rarely demonstrable in the blood and were much less persistent in the genital tract than in the respiratory tract after nasal instillation. They were recovered,

however, from the middle ears during the 12th week and were actively pathogenic for susceptible mice by the nasal route.

PPLO were obtained from one of 20 blood cultures made between the 2nd and the 30th day. The single positive culture came from a mouse killed 48 hours after injection. Peritoneal washings cultured from mice killed during this interval showed a scanty declining growth (30 colonies or less per plate) through the 15th day but were sterile thereafter. PPLO were first isolated from uterine washings on the 5th day. Subsequent cultures showed a heavy growth through the 30th day. Lung cultures were uniformly negative.

One of 5 mice held for extended observation died during the 10th week. The 4 survivors were killed after 12 weeks. At autopsy the ovaries were abnormal in appearance but not purulent. PPLO were not obtained in cultures from the genital tract. The lungs were normal and sterile. Two of these mice showed purulent exudate in the middle ears but not in the nasal passages. PPLO were demonstrable in the middle ear exudate on culture and also in washings from the seemingly normal nasal passages. Five susceptible mice injected intranasally with the pooled middle ear exudate were killed after an interval of 4 weeks. Chattering was observed prior to death. At autopsy all the 5 mice showed consolidation of one or more lobes of the lung, otitis media, and rhinitis. Cultures from the middle ear exudate were uniformly positive.

*The C Strain.*—As indicated in Table II this strain likewise showed a selective affinity for the female genital tract on injection in the peritoneal cavity of mice. Unlike the J strain, however, it also tended to produce small local extragenital abscesses. Otitis media was less commonly encountered in these mice but evidence of carriage to the joints, with ensuing arthritis, was observed not infrequently.

Two males and 5 females of the mice in this series showed swelling of the joints and 4 additional cases were observed in the subsequent experiments. The reaction was generally limited to one knee joint and was first noted between the 7th and 14th day after injection. In most cases the local edema had subsided by the time the mice were killed. In 2 mice, however, stiffening of the affected joints was observed at autopsy. The only additional outward sign of disease in these mice was chattering, detected in one female.

At autopsy a number of the injected mice, both males and females, showed small, pale yellow, grain-like pellets loosely attached to the peritoneal wall or the supporting tissues. In several mice larger nodules with a purulent contents were also noted. The former were commonly sterile on culture whereas the latter yielded a pure growth of PPLO. The livers, spleens, and lungs were uniformly normal.

The genital organs of the males showed no evidence of involvement. Cultures from the testis and seminal fluid were all negative in respect to PPLO. One male showed otitis media without rhinitis and a positive culture was obtained from the middle ear exudate. Well defined inflammatory changes were observed in the genital tract of 13 females and PPLO were regularly isolated from the uterine washings. Three of the females showed otitis media with demonstrable PPLO, but the nasal passages were uniformly normal.

Similar involvement of the genital tract, with recovery of PPLO, was observed in each of the 5 female mice injected intraperitoneally with PPLO of the C strain in pure culture. In these mice there was no involvement of the middle ears but in one individual arthritis was noted.

Supplementary experiments, similar to those made with the J strain, revealed a high percentage of positive blood cultures during the first week after

injection. PPLO were also recoverable from ovarian suspensions as early as the 2nd day, in the absence of macroscopic lesions. Viable organisms persisted in the female genital tract through the 4th week but not through the 12th. At this time, however, actively pathogenic PPLO were isolated from middle ear exudate.

Thirteen of 14 blood cultures made during the 1st week after injection showed a pure growth of PPLO. Subsequent blood cultures were negative. PPLO were also recovered from peritoneal

TABLE II

*The Examination of Mice Injected Intraperitoneally with the C Strain of PPLO*

| No. of experiment | Inoculum | Sex | Autopsy findings with each group of 5 mice | | | | Presence of PPLO in cultures |
|---|---|---|---|---|---|---|---|
| | | | Peritoneal cavity | Genital organs | Middle ears | Nasal passages | |
| 8 | Middle ear exudate | Male | 3 with pellets<br>2− | 5− | 1+ 4− | 5− | 5 from peritoneal washings −<br>1 from middle ear − |
| 9 | " " | " | 1 with nodule<br>4− | 5− | 5− | 5− | 1 from peritoneal nodule +<br>5 from seminal fluid − |
| 10 | " " | " | 1 with pellets<br>4− | 5− | 5− | 5− | 5 from testes − |
| 11 | " " | Female | 5− | 5+ | 3+ 2− | 5− | 5 from uterus +<br>3 " middle ears + |
| 12 | " " | " | 5−<br>1 with nodule | 4+ 1− | 5− | 5− | 4 from uterus +<br>1 " nodule + |
| 13 | " " | " | 4 with pellets<br>1− | 3+ 2− | 5− | 5− | 3 from uterus +<br>2 " uterus and ovary − |
| 14 | Culture | Female | 5− | 5+ | 5− | 5− | 5 from ovary + |

washings, in declining numbers, through the 15th day, while from uterine washings they were isolated in increasing numbers through the 30th day.

One mouse in the long duration experiment died before the test was completed. At autopsy during the 12th week after injection the 4 survivors showed cystic alteration of the ovaries. PPLO were not demonstrable on culture. The lungs and nasal passages of these mice were normal. A single mouse showed otitis media with the presence of PPLO. Intranasal injection of the middle ear exudate in 5 normal mice was followed by chattering. At autopsy, during the 4th week, 3 of these mice showed pneumonia, 3 otitis media with positive cultures, and 2 rhinitis.

*The P Strain.*—Since the behavior of this strain was essentially similar to that of the 2 preceding ones the experimental findings are not tabulated.

One death occurred in the mice of this series. An occasional peritoneal abscess was observed in both males and females. The spleens, livers, lungs, and joints were uniformly normal. The males showed no involvement of the genital tract and their cultures were negative. Otitis media and rhinitis were limited to one animal. PPLO were isolated from both loci. Inflammation of the genital tract occurred in 14 of the 15 females and cultures from the uterine washings were positive. Six of the females also showed otitis media without rhinitis. PPLO were again obtained from the middle ear exudate.

Of 18 blood cultures only one, made on the 7th day, was positive. The ovaries of 5 mice killed during the 12th week showed degenerative changes and were bacteriologically sterile. PPLO were recovered, however, from the middle ears of 3 mice. These organisms were pathogenic on nasal instillation in normal animals but gave some evidence of reduced virulence.

*The F Strain.*—Swiss mice, in groups of 5, were used in most of the experiments with this strain. A passage series was begun in females by the intraperitoneal injection of a pure culture. At autopsy, 15 days later, all of the animals showed obvious involvment of the genital tract. A composite suspension from the ovaries yielded a pure culture of PPLO and supplied the inoculum for the subsequent passage. Five additional passages were then made, in the same way, at intervals of 3 to 4 weeks. The results of the 7 tests were in essential agreement with those of the 3 preceding strains.

Signs of disease, including chattering, were not observed in any of the 35 mice. At autopsy 6 of them showed small pellets or nodules in the peritoneal cavity, outside the genital tract. Inflammation of the genital tract occurred in 34 of the 35 animals. Individual cultures were not made but a pure growth of PPLO was obtained from each of the 7 composite suspensions. Eight mice showed a cystic distention of one or both ovaries in addition to the inflammatory involvement. The lungs were regularly normal. The middle ears and nasal passages were examined only in the mice of passages 5, 6, and 7. Six of the 15 mice showed purulent otitis media with positive exudate cultures. Their nasal passages were normal.

The ovarian suspension from passage 6 was also injected intraperitoneally in 5 Princeton females. Four weeks later all of them showed inflammation of the genital organs but the ovaries were not cystic. In 4 mice extragenital nodules, attached to the liver in one animal, were present in the abdominal cavity. The middle ears of 3 mice contained a purulent exudate with PPLO on culture.

### *Vaginal Examination of Mice Injected with the F and J Strains of PPLO*

Cultures and also Gram-stained films were made of vaginal washings from 20 of the preceding Swiss mice. Ten Princeton mice injected with the J strain were similarly examined. None of these animals showed a discharge from the vulva or soilage adjacent to it. PPLO were recovered from 26 of the 30 vaginal cultures and leucocytes, in significant number, were present in 17 of the films. A few films also showed extracellular groupings of PPLO.

Five Swiss females were injected directly into the vagina with an ovarian suspension from the F strain passage series. Three weeks later PPLO were isolated from the vaginal washings of 4 but there was no indication of carriage to the internal portions of the genital tract or to the middle ears.

A discharge from the vulva was not observed even though exudate containing PPLO was present in the vagina. It was possible, nevertheless, that the organisms were excreted in suffi-

cient numbers to infect exposed normal mice. Accordingly, 2 contact experiments were carried out with Princeton females known to be vaginal carriers. Mature males (individually) and weanling females (in a group) were placed in direct contact with these carriers for a period of 2 to 3 weeks. The exposed mice, 10 in number, were uniformly normal at autopsy. PPLO were not demonstrable in cultures from any of them.

### *The Pathological Changes Produced by PPLO in the Female Genital Tract*

Inflammation of the Fallopian tubes, salpingitis, and of the ovaries, referred to as oophoritis, although the ovary proper was not involved, were the principal genital manifestations at autopsy.

The ovary of normal immature mice is a small, roughly oval body, measuring about 2 by 3 mm. It is partially encircled by the periovarian space and enveloped by a thin mesothelial lined membrane, the ovarian capsule. A narrow, much coiled tube, the oviduct or Fallopian tube, connects the periovarian space with the uterine horn.

Most of the experimental mice were autopsied during the 3rd to the 4th week after injection (about the 8th week of life). At this time the genital organs commonly showed macroscopic changes which varied only in degree and location. The maximum change. illustrated in Fig. 1, involved both the ovary and the Fallopian tube. These organs were much enlarged and contained a copious, pale yellow, purulent exudate. A variation of this change, shown in Fig. 2, occurred in mice injected with the F strain. In addition to a purulent enlargement of the oviduct the periovarian space was greatly distended by a fluid containing blood. With all 4 strains some degree of inflammation undoubtedly occurred in the uterus but was often obscured by early estrus.

The ovary, with attached uterus, was removed from each mouse and placed in a Petri dish for examination with a dissecting microscope at a magnification of about 2×. In some instances changes not visible macroscopically were seen. In doubtful cases the ovary was sectioned.

The examination of sections indicated that inflammation occurred before any macroscopic change was apparent in the ovary. Migratory cells, comprising polymorphonuclear leucocytes and large mononuclear cells, were observed in the periovarian space as early as the 2nd day after injection. At this time, as illustrated in Fig. 3, the lumen of the oviduct coils was relatively free from cells. By the 7th day, indicated in Fig. 4, the migratory cells had increased in number within the periovarian space and were also present in the lumen of the oviduct. The inflammatory reaction progressed for several weeks, ultimately converting both ovary and oviduct into a multilocular abscess. Fig. 5 is characteristic of the ovarian reaction during the 3rd to the 5th week. The massive cellular exudate within the capsular space and the oviduct show central necrosis. The ovary proper is compressed but appears uninjured. This intense purulent reaction did not extend to the uterine horns. In some sections, however, the uterine wall showed cellular infiltration and exudate was present in the lumen.

Repair ultimately occurred. By the 12th week PPLO were no longer detectable and the purulent reaction had subsided. At this time the ovaries were reduced in size but tended to show cystic changes and a brownish discoloration. In sections the ovarian capsule and the oviduct walls were thickened by connective tissue. Leucocytes were rarely observed. The periovarian space was sometimes distended by a finely granular pink-staining material, presumably fluid, in which large mononuclear cells containing a brown pigment were embedded.

### *The Intraperitoneal Injection of Conjunctival Type PPLO in Mice*

Negative findings were obtained throughout from 30 Princeton mice (15 of each sex) injected intraperitoneally with ocular washings containing PPLO

of the conjunctival type. None of the mice showed any indication of inflammation in the peritoneal cavity, genital tract, or respiratory tract, including the middle ears and conjunctivae. PPLO were not obtained in cultures from the peritoneal and nasal washings of males during the 4th week after injection, nor from the uterine and nasal washings of females. Injection of the conjunctival type of PPLO in pure culture was omitted since there was no reason to believe that the isolated organisms would be more pathogenic than those contained in ocular washings.

## DISCUSSION

PPLO are known to inhabit the genital tract of goats, cattle, dogs, and man. They have been recovered from suppurative lesions but also occur in normal individuals. An appraisal of their precise relation to genital disorder has been handicapped by a nearly complete lack of experimental observation. In man the role of PPLO in the etiology of non-specific urethritis has long been debated. Nicol and Edward (1953) have come to regard them as commensals, in this connection, but do not exclude the possibility that they may produce suppuration under certain circumstances (10).

The results of the present experiments in mice cannot be extended, by inference, to other hosts. These findings do provide proof, however, that organisms of the pleuropneumonia group are capable of provoking an acute inflammation in the genital tract of at least one animal species. No explanation is offered for the limitation of this reaction to female animals.

Invasion of the female genital tract by PPLO seems not to occur in naturally infected mice or in mice infected by nasal instillation. An extensive series of autopsies has failed to reveal a single instance of genital involvement in mice with infectious catarrh. This observation is somewhat surprising in view of the demonstrable carriage of PPLO in the blood. It is possible that prolonged survival in the middle ear results in a low grade immunity with restriction of the organisms to the respiratory tract.

The conjunctival type of PPLO, which in nature produces at most an inconspicuous conjunctivitis, was completely inactive in the peritoneal cavity of both male and female mice. The 3 catarrhal strains that were studied in detail produced nearly identical reactions in the female genital organs but otherwise differed considerably in their behavior. It is probable that they were carried to the ovary in the blood, despite the low rate of isolation with 2 of the strains. Penetration through the outer surface of the capsular membrane might occur but seems a less likely route. It is also probable that carriage to the middle ears and in case of the C strain to the joints occurred by way of the blood. It is noteworthy that the catarrhal strains invaded epithelium derived from all 3 germ layers: namely, ectodermal (nasal passages), mesodermal (genital tract), and endodermal (middle ears).

SUMMARY

Acute oophoritis and salpingitis were commonly observed in weanling mice injected intraperitoneally with murine pleuropneumonia-like organisms of the catarrhal type (4 strains). Organisms of the injected strain were regularly recovered in cultures from the ovary or uterus during the 3rd to the 5th week but not after the 12th week. They were also obtained from the vagina through the 5th week and with varying results, depending on the strain, from the heart's blood through the 1st week.

PPLO injected into the vagina survived for some weeks but did not migrate inwardly nor were they transmitted outwardly to exposed mice. In male mice peritoneal abscesses were sometimes present but the genital organs were normal and free from PPLO. Otitis media, with positive exudate cultures, occurred more often in females and varied in rate with the strain of PPLO. PPLO of the conjunctival type failed to survive in the abdominal cavity of mice and produced no reaction in either the genital or the respiratory tract.

BIBLIOGRAPHY

1. Nelson, J. B., *J. Exp. Med.*, 1937, **65,** 833.
2. Nelson, J. B., *J. Infect. Dis.*, 1948, **82,** 169.
3. Klieneberger, E., *J. Hyg.*, 1938, **38,** 458.
4. Sabin, A. B., *Science*, 1939, **89,** 228.
5. Sullivan, E. R., and Dienes, L., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41,** 620.
6. Edward, D. G. ff., *J. Path. and Bact.*, 1940, **50,** 409.
7. Pearson, H. E., *J. Bact.*, 1942, **43,** 229.
8. Mooser, H., *Arch. ges. Virusforsch.*, 1951, **4,** 207.
9. Nelson, J. B., *J. Exp. Med.*, 1950, **91,** 309.
10. Nicol, C. S., and Edward, D. G. ff., *Brit. J. Venereal Dis.*, 1953, **29,** 141.

## EXPLANATION OF PLATE 40

Unless otherwise indicated the tissues illustrated in the following figures were removed from Princeton mice injected intraperitoneally with PPLO of the J strain. The sections were stained with eosin–methylene blue.

FIG. 1. Paired ovaries and uteri removed on the 22nd day after injection. The coils of the oviducts and the periovarian spaces are distended by a purulent exudate. × 1.5.

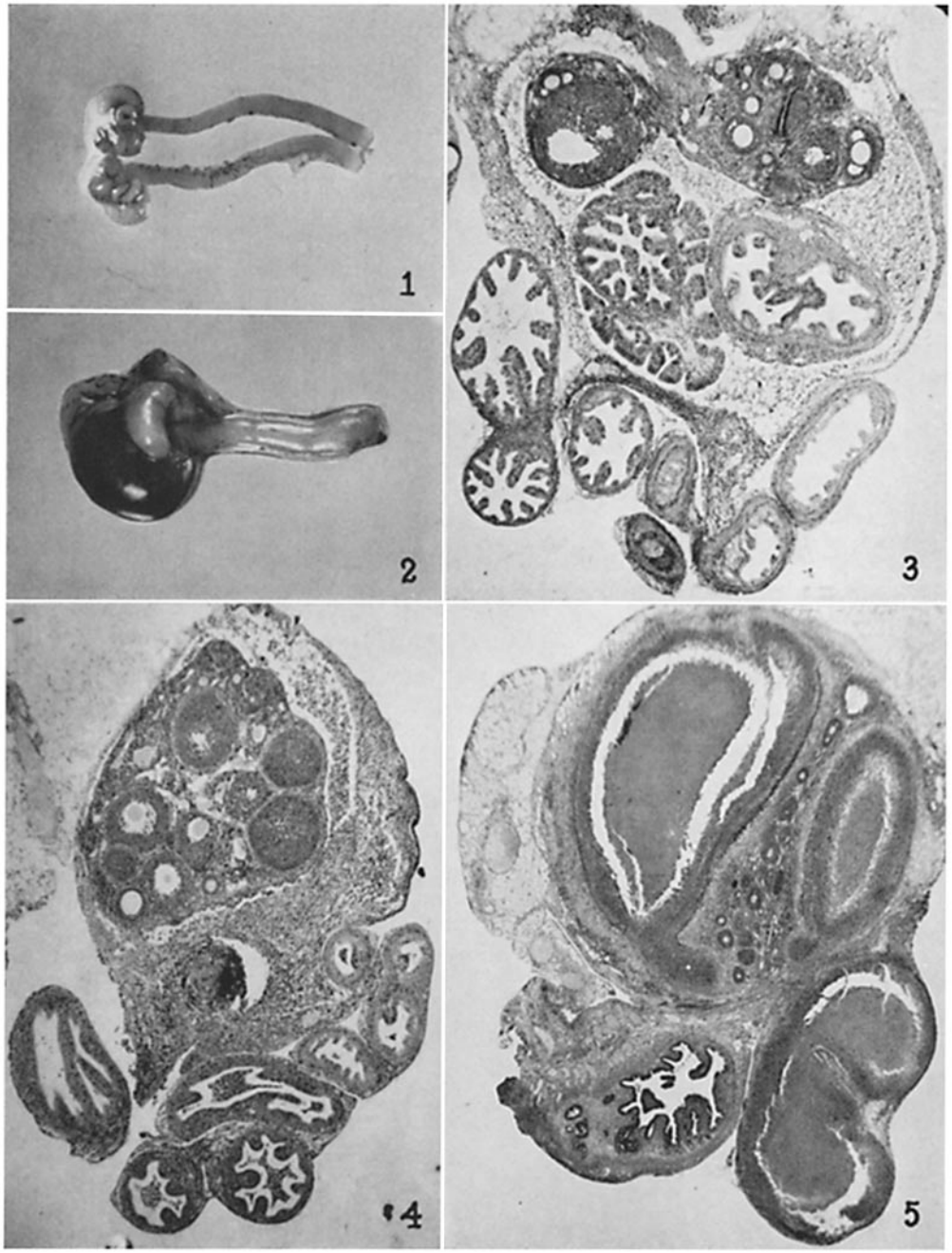
FIG. 2. Right ovary and uterus removed on the 28th day from a Swiss mouse injected with the F strain. The oviduct is purulent. The periovarian space is cystic, greatly distended by a fluid containing blood. Exudate, rich in leucocytes, is also present in the uterus. × 1.8.

FIG. 3. Section of ovary removed on the 2nd day. The ovary proper is in the upper portion of the capsular space and below it 2 oviduct coils. The space contains an exudate with numerous leucocytes and mononuclear cells. Several of the outer oviduct coils show a few cells. × 49.

FIG. 4. Section of ovary removed on the 7th day. There is an increased amount of exudate in the capsular space and most of the oviduct coils contain a cellular plug. × 49.

FIG. 5. Section of ovary removed on the 30th day. The ovary and attached oviduct is now a multilocular abscess. The ovary proper is compressed between the exudate of the capsular space and that of the one oviduct coil. Two outer coils are also greatly distended by exudate. The central area of the confined exudate is necrotic. × 13.8.

Photographed by Mr. J. A. Carlile.




(Nelson: Pleuropneumonia-like organisms in mice)