INFECTIOUS MYXOMATOSIS (SANARELLI) IN PREGNANT RABBITS

By DOUGLAS H. SPRUNT, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

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Viruses exhibit an intimate type of parasitism. This phenomenon is emphasized by the fact that many of the active agents multiply only in one species of host and only in certain tissues of that host. In the course of some experiments with infectious myxomatosis it was noticed that the clinical picture and morbid anatomy of the disease in pregnant rabbits are different from those in non-gravid animals. These differences indicate a line of approach to a better understanding of the host-virus complex and are sufficiently striking to be recorded.

Many investigators have observed that the course of certain tumors is influenced by pregnancy. Inasmuch as the reports and views regarding this matter are contradictory, some of the more prominent ones are cited. Morau (1), Ehrlich (2), Uhlenhuth and Weidanz (3) reported that pregnancy retards the growth of transplantable tumors in mice. Cuénot and Mercier (4), however, observed an acceleration of growth of similar tumors, while Albrecht and Hecht (5) stated that pregnancy has no effect on such neoplasms. Askanazy (6), Jentzler (7), and Herzog (8) reported that the growth of tumors in rats was accelerated by pregnancy. Kross (9), however, claimed that pregnancy has no effect on the rate of growth of neoplasms in rats.

Rous (10) in studying the effect of pregnancy on embryonic transplants, which he had shown to behave in many respects like transplantable tumors, reported the following experiments. Several loops of the uterus of a pregnant mouse were removed. A small fragment from one of the embryos was injected beneath the skin of the mother. This piece of tissue became vascularized but did not grow until the remaining part of the uterus was emptied. In other experiments he found that embryonic transplants grew equally well in non-gravid mice and in pregnant mice but that the transplants became differentiated into a larger number of tissues in the latter group of animals. Fischera (11) made similar observations concerning embryonic transplants in rats. Shattock, Seligmann, and Dudgeon (12), however, reported that gestation had no effect on the growth of transplanted fetal cartilage.

The effect of pregnancy on spontaneous tumors in animals has not been investigated to any extent. Slye (13) stated that neoplasms occur less frequently in gestating mice than in virgin mice. Krotkina (14) observed that in a rabbit with a tar cancer the neoplasm became smaller during each pregnancy. Rous (10) observed that the growth of a spontaneous tumor in one mouse was accelerated during pregnancy.

The reports concerning neoplasm in pregnant women are with a few exceptions in accord. Bainbridge (15) in an excellent review of the literature concluded that pregnancy exerts a stimulating and hence malign influence upon coexistent cancer. This he believes to be true not only of cancer of the breast and uterus where an increase in the blood supply might account for the accelerated growth but also of neoplasms in other parts of the body with the possible exceptions of certain epitheliomas.

Taliaferro (16) and his associates found that during the latter stages of pregnancy the resistance of rats to *Trypanosoma lewisi* is lowered. No other reports of experimental work dealing with the effect of pregnancy on diseases due to microorganisms have been encountered.

The mortality in acute infections is generally considered to be increased by pregnancy. This change is attributed by Williams (17) to the extra strain of the abortion which accompanies the infection. Weintraub (18) pointed out in his review of the literature on influenza that women may not die because they abort but abort because they are dying. Many authors have claimed that the susceptibility to influenza is increased by pregnancy. Weintraub (18) is of the opinion that this may be only apparent since influenza is most common in early adult life, the period during which women are most frequently pregnant. It has been maintained that the incidence of acute infections among pregnant women is lower than among the non-pregnant. With the exception of scarlet fever (19) there appears little evidence to support this hypothesis.

Methods and Materials

Virus.—The virus employed in these experiments is the strain used by Rivers (20). Its virulence may have been somewhat enhanced by numerous passages through animals. The virus was prepared by dissecting the myxomatous material, occurring at the point of an intradermal inoculation of the virus, free from skin and muscle. The infectious tissue was then ground thoroughly with alundum and diluted with sufficient distilled water to make a 10 per cent suspension. The virus was found to be active in such suspensions diluted 1.0×10^{-4} and 1.0×10^{-5}.

Animals.—Pregnant and non-pregnant female rabbits from the same source were used. The gestation period of the gravid animals had proceeded for about 20 days—the normal gestation period is about 30 days.

Inoculation.—The rabbits were inoculated intradermally. The points chosen for inoculation were well up on the rabbit's side in order that the mammary glands would not be involved. This precaution was considered necessary because the

mammary tissue has an increased blood supply during pregnancy which might affect the local lesion and the dissemination of the virus. The virus was diluted with Locke's solution in multiples of 10 up to 1.0×10^{-6}, 0.25 cc. of the different dilutions being injected as indicated below.

Fixation and Stains.—Tissues used for histological study were removed from the animals within an hour after death except in the instances where it appeared that a rabbit would not live through the night. Under such conditions the animal was killed and necropsy was performed immediately. Tissues from the heart, lungs, spleen, liver, adrenals, kidney, ovaries, intestines, bone marrow (shaft of the femur), skin, lymph nodes, and brain were fixed in Zenker's fluid (5 per cent acetic acid) and in formalin (10 per cent or 3.7 per cent formaldehyde gas) and stained with hematoxylin and eosin, with Mallory's phosphotungstic acid hematoxylin, and according to the Giemsa method. Sections of the liver were stained with Sudan III for fat.

EXPERIMENTAL

Experiments were designed to ascertain whether pregnancy altered the resistance of rabbits to the virus. Hence some of the animals were given relatively small amounts of the active agent.

Experiment 1.—Each of 3 pregnant and 2 non-pregnant female rabbits received intradermal inoculations, 0.25 cc., of dilutions of the same virus emulsion ranging from 1.0×10^{-2} to 1.0×10^{-6}.

Experiment 2.—Each of 6 pregnant rabbits and 1 non-pregnant female rabbit were given 3 intradermal injections, 0.25 cc., of dilutions of the virus emulsion ranging from 10^{-4} to 10^{-6}.

Experiment 3.—Each of 4 pregnant and 8 non-pregnant female rabbits received 6 intradermal injections, 0.25 cc., of dilutions of the virus emulsion ranging from 10^{-1} to 10^{-6}.

In the 3 experiments, 13 pregnant and 11 non-pregnant female rabbits were inoculated with the virus of infectious myxomatosis and the resulting clinical picture and morbid anatomy were observed. Inasmuch as the results of the experiments were similar, they will not be described separately.

Clinical Picture in Non-Pregnant Rabbits.—On the 3rd day slightly elevated skin lesions appear at the points of inoculation. These lesions increase in size and on the 5th day secondary lesions in the skin are usually visible. The primary lesions at this time become hemorrhagic and are capped by small vesicles. The subcutaneous tissues of the eyelids, ears, and genitalia as a rule show involvement on the 6th day and by the 9th the eyes are closed. On the day of the disease on which the animals die, usually the 9th after inoculation, they are dyspneic.

Clinical Picture in Pregnant Rabbits.—The course of the disease in the pregnant animals is similar to that described above with the following exceptions. The skin lesions are less elevated. Secondary lesions of the skin and involvement of the subcutaneous tissues of the ears are frequently absent or small. Approximately half of the animals abort on the 8th or 9th day of the disease. The remainder carry the young until death which usually occurs on the 9th day. The pregnant and non-pregnant animals responded in like manner to the various dilutions of the virus.

Morbid Anatomy of Non-Pregnant Rabbits.[1] *Liver.*—Small areas of focal necrosis are seen in the liver but such lesions are not uncommon in "normal" rabbits. In 2 animals a few necrotic cells are seen around the central vein.

Skin.—The lesions are slightly elevated, ovoid, firm, purplish masses that extend through the superficial muscles into the deeper fascial layer. A cross-section reveals a myxomatous-like tissue which contains numerous small hemorrhagic areas and dilated blood vessels. Histological preparations reveal hyperplasia, necrosis, and vesiculation of the epithelium. The cytoplasmic inclusions described by Rivers (20) are seen. Beneath the epithelium are some degenerating polymorphonuclear cells. Extending throughout the subcutaneous tissue are numerous small strands of fibrin. The superficial muscle layer is atrophied and in places necrotic. Around the hair follicles, glands, and blood vessels are a number of proliferating stellate cells, characteristic of the disease.

Lungs.—Only 4 animals in this group have secondary myxomatous lesions in the lungs. The lesions first appear in the lymphoid tissue around the bronchi and are frequently infiltrated with polymorphonuclear leucocytes. From this point of origin the pathological process extends around the blood vessels and into the interstitial tissue. The epithelial cells of the bronchi in close proximity to the myxomatous tissue are hyperplastic.

Spleen.—In only a few instances are the spleens enlarged. Myxomatous secondary lesions, however, are found in the Malpighian corpuscles of all the rabbits (Fig. 1). In most of the animals only a few follicles are involved while in others only a few normal lymphoid cells remain.

Lymph Nodes.—The architecture of the lymph nodes draining the region of inoculation is destroyed. The lymphoid cells are replaced by a large number of stellate elements among which are scattered numerous red blood cells, some fibroblasts, and fibrin. The endothelial cells lining the blood vessels are swollen and granular. In places such changes are sufficiently extensive to cause a loss in the continuity of the vessel wall.

Heart, Adrenals, Kidney, Intestines, Bone Marrow, Brain, Ovary, and Uterus.—No significant changes are found in these organs.

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[1] The findings in the non-pregnant group are in agreement with the observations of Rivers (20) and Stewart (21), and for a more detailed description of the pathology the reader is referred to their papers.

Morbid Anatomy of the Pregnant Rabbits. Liver.—All the rabbits, except one, shown an acidophilic necrosis of the hepatic cells around the central vein (Fig. 3). In some instances the destruction involves practically the entire lobule but there is never any cellular infiltration. In the periportal region of one liver there are a number of stellate elements that resemble the myxomatous cells seen in the spleen. Microscopic examination of the livers from 2 normal rabbits which had just given birth to young reveals that the hepatic cells are filled with fat but are not necrotic.

Skin.—The lesions are not elevated above the surrounding tissue as much as are those in the non-pregnant animals. Histological preparations reveal lesions similar to those observed in the non-pregnant group except that there is less fibrin and the muscle layer appears to be less severely damaged.

Lungs.—All the pregnant animals have secondary lesions in the lungs similar to those described in the non-pregnant animals with the exception that they are much more extensive (Fig. 4). One lung showed a generalized infiltration of the interstitial tissue with large mononuclear cells.

Spleen.—All the spleens are increased in size and the structure is greatly distorted (Fig. 2). Practically no lymphocytes are seen and the Malpighian corpuscles are represented by nests of large stellate cells. Many small foci of necrosis are seen. In the sinuses of the splenic pulp are large amounts of fibrin. The endothelium of the blood vessel is swollen and granular. Macrophages have phagocytized a number of lymphocytes. A few polymorphonuclear cells and some lymphocytes with pyknotic nuclei are seen, but the majority of the cells in the splenic pulp are large stellate elements similar to those seen in the Malpighian corpuscles. Throughout the spleen there is an increase in the number of fibroblasts.

Lymph Nodes.—The amount of endothelial damage and hemorrhage and the number of fibroblasts seem to be greater in the lymph nodes of the pregnant rabbits than in similar structures of the non-pregnant animals.

Ovary and Uterus.—In the muscular layer of the pregnant uteri are seen a few stellate cells resembling the myxomatous cells in the spleen. Around and in the Graafian follicles of the ovaries similar cells are found.

Heart, Adrenals, Kidney, Intestines, Bone Marrow, and Brain.—No significant changes are present in these organs.

From the results of the experiments described above it is obvious that there are differences in the phenomena occurring in pregnant and non-pregnant rabbits infected with the virus of infectious myxomatosis. In pregnant rabbits the lesions at the point of inoculation are less elevated and the secondary lesions of the skin and involvement of the subcutaneous tissues of the ears are either entirely absent or much smaller than are those observed in the control group. More extensive lesions in the spleen and the presence of secondary lesions in the lungs of all the pregnant animals contrast sharply with the involvement of only

a few of the Malpighian corpuscles of the spleen and only an occasional secondary lesion in the lungs of the non-pregnant group. A necrosis of the hepatic cells around the central vein occurred in all except one of the pregnant animals, while only a few small areas of central necrosis were seen in 2 of the non-pregnant rabbits. In spite of these differences the animals in the 2 groups lived the same number of days after inoculation.

DISCUSSION

Pregnancy apparently does not change the general resistance of the rabbit to infectious myxomatosis because all the animals, both the pregnant and the non-pregnant, lived the same number of days following inoculation of the virus. The resistance, however, of the various organs in the pregnant animals appears to have been altered—that of the lungs, spleen, and liver is lowered while that of the skin is elevated. This interpretation is of course open to question, as one might maintain that the spleen of the pregnant animal, for example, is more resistant since it evidenced a greater degree of reaction.

In passing one should note that a similar decrease in the resistance of the internal organs of pregnant women to invasion by malignant neoplasms is generally found. The apparent increase in resistance of the skin of pregnant women to certain epitheliomas (15), however, is also of interest in connection with the increased resistance of the skin and subcutaneous tissue of the pregnant animal to infectious myxomatosis.

The conflicting reports of the results of investigation on the influence of pregnancy on the various experimental tumors in animals make a comparison of them with the findings reported here of little value. Furthermore, only the last third of pregnancy was investigated in the present experiments whereas the tumor experiments of other workers extended throughout the whole reproductive cycle, gestation, lactation, and resting phase. It is important to keep this fact in mind, because 2 rabbits inoculated in the earlier period of pregnancy reacted like non-pregnant animals.

The results of this investigation are more comparable to the observations on the effect that acute diseases have on pregnant women, and it is here that we find the most striking similarity, particularly in regard

to the reaction of the liver of the pregnant rabbit and the pregnant woman. Acute yellow atrophy occurs more frequently among pregnant than among non-pregnant women. The amount of arsenic that a non-pregnant woman can take with impunity results in an extensive central necrosis of the liver when given to a pregnant woman. The lesion is similar to that seen in the gravid rabbit with myxoma. Such observations indicate that the resistance of the hepatic cells to toxic substances is lowered during pregnancy. A pregnant woman with influenza is much more likely to have pneumonia than is a non-pregnant one (18). In like manner the pregnant rabbit has numerous secondary lesions of the myxoma in the lungs. The decrease in the resistance of the spleen to infectious myxoma resembles the lowered resistance of the spleen of pregnant women to leukemia (22).

SUMMARY

Pregnancy in rabbits alters the reactivity of the tissues to the virus of infectious myxomatosis. The livers of pregnant animals with the myxoma have a central acidophilic necrosis. Secondary lesions in the lungs are much more numerous and larger in the pregnant than in the non-gravid animals. In like manner the lesions in the spleen are more extensive in the pregnant rabbit. On the other hand the skin lesions of the pregnant animal are decreased in size.

REFERENCES

1. Morau, H., *Arch. méd. exp. et anat. path.*, 1894, **6**, 677.
2. Ehrlich, P., *Verhandl. deutsch. path. Ges.*, 1908, **12**, 13.
3. Uhlenhuth and Weidanz, *Arb. k. Gsndhtsamte*, 1909, **30**, 434.
4. Cuénot, L., and Mercier, L., *Compt. rend. Acad.*, 1909, **149**, series 2, 1012.
5. Albrecht, H., and Hecht, V., *Wien. klin. Woch.*, 1909, **22**, 1737.
6. Askanazy, M., *Verhandl. deutsch. path. Ges.*, 1907, **11**, 39.
7. Jentzler, F., *Rev. méd. Suisse romande*, 1908, **28**, 329.
8. Herzog, M., *J. Med. Research*, 1902, **3**, N. S., 74.
9. Kross, I., *J. Cancer Research*, 1921, **6**, 245.
10. Rous, P., *J. Exp. Med.*, 1911, **13**, 248.
11. Fischera, G., *Policlinico, sez. prat.*, Rome, 1909, **16**, 692.
12. Shattock, S. G., Seligmann, C. G., and Dudgeon, L. S., *Proc. Roy. Soc. Med.*, 1910, **3**, path., 127.
13. Slye, M., *J. Cancer Research*, 1920, **5**, 25.
14. Krotkina, N., *Z. Krebsforsch.*, 1924, **21**, 450.

15. Bainbridge, W. S., *Am. J. Obst. and Dis. Women and Child.*, 1918, **77**, 21.
16. Taliaferro, W. H., personal communication.
17. Williams, J. W., Obstetrics, New York, D. Appleton and Co., 5th edition, 1926, 545.
18. Weintraub, S. A., *Mod. Med.*, 1920, **2**, 219.
19. Williams, J. W., Obstetrics, New York, D. Appleton and Co., 5th edition, 1926, 546.
20. Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 965.
21. Stewart, F. W., *Am. J. Cancer*, 1931, **15**, suppl., 2013.
22. Senator, H., and Kaminer, S., Health and disease in relation to marriage and the married state, translated by Dulberg, J., New York, Rebman Co., 1904, 316.

EXPLANATION OF PLATES

PLATE 32

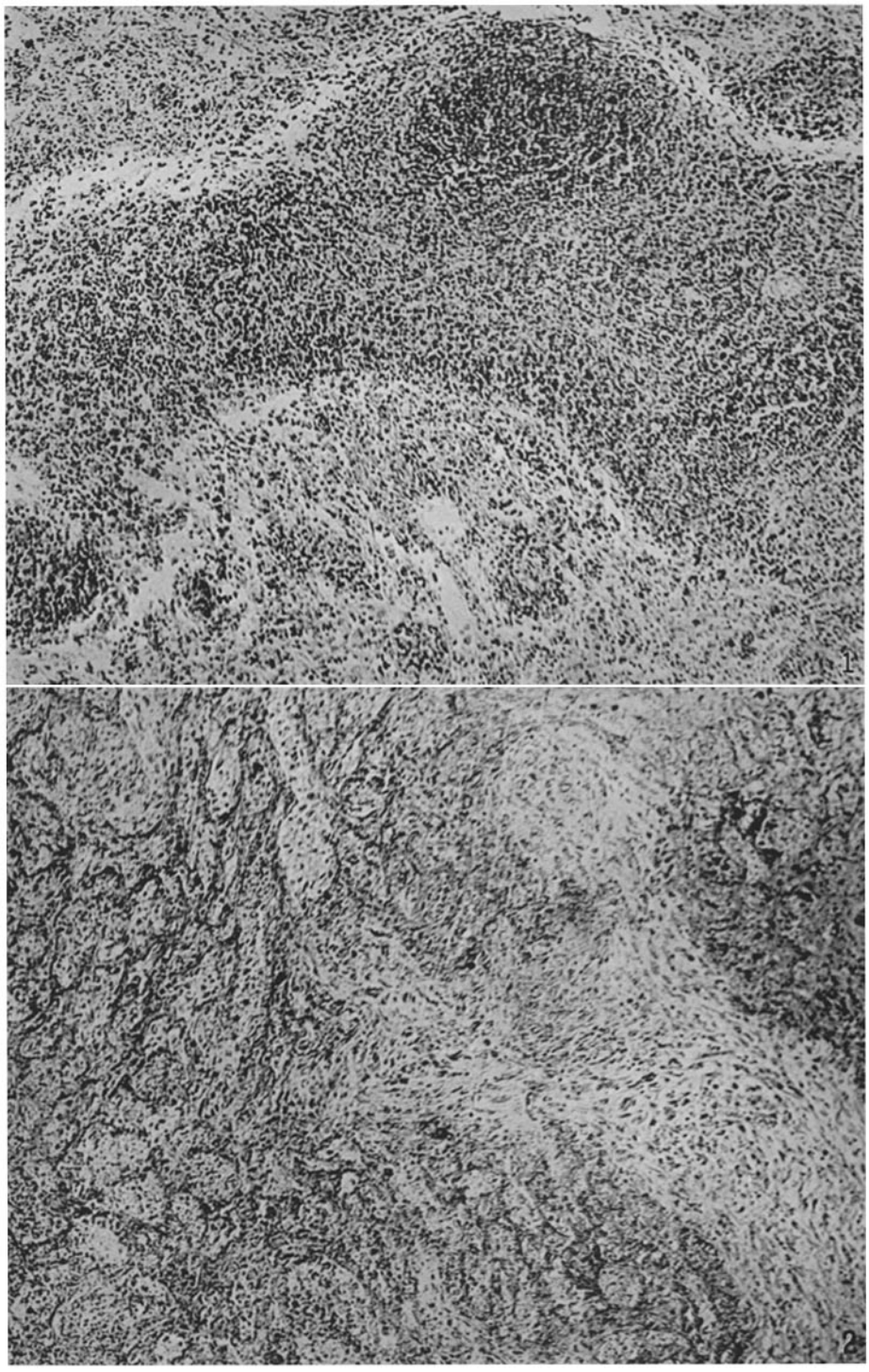
FIG. 1. Histological preparation of a spleen from a non-pregnant rabbit with infectious myxomatosis showing only a slight myxomatous change. Hematoxylin and eosin. × 90.

FIG. 2. Histological preparation of a spleen from a pregnant rabbit with infectious myxomatosis showing marked distortion and obliteration of splenic architecture. Hematoxylin and eosin. × 90.

PLATE 33

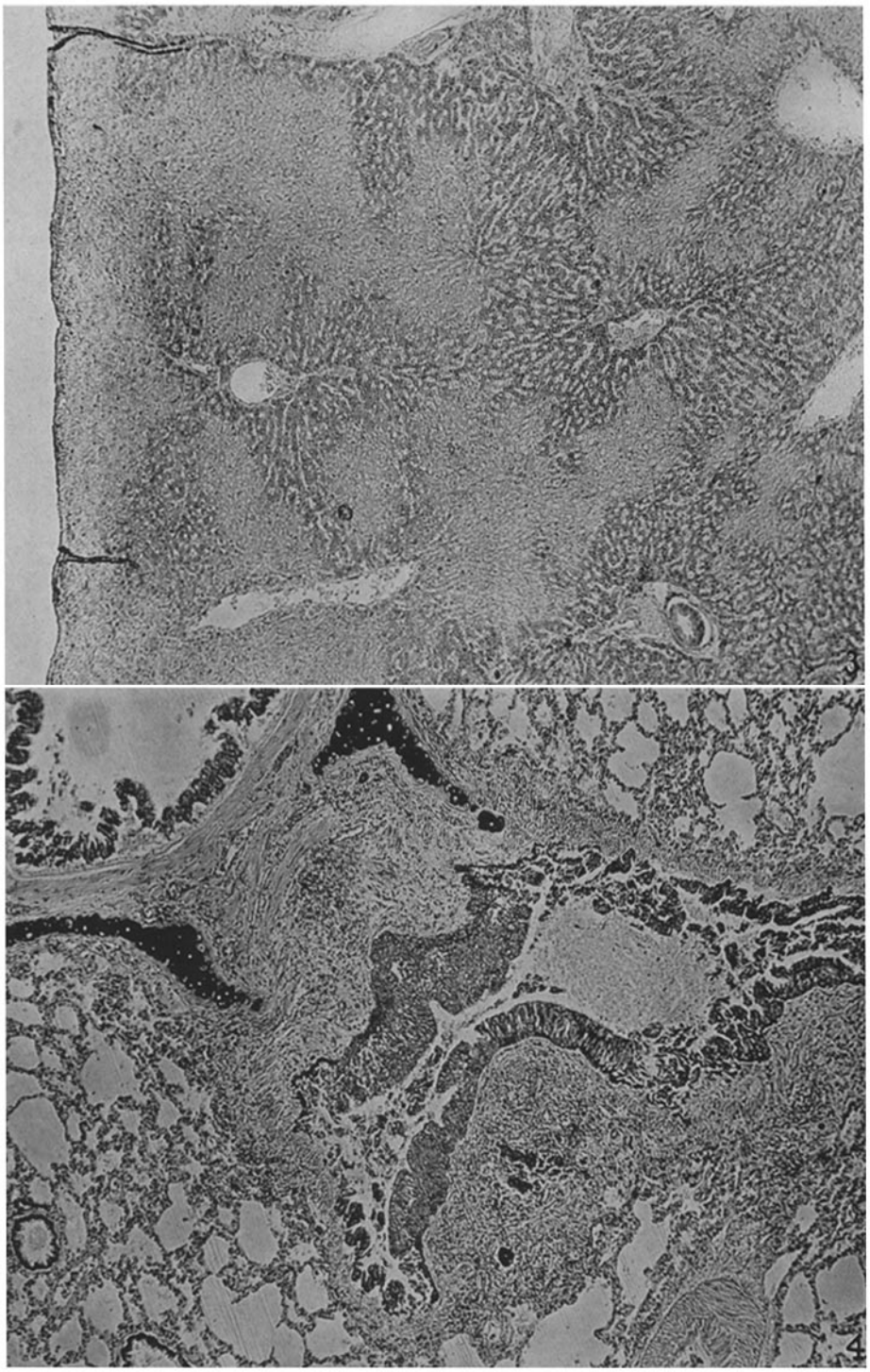
FIG. 3. Histological preparation of the liver of a pregnant rabbit with infectious myxomatosis showing a central necrosis of the hepatic cells. Hematoxylin and eosin. × 47.

FIG. 4. Histological separation of a lung of a pregnant rabbit with infectious myxomatosis showing secondary lesions around a bronchiole and a hyperplasia of the bronchial epithelium. Hematoxylin and eosin. × 47.



Photographed by Louis Schmidt

(Sprunt: Infectious myxomatosis in pregnant rabbits)



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