THE VISCERAL LESIONS PRODUCED IN MICE BY THE SALIVARY GLAND VIRUS OF MICE*

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Intranuclear inclusions have been described in the cells of the salivary glands of many species and, in several instances, have been shown to be due to a filterable virus. In all these species the virus has been looked upon as a harmless one which, although responsible for the salivary gland lesion, produces no clinical evidence of disease in the natural infection.

Experimentally, when an emulsion of salivary gland tissue of adult guinea pigs, mice, hamsters, or rats is injected into the same species, characteristic lesions in the salivary gland are produced whether the inoculation is intraperitoneal, subcutaneous, or intraglandular.[1,2,3] The production of lesions in other organs except at the site of inoculation has not been described. When Cole and Kuttner[1] injected the guinea pig salivary gland virus into the tongue, testicle, or lung of the guinea pig, they produced a local subacute inflammation with intranuclear inclusion bodies in these organs. Kuttner[2] and Kuttner and Wang[3] were also able to produce with the virus specific for the species a meningitis, fatal in some instances, in guinea pigs, mice, hamsters, and rats. Generalized visceral lesions were not produced in any of these experiments and only in the case of the intracerebral injections was a fatal disease produced in any species.

We have produced extensive visceral lesions in mice of the Buffalo

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[1] Cole, R., and Kuttner, A. G., *J. Exp. Med.*, 1926, **44**, 855.

[2] Kuttner, A. G., *J. Exp. Med.*, 1927, **46**, 935.

[3] Kuttner, A. G., and Wang, S., *J. Exp. Med.*, 1934, **60**, 773.

strain with the mouse salivary gland virus by intraperitoneal and intracerebral injections. Also after subcutaneous inoculations focal areas of inflammation with intranuclear inclusion bodies were occasionally found in the pancreas in addition to the usual salivary gland lesion. In a series of four experiments, an infection fatal in 4 to 7 days with extensive visceral lesions was produced in more than half of the animals by intraperitoneal inoculation of the virus. Fatal infections were produced less often by intracerebral injections.

Methods

The material for the inoculations was prepared as follows: Our stock Swiss mice have shown intranuclear salivary gland inclusions in a high percentage of the adult animals examined and, on the other hand, mice from the Buffalo strain have failed to show similar inclusions either in the adult or the young animals examined. Therefore the salivary glands of several adult Swiss mice were used as the source of the virus and young mice of the Buffalo strain approximately 3 weeks of age were used for the remainder of the experiments. Since neither the adult nor the young animals which we examined from the Buffalo strain have shown inclusions it seems safe to assume that these young animals were not infected.

The salivary glands of several adult Swiss mice were emulsified in broth (approximately one gland to ¾ cc. broth) and the emulsion centrifuged. With ¼ cc. of the supernatant fluid young mice of the Buffalo strain were injected subcutaneously. After 2 weeks, the salivary glands of these animals prepared in the same way were used for a series of experiments and also to inoculate subcutaneously another group of young Buffalo mice to carry on the virus. In each instance the amount of solution injected either subcutaneously or intraperitoneally was ¼ cc. For intracerebral inoculations 0.02 cc. of solution was used. For both the intraperitoneal and intracerebral inoculations, the centrifuged solution was also passed through a Berkefeld V filter to avoid transferring the paratyphoid infection which was endemic among the stock mice.

RESULTS

In four experiments forty-two young mice of the Buffalo strain were inoculated intraperitoneally. Twenty-eight of these animals died or were killed when moribund between the 4th and 7th day. Some of the animals which survived were killed and autopsied later. In the animals autopsied between the 4th and 7th day after inoculation, the most extensive lesions were in the liver, spleen, adrenals, lymph nodes, and the subperitoneal connective tissue and fat. Less extensive changes were found in the lungs, kidneys, intestines, and pancreas.

Lesions were not found in the salivary glands of animals autopsied before the 8th day but after the 8th day intranuclear inclusion bodies were found in some of the acinar cells of the salivary glands whenever they were examined.

In the gross, the liver was enlarged, very pale, and at times showed small areas of hemorrhage. The spleen was somewhat enlarged and friable. The lungs were more congested than normal and sometimes showed small hemorrhagic areas. There was no peritoneal exudate and the peritoneal lining was smooth. Cultures from the peritoneal cavity were negative. The serous surface of the intestines was smooth but individual intestinal loops were occasionally very red and the mucosa in these areas was intensely congested.

Microscopically, the liver lesion varied in intensity. In many liver cells, but especially in those nearest the portal zones, the nuclei were hypertrophied and contained large eosinophilic intranuclear inclusions. Most of these inclusions were larger than those usually found in the mouse salivary gland. However, the inclusions and also the nuclei varied in size. In the smaller nuclei which contained small inclusions the chromatin was not so distinctly marginated as it was when the nucleus and its inclusion were larger. With hematoxylin and eosin the staining of the inclusions varied but they were always less basophilic than normal nuclear chromatin. Occasionally intranuclear inclusions were seen in Kupffer cells, in connective tissue cells about the large bile ducts, and in the multiple nuclei of megalokaryocytes in liver sinusoids. Liver cells with two nuclei each containing an inclusion were also found.

The inclusions in the liver cells were round or oval, of various sizes, and slightly irregular in outline. In the well preserved cells they seemed to be coarsely granular or made up of masses of material molded together. In some nuclei, the irregularly outlined inclusions seemed lobulated and in other nuclei two or three distinct masses of eosinophilic material were present. The large number of inclusions produced in a short time seems to offer an excellent opportunity for further detailed study of the structure of these bodies. The cytoplasm of the liver cells containing inclusions was finely granular and stained purplish red with hematoxylin and eosin. No basophilic cytoplasmic inclusions occurred.

Necrosis of the liver cells occurred, involving single cells, only a few cells, or a large section of a lobule. Large areas of necrosis were usually midzonal in position and were often associated with hemorrhage (Fig. 1). Polymorphonuclear leukocytes accumulated about the liver cells which contained inclusions even before necrosis of the cells occurred (Fig. 2), but they were especially numerous about necrotic cells or groups of cells. The cells of this exudate, which consisted principally of polymorphonuclear leukocytes but also some mononuclear cells, later became necrotic also. On the 5th day after inoculation, many cells with intranuclear inclusion bodies were seen and areas of necrosis with hemorrhage were present. However, these changes were at times found as early as the 3rd day. On

the other hand, in animals autopsied on the 6th or 7th day, there were, as a rule, fewer inclusions but larger groups of liver cells were necrotic and the inclusions were found chiefly in cells about the edge of the necrotic zones. There were also groups of large, pale staining liver cells without inclusions, usually near the central veins, which had vacuolated cytoplasm and sometimes contained fat droplets. In a few animals autopsied later small areas remained where necrotic liver cells seemed to have been replaced by a loose stroma infiltrated with wandering cells. All the intranuclear inclusions had disappeared.

The lesion in the spleen also varied in degree. In animals dying on the 4th and 5th day the spleen was often almost entirely replaced by necrosis and hemorrhage with a few large mononuclear cells containing large intranuclear inclusions still visible where the splenic tissue was not entirely necrotic (Fig. 5). In other animals with less advanced lesions, the hemorrhage and necrosis were most marked about the Malpighian bodies, and there were innumerable large mononuclear cells with pale blue, finely granular cytoplasm and a large nucleus containing a large oval, or round, or greatly elongated inclusion body. The shape of the inclusion usually corresponded to that of the nucleus in which it occurred. There were many polymorphonuclear leukocytes where necrosis had taken place. In spleens in which necrosis had not occurred, there was a marked proliferation of large mononuclear cells, probably reticulum cells. These were chiefly at the margin of the Malpighian bodies and it was in these cells that the inclusions were most conspicuous. Multinucleated cells with an inclusion in each nucleus were occasionally seen.

Lesions were always present in the adrenals. In some animals only a few groups of cells in the cortex contained intranuclear inclusions. These cells became necrotic and surrounded by polymorphonuclear leukocytes. In the most extreme lesions there were extensive necrosis and hemorrhage in the inner half of the cortex (Fig. 3) and almost every remaining cell of the cortex had a hypertrophied nucleus containing an inclusion (Fig. 4). In some animals which survived and were killed later there were found areas of degeneration in the cortex containing a few mononuclear phagocytic cells.

In the kidney, inclusions were not found in the cells of the tubules, but a few glomeruli contained one or more large cells with an intranuclear inclusion. These cells usually occurred within the tuft and it was impossible to determine whether they were endothelial cells of the capillaries, or epithelium covering loops of the tuft.

Sections made through loops of intestine congested in the gross showed an extremely hyperemic mucosa. A few intranuclear inclusions were found in connective tissue cells of the mucosa and in epithelial cells deep in mucosal glands.

In lobules of fat tissue and loose connective tissue of the omentum and of the retroperitoneal tissue about the pancreas and kidney there was an inflammatory process with an increase in mononuclear cells and proliferation of fibroblasts. The capillaries were engorged and in addition there seemed to be newly formed capillaries. Many of the large mononuclear wandering cells and fibroblasts

contained intranuclear inclusions. They were also seen a few times in the swollen endothelium of capillaries. Some polymorphonuclear leukocytes were present especially where necrosis of cells occurred. Occasionally cells of the serous lining of the peritoneal cavity were enlarged and contained intranuclear inclusions.

Small lymph nodes near the duodenal end of the pancreas, near the kidney, and also at the hilum of the lungs showed foci of necrosis and hemorrhage just inside the peripheral sinus. Large mononuclear cells with intranuclear inclusions were seen in or near these foci.

In two instances sections of the ovary were made and in each case intranuclear inclusions were found in some of the cells of the theca interna.

Pathological changes were found in the lung in more than half of the animals that died. These consisted of focal areas of cellular infiltration about small vessels and in the alveolar walls with thickening of the latter. The infiltrating cells consisted of both polymorphonuclear leukocytes and mononuclear cells. Cells with intranuclear inclusions were frequently found in these foci. Whether a cell containing an inclusion lined a capillary or was a wandering cell could not always be determined. At times, however, the inclusions were definitely in cells lining alveoli. Intranuclear inclusions were also found in connective tissue cells about large bronchi and where they were present there was also an inflammatory exudate composed of polymorphonuclear and mononuclear cells. Rather large areas of alveolar hemorrhage occurred in some lungs in which the intranuclear inclusions were numerous.

In the pancreas lesions were found in more than half of the animals autopsied between the 4th and 7th day after intraperitoneal inoculations. There were intranuclear inclusions in a few acinar cells and there were small foci of wandering cells, both polymorphonuclear and mononuclear cells. In one instance, intranuclear inclusions were found in the cells of Brunner's glands in the duodenum immediately adjacent to the pancreas. The inclusions in the pancreatic cells were usually smaller than those in the other viscera. They resembled more those found in the salivary gland. It is of interest that the pancreatic lesions were less intense than those in other abdominal organs after intraperitoneal inoculations and yet pancreatic lesions were the only ones found outside the salivary glands 2 weeks after subcutaneous inoculation.

Cellular inclusions were not found in the salivary gland of mice dying between the 4th and the 7th day after intraperitoneal injection. But they were found in the salivary gland of all animals that survived and were autopsied after the 8th day. They were almost always found in acinar cells of the serous acini in a part of the gland which contains both mucous and serous glands, rarely in duct cells. In addition small basophilic cytoplasmic bodies were occasionally present in the affected cell. The inclusions in mice were more acidophilic than those found in the salivary glands of infants. The cells containing these inclusions and their nuclei were hypertrophied but usually not to such a marked degree as seen in the guinea pig and in the human salivary gland. However, both the size of the inclusions and the degree of hypertrophy of the cells varied considerably. Our observa-

tions in regard to the character of the inclusions and the cells containing them agree exactly with the descriptions given by Kuttner and Wang.[3] Some degree of lymphocytic infiltration was always present in the glands in which the inclusions were found but not always near the cells which contained them. The cellular infiltration was usually more extensive and diffuse than that seen in the salivary glands of the stock Swiss mice which showed inclusions.

In spite of the fact that such extensive and fatal lesions were produced in the liver and spleen, we were unable to pass the virus in series with material from these organs. In a few instances a small number of intranuclear inclusions were found in the spleen of an animal receiving this liver and spleen material but extensive lesions were never produced and the animals did not become sick. However animals of this group which were autopsied 2 weeks after inoculation showed intranuclear inclusions and an infiltration of mononuclear cells in the salivary glands.

Following intracerebral inoculation of mice of the Buffalo strain extensive generalized lesions were at times produced with the same distribution and character as those produced by intraperitoneal inoculation. These animals died between the 3rd and 7th day. At other times, perhaps because the solution of virus was less concentrated, the animals survived 7 days and when autopsied showed no lesion except a slight mononuclear meningeal exudate with a few intranuclear inclusions in the cells of the exudate.

Mice injected subcutaneously did not become sick. Those autopsied after 2 weeks showed the characteristic salivary gland lesions. Organs other than the salivary glands from these animals were not examined as a routine, but in the few instances in which they were examined inclusions in organs other than the salivary glands were found three times. These were in the acinar cells of the pancreas.

DISCUSSION

The failure of others to produce generalized visceral lesions in mice with the salivary gland virus may have been due to the fact that they have used smaller amounts of the virus for inoculation, but there is also the possibility that there is some difference in the susceptibility of different strains of mice. We have not been able to produce extensive lesions so readily in black mice of the C57 strain, while we have

some indication that the Swiss mice are even more susceptible than those of the Buffalo strain.

The inability to transmit the virus in series with liver and spleen in spite of the innumerable intranuclear inclusions and extensive damage produced in these organs is puzzling. Cole and Kuttner[1] reported the same difficulty in transferring the salivary gland virus of the guinea pig with material other than the salivary gland but they had not produced lesions of such intensity in the organs which they had inoculated locally and then attempted to use for passing the virus. Kuttner[2] believed that the guinea pig virus lost virulence after repeated transfers even when a salivary gland emulsion was used to pass the virus. In our experiments we were able to produce extensive visceral lesions by intraperitoneal inoculation of salivary gland emulsions after the sixth transfer by the subcutaneous route. No decrease in virulence was detected.

The salivary gland, although it becomes the site of a persistent infection in mice which survive, does not show inclusions or cellular infiltration at the time when the mice inoculated intraperitoneally die, showing many intranuclear inclusions in the abdominal organs, lungs, and lymph nodes. Whether the virus can be passed by salivary gland emulsions at this early period has not been determined.

The experimental production of visceral lesions with mouse salivary gland virus, possessing a low natural pathogenicity, suggests the possibility that the natural infection may at times become generalized, perhaps in especially susceptible strains or individuals. By analogy the suspicion is strengthened that the generalized lesions seen in infants with intranuclear inclusions similar to those occurring in the salivary gland are due to a generalization of the salivary gland virus. Kuttner and Wang,[3] in their review of the literature, call attention to the fact that intranuclear inclusions have not been described in the salivary glands of infants when they have been found in various other organs. For this reason, they raise the question as to the identity of the virus concerned in the two groups of cases. However, the failure to describe salivary gland lesions in infants in association with visceral lesions containing this type of inclusion may be due in large part to the failure to make routine examinations of the salivary gland. In our material from the St. Louis Children's Hospital and the St. Louis

Isolation Hospital we have found in eight infants intranuclear inclusions in the salivary glands together with similar inclusions in other organs. The salivary gland was examined in only one of twenty-nine other infants and young children who showed such inclusions in other organs.

CONCLUSIONS

Extensive visceral lesions containing intranuclear inclusions have been produced in mice by intraperitoneal and intracerebral inoculations of the homologous salivary gland virus. Rarely small pancreatic lesions containing inclusions have been encountered 2 weeks after subcutaneous inoculation.

Many of the animals injected intraperitoneally died between the 4th and 7th day after inoculation.

In spite of the extensive lesions produced in the liver and spleen, the virus could not be transferred with an emulsion of these organs.

EXPLANATION OF PLATES

All sections have been stained with hematoxylin and eosin.

PLATE 24

FIG. 1. Low power of mouse liver, showing destructive lesions caused by salivary gland virus. The black areas represent necrosis of liver cells with inflammatory exudate. ×30.

PLATE 25

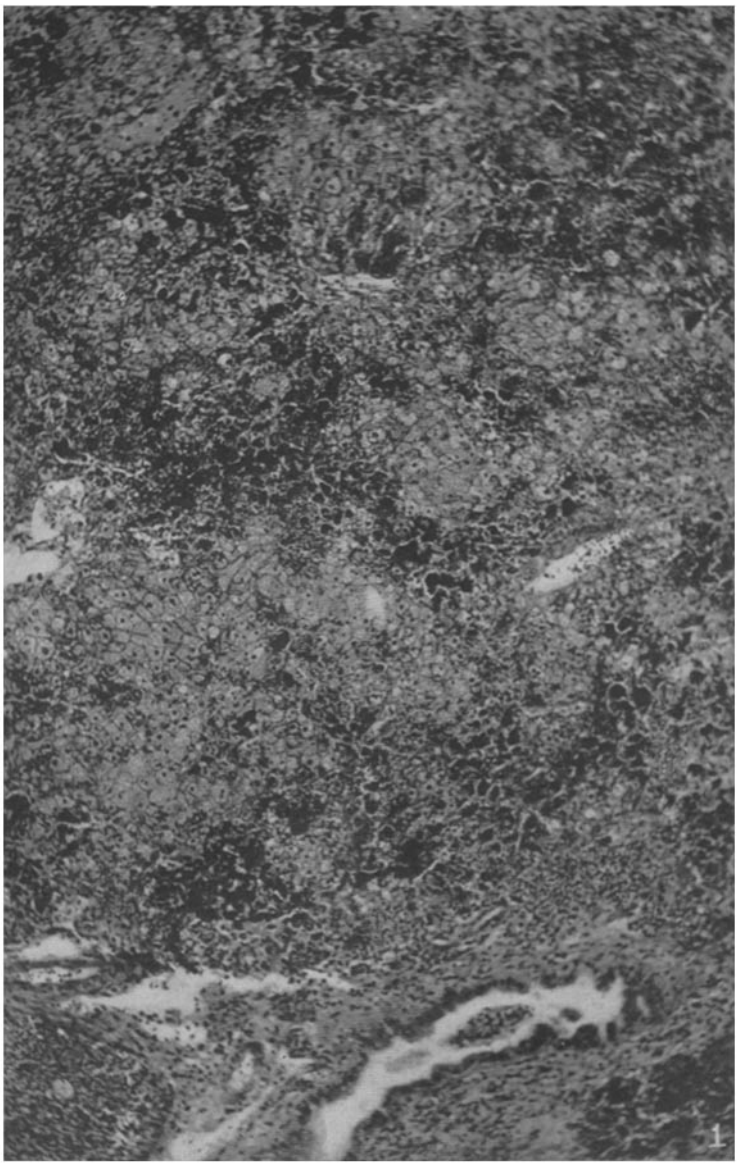
FIG. 2. High power of liver lesion with necrosis and cellular infiltration. An intranuclear inclusion can be seen in a liver cell near the center of the field. ×300.

FIG. 3. Adrenal gland showing areas of necrosis and hemorrhage in the inner cortical layer. ×20.

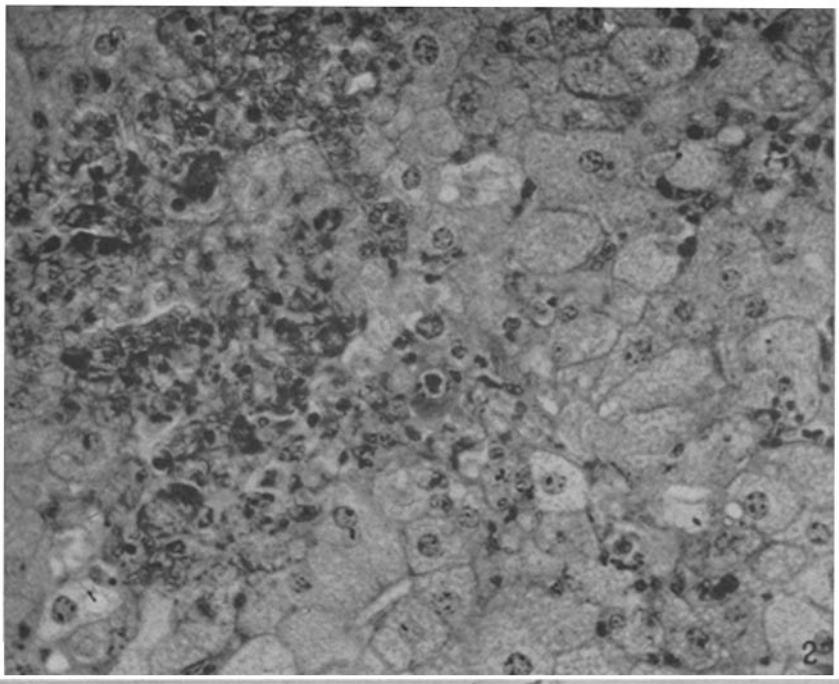
PLATE 26

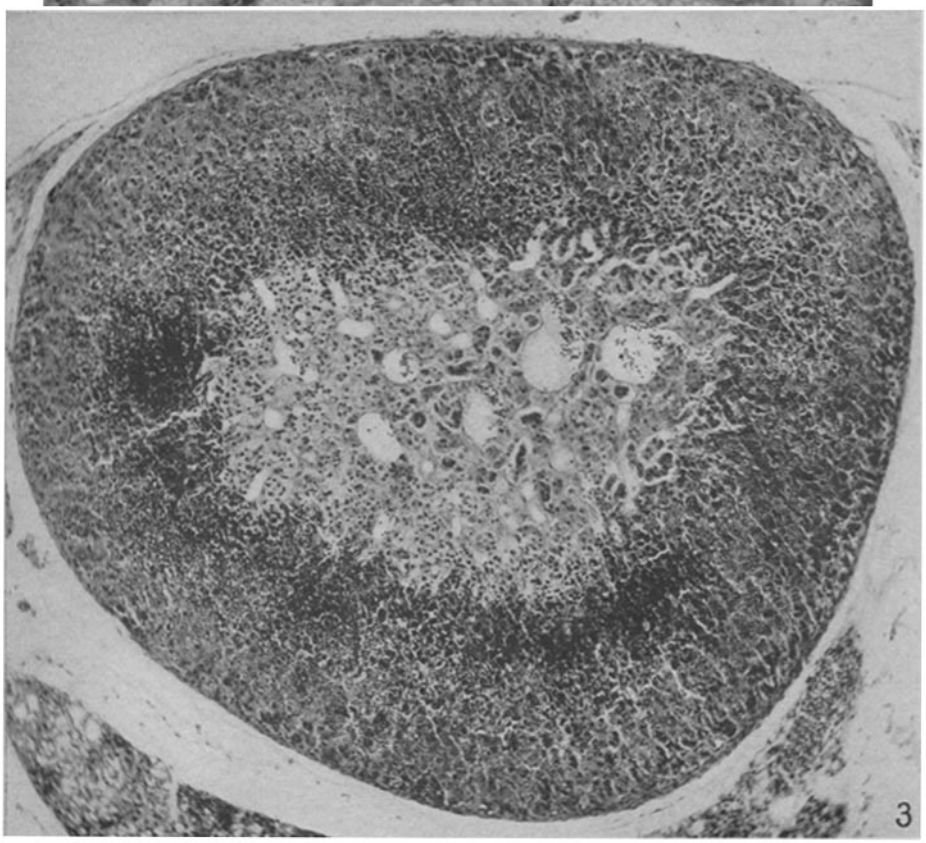
FIG. 4. High power of adrenal with numerous intranuclear inclusions and area of necrosis and hemorrhage in upper left hand corner. ×300.

FIG. 5. Area from a section of mouse spleen. The black masses are collections of fibrin about necrotic cells. Many inclusion bodies are present in the nuclei of the large mononuclear cells. ×300.



(McCordock and Smith: Salivary gland virus lesions)





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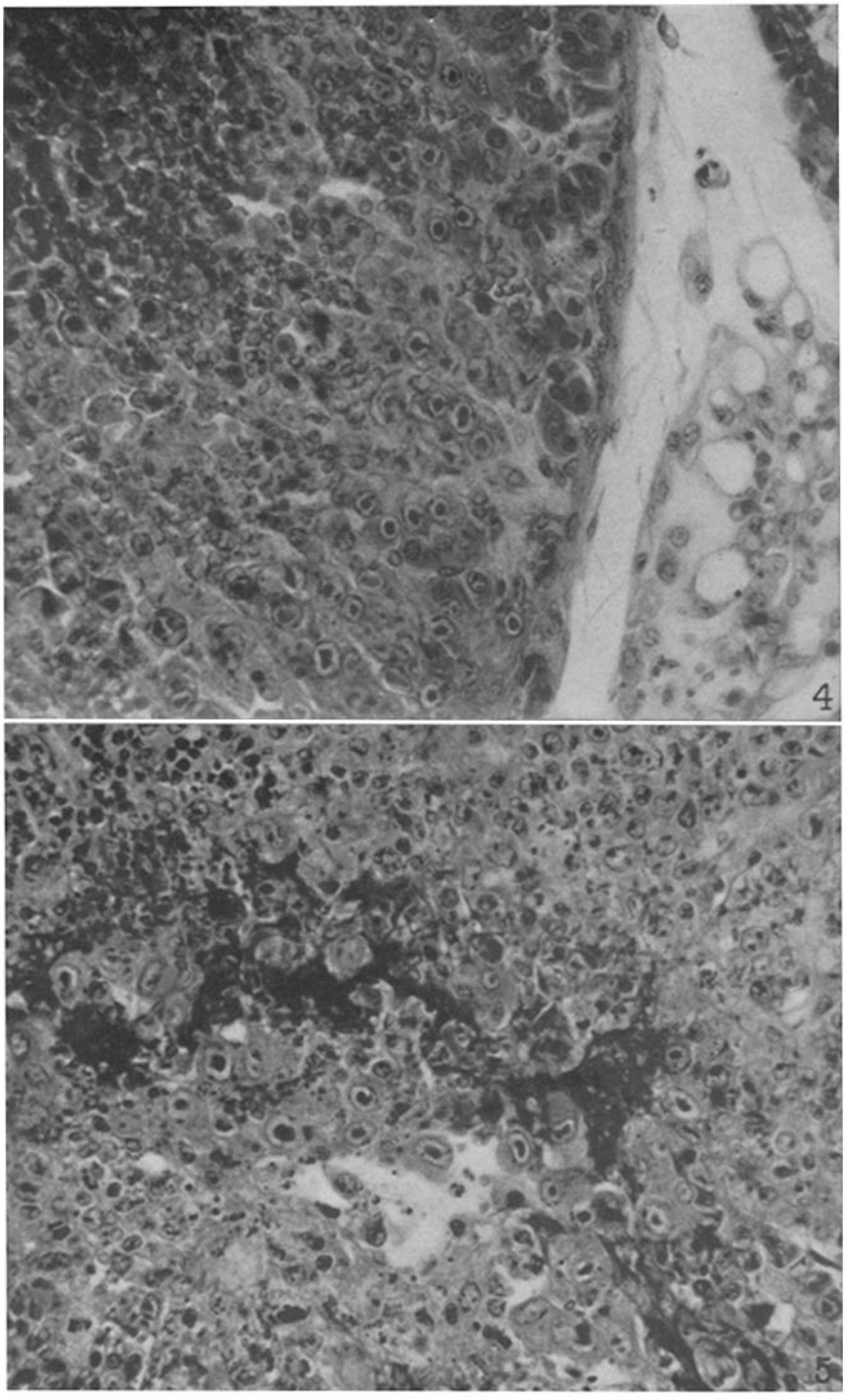

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