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SECTION MEETINGS

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Cytoplasmic Inclusion Bodies in the Pancreas and Salivary Glands of Small Laboratory Animals.

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During the examination of microscopic sections of the viscera of hooded rats that had been used in other experiments, large cytoplasmic inclusion bodies were noted in the acinus cells of the pancreas and of the salivary glands of a large proportion of the animals. The presence of the inclusions in untreated control animals and the resemblance of the inclusions to those described in mice affected with the virus of infectious ectromelia[1] made it seem worth while to survey our own and other colonies of small experimental animals and to attempt to influence the incidence of the inclusions by the means at our disposal.

Materials and Methods. Small experimental animals of 3 separate laboratories (labeled B, C, and D in Table I) of the Faculty of Medicine of McGill University were examined in addition to animals housed in our own department (labeled A in the table). The 4 laboratories were located in 3 separate buildings and there was no direct contact between the several animal colonies. The animals examined were apparently healthy and all were killed by exsanguination or illuminating gas. Blocks of tissue were removed from all of the organs, preserved in formalin and his-

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1 Marchal, J., *J. Path. and Bact.*, 1930, **33**, 718.

TABLE I.
Incidence of Inclusion Bodies in Pancreas and Salivary Glands.

Colony	Animal	No.	Age	Incidence of inclusions: Pancreas, %	Incidence of inclusions: Salivary glands, %
A	Hooded rats	120	2-20 mo.	75	12
	White "	10	2 yrs.	80	10
	White mice	10	3-12 wks.	50	0
	Wild " (trapped in animal quarters)	2	unknown	100	0
	Rabbits	3	"	66	0
	Guinea pigs	5	6-12 mo.	0	0
B	Hooded rats	15	3 wks.-6 mo.	47	20
	White mice	10	2 wks.-4 mo.	20	0
C	Hooded rats	10	adult	40	30
	Rabbits	3	unknown	66	0
D	Hooded rats	13	1-8 mo.	23	15

TABLE II.
Incidence of Inclusions in Hooded Rats Treated with Pilocarpine or Atropine.

Drug	No. of animals	Incidence of inclusions: Pancreas, %	Incidence of inclusions: Salivary glands, %
Pilocarpine nitrate (100 mg/kilo subcutaneously)	10	80	10
Atropine sulfate (60 mg/kilo intraperitoneally)	10	50	0
Untreated controls	5	80	20

tological sections stained with hematoxylin and eosin. All organs were examined microscopically until it became apparent that inclusions were present only rarely in sites other than the pancreas or salivary glands. The relative abundance of the inclusions in these latter organs was graded on a scale of zero to 4 plus.

Results. The results of the survey are set forth in Table I. A factor too cumbersome to be included in the table, but one which may be of importance is the date on which each animal was killed. A scatter graph constructed by plotting the relative abundance of inclusions in the pancreas of the hooded rats from our own laboratory against the date of examinations showed a definite peak of incidence and abundance of the inclusions in May and June for the 12 months embraced by the survey. The fact that no large number of animals was killed in April, September, or October made the peak less than absolute, but did not deny the marked variation in the months when large and comparable groups of animals were killed.

As shown in the table, the range of ages in each group is rather wide. When the animals whose ages were known exactly were studied individually, it was noted that no animal under 30 days old exhibited inclusions in either the pancreas or salivary glands, and that the oldest animals had the highest incidence. A litter of newborn hooded rats was sampled at weekly intervals, and no inclusion bodies appeared until the litter was exhausted at 8 weeks. In the last animal sacrificed, inclusion bodies were found in small numbers, but in the pancreas only.

The inclusion bodies (Fig. 1) as seen in the pancreas and in all 3 histological types of salivary gland tissue were found only in the acinus cells of these glands. Although frequently a single inclusion was seen in only one cell of an acinus, more often the inclusions appeared in clusters with from one to 3 bodies contained in each of 3 or 4 contiguous cells. The inclusions were cytoplasmic and generally located in that part

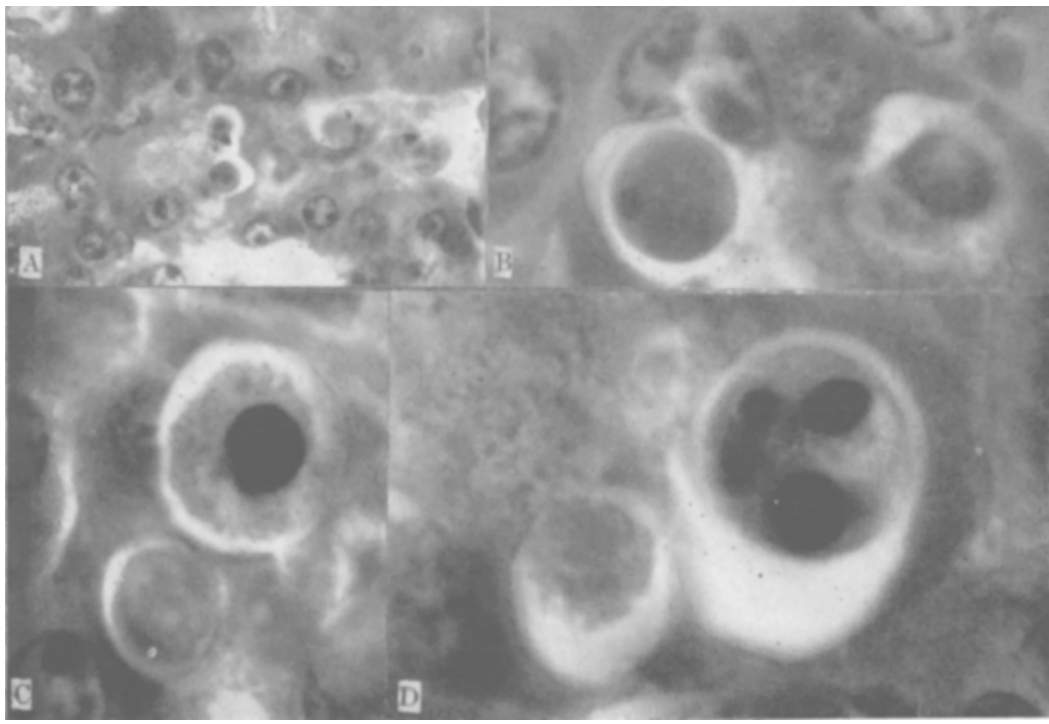

FIG. 1.

Photomicrographs of Cytoplasmic Inclusion Bodies in the Pancreas of Hooded Rats.

A. A cluster of inclusions in adjoining acini. Three large and several small inclusions may be seen. × 810.

B. One large hyaline appearing inclusion, clearly compressing the nucleus. Another inclusion to the right is slightly out of focus. × 2800.

C. A cluster of inclusions, one of which has a single basophilic granule. × 2800.

D. Two inclusion bodies, one of which contains 4 basophilic granules. The smaller inclusion consists of granules which resemble the zymogen granules visible in the left upper corner. × 3300.

of the cell nearest the lumen of the acinus. They were remarkably uniform in appearance in both pancreas and salivary glands in all of the animals regardless of species. They were spherical and large, often reaching a diameter of 15-20 micra, but in each animal varied widely in size. Their staining reaction was also variable, but tended in the main to be strongly eosinophilic. The largest inclusions usually contained from one to 5 small round basophilic granules. The eosinophilic part of the inclusions had either a homogeneous hyaline appearance or was finely granular. In some sections of the pancreas these granules strongly resembled the adjacent zymogen granules. In the salivary glands, the zymogen granules were slightly paler than those of the inclusions. Each of the inclusions was contained in an otherwise empty vacuole slightly larger than the inclusion body itself. The nuclei of the affected cells were pushed aside and were occasionally pyknotic, but showed no other sign of degeneration. Evidence of inflammatory response was lacking. Intranuclear inclusions were not seen, nor were inclusions of any kind found in the duct cells of either pancreas or salivary glands. It is noteworthy that the cytoplasmic inclusions in salivary glands were encountered only in rats.

Because facilities for virus research were not available, it was not possible to investigate by direct experiments the question of the possible virus etiology of the cytological changes described. The resemblance of the eosinophilic granules contained in some of the inclusions

to the zymogen granules in neighboring cells suggested the possibility that the formation of the cytoplasmic bodies might depend on a secretory disturbance. If this were true it seemed possible that their incidence, abundance, or appearance could be influenced by pilocarpine or atropine. Accordingly, the effects of these drugs were studied.

Hooded rats of comparable ages were given pilocarpine or atropine and killed in pairs at hourly intervals for 5 hours after injection. The details and results of this experiment are contained in Table II. It is evident from the figures presented in the table that the injection of pilocarpine produced no difference in the incidence of the inclusions from that observed in the control animals, nor was there any difference in the abundance or appearance of the inclusions in the affected animals. The incidence of the inclusion bodies was distinctly lower in the animals treated with atropine than in the controls, but it is noteworthy that there was no decrease in the abundance of the inclusions in the animals that presented them nor any change in their microscopical appearance. It is questionable, therefore, whether the decrease in incidence is really significant.

Discussion. Histological sections of pancreatic tissue containing typical inclusions were sent to Professor E. V. Cowdry for his opinion. He was kind enough to comment that he had never before seen similar cytoplasmic bodies in the pancreas. He noted the fact that there was no evidence of destruction of the affected cells and no sign of any inflammatory reaction, and expressed the opinion that a virus etiology was very questionable. Pointing to the similarity between the granules in the inclusions and the zymogen granules in neighboring cells, he proposed that the inclusions might represent some sort of temporary secretory retention and suggested trying the effects of pilocarpine and atropine. The essentially negative results of the experiments with these drugs offer no support for the idea that the inclusions were caused by a disturbance of secretory function, but neither do they completely exclude this possibility.

The possibility of a dietary factor being responsible for the formation of the inclusion bodies was considered. All of the rats examined, except for those from Laboratory D, were fed with Purina fox chow, which is commonly employed as the standard diet for small laboratory animals. This diet was supplemented with whole wheat bread in two laboratories. In Laboratory D, half the rats were raised on another brand of chow, and their growth was believed to be slower than usual. Among these latter animals inclusions were found more frequently than in other animals from the same laboratory, but the incidence in this sub-group still was lower (33% in the pancreas) than that in the other colonies. The histological criteria of avitaminoses (*i.e.*, epithelial metaplasia, muscle degeneration, etc.) were lacking in these and all the other animals. These facts offer no evidence to indicate that any dietary deficiency was a factor.

Search of the literature of the last 20 years failed to reveal any mention of the occurrence of inclusions similar in morphology and distribution to those described above. The closest resemblance to the lesions in the pancreas is shown by the cytoplasmic inclusion bodies described in this organ in association with infectious ectromelia of mice.[1] In this spontaneous, and often fatal, virus disease, similar, but smaller, inclusions are also found in the skin, intestinal glands, and salivary glands. A spontaneous disease comparable to infectious ectromelia of mice is unknown in rats, but inapparent ectromelia has been induced in rats by Reames[2] and by Burnet and Lush[3] by nasal inoculation. The latter investigators were able to demonstrate inclusion bodies in the nasal mucous membranes of some of the rats, but no inclusions were described in other tissues.

Pearson[4] and Cowdry,[5] among others, reported the presence of cytoplasmic inclusions in the duct cells of the salivary glands in animals affected by the salivary gland virus, in association with the more typical intra-

[2] Reames, Harold R., *J. Infect. Dis.*, 1940, **66**, 254.

[3] Burnet, F. M., and Lush, Dora, *J. Path. and Bact.*, 1936, **42**, 469.

[4] Pearson, E. F., *Am. J. Path.*, 1930, **6**, 261.

[5] Cowdry, E. V., and Scott, G. H., *Am. J. Path.*, 1935, **11**, 647.

nuclear inclusions. The cytoplasmic bodies, however, are smaller than those we have seen, are basophilic, and do not contain granules. In Pearson's cases, the cytoplasmic inclusions were seen in the acinus cells of the salivary gland occasionally, but most often in the duct cells, where the nuclear inclusions are characteristically present. McCordock and Smith[6] reported visceral inclusions in mice affected with the salivary virus. The inclusions were present in the acinar cells of the pancreas, as well as in other organs, but were chiefly intranuclear in type, although occasional basophilic cytoplasmic inclusions were found. From consideration of these facts, it is apparent that there is no morphological evidence of relationship between the inclusions we have seen and the salivary virus disease.

Inclusion bodies similar to those found in our animals were seen in the livers of 3 mice by Campbell[7] who thought they resembled the inclusions of ectromelia, but he was unable to prove their etiology. Shortt and Lahiri[8] described bodies which they called "eosinophilic vacuole-like structures" in the serous acini of the salivary glands of dogs affected by rabies. Later investigation showed similar inclusions to be present in non-rabid animals. These workers considered the bodies they observed to be identical with ectromelia inclusions, but were convinced that the inclusions in their animals resulted from hypersecretion. They attempted to prove this by showing a vast number of inclusions in dogs given pilocarpine, but unfortunately, the salivary glands were not biopsied before the drug was given, and their results are therefore inconclusive. Rosin and Doljanski[9] reported the presence of erythrocytes in the cytoplasm and nuclei of liver cells in rats given urethane and allyl formate. In late stages of degeneration, the red cell aggregates in the cytoplasm resembled the inclusion bodies seen in our animals, but there is no reason to believe that they were similarly formed, since hemorrhage was prominent in their animals and absent in ours.

Summary. Hitherto undescribed cytoplasmic inclusion bodies have been found in the pancreas of apparently healthy mice, rats, and rabbits and in the salivary glands of the rats. In both organs the inclusions were confined to the acinus cells. They were not encountered in guinea pigs. Their etiology is unknown. The possibility that the inclusions were caused by disturbance of secretory function was not supported by the results of experiments to test the effects of pilocarpine and atropine. Pilocarpine provoked no change in the incidence, abundance, or appearance of the inclusions and atropine, if it had any effect at all, was only mildly inhibitory. There is no evidence that dietary factors were responsible. Evidence for viral origin is only suggestive and consists of the following points: 1. The presence of inclusion bodies in the absence of nonviral factors known to produce them. 2. Strong resemblance of the inclusions to those described in infectious ectromelia of mice, a disease of proved virus etiology. 3. Absence of inclusions in animals under 30 days of age and an increasing incidence thereafter with increasing age. 4. A seasonal variation in the incidence and abundance of the inclusion bodies.

[6] McCordock, H. A., and Smith, M. G., *J. Exp. Med.*, 1936, **63**, 303.

[7] Campbell, J. A., *J. Path. and Bact.*, 1939, **48**, 223.

[8] Shortt, H. E., and Lahiri, B. N., *Indian J. Med. Res.*, 1934, **21**, 587.

[9] Rosin, A., and Doljanski, L., *Brit. J. Exp. Path.*, 1944, **25**, 111.