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Certain Affections of the Liver That Arise Spontaneously in So-Called Normal Stock Albino Mice.*

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During the course of attempts to find an agent possibly present in human infectious hepatitis, certain spontaneous lesions were encountered in the liver of "normal" stock albino mice which could confuse workers in this field.

The present paper reports on the pathological changes observed in young and old mice used in this investigation. Another phase of the problem, the application of the thymol turbidity test[1] for detection of hepatic dysfunction in the mouse, is still being studied and the results will be described in another article.

The disclosure of the hepatic lesions came about in this way: Serum or stool deriving from patients in the acute phase of infectious hepatitis was prepared as filtrates, ultracentrifugates, or left unchanged, and the material was injected into mice of the Swiss strain by a variety of routes. The mice generally (with few insignificant exceptions) showed no signs of disturbed health for periods of observation lasting beyond 5 months. Certain ones were sacrificed at different intervals beginning at 2 weeks after injection and continuing at 1 to 2 weeks' intervals for 3 months or longer after inoculation. When it was found that the liver of every animal exhibited changes no matter whether it had received test or control material, suspicion was aroused that the pathological reaction was nonspecific and a study was therefore undertaken based on the histopathology of the organ of normal mice of the Swiss as well as of the Rockefeller Institute strain.†

Two affections of the liver have been encountered in so-called normal stock mice. Whether they are different aspects of the same affection or two pathological entities, is not known. One was characterized by infiltration and necrosis, and the other by intranuclear inclusion bodies. Both conditions were distinct from the familiar cyst formation, atrophy, congestion and secondary involvement of the liver in experimental or natural infections with various microorganisms or viruses, or other changes met with heretofore in the laboratory. In both, the gross anatomy of the liver and other organs was normal and no associated microorganism or causal agent has as yet been recovered. To all appearances, animals bearing the lesions have been in good health.

Infiltrative and Necrotic Lesions. (Fig. 1 and 2). This type is commonly present in both strains of mice to varying degrees and more pronounced with increasing age, up to 1 year or older. Nurslings as young as 6 days exhibited the changes in all of 6 examined.

The histopathology consists of an infiltration, collar-like, chiefly about the smaller bile ducts, sometimes also about blood vessels, of cells of which polymorphonuclear leucocytes are predominant; monocytes, lymphocytes and plasma cells are also seen. There may be a few cells in the collar, or as many as a dozen rows. No bacteria, protozoa, or other microorganisms are detectable in the infiltrated areas. Here and there a small or large focus of these cells lies by itself in the parenchyma. The capsule of the liver is not

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1 Maclagan, N. F., *Brit. J. Exp. Path.*, 1944, **25**, 234.

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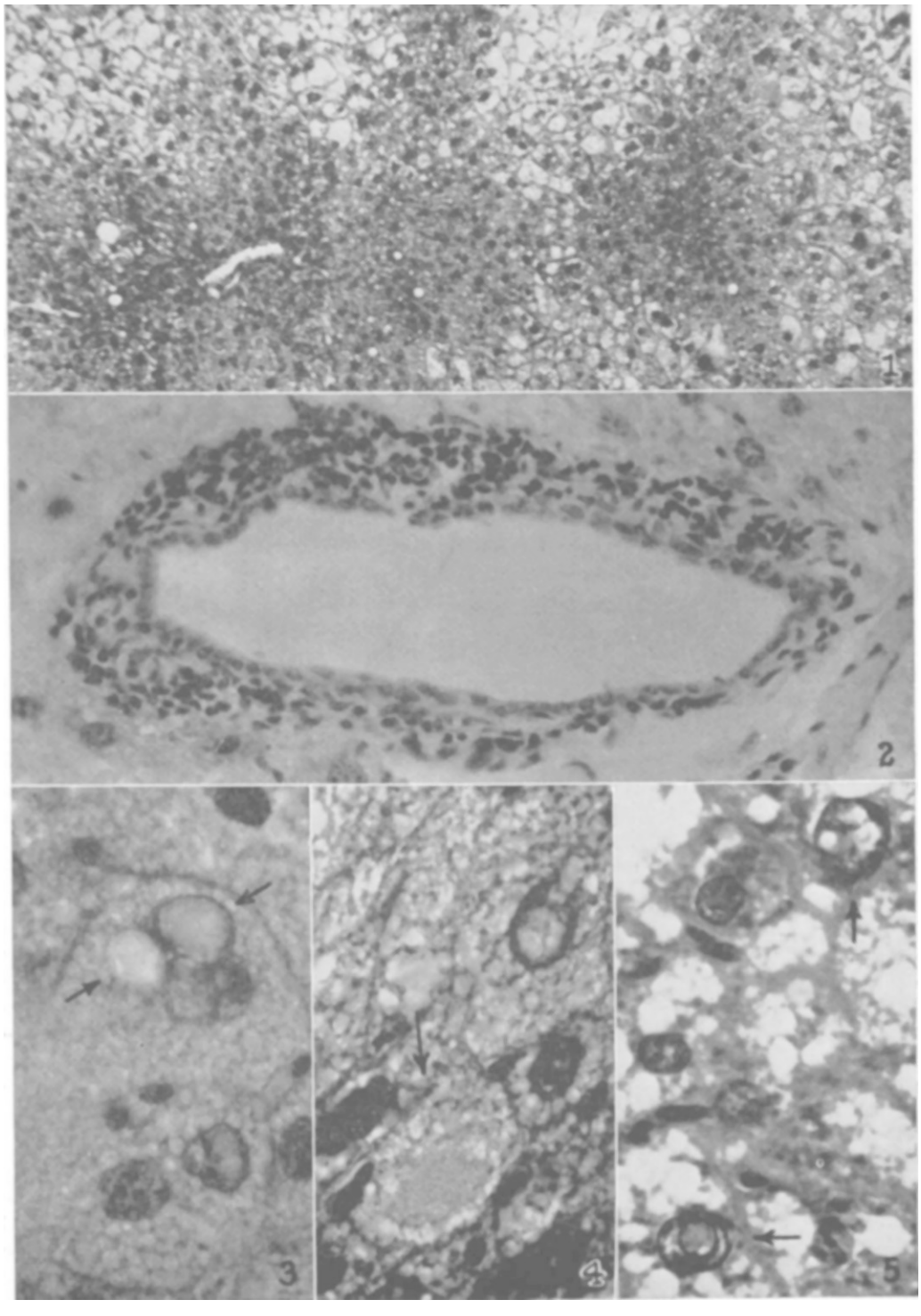

FIG. 1. Normal, 15-months-old mouse; necrosis of parenchymal cells of liver. Hematoxylin and eosin stain. × 275.

FIG. 2. *Idem.* Cellular infiltration of bile duct. Hematoxylin and eosin stain. × 425.

FIG. 3. Mouse about 3 months old, given infectious hepatitis material intranasally and examined 70 days later. To be noted are multinucleated giant hepatic cells with intranuclear inclusion bodies, and margination of nuclear chromatin. The topmost nucleus has an inclusion filling the entire nucleus, which is vacuolated, and next to it is an "empty" or "ghost" nucleus. Eosin-methylene blue stain. × 1000.

FIG. 4. *Idem.* A Councilman body (only rarely seen in these tissues) and nearby an intranuclear inclusion body surrounded by basichromatin, also margination of nuclear basichromatin. Giemsa stain. × 1000.

FIG. 5. Normal, 15-months-old mouse. Intranuclear inclusion with margination of basichromatin. Above, hypertrophied nucleus with inclusions and margination. Hematoxylin and eosin stain. × 950.

thickened; occasionally sinusoids are engorged; the architecture of the lobule is generally retained (Fig. 1). Necrosis of individual parenchymal cells is noted, or of larger zones usually about the central or about the intercalated (sublobular) veins, or sometimes midzonal. There is more variation in the degree of cellular necrosis than of infiltration. Of 97 mice examined, only 6 failed to reveal the infiltrative and 12 the necrotic or necrobiotic lesions. The liver cells lose their cytoplasmic contents, the nucleus is not visible and the cell body may disappear completely—yet the arrangement of the lobules is retained—a cell or a collection of cells becoming necrosed in its own site. Thus the necrosis is generally focal, not diffuse, and the areas are not surrounded by or involved in an inflammatory reaction or subject to autolysis. It would therefore appear that these changes are compatible with the seemingly good health of the animal and the liver can be regenerated to a normal appearance. Associated is the nonspecific nuclear degeneration in otherwise normal cells or in early stages of cellular necrosis. There may be a certain degree of chromatolysis and of margination of the chromatin with one or more nucleoli colored lavender or purple, instead of blue or blue-black, by Romanowsky-type and Giemsa, or hematoxylin-eosin dyes respectively. When a single nucleolus is present, it is often increased in size and still so stained. Such changes are not similar to true viral inclusions and are often seen in other conditions in various tissues.

Lesions Characterized by Intranuclear Inclusion Bodies (Fig. 3, 4 and 5). In older mice, from 3½ months to one year or more of age, and especially in the oldest of these, lesions occur in the liver, characterized by the presence of intranuclear inclusions which have the features of typical viral inclusion bodies. The inclusions have been noted in Swiss and Rockefeller Institute strains of mice, in one of 2 aged 3½ months and in 13 of 14 at least one year old, although they were not detected in any of 6 aged 4 months. Associated generally were the infiltrative and necrotic changes just described. At this stage it is difficult to say that the latter and the inclusion-body affection are independent manifestations—the only available evidence, *viz.*, that the inclusions have been observed to be free from the infiltrative and necrotic type and younger animals having these lesions are free, as a rule, from the inclusions, is not sufficient basis for their difference.

The bodies may occur in prodigious numbers with the majority of the hepatic cells involved but more often in an occasional cell nucleus. With eosin-methylene blue, Giemsa or hematoxylin-eosin, they stain bright pink and stand out in a nucleus in which the nucleoli are dislocated to the margin and are shrunken, and the nuclear chromatin is also marginated. The nucleus is often considerably hypertrophied. A typical picture of an involved nucleus shows a dark-bluish, wide and irregular margin enclosing a clear space in which are set one or more pink-stained bodies, smaller in size when multiple, but often when one body is present it occupies the entire space (Fig. 3, right). Other nuclei exhibit no structure whatever within their membranes (Fig. 3, left), similar to the "empty" or "ghost" nuclei seen in the liver of yellow fever[2] and in that organ following burns.[3] The inclusions do not, however, resemble those of the latter two maladies, since they are not wholly granular and often reveal a single large or several small spherical vacuoles. A single vacuole may have a well-defined, whole, regular margin (Fig. 4, upper right). The inclusions are surrounded now and again by a border of basichromatin (Fig. 5, lower arrowhead). A halo or clear zone encircles the entire inclusion body which is found, as a rule, in a cell the cytoplasmic structure of which is damaged. Certain inclusions are granular with marginated chromatin corresponding to Cowdry's Type A; others exhibit the hyaline, droplet-like appearance of Type B; a classification on this basis is not possible, moreover, because still others differ from both types.

Associated with these bodies may be seen the nonspecific nuclear changes already de-

[2] Cowdry, E. V., and Kitchen, S. F., *Am. J. Hyg.*, 1936, **23**, 55.

[3] Belt, T. H., *J. Path. and Bact.*, 1939, **48**, 493.

scribed. Rarely one encounters a Councilman lesion—acidophilic, hyaline necrosis of hepatic cells[4] (Fig. 4)—binucleated, and multinucleated giant (Fig. 3) cells. Finally, the infiltrative and necrotic lesions can, as already stated, exist together with the inclusion bodies.

Discussion. Even though the studies on the causal agent of the two described types of lesions are incomplete and the relation of one to the other is as yet to be determined, a report is made at this time in an effort to assist in the problem now being pursued by several workers on the possible transmission of human infectious hepatitis to mice.

Apart from the various pathological states which may arise in the livers of mice, mentioned in this paper, others have been reported; these have been published by Dingle in his valuable monograph.[5] In addition, Dubos[6] has recently observed in several strains of "normal" stock mice, especially in older ones, hepatitis characterized grossly by whitish, circumscribed areas scattered over the surface of liver and microscopically by areas of parenchymal necrosis infiltrated by cells surrounded by an inflammatory reaction. The causal agent of this affection has not as yet been determined by Dubos, but it is apparently wholly independent of *B. piliformis* infection of the mouse liver as described by Tyzzer.[7]

The inclusion bodies here reported conform with the three terms of the definition, stated by Cowdry,[8] of inclusions due to viral action: (a) their arrangement to represent developmental stages; (b) their characteristic appearance and their association with the ultimate death of the cell, and (c) a concomitant cellular reaction of hyperplasia, hypertrophy or necrosis. Further work is needed to determine their agreement with another definition of inclusions as viral in origin, as given by Lucas and Riser:[9] their specific association with the agent or pathological condition which produces them.

The published record on this subject is scant. Twort and Twort[10] reported no characteristic inclusion bodies in 12,000 mice employed in experiments with carcinogenic agents. On the other hand, Findlay[11] described briefly intranuclear bodies in hepatic cells in all of 25 Clacton-strain mice but in none of 60 other normal mice of 7 other strains. He was able to transmit an inclusion-body malady with positive liver tissue and thus suggested that the bodies are induced by a virus of low pathogenicity. There is a resemblance between the picture of the present inclusions and the description offered by Findlay—their identity is a matter for further study. Recently somewhat similar cellular inclusions in mice have been reported by Nicolau *et al.*[12] from Roumania. They encountered these lesions in the course of their attempts to transmit the agent of infectious hepatitis to mice. The Roumanian workers have stated that certain (undescribed) lesions can be found in livers of mice before inoculation.

Conclusion. Certain pathological states which arise spontaneously in the livers of so-called normal stock mice, especially with increasing age, have been described. Of particular interest are the hepatic lesions of (a) infiltration and necrosis, and (b) of intranuclear inclusion bodies—both occurring in Rockefeller Institute and Swiss strains of albino mice. The relation of the two types of changes is still to be studied as well as their etiology.

[4] Councilman, W. T., *Report on the Etiology and Prevention of Yellow Fever*, U. S. Marine Hosp. Service, 1890, p. 151.

[5] Dingle, J. H., in *Biology of the Laboratory Mouse*, G. D. Snell, Editor, Blakiston Co., Philadelphia, 1941, Chapter 12, p. 380.

[6] Dubos, R. J., personal communication.

[7] Tyzzer, E. E., *J. Med. Res.*, 1917, **37**, 307.

[8] Cowdry, E. V., *Am. J. Clin. Path.*, 1940, **10**, 133.

[9] Lucas, A. V., and Riser, W. H., *Am. J. Path.*, 1945, **21**, 435.

[10] Twort, J. M., and Twort, C. C., *J. Path. and Bact.*, 1932, **35**, 219.

[11] Findlay, G. M., *Brit. J. Exp. Path.*, 1932, **13**, 223.

[12] Nicolau, S., *et al.*, *Analele Institului Victor Babes*, 1944, **14**, 266; *Experiments Dealing with Epidemic Hepatitis Virus*, Bucarest, 1944, 34 pp. (Roumanian language).