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Difference in Susceptibility of Mice and Rats to Experimental Production of Amyloidosis.

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The chemical nature of amyloid, as well as the mechanism of its formation, has been very obscure. Experimental investigation of the problem has until recently encountered the difficulty that amyloid could not regularly be produced in animals. Thanks to the work of Kuczinski,[1] Jaffé,[2] Letterer[3] and others, this difficulty no longer exists. By parenteral injection of sodium caseinate and various other proteins and non-protein substances, as well as by feeding excessive amounts of protein, amyloidosis is produced *in mice* in the majority of those animals. In the course of the work of these investigators, certain observations have been made that greatly increase the interest of the problem of amyloid disease.

In experimental amyloidosis as well as in the spontaneous condition as it occurs in man, excessive breakdown of tissue proteins is known to take place. There is evidence that this is followed by an increased production of serum globulin, which apparently acts as the mother substance of amyloid.[3] It has been stated that on further injection of casein or other substances employed, globulin is precipitated in the tissues in altered form and that the resulting deposits constitute amyloid. The well recognized close relation of globulin to antibodies suggests that the whole process of amyloid formation is akin to an immunological reaction. This suggestion is at least worth testing, since, if true, experimental production of

[1] Kuczniski, M. H., *Virchow's Arch.*, 1922, ccxxxix, 185; *Klin. Wochenschr.*, 1923, ii, 727, 2193.

[2] Jaffé, R. H., *Arch. of Path. and Lab. Med.*, 1926, i, 25; 1926, ii, 149.

[3] Letterer, E., *Beiträge zur path. anat. u. z. allg. Path.*, 1926, lccv, 486.

amyloid would be a most useful method in studying the mechanism of certain immunological reactions, having the unique advantage of producing easily recognizable and characteristic morphological changes.

The best line of attack appears to be study of changes in blood chemistry during the course of production of amyloidosis. For this purpose an animal larger than the mouse is highly desirable. Guinea pigs and rabbits have, in the hands of other workers, given negative results. The rat was, therefore, selected as a possibly suitable animal.

Sixty adult mice and 16 rats were given daily intramuscular injections of pure casein (Hammersten), in 5% concentration with the addition of sufficient NaOH to give a clear solution. Since alkali caseinates may be denatured by heat sterilization and since it had not been determined whether denaturization affects its amyloid producing properties, different groups of animals received casein that had been prepared by boiling, or by autoclaving, or merely by addition of thymol. However, no constant difference in the effects of these various preparations was found. The dose given the mice was 15 mg. (0.3 cc.) in the gluteal region, while the rats received 15 mg. per 50 gm. of body weight for the first 35 injections and thereafter the same amount per 20 gm. body weight (*i. e.*, the same dose per body weight as was used in the mouse). The highest number of injections made was 91. The animals were kept on a constant diet of cracked corn and other cereals, fresh vegetables and bread (no additional water was given).

Animals were killed at intervals of 5 to 10 days. The spleen, liver, and kidneys were fixed in alcohol and in formalin. Paraffin sections were stained with haematoxylin and eosin and with cresyl-violet, the latter giving the clearest metachromatic reaction. Frozen sections were used for the iodine reaction and for specific staining with congo-red. Bielschowski's method was occasionally used to bring out the reticulum of the spleen.

A noteworthy difference was found in the effect of injections on the health of rats and mice. The former gave no evidence of illness, increased normally in weight and all survived to the end of the experiment. The mice, on the other hand, became emaciated and obviously ill. 29 out of 60 died during the experiment, but at autopsy no lesions (including suppuration) were found; these animals were not included in the study.

An equally striking difference was found in the tissues of the 2 species. No amyloid was found in any of the rats. 3 rats had received 20 to 34 doses, and 10 had received from 35 to 91.

FIG. 1.
Amyloid infiltration of the splenic follicles. The mouse had received 25 injections of casein solution. (Magnification X20.)

The mice, on the contrary, showed positive results in 75% of 25 animals that survived for 20 or more injections. The deposits appeared first and were always more marked in the spleen, the liver showed moderate amounts, while in the kidneys no amyloid or traces only were found. The various characteristic microchemical reactions for amyloid were positive whenever the material was found. In the spleen the earliest deposits had a fibrillar or shred-like appearance coating the fibrils and capillaries in the outer rim of the follicles. After 25 or 30 injections all but the inner third of the follicles were generally involved (Fig. 1), the deposition then was nearly homogenous, but remnants of lymphoid tissue were present in a few places. The central arterioles showed typical involvement in about one-half of the follicles. The splenic pulp never contained any amyloid. In the liver the deposition occurred first in the walls of hepatic arteries, then in the branches of the portal veins and in the intralobular capillaries, especially those near the lobular peripheries. The amyloid was found between the capillary endothelium and the cords of liver cells, eventually compressing both and occluding the capillary (Fig. 2). The character of the deposits was somewhat different from those in the spleen, for nearly all of them consisted of delicate needles with the points directed towards the cords of liver cells. After complete occlusion of the capillaries the crystals appeared as spherical masses (Fig. 3). In a few kidneys traces only of amyloid were found, occurring in the glomerular loops and in the fibrous stroma.

It has been seen that under identical conditions of experimentation amyloidosis was produced in 75% of mice, but that entirely

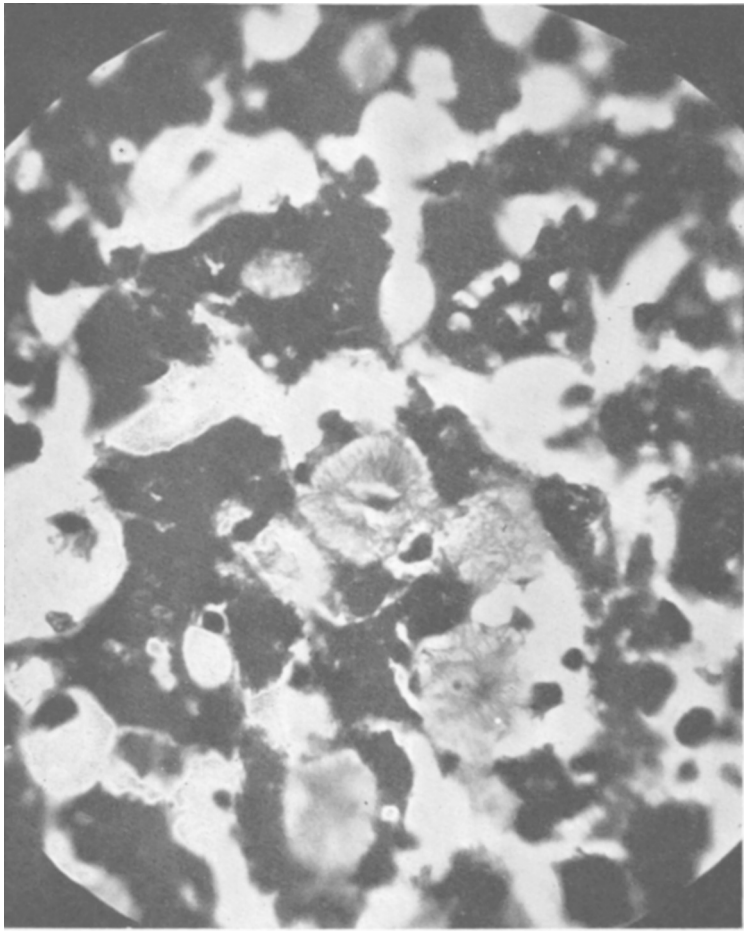

FIG. 2.

Amyloid infiltration of the liver. The amyloid occurs here in the form of crystalline needle-shaped deposits which surround and compress the sinusoids. An incompletely obliterated sinusoid, cut in transverse section, is seen as a dark spot in the center of one of the deposits. The mouse had received 25 injections of casein solution. (Magnification X920.)

negative results were obtained in the rat, a related species. Also that the mice suffered in health from the injections, while the rats thrived. It seems probable that in the latter, breaking down of tissue proteins is not sufficiently severe to lead to amyloid formation. No explanation of this difference in species susceptibility is known. That such difference exists is not, however, surprising since it is known that in man even individual susceptibility varies widely, in that comparable chronic suppurative conditions may or may not lead to amyloidosis.

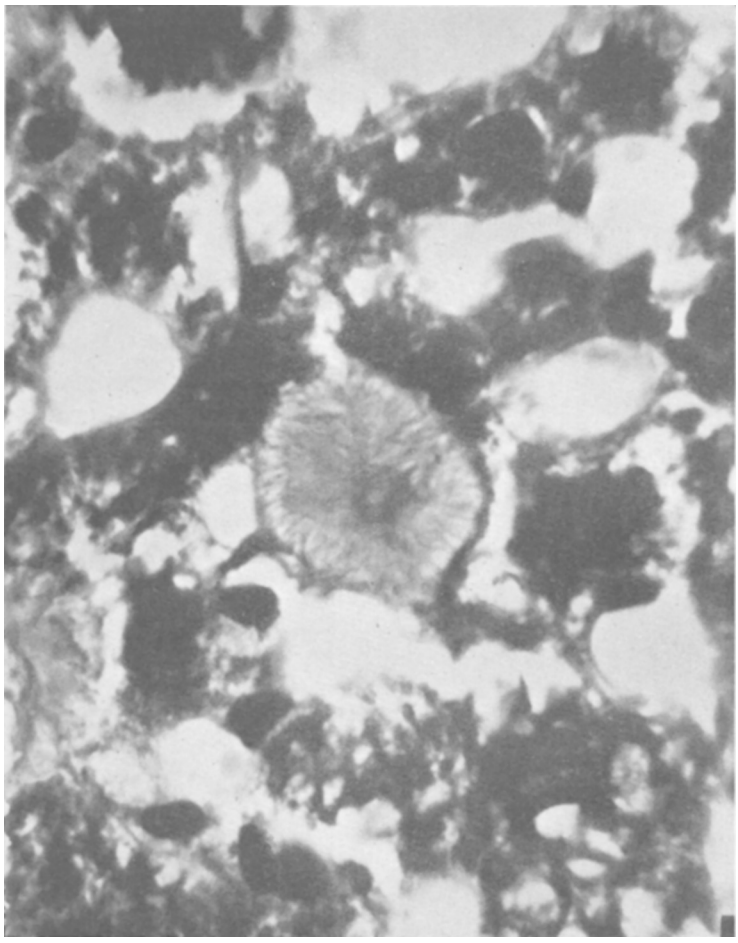

FIG. 3.
A higher magnification (X1840) of preceding photograph showing more clearly the crystalline structure of the amyloid, and the obliteration of the sinusoid.

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Histologic Effects of a Cholesterol-Free Diet on Adult White Rats.

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A series of white rats was raised upon a cholesterol-free diet over a period of 3 generations since the question had arisen as to whether or not cholesterol was synthesized in these rats in the absence of