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Experimental Studies of Amyloidosis.

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(Introduced by Benjamin Kramer.)

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Amyloidosis in humans is a secondary condition, resulting from a long continued suppurative infection or wasting disease. It is believed by some that it is not a degenerative process, but a physico-chemical disorder. The amyloid substance is deposited in the organs, initiating changes which interfere with the function of the organ. The theoretical aspects of this question will be discussed in a subsequent paper.

Some favorable clinical results obtained in a group of patients suffering with amyloidosis secondary to bone tuberculosis, as a result of administering a concentrated powdered whole liver preparation, led us to study this matter experimentally. White mice were used as the experimental animals.

The mice were set into 3 groups. Thirty mice of Group I received diet No. 1, which consisted of oats, powdered whole milk, bread and water. Forty mice of Group II were fed diet No. 2, which contained chopped raw meat in addition to the constituents of diet No. 1. Diet No. 3, which included the liver preparation* plus diet No. 1, was given to the 40 mice of Group III.

Amyloidosis was produced in the white mice by subcutaneous or intramuscular injections of a 5% aqueous suspension of sodium caseinate (Pfannstiehl) which is also designated as nutrose solution. The inoculations were given daily for 6 days, omitted on the seventh day. This procedure was repeated weekly. The amounts given varied from 0.3 cc. to 0.5 cc. per day.

To detect the earliest possible traces of amyloid formation, differential staining, intra-vitam with 1% Congo Red was used. This was in accordance with the methods of Smetana[1] and Jaffe[2], and stained only the amyloid.

The following procedure was used: Under ether anesthesia, the thoracic bony wall was exposed but not opened, and 0.5 cc. to 1.0 cc. of a 1% aqueous solution of Congo Red was injected directly into

* This liver preparation was generously supplied by Livermeal Corporation, Hoboken, N. J.

[1] Smetana, H., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 383.

[2] Jaffe, R. H., *Arch. Path. and Lab. Med.*, 1926, **1**, 26.

the heart. The fluid was allowed to run into the heart without extraneous pressure. The mouse was sacrificed from 3 to 6 hours thereafter.

The spleen, liver and kidneys were examined grossly. Blocks were hardened in Zenker's solution, imbedded in paraffin, cut, and the nuclei were stained with Delafield's haematoxylin. No cytoplasmic stain was used.

By this method, very early amyloid appeared as faint pink or violet intracellular inclusions, granular, homogeneous, or fibrillar in character. This was also observed by Smetana.[1] Adult amyloid appeared as a bright pink or red substance. In many instances, when stained according to Bennhold,[3] the microscopic findings were exactly as those seen in human amyloid. This amyloid, when in small amounts, appeared intracellular. In larger amounts, no cell structure could be detected, the amyloid appearing as homogeneous red masses surrounding vessels (as in the liver) or distending sinuses (as in the spleen), with a few shrunken, deeply pigmented and dense, or fragmented nuclei scattered within it. In no instance were we able to detect any cells or cell membranes separating the vessel lumen from the amyloid.

In the preliminary experiments, amyloidosis was produced successfully in the white mice after the 30th injection. Similarly in the present series of experiments, it occurred in the same period of time in Groups I and II. The mice in both groups showed essentially similar gross and microscopic changes at this stage and subsequently throughout the experiment. The first suggestive signs of amyloid formation in Group III appeared after the 58th injection. Definite evidence was found at the 66th injection. With continued inoculations, the amyloid changes became more marked in all groups. However, comparative studies (made at corresponding number of injections) showed strikingly less marked changes in Group III, definitely indicating a significant retardation in the development of amyloidosis.

Summary. 1. Amyloid first appeared in the spleen, then in the liver and later in the kidneys. In each instance this substance was first noted within cells of the reticulo-endothelial system. 2. Feeding concentrated powdered liver protects the animal against the formation of amyloid, delaying its formation markedly. In mice on ordinary laboratory diets or with chopped meat added to the diet, amyloid appeared after 30 injections of nutrose, in those kept on a laboratory diet plus powdered liver, no amyloid appeared until the

[3] Bennhold, H., *Klin. Wchnschr.*, 1924, **3**, 1711.

58th injection, at which time the animals kept on the other diets showed amyloid grossly and in large amounts, as well as the secondary pressure effects of the amyloid.

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Influence of Age on the Effect of Parathormone on Guinea Pig Bones.

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We have already indicated the striking difference in the response of young and adult guinea pigs to relatively large single doses of parathormone.[1, 2] *For the purposes of this study we consider a guinea pig fully adult when the major epiphyseal cartilage plates of the humerus, tibia and femur are closed.*

We produced the essential lesions of ostitis fibrosa in young guinea pigs by daily administration of 10 to 20 units of parathormone for 10 to 34 days. It is usually assumed that a more prolonged period of treatment would produce a greater effect. However, the animals' increasing age during the period of treatment had to be considered. Four guinea pigs were treated daily, beginning at the age of 2 to 7 days, with daily doses increasing to 20 units during the last 2 to 3 months. The total length of the treatment was about 110 days, at the end of which time the epiphyses were not closed. In the treated animals the bones showed no marked changes when compared with their normal litter-mate controls, and the changes were insignificant when compared with those previously reported in younger animals. While the number of animals was small, the data were consistent enough to warrant the conclusion that the serum calcium is lowered toward the end of a long course of treatment of guinea pigs with parathormone.

We believe that for a time the severity of the lesions increased in these animals. However, when the animals reached a certain age the effect of parathormone on the bone was decreased, and healing actually occurred after that time in spite of the continued administration of parathormone. This is not to be interpreted as evidence of immunity developed in the course of the treatment.

[1] Bodansky, A., Blair, J. E., Jaffe, H. L., *J. Biol. Chem.*, 1930, **88**, 629.

[2] Jaffe, H. L., Bodansky, A., Blair, J. E., *Arch. Path.*, in press.