

Changes in Adrenal Medulla Following Treatment with an ACTH-Preparation Containing Carboxymethylcellulose.* (22132)

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Several investigators believed to have produced changes in the adrenal medulla by treatment with certain pituitary preparations. It was thought, for instance, that injections (1) or even oral administration (2) of a special "medullotrophic hormone," as well as parenteral administration of purified somatotrophic hormone (3), can stimulate growth of the chromaffin-cells in adrenals of laboratory rodents. However, none of these changes can be regularly reproduced and definite proof is lacking that any pituitary-preparation exerts a specific effect upon the function or structure of medullary cells. The generally recognized adrenotrophic hormone of the pituitary is called "adrenocorticotrophic" precisely because of its selective effect upon the cortex. We have long been searching for possible medullotrophic actions of ACTH-preparations. We were never able to demonstrate any such effects however, excepting the dilatation of the medullary blood-vessels and a suggestion of hypertrophy in the chromaffin-cells, which were thought to be merely secondary results of the increased blood-flow and the consequent improvement in the nutritional status of the gland as a whole. On the other hand, recently, using the PAS-technic (4), we quite regularly observed definite changes in the adrenal medulla of rats treated for various purposes with certain "long-acting" ACTH-preparations. (5). To illustrate the histologic characteristics of these changes it will suffice to present one such experiment here, since the results were uniformly the same whenever 20 I.U. per day or more was given to rats during 2 weeks or more.

Experimental. In a typical experiment, 20 I.U. of "Duracton" (Nordic Biochemicals Ltd., Montreal) was given subcutaneously, twice daily, to 10 female Sprague-Dawley rats having an average initial body-weight of

100 g. This ACTH-preparation contains 24 I.U./mg, and is made available with carboxymethylcellulose (added as a retarding agent) in an aqueous solution containing 20 I.U./ml. This treatment inhibited growth, so that the treated animals gained only an average of 14 g, while a group of 10 normal control rats gained an average of 42 g during the period of observation. All animals were killed after 14 days, at which time the mean adrenal-weight was 42 mg (range 37-47) in the controls and 92.5 mg (range 90-104) in the ACTH-treated animals. Immediately after autopsy the adrenals were fixed in susa-mixture, embedded in paraffin and stained with hematoxylin-eosin.

The slides again gave the impression that only the cortex participates in the pronounced adrenal enlargement. The individual medullary cells were not conspicuously hyperplastic, although the medullary vessels were markedly dilated. However, slides subsequently stained with the PAS-method revealed numerous rust-colored darker cell-elements throughout the medulla. Some of these cells are elongated or roundish, while others have a typical epithelioid appearance and resemble the adjacent chromaffin-cells. Occasional rust-colored granules were also seen in the cortical cells, especially in the ectopic cell-nests within the medulla, as well as in the cells of the glomerulosa and reticularis layers. Some of the reticulo-endothelial cells, which line the cortical sinusoids, likewise contain such granules (Fig. 1).

The general appearance of many among these cells is reminiscent of mast-cells. We have therefore stained additional sections of these adrenals with such selective mast-cell dyes as neutral red, acridine yellow, toluidine blue and Unna's mixture. The many granules in the mastocytoid cells of the medulla, as well as the individual granules within the cortical cells, stained uniformly, like mast-

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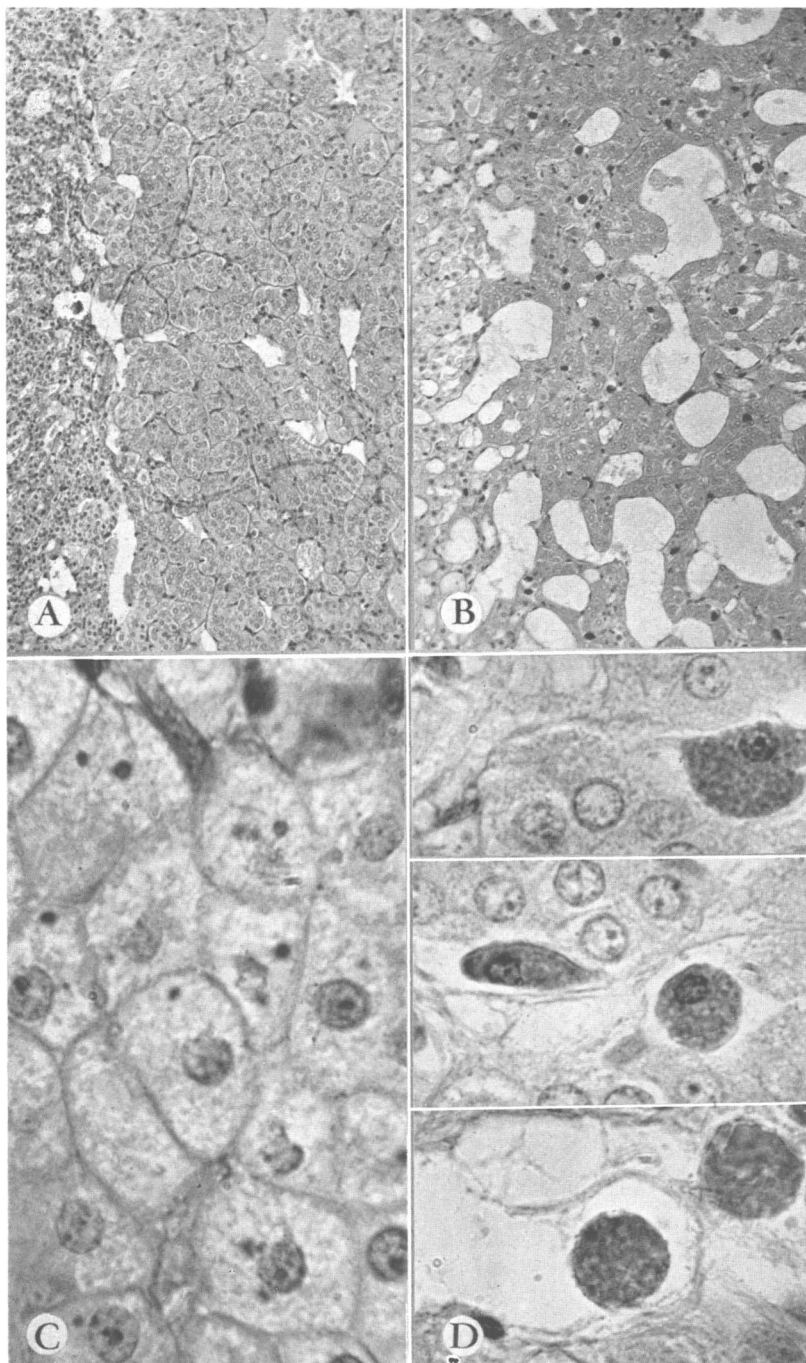


FIG. 1. A. Inner cortex (left) and medulla, under low magnification, in an untreated control rat. B. Same region in an ACTH ("Duracton") treated rat. Black mastocytoid elements are distributed throughout the chromaffin-tissue of the medulla. The medullary veins are greatly dilated. C. High magnification of a cell-region from the fasciculata of an ACTH ("Duracton") treated rat. Note individual small black granules (which stain like mast-cell granules) in the cortical cells. Their size is usually approximately that of a nucleolus and only a few such granules tend to appear within any one cortical cell. D. Three fields from the medulla shown in "B." Note various types of mastocytoid elements, some of which are epithelioid, some spindle-shaped and some rounded. All the sections in this plate were stained with the PAS-stain, which lends itself particularly well for black-and-white photography.

cells (selectively, but orthochromatically, with neutral red and acridine yellow, metachromatically with Unna's mixture and toluidine blue). Some material with similar staining properties was also found in the blood of the adrenal venules; here it usually adhered to erythrocytes.

A comparison with typical mast-cells was then made by staining sections of the thymus of the same animals on the same slide with their adrenals. It was found that the ordinary mast-cells of the connective tissue in the thymus are smaller, their shape is more regularly ovoid and they contain much smaller and more intensely staining granules than the medullary mastocytoid elements.

Discussion. On the basis of the evidence available to us, it is impossible to advance any hypothesis concerning the possible histogenesis or function of the mastocytoid cells. It should be pointed out however that, after treatment with ACTH-preparations without added carboxymethylcellulose, we have not observed any such mastocytoid elements in the medulla, nor metachromatic granulations in the cortical cells. On the other hand, control experiments with the carboxymethylcel-

lulose-containing solvent alone, gave positive results. Presumably, the mastocytoid elements in the adrenal medulla are due to ingestion by histiocytes of metachromatically staining carboxymethylcellulose granules.

Summary. Observations on rats indicate that treatment with large doses of an ACTH-preparation, to which carboxymethylcellulose was added as a retarding agent, results in the development of mast-cell-like elements within the adrenal medulla. At the same time, in some cortical cells and in adrenal-vein blood, occasional granules appear, which give the characteristic staining reactions of mast-cell granules. Carboxymethylcellulose-solutions containing no ACTH produce similar changes.

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Further Observations on L Forms of *Alpha*-hemolytic Streptococci.*† (22133)

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Isolation of L-type cultures from 2 strains of *alpha*-hemolytic streptococci was described in 1953(1). Many attempts at isolation of

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these forms from *alpha*-hemolytic streptococci in previous years had been unsuccessful. This paper contains the description of two methods which permit with some regularity the finding of strains of *alpha*-hemolytic streptococci which produce L forms. Data are also presented concerning the occurrence of such strains in humans and observations on a pleomorphic strain of *alpha*-hemolytic streptococcus which produced L forms spontaneously without the influence of penicillin.

After the characteristic appearance of L colonies of *alpha*-hemolytic streptococci and the conditions under which they grow became