

clarify the point. No evidence has been obtained to indicate which of several conceivable biochemical pathways might be involved in the formation of BAIB from a 5-methylpyrimidine.

The hypothesis(2) of a genetically determined error in kidney resorption seems logical as an explanation of the high BAIB levels in some normal individuals, but it does not appear to be consistent with clinical data showing marked lowering of the BAIB excretion following therapeutic regimes more likely to injure than to improve kidney function. The studies described suggest, as one alternative hypothesis, that a high BAIB level might indicate an abnormal rate or type of 5-methylpyrimidine catabolism.

Summary. (1) A compound frequently noted in a chromatographic study of urinary amino acids has now been isolated from the urines of two cancer patients and identified as

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beta-aminoisobutyric acid (BAIB). (2) In a group of 140 apparently healthy subjects, 52 individuals showed detectable urinary levels of BAIB (>0.15 millimole per liter), and three of these showed concentrations over 1 millimole per liter. Individual control subjects studied at intervals for periods up to $2\frac{1}{2}$ years showed little fluctuation in BAIB excretion. (3) In individual patients with neoplastic disease, the urinary BAIB level occasionally varied markedly and appeared to bear some relationship to the neoplastic process. Such variations are illustrated by a report of a case of chronic myelogenous leukemia. (4) Theoretical considerations pointed to a 5-methylpyrimidine as a likely precursor of BAIB, and evidence favoring this view was obtained by rat experiments in which BAIB excretion was produced by a diet containing large amounts of desoxyribonucleic acid but not by one similarly enriched in ribonucleic acid.

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Effect of Cortisone on the Mast Cells of the Rat. (19001)

CESARE CAVALLERO AND CARLO BRACCINI. (Introduced by C. G. Tedeschi.)

From the Anatomico-pathological Institute, University of Milan, Italy.

Recent observations have stressed the important role played by cortical hormones, especially cortisone, in the collagen diseases and in the biochemistry of the mesenchymal changes in dermal connective tissue both in man and in laboratory animals under normal and pathological conditions. These changes concern both the fibrillar and cellular constituents of the connective tissue and the homogeneous interfibrillar ground substance. There are two prevalent opinions concerning the physiological significance of the mast cells: some authors believe that these cells produce the hyaluronic acid of the ground substance; according to others the mast cells produce heparin.

In view of the evident changes caused by cortisone on acid muco-polysaccharides (hyal-

uronic acid) of the tissues, we wished to ascertain whether these histochemical and biochemical changes were related to the morphology of the mast cells.

Material. For this purpose, 36 unselected adult male rats, fed on a fixed and balanced standard diet, were divided in groups of 6 animals. Three groups were injected subcutaneously with cortisone ("Ciba") 10-5-1 mg/day per animal for 3-20-70 days respectively. For the same period 3 groups of control animals were given similar doses of physiological saline alone. At the end of experiment all animals were killed with a sharp blow on the head. Specimens were taken from the skin of the neck and from the muscles of the thigh and myocardium; these were fixed in Carnoy fluid, cut in series, and stained with an aqueous

TABLE I. Effect of Cortisone on the Mast Cell Counts in the Skin, Muscle and Myocardium of the Rat.

Group	Dosage	No. of mast cells per mm ² of tissue		
		Skin	Muscle	Myocardium
Controls	saline 3 days	68.13 ± .57*	5.55 ± .50	4.41 ± 1.24
	20	70.64 ± 2.70		
	70	81.13 ± 2.30	7.09 ± .23	3.73 ± .34
Cortisone	10 mg 3	30.90 ± 2.16	1.50 ± .39	1.22 ± .60
	5 mg 20	30.58 ± 4.50		
	1 mg 70	25.31 ± 5.20	3.30 ± .15	.59 ± .10

* Stand. error calculated following the formula $\sqrt{\frac{S \sum x^2}{n(n-1)}}$.

0.05% toluidin blue solution for 18 to 20 hours according to Bunting(1). Microscopical examination of each specimen was carried out and the mast cells were counted. For this purpose we used a magnification of 200 diameters, counting the mast cells in 300 microscopic fields in each specimen and in each animal. The values reported in Table I represent the calculated number of mast cells per mm² of tissue examined. Hyaluronic acid in the connective tissue was identified by means of bull testis hyaluronidase and subsequent staining with periodic acid-leucofuchsin according to Hotchkiss(2); the enzyme was employed in a concentration of 15 turbidity reducing units per cc in 0.3% NaCl solution at 37°C per 18 hours.

Results. Cortisone treated rats showed a marked fall of the body weight, a decrease of the eosinophil and lymphocyte count in the peripheral blood, and atrophic changes of the spleen, thymus, and bone marrow as well as a rise of the liver glycogen. In these rats the skin was atrophied and presented a clear decrease in the dermal hyaluronic acid; fibroblasts were scanty, and the fibrils reduced in number. The deeper layers of the corium showed an evident reduction of the loose connective tissue. In all the experimental series the mast cells were clearly reduced in number (see table) and in size, and the granules were similarly affected; many of the cells were disintegrated and the granules dispersed in the

ground substance. As in normal animals, these cells showed mainly a perivascular distribution. The metachromatic substance of the granules was refractory to the action of hyaluronidase, in contrast with the ground substance of the connective tissue.

The examination of the muscular tissue and myocardium gave similar results: metachromatically stainable material had practically disappeared from the connective interfibrillar tissue and the mast cells were markedly decreased in number (see table).

Discussion. It has been claimed that cortisone and ACTH reduce the hyaluronic acid content of the mesenchymal tissues both in normal and pathological conditions(3,4). On the other hand it has been demonstrated that the administration of cortisone in man is rapidly followed by a rise of the circulating heparin-like substances(5,6).

It is very probable that both these changes are intimately correlated with the inhibitory effect of cortisone and the mast cells. In our experiments the decrease in number of these elements was very marked and at the same time a loss of the metachromatically staining material of the connective tissue could be observed. Similar results were obtained by Asboe-Hansen(7) in skin biopsies from pa-

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4. Cavallero, C., Bertazzoli, C., Rossi, L., and Sala, G., *Sperimentale*, 1951, in press.

5. Monto, R. W., Brennan, M. J., Margulis, R. R., and Smith, R. W., *J. Lab. Clin. Med.*, 1950, v36, 1008.

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tients treated with ACTH; this author was of the opinion that hyaluronic acid is a product of the mast cells and that these cells are the peripheral transmitters of the cortisone hormonal action.

In other experiments, we have been able to show that the inhibitory effect of cortisone is well marked on all the cellular constituents of the connective tissue: fibroblast, reticulo-endothelial system, plasma cells(8,9). The reduction in number of mast cells may be regarded as a partial manifestation of this general inhibitory effect of the hormone. In other unpublished experiments we have also observed that in agreement with others(10), the hormone, also when locally applied de-

creases the mast cells and other cellular constituents in the dermal tissue. It is very likely therefore that the action of cortisone is more pharmacological than hormonal in nature.

Summary. Adult rats, injected with cortisone in various doses and for a different period, constantly showed a marked reduction in the mast cell counts of the dermal, muscular and myocardial tissue, together with a strong decrease of hyaluronic acid of the connective tissue. Similar results were observed in the skin when the hormone was applied locally. It is pointed out that a close correlation exists between the mast cells and the hyaluronic acid and that cortisone acts as an inhibitory factor on these cells, just as on the other cellular constituents of the mesenchymal tissues.

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Effect of Soybean Sterols in the Diet on Plasma and Liver Cholesterol in Chicks. (19002)

D. W. PETERSON. (Introduced by Ruth Okey.)

(With the technical assistance of R. Robbins, E. A. Shneour, and W. D. Myers.)

From the Division of Poultry Husbandry, College of Agriculture, University of California, Berkeley.

It is generally agreed that the absorption of sterols from the intestinal tract is highly specific. Cholesterol is readily absorbed by animals, especially in the presence of fat. Schoenheimer and associates(1-3) and others (4) have shown that a number of plant sterols are not absorbed.

The experiments described in this report were suggested by the author's observation that simultaneous addition of soybean sterols and cholesterol to the diet did not produce the rise in plasma cholesterol which was obtained

when cholesterol alone or in oil was added to the chicks' diet.

Animals. The experimental animals were 14-day-old single comb White Leghorn chicks that had been maintained on a standard stock diet from the time of hatching. In the preliminary experiments (Fig. 1) chicks of both sexes were used. In the later experiments (Tables I and II) only males were employed.

Diets. The basal diet used in these experiments was the same as that previously reported by Peterson(5). Additions of cholesterol, soy sterols* and cottonseed oil were made by substitution for a like amount of

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5. Peterson, D. W., *J. Nutrition*, 1950, v42, 597.

* Mixed Soy Sterols containing a minimum of 95% sterols, principally sitosterols and stigmasterol, were supplied through the courtesy of Mr. C. N. Behrmdt of Distillation Products Industries.