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Mucopolysaccharides of Renal Collecting Tubule Cells in Potassium Deficient Rats.* (28194)

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Striking cytoplasmic granules appear in the cells of the renal collecting tubules in potassium deficient rats. These granules are PAS positive(1), and there is evidence that some components of the serum proteins which pass through the glomerular filter enter into their composition(2). This report is concerned with further investigations dealing with the nature of the amino-sugar component of these granules. The Hale iron method(3) was employed as well as the well-known PAS technique. This Hale procedure involved exposure of the tissue to a colloidal ferric hydroxide. When acid groups are present in the mucopolysaccharide, a colorless compound is formed, which becomes blue after potassium ferrocyanide treatment.

Methods. Male Wistar rats, about 200 g in weight, were made potassium deficient by feeding a commercial low-potassium diet (Nutritional Biochemicals Corp., Cleveland). Control animals were pair-fed this diet adequately supplemented with potassium. After 4 weeks of feeding, blood was withdrawn by aortic puncture, the rats were killed, and portions of the renal papillae were fixed with a formalin-sublimate solution(4) and embedded in paraffin for sectioning at 3 μ. Sections were stained with the Hale iron technique(3), the PAS method, Azure A at low pH(5), and with hematoxylin and eosin. Other procedures used before applying the Hale or PAS method included mild methylation(5), extraction with hot chloroform-methanol(5), or incubation at 37°C in the presence of one of the following enzymes, according to the directions given in Pearse(6): amylase-alpha, malt diastase, crystalline pepsin, crystalline trypsin, chymotrypsin (Nutritional Biochemicals Corp.), cathepsin (Bios Laboratories, Inc.), testis hyaluronidase (Mann Research Laboratories), receptor destroying enzyme (Beringwerke Ag., Marburg-Lahn, Germany), or a highly purified neuraminidase preparation (1.1 mg of protein/ml), kindly supplied by Dr. Leonard Warren, Nat. Inst. Health. Analyses of the serum for potassium were made by flame photometry.

Results. Rats fed the low potassium diet failed to gain in weight during the 4 weeks of the experiment. The mean serum-potassium of the experimental rats was 3.1 $\pm$ 0.2 meq/l compared to 4.9 $\pm$ 0.1 meq/l in the controls. These findings are consistent with a satisfactory induction of potassium deficiency in the experimental animals.

Characteristic PAS positive granules were seen in the collecting tubule cells of the potassium deficient rats. Since chloroform-methanol extraction or pretreatment of the sections with 1% amylase-alpha in saline at 37°C for one hour did not affect the staining capacity of the granules with the PAS, the granules presumably contain a polysaccharide other than glycogen. The granules were also positive with the Hale iron method which

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indicated the presence of acid groups. The absence of metachromasia in the granules when sections were treated with 0.02% Azure A at a low pH and the fact that mild methylation blocked the Hale staining indicate that the ferric hydroxide, which is used in the Hale method, is combining with carboxylic rather than sulfonic groups.

Since carboxylic groups are present everywhere, a positive Hale reaction may be meaningless, unless, as shown by Mowry(3), the procedure is performed at a very low pH. Therefore, in order to rule out any possibility of nonspecific effect, further tests were made. The Hale reaction was unmodified after hot chloroform-methanol extraction (at 37°C for 24 hours) and uninfluenced by amylase or diastase treatment. Lipoid substances or simple polysaccharides were thus not responsible for the positive Hale staining.

Cathepsin incubation at pH 5 and 37°C for 1-2 hours did not interfere with Hale staining. On the contrary, pepsin, and in less degree trypsin and chymotrypsin(6), increased its intensity. We believe this results from removal of "masking" proteins. From all these tests, it is concluded that the Hale positive material of the granules is an acid mucopolysaccharide.

The particular groups in the mucopolysaccharide were studied by exposing the sections to the action of various mucolytic enzymes. It was observed that testis hyaluronidase in buffer phosphate of pH 6.8 (1 mg/ml) at 37°C for 3 hours was inactive, even after the section had been incubated with pepsin to remove the masking proteins. On the other hand, receptor destroying enzyme was highly effective in removing the Hale positive material. In this reaction, 3 drops of the enzyme solution obtained from the manufacturer were added to each milliliter of a Tris-maleate buffer at pH 5.6 which contained 0.01 M of $CaCl_2$, and sections were incubated in the solution at 37°C for 5 hours. Neuraminidase, which was used according to Warren's technique(7), was also active in removal of Hale positive material in the granules. The PAS positive component was not affected by this last enzyme.

Comment. These results support the idea that one of the components of the granules which appear in the collecting tubule cells of the potassium deficient rats is likely to be a sialic acid-containing mucopolysaccharide. The sialic acid may be incorporated as a side group in the same polymer responsible for the PAS staining. The fact that the granules continue to give a positive PAS reaction after receptor destroying enzyme treatment indicates that this basic polymer does not suffer extensive degradation after enzymatic elimination of the sialic acid.

The finding that pepsin, trypsin, and chymotrypsin may uncover masked mucopolysaccharides and thus allow increased Hale staining capacity, as happened to the granules in our experiments, confirmed similar observations made by one of us on the surface of free tumor cells(8,9) and on the ground substance of solid tumors(10).

It has been shown elsewhere that sialomucins from normal tissues in rats are not susceptible to the action of neuraminidase, but sialomucins from abnormal tissues of the rat may be successfully attacked by such an enzyme(11). The observation reported here appears to be another instance of this phenomenon.

Summary. The granules which appear in the collecting tubule cells of potassium deficient rats give a positive reaction with both the Hale iron technique and the PAS stain. The capacity for Hale staining is removed after exposure to receptor destroying enzyme or neuraminidase, but not by hyaluronidase, cathepsin, hot chloroform methanol extraction, or exposure to amylases. The Hale staining was intensified after pepsin, trypsin, or chymotrypsin treatment. It is concluded that the granules contain an acid mucopolysaccharide, which probably contains sialic acid side groups, and that the acid mucopolysaccharide may be masked by protein, which can be uncovered by the action of pepsin, trypsin, or chymotrypsin.

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Development of Malignant Lymphomas by Cell-Free Filtrates Prepared from a Chemically Induced Mouse Lymphoma.* (28195)

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It is known that Ak mouse malignant lymphomas can be transmitted by injection of cell-free filtrates into newborn C3H mice(1). Malignant lymphomas have also been induced following inoculation into newborn hosts of cell-free filtrates, prepared from x-ray-induced malignant lymphomas of C57Bl/Ka and C3H mice(2,3). Recently, several unsuccessful attempts have been made to transmit chemically-induced mouse lymphomas by cell-free filtrates(4,5,6).

In the present investigation a further effort is made to demonstrate a viral component by cell-free filtration in malignant lymphomas induced with a single subcutaneous injection of 100 μg of 7,12-dimethylbenz(a)anthracene (DMBA) in Swiss mice.

Materials and methods. Malignant lymphomas have been induced in our laboratory by subcutaneous injection of 100 μg DMBA in Swiss mice at birth with an incidence of 50.0 % in the females and 63.6% in the males. The clear distinction between induced and spontaneous malignant lymphomas both on the basis of histological type and time of origin has been described(7). At the age of 16 weeks one male mouse from the above mentioned group was sacrificed with ether and showed a marked enlargement of the thymus. Small sections from the thymus and other organs of this mouse were fixed in 10% buffered formalin and stained with hematoxylin eosin. The cytologic diagnosis revealed a malignant lymphoma, stem-cell type.

A cell-free extract from the thymus was prepared by Gross technique(1), using a 20% cell suspension in physiological saline, centrifuged at 0°C, first at 3000 rpm (1400 $\times$ *g*), for 15 minutes, then at 9500 rpm (7000 $\times$ *g*) for 7 minutes; the supernate was passed through a Selas, porosity 02, porcelain filter candle. The filtrate was used for injection as follows: 0.05 ml was injected subcutaneously to each of 8 newborn (less than 24 hours old) Swiss mice and 0.1 ml was injected intraperitoneally into each of 10 eight-week-old Swiss mice. As a control, 29 female and 28 male Swiss mice were kept untreated.

The mice used were all Swiss albino of the colony bred randomly in this laboratory. They were housed in plastic cages with wood shavings, separated according to sex at weaning, fed Rockland diet in pellets and tap water *ad libitum*. The animals were carefully checked and weighed at weekly intervals and the changes recorded. They were allowed to die spontaneously or were killed when found in poor condition. Complete necropsies were performed on all animals. All organs were fixed in 10% buffered formalin and sections from these tissues were stained with hematoxylin-eosin.

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