marrow cells of 8-month-old radiochimeras were virtually entirely of donor origin, whereas about 50% of the bone marrow cells from the 2-month-old heterotopic grafts were of recipient origin, in agreement with results obtained by other workers [6].

All controls of specificity of the immunofluorescence reaction were positive, i.e., the fibroblasts and histiocytes did not fluoresce in the control reactions. The results of the immunofluorescence test indicate that bone marrow fibroblasts are histogenetically independent of macrophage-histiocytes and hematopoietic cells. In fact, all fibroblasts in cell cultures from radiochimeras bound antibodies against H-2 antigens of (CBA  $\times$  C57BL/6)F<sub>1</sub> recipients but no histiocytes did so. All bone marrow fibroblasts of the radiochimeras were thus of recipient origin and all the histiocytes of donor origin. All fibroblasts growing from cells of the heterotopic grafts were of donor origin, whereas 80% of the histiocytes were recipient's cells and 20% donor's cells.

## LITERATURE CITED

- 1. Yu. F. Gorskaya, A. Ya. Fridenshtein, and N. N. Kulagina, Byull. Eksp. Biol. Med., No. 5, 614 (1976).
- 2. M. S. Didukh and A. Ya. Fridenshtein, Tsitologiya, No. 12, 901 (1970).
- 3. A. I. Kuralesova, Ontogenez, No. 6, 581 (1973).
- 4. A. Ya. Fridenshtein and A. I. Kuralesova, Transplantation, 12, 99 (1971).
- 5. A. Ya. Fridenshtein, K. V. Petrakova, A. I. Kuralesova, et al., Transplantation, 6, 230 (1968).
- 6. S. Amsel and E. S. Dell, Proc. Soc. Exp. Biol. (New York), 138, 550 (1971).
- 7. A. H. Coons and H. H. Kaplan, J. Exp. Med., 91, 1 (1950).
- 8. A. E. Reif and G. M. V. Allen, J. Exp. Med., 120, 413 (1964).

ACID MUCOPOLYSACCHARIDES OF THE INNER ZONE OF THE RENAL MEDULLA IN ALBINO RATS KEPT UNDER DIFFERENT CONDITIONS

T. L. Dubynin

UDC 612.46.015.32-06:613.2+613.162

In albino rats kept without water reactions for acid mucopolysaccharides in the interstitial tissue of the distal part of the inner zone of the renal medulla remained highly stable in experiments carried out under conditions of higher relative atmospheric humidity.

KEY WORDS: renal medulla; interstitial tissue; acid mucopolysaccharides.

Dry feeding experiments on rats have shown that the morphological picture of the inner zone of the renal medulla differed considerably depending on the relative atmospheric humidity. Corrections thus had to be introduced to the morphological picture of the renal medulla described by the writer previously [2, 3], given the concentration character of diuresis in rats.

The object of this investigation was to study the distribution of acid mucopolysaccharides (AMPS) in the renal medulla of albino rats during dry feeding at different levels of relative atmospheric humidity  $(\varphi)$ .

## EXPERIMENTAL METHOD

Male rats weighing 250-300 g were kept in single cages at 20-22°C without water, with an excess of dry food (cereals), with a water content of 4.8-5%. The 100 rats of group 1 were kept in a relative humidity of

Department of Geographic Pathology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1271-1274, October, 1976. Original article submitted March 15, 1976.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$7.50.

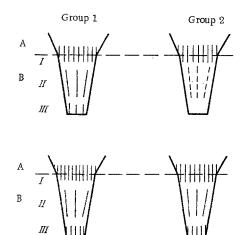


Fig. 1. Scheme of distribution of AMPS in renal medulla of rats. Group 1:  $t = 20-22^{\circ}$ ,  $\varphi = 80-90\%$ . Group 2:  $t = 20-22^{\circ}$ ,  $\varphi = 60-70\%$ . Top row is experiment, bottom row control. A) Outer zone; B) inner zone. Strips: I) outer; II) middle; III) apex. Closeness of shading reflects intensity of reaction for dialyzed iron in different zones and strips of medulla.

80-90%, and four rats acted as the control. The 24 rats of group 2 were kept in a relative humidity of 60-70%, and five rats served as the control. The rats were decapitated from 4 to 12 days later. The kidneys were fixed in 10% acid formalin at 4°C. Blocks containing the central part of the medulla were embedded in paraffin wax and serial sections were cut in two mutually perpendicular planes: parallel and perpendicular to the long axis of the pyramid. The sections were stained with hematoxylin-eosin, picrofuchsin, Heidenhain's iron hematoxylin, Heidenhain's azan, and the combined reaction of Ritter and Oleson, with salivary amylase and testicular hyaluronidase controls.

## EXPERIMENTAL RESULTS

The rats in the experimental groups continued to excrete urine for not more than 4 days. At the same time the animals lost interest in the food, became disinclined to move and aggressive, and their hair was untidy. Their mucous membranes were dry and developed ulcers after 8 days. At autopsy the blood was thick and dark, the tissues flabby and dry, and the intestine and urinary bladder were empty.

Distribution of AMPS in Interstitial Tissue. The intensity of the reaction for AMPS in the outer strip of the inner zone of the medulla remained unchanged in both groups of animals (Fig. 1). In transverse sections through the middle strip of the inner zone in the animals of group 1 AMPS consisted of masses of large granules in the rays of the delta-shaped areas of interstitial tissue separating the thin limb of Henle's loop and the collecting tubule. Hale- and PAS-positive structures of the interstitial tissue had very distinct boundaries. In the animals of group 2 small elongated granules of AMPS remained in the middle strip of the inner zone, parallel to the long axis of the nuclei of the interstitial cells. The density of these granules varied. They were arranged in groups in close contact with the collecting tubules and the epithelium covering the papillae. As the duration of the experiment increased, a clear tendency was observed for them to increase in number and density. The reaction for dialyzed iron was partly preserved even in the latest stages of the experiment in the interstitial tissue of the distal portion of the inner zone of the medulla (apex) in the animals of group 1 (Fig. 2a). Layers of AMPS, considerably reduced in intensity and apparently fragmented, exposing area of PAS-positive basement membranes and their projections into the interstitial tissue. In 12 of the 13 animals in group 2, after 8 days of dry feeding, the reaction for AMPS disappeared completely from the interstitial tissue of the apex (Fig. 2b). The vessels of the inner zone of the medulla in both groups of rats on a dry diet were dilated and gave the interstitial tissue a honeycomb appearance. The endothelium was high and the lumen of the vessel, filled with blood, resembled a broken spiral in appearance. Because of swelling of the endothelium sections passing through the hydrated areas of endothelium could give the impression of an empty space in the lumen of the vessel. The plasma in the lumen of the vessels in this zone was PAS-positive and stained darkly with picric acid, eosin, iron hematoxylin, and aniline blue. Sometimes areas of plasma with a positive reaction for AMPS appeared. In the middle strip of the inner zone in some vessels erythrocytes formed "rouleaux" of

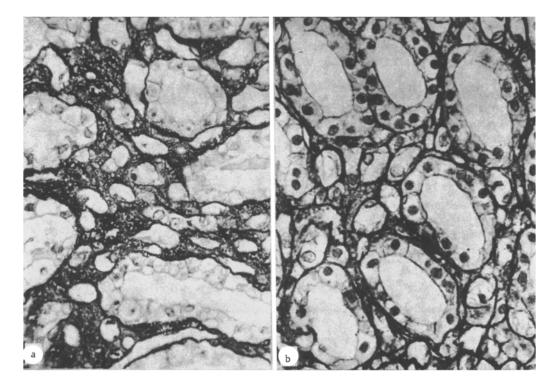


Fig. 2. Interstitial tissue of apex of renal medulla of albino rats. Dry feeding for 12 days: a)  $\varphi = 80-90\%$  (group 1); b)  $\varphi = 60-70\%$  (group 2). Ritter-Oleson, objective  $40 \times$ .

different lengths, becoming denser toward the apex, and the quantity of plasma in the lumen was small. Other vessels contained plasma among which separate erythrocytes could be distinguished. In the capillaries of the "rete mirabile" at the apex of the papilla the distribution of erythrocytes and plasma was more uniform. No difference was found in the histological picture of the microcirculation in the animals of groups 1 and 2.

Two types of abnormalities of the morphological picture of concentration diuresis could be distinguished in the medulla: a) highly individual abnormalities found in particular animals and observed also in the control rats, b) the presence of a residual reaction for AMPS in the interstitial tissue of the apex of the renal medulla of animals kept on a dry diet and in a high relative atmospheric humidity (80-90%) but the absence of this reaction in animals kept without water and in a relative humidity of 60-70%.

At a temperature of 21°C and with mean values of  $\varphi$  (65 and 85%) the weight of water vapor in 1 cubic meter of air (absolute humidity) is  $13 \cdot 10^{-3}$  and  $17 \cdot 10^{-3}$  kg respectively. It may naturally be assumed that the difference of  $4 \cdot 10^{-3}$  kg water vapor/m³ air (amounting to more than 23% of the second figure quoted) is a significant factor for rats kept in a state of acute water deprivation.

The deviations or, more accurately, the difference between the results of the two groups of experiments, must in the writer's view be linked with the loss of water by the animals in thermoregulation. With a higher moisture content in the atmosphere (for the temperature range from 20 to 22°C) the heat output of the animal is higher and, correspondingly, the loss of water in thermoregulation is reduced.

These results confirm once again Ginetsinskii's view [1] of the extraordinary sensitivity of the polysaccharide system of the renal medulla to factors changing the water balance of the body. It follows from these results that atmospheric humidity affects the distribution of AMPS in the inner zone of the renal medulla of rats kept on a dry diet but has no appreciable effect on the distribution of AMPS in animals with an ordinary intake of food and water.

## LITERATURE CITED

- 1. A. G. Ginetsinskii, The Physiological Mechanisms of Water and Salt Balance [in Russian], Moscow-Leningrad (1963).
- 2. T. L. Dubynin, Byull. Éksp. Biol. Med., No. 1, 91 (1969).
- 3. T. L. Dubynin and I. I. Todris, Byull. Eksp. Biol. Med., No. 11, 105 (1969).