Response of Rat Zona Glomerulosa in Experimental Nephrosis.* (25858)

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Hypertrophy and activation of the adrenal zona glomerulosa occur in experimental situations designed to increase output of electrolyte-regulating corticosteroid; Na^+ deprivation was an excellent stimulus(1). Subsequently aldosterone was found to be the most active mineralocorticoid(2); its synthesis occurs almost exclusively in the glomerulosa (3). Secretion of aldosterone is enhanced by reduction of Na^+ intake and also in diseases characterized by edema and ascites(4), including experimental nephrosis in the rat(5,6). Such diseases generally exhibit a demonstrable reduction in plasma Na^+/K^+ ratio(7,8). The present experiment was performed to follow response of glomerulosa during development of and recovery from the nephrotic syndrome induced in rats by aminonucleoside of Puromycin, 6 - dimethylamino - 9 - (3′ - amino - 3′-deoxy-β-D-ribofuranosyl) purine(9).

Methods. Twenty-one young male rats (100 g, Holtzman strain) were given 13 daily subcutaneous injections of 0.5% solution of aminonucleoside‡ in physiological saline, 0.03 ml/10 g body weight(9). Four controls received equivalent injections of saline. Experimental rats were killed at 13, 15, 19, 26, 36 and 61 days after beginning of treatment; controls were killed at 13, 19, 26 and 36 days. The animals were weighed, blood drawn for plasma cholesterol determination(10), and pieces of kidney fixed for microscopic examination. The 2 adrenal glands were trimmed, weighed, and fixed (a) in alcohol-formalin-acetic acid, sectioned serially at 10 μ in paraffin and stained with hematoxylin and eosin, and (b) in formalin, sectioned at 15 μ on freezing microtome and stained with Oil red O.

Results. Weight gain in experimental rats became slower than normal on day 6. They developed gross edema by day 11 and visible ascites by day 13. Maximal accumulation of ascitic fluid occurred between days 20 and 25. From one animal sacrificed on day 19, 21 ml fluid was easily withdrawn from abdominal cavity, and there was additional fluid in thoracic and pericardial cavities. Nine animals died between 15 and 22 days. The remaining ones had diuresis. Lowest body weights were recorded between days 22 and 28, after which the animals regained weight.

Kidney lesions (swelling and pallor, cast formation and internal hydronephrosis, disruption of brush borders, appearance of free lipide droplets in proximal tubular cells) were maximal in rats killed at 19 days and had essentially disappeared by 2 months. At this time, however, some glomeruli appeared contracted and cells therein exhibited fatty infiltration. Plasma cholesterol levels were above normal in all experimental animals, reaching a peak in 15-26 days and remaining above normal after 2 months (Table I).

Width of zona glomerulosa in each animal was determined by making 4 measurements with an ocular micrometer at 560 X on each of 2 sections (paraffin) in mid-region of the gland. At 15 days average width was over 50% above that in controls; thereafter it slowly declined to normal (Table I). Even when most enlarged, the glomerulosa retained abundant lipide (Fig. 1).

Discussion. If administered for longer

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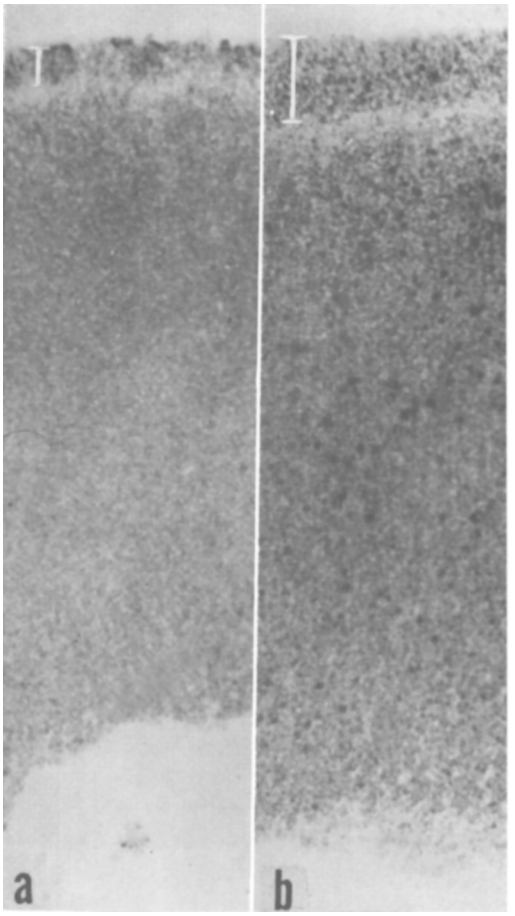

FIG. 1. Frozen sections of adrenal glands, stained with Oil red O, × 100. Width of glomerulosa is indicated on each micrograph. (a) From control killed on day 19; (b) from experimental killed on day 15. In (b) the glomerulosa is significantly broader than normal but its cells contain abundant lipide droplets.

than 12-13 days, the dose of aminonucleoside used is invariably fatal. In this experiment, about half the treated rats died in the severe stage of nephrotic syndrome.

Greatest hypertrophy of glomerulosa was recorded at 15 days after initiation of treatment with aminonucleoside, several days prior to maximal accumulation of ascitic fluid in the surviving group. This result agrees with current evidence that increased secretion of aldosterone may be largely responsible for retention of fluid in diseases like nephrosis (6). Administration of a spirolactone antagonist of aldosterone action on the kidney induces diuresis in nephrotic rats with ascites (11), although it has little or no effect on other important aspects of the disease, such as renal protein loss, shift in plasma albumin/globulin ratio or increased plasma cholesterol. Our results also suggest that regression of the glomerulosa and hence reduction of aldosterone secretion precedes spontaneous diuresis (12). The stimulus to enhanced aldosterone secretion may well lie in the depressed Na^+/K^+ ratio in the plasma (8), although the origin of this depression remains unclear.

Increase in glomerulosal width cannot represent passive response to reduction in width of the entire cortex, as it does after hypophysectomy (1). Indeed, the glands were slightly enlarged, relative to body weight, in experimental rats. Because of fluid retention, this enlargement would have been even more conspicuous if dry body weight had been used for determination of relative size of the glands. Hypertrophy of the glands correlates well with the increased secretion of corticosterone demonstrated by Das Gupta and Giroud (6, 11) and is in contrast with the conclusion of Fiegelson *et al.* (13).

Hypertrophy of the glomerulosa was not so great as found in other, more chronic experimental situations (1). Nor did the zone become depleted of lipide. It may therefore be concluded that the stimulus to secretion was less than maximal.

Recorded for the first time is the gradual morphological repair of the kidney in animals recovered from experimental nephrosis. In this experiment, possibly either because animals were treated for 13 rather than 12 days or because they were older and heavier when the experiment began, plasma cholesterol levels rose higher and dropped more slowly than in an earlier study (14).

Summary. In rats rendered nephrotic by repeated injections of the aminonucleoside of Puromycin, the adrenocortical glomerulosa became hypertrophied. Greatest enlargement occurred on 15th day of experiment, prior to maximal accumulation of ascitic fluid in other animals in the group. Diuresis in surviving animals occurred after glomerulosa had begun to shrink.

TABLE I. Plasma Cholesterol and Adrenal Data for Control and Experimental Rats.

No. animals	Day	Wt, g	Plasma cholesterol, mg/100 ml	Wt adrenals mg	Wt adrenals mg/100 g	Glomerulosal width, ocular micrometer units
			Control animals			
1	13	181	75	37	20	18
1	19	180	64	28	16	18
1	26	214	58	39	18	18
1	36	365	61	54	15	18
			Experimental animals			
1	13	147*	324	31	21	26
2	15	154*	452	34	22	30
3	19	175*	401	39	22	25
2	26	162†	492	41	25	22
2	36	193	161	44	23	18
2	61	301	98			20

* These animals exhibited ascites and tissue edema, so that body weights are too high and relative adrenal weights too low.

† One of these animals had a diuresis on day 25 and still exhibited considerable tissue edema.

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Survival of Polioviruses at Elevated Temperatures (60°-75°C).* (25859)

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It is generally stated that poliovirus is rapidly destroyed at temperatures slightly exceeding 60°C(1,2). Kaplan and Melnick (3) reviewed the relevant literature and added results of their own showing that even when suspended in milk or cream, which exhibit a stabilizing effect, poliovirus from human feces as well as stock strains of rodent-adapted viruses were inactivated after heating for 30 minutes at 61.7°C. Infectivity of such preparations was also eliminated by heating at 71.1°C for much shorter intervals

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