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Effect of Hypophysectomy on Serum Lipids in Aminonucleoside Nephrosis.* (31198)

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Pituitary hormones are known to have an important influence upon lipid metabolism (1) and the concentration of lipids in the serum(2). It has been suggested that the pituitary plays a role in mediating the lipemia of nephrosis(3). Hypophysectomy reduced the lipemia and proteinuria(4) in rats made nephrotic by injections of anti-kidney serum (4).

Anatomic and physiologic disturbances similar to those of the nephrotic syndrome in man have been produced in rats by injection of the aminonucleoside of puromycin, 6 dimethylamino purine, 3-amino-d-ribose(5). In addition, this compound produces changes in oxidative phosphorylation(6) and influences the hormonal control of fatty acid release from adipose tissue(7).

This study was performed to evaluate the effect of hypophysectomy upon serum lipids in rats made nephrotic by aminonucleoside, and to correlate the lipemia with other findings in this type of experimental nephrosis.

Materials and methods. Normal and hypophysectomized Charles River rats, weighing 130-150 g, were used. All were housed in metabolic cages, and were maintained on Purina Lab Chow and 5% glucose water. The animals were weighed daily. A period of 7 days was allowed for stabilization before the aminonucleoside injections were started. Adequacy of hypophysectomy was confirmed by failure of weight gain, lack of testicular growth, and the absence of pituitary tissue when the pituitary fossa was examined after the rat was killed. The experimental animals were given daily subcutaneous injections of the aminonucleoside,† 1.5 mg/100 g body wt as 0.5% solution in water. Control animals received daily injections of equivalent amounts of distilled water. The nephrotic rats developed signs of edema and ascites 10 to 12 days after the start of the aminonucleoside injections. At this time they were anesthetized with ether, and were bled to death from the aorta. Control rats were bled and killed in the same manner.

Serum cholesterol was measured by the Zak method(8) and serum triglycerides were determined by the method of Van Handel and Zilversmit(9). Blood urea nitrogen determinations were made by the diacetyl method (10). Total serum proteins(11) and urinary protein(12) were measured by the biuret method.

Results. Aminonucleoside produced nephrosis in both normal and hypophysectomized rats. The hypophysectomized rats did not gain weight until ascites and edema appeared 7 to 8 days after the start of the aminonucleoside injections. Normal rats, made nephrotic, gained weight throughout the experimental period but had accelerated weight gain with the onset of fluid retention. Typical weight curves are shown in Fig. 1.

The results of the various biochemical determinations are given in Table I. Significant elevation of both cholesterol and tri-

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† Obtained from Nutritional Biochemicals Corp., Cleveland.

TABLE I. Blood and Urine Values of Nephrotic, Hypophysectomized and Control Rats.

Group	No. of rats	Serum cholesterol, mg %	Serum triglyceride, mg %	Total serum protein, g %	mg protein lost in urine per 24 hr	Blood urea nitrogen, mg %
Nephrotic						
Normal	8	267 (± 15)S	251 (± 28)S	4.3 (±.04)	513 (± 93)S	80 (±16)S
Hypophysectomized	16	124 (± 21)NS	89 (± 15)NS	4.6 (±.25)	123 (± 15)	174 (± 8)S
Control						
Normal	8	72 (± 4)	98 (± 19)	6.9 (±.15)	120 (± 10)	19 (± 2)
Hypophysectomized	10	96 (± 5)	73 (± 8)	7.2 (±.28)	89 (± 17)	26 (± 2)

Numbers shown represent mean values for the respective groups.
± = Standard error.
S = Significant difference from other 3 groups, $p < .05$.
NS = No significant difference from control.

glyceride concentrations was found in the normal rats with nephrosis, but not in any of the other groups. Although both groups of rats with nephrosis had lower serum protein levels, there was no significant difference between them. The normal rats with nephrosis lost considerably greater amounts of protein in the urine than did the hypophysectomized rats with nephrosis or the control rats.

The hypophysectomized rats with nephrosis had higher mean blood urea nitrogen values than did any of the other groups. Only the hypophysectomized rats with nephrosis had signs associated with the uremic state. Many appeared weak and lethargic and ate poorly during the last 2 days of the experiment.

Discussion. These results suggest that hypophysectomy prevented the development of the usual rise in serum cholesterol and triglycerides found in rats with aminonucleoside-induced nephrosis. Tracy found that total serum lipids decreased in nephrotic rats following hypophysectomy. In his study nephrosis was produced by injections of anti-kidney serum(4). Oliver and Kelsch found contrary results in a small group of hypophysectomized rats with aminonucleoside-induced nephrosis(13). In their hypophysectomized rats with nephrosis, the mean serum cholesterol was 307 mg% compared to 122 mg% for the control group. Serum triglycerides were not determined. We have no explanation as to why Oliver and Kelsch did not find an "antilipemic" effect from hypophysectomy in their nephrotic rats. We used 16 hypophysectomized nephrotic rats in contrast to their 5 animals and found that both serum cholesterol and triglyceride levels remained low.

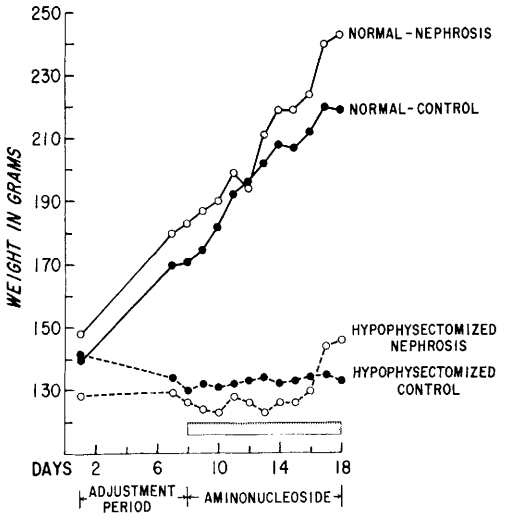

FIG. 1. Typical weight changes nephrotic, hypophysectomized, and control rats.

A significant positive correlation(14) was found between the amount of proteinuria and the serum cholesterol level ($p<0.01$) in the nephrotic animals. Thus, as the amount of proteinuria increased, the serum cholesterol tended to be higher. A significant negative correlation was found between the BUN and the serum cholesterol ($p<0.05$) and the serum triglycerides ($p<0.01$). That is, the higher the BUN, the lower were the serum lipids. A significant correlation was also found between the serum cholesterol and serum triglyceride levels ($p<0.01$) of all nephrotic rats.

There was no significant difference between

the total serum protein concentrations of the 2 groups with nephrosis. Since there were significant differences between these 2 groups in regard to the urinary protein loss and the BUN, the lower lipid values in the hypophysectomized rats with nephrosis might have been related to the degree of proteinuria or to the effects of the uremic state.

It is known that pituitary hormones increase renal clearance(15) and the lack of this effect in the hypophysectomized rats may have been responsible for decreased urinary protein loss which led to lower serum lipid concentrations.

While the exact reasons responsible for the lipemia of nephrosis remain to be clarified, these results suggest a relationship between pituitary hormones and the capacity of the nephrotic rat to develop hyperlipemia. If the lipemia of nephrosis develops because of enhanced endogenous synthesis of lipoproteins in the liver, as seems likely, then pituitary hormones might well be the mediator of this effect.

It is known that cholesterol synthesis in the liver is enhanced in rats having aminonucleoside nephrosis(16,17). A depression of cholesterol synthesis has been found in liver slices from hypophysectomized rats(18).

Another action of pituitary hormones is to mobilize fatty acids from adipose tissue triglyceride(19,20). These fatty acids are then transported to the liver bound to albumin and serve as a substrate for lipoprotein synthesis. In nephrosis the low serum albumin would not facilitate the transport of fatty acids to the liver and tissues. Perhaps it is because of the low serum albumin and the resulting disturbance of energy metabolism that the liver in nephrosis synthesizes more lipoproteins (beta lipoproteins) to supply an alternative pathway for fat transport. Of course, there is the possibility that the effects of hypophysectomy in preventing the hyperlipemia of nephrosis might be mediated through reduced lipid mobilization from adipose tissue. The factors of food intake and growth may have influenced our results. Hypophysectomized rats showed a lack of growth and ate less than did the control rats made nephrotic.

It appears that the pituitary gland plays a significant role in the development of lipemia in nephrosis but the lipid lowering influence of hypophysectomy may be a secondary effect rather than a primary one.

Summary. The effect of hypophysectomy upon the serum lipids was studied in rats in which nephrosis was produced by the aminonucleoside of puromycin. Hypophysectomy prevented the usual rise of the serum cholesterol and triglyceride in the nephrotic rats. It is postulated that the hyperlipemia of nephrosis may result from enhanced lipoprotein synthesis in the liver from an effect of pituitary hormones. The hypophysectomized rats with nephrosis had higher blood urea nitrogen values and less proteinuria than did the nonhypophysectomized rats with nephrosis.

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Hemagglutination Studies of the Viral Antigen in a Murine Leukemia (Rauscher).* (31199)

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Hemagglutination (HA) and hemagglutination-inhibition (HAI) reactions with tannic acid, protein treated sheep red blood cells (SRBC) have been employed for the study of a wide variety of immunological problems (1). Although these methods are potentially suited for detection of viruses and virus antibodies, relatively few viruses have been studied(2). The recent availability of highly purified tissue culture virus has made it possible to adapt the HA method to measure antibodies to a murine leukemia virus (Rauscher)(3). The present report has two aims: to determine the sensitivity of this method for detection and estimation of small amounts of virus and of antibody, and to study the primary and secondary antibody responses of Rhesus monkeys immunized with Rauscher virus.

Materials and methods. Viruses. For sensitization, the supernatant fluids from embryonic Balb/c spleen/thymus cultures inoculated *in vitro* with Rauscher virus (JLS-V5) were used(4). A similar preparation (JLS-V6) from the same culture, but not inoculated with Rauscher virus, served as a control(4). These antigens were enzyme treated and concentrated by zonal ultracentrifugation as previously described(5). The virus preparation had a nitrogen concentration of 30 μg/ml, an RNA concentration of 47 μg/ml, and a relative virus concentration of approximately 500 particles/square on a standard electron microscope grid. All of these materials and assays were obtained from Mr. Irving Toplin, John L. Smith Memorial Laboratory, Charles Pfizer & Co., under contract to the National Cancer Institute.

Rauscher virus preparations and their controls tested in HAI studies included the following: 10% homogenate of spleen from infected and from normal Balb/c mice(6); and 10-fold concentrate of viremic and of normal Balb/c mouse plasma prepared by differential centrifugation(3); and the 1.16 density isolates obtained on centrifugation of these 10-fold concentrates of viremic and of normal plasmas on sucrose gradients(7).

Sera. Monkey anti-Rauscher serum was prepared by the inoculation of 10 times concentrated plasma from Balb/c mice as previously described(8). The course for immunization consisted of a primary intraperitoneal inoculation of 1.0 ml or 2.0 ml of virus emulsified with an equal quantity of complete Freund's adjuvant. This was followed 28 days later by a subcutaneous inoculation of 0.25 ml of virus without adjuvant. Sera were obtained 7-10 days after the booster inoculation and stored individually at −20°C.‡ Rabbit anti-mouse plasma serum was prepared by a similar procedure using a 1:2 dilution of mouse plasma as the antigen.

All of the serums used in these experiments were inactivated for 30 minutes at 56°C and absorbed with washed SRBC until no reactions were observable by HA. Monkey anti-Rauscher virus serums were then absorbed to completion with normal Balb/c erythrocytes (MoRBC). This was followed by absorption with normal Balb/c plasma (NMoP) until an excess of Balb/c plasma could be

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