(4) locate ascorbic acid in the cytoplasm of acinar cells, along with sudanophilic lipids. Since the aged sebum contains steroids, the preputial glands may be a more favorable site for elucidation of the relationship between ascorbic acid and steroid formation than the more complex adrenal glands.

The preputial content of ascorbic acid is lower in rats hypophysectomized 24 hours previously than in controls. This may be a result of depletion due to trauma associated with the operation without return to the normal level, or may be a result of decreased pituitary hormones.

Summary. 1. The normal level of ascorbic acid in the preputial glands of the young male rat is 60 mg %. 2. Adrenalectomy (3 wks) induces preputial gland hypertrophy, which is partially prevented by DCA. 3. Severe stress produces a depletion of preputial ascorbic acid, which is accentuated in hypothyroid animals. 4. Hypophysectomized rats (24 hr) have a level of preputial ascorbic acid which is 78% that of controls.

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Metabolism of Renal Cortex in Nephrotic Syndrome of Rats.* (19356)

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The chronic renal disease induced in rats by the intravenous injection of nephrotoxic sera obtained from rabbits(1) simulates the nephrotic syndrome as it is observed in infants and children(2). The site of the antibody-antigen reaction is in the renal cortex(3,4), probably in the basement membrane of the glomerulus(5,6). The initial injury elicits a chronic disease that may continue for 12 to 15 months or longer, without further injection of nephrotoxic antibodies.

It is impossible that heteronephrotoxins may have this prolonged effect by virtue of their continued presence. It appears more likely that the initial antibody-antigen reaction produces a cellular injury that may be irreversible. It thus seemed of interest to investigate whether this lesion is associated with metabolic alterations in the renal cortex of rats in which this disease had been induced.

Methods. Rats of the Long-Evans and Whelan strains were used. They were kept on Friskies and water. Renal disease was induced according to the technic previously described(2) when they were 4 to 5 weeks of age. Animals were chosen that had a well established disease for various lengths of time. Fourteen had had renal disease for less than 8 days, 5 for 1-4 weeks, 14 for 1-6 months, and 9 for 6-14 months. They were sacrificed by decapitation, blood being obtained for chemistry studies. One-fourth to one-half of each kidney was preserved for histological examinations. Immediately thereafter the remaining kidneys were freed of non-cortical tissue and placed in ice-cold Krebs-Ringer

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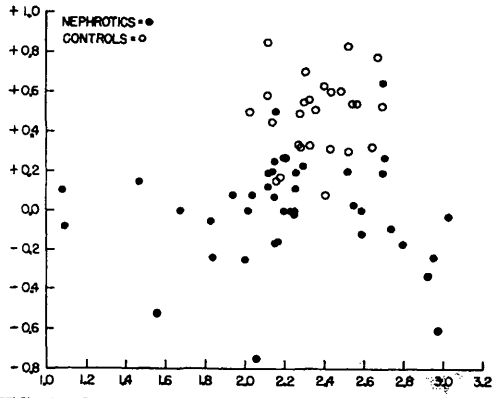

FIG. 1. Oxygen uptake of renal cortex slices from control and nephrotic rats. Abscissa: Qo_2 in Krebs-Ringer solution. Ordinate: Changes in Qo_2 produced by addition of glucose. The circles (○) represent the values obtained with controls, the dots (•) those with nephrotic animals.

solution(7). Slices 0.4 to 0.5 mm thick were prepared with a double blade tissue slicer. They were blotted with No. 50 Whatman filter paper, weighed on a torsion balance and placed in Warburg respirometer vessels (total volume 4 to 5 ml) containing 0.8 ml of Krebs-Ringer solution. Their respiration was measured in an atmosphere of 100% oxygen for a period of 90 minutes at 38°C, by the conventional Warburg technic(7). The time interval between decapitation and the initial manometric reading varied between 30 and 45 minutes. All solutions of substrates were prepared immediately before each experiment and were used in a final concentration of 0.02 molar.

Results. In the absence of substrate the average oxygen uptake of cortex slices of nephrotic rats did not differ significantly from that of the controls (Table I). In 29 control rats the average Qo_2 was 2.39 as compared to 2.28 obtained in 42 nephrotic animals. It should be pointed out, however, that in the experimental group the oxygen uptake varied within a wider range than in the control group (Fig. 1).

The addition of glucose invariably resulted in an increase in the oxygen uptake in the control slices, varying from +3 to +40%. The average value obtained from 27 animals was +22%. No consistent increase of the oxygen uptake after addition of glucose was noted when the kidney slices were obtained from nephrotic rats (Fig. 1). In this group of 41 animals a rise in the oxygen uptake by glucose was noted in 16. In 13 there was a decrease varying between −5% and −37%. In the 12 remaining rats the differences in the oxygen uptake in the presence and in the absence of glucose were not greater than ±3%. In this group as a whole the average change in Qo_2 produced by glucose was less than +1%.

When fructose was used instead of glucose, a somewhat greater increase in the oxygen uptake of the controls was noted. It varied between +22% and +45%; the average value obtained from 8 rats was +31%. Again there was no consistent increase after addition of fructose to the kidney slices of nephrotic animals (Fig. 2). In 6 of 11 experiments an increase varying between +9% and 30% was observed. In 5 this substrate produced a decrease in Qo_2 varying between −4% and −16%. The average change obtained in 11 rats was +5%.

In contrast to the above 2 substrates, the increase in Qo_2 of the nephrotic slices produced by the addition of lactate, succinate, glutamate, acetoacetate and butyrate was equal to or only slightly less than in the corresponding control experiments. (Table I). The

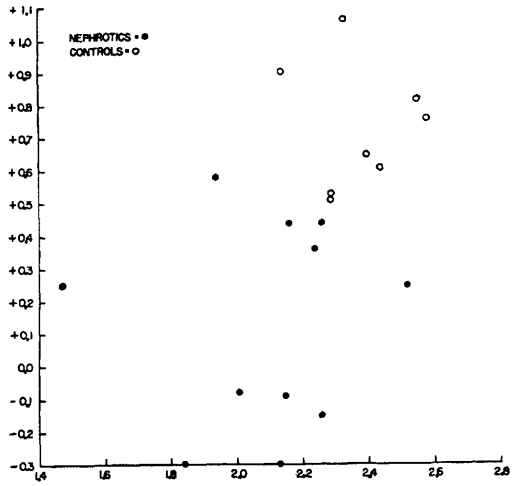

FIG. 2. Oxygen uptake of renal cortex slices from control and nephrotic rats. Abscissa: Qo_2 in Krebs-Ringer solution. Ordinate: Changes in Qo_2 produced by addition of fructose. The circles (○) represent the values obtained with controls, the dots (•) those with nephrotic animals.

TABLE I. Oxygen Uptake of Renal Cortex Slices from Control and Nephrotic Rats in the Presence and Absence of Various Substrates.

Rats	No substrate		Na lactate		Na succinate		Na glutamate	
	No.	Q_{O_2}	No.	Q_{O_2} % change	No.	Q_{O_2} % change	No.	Q_{O_2} % change
Control	29	2.39	11	+50	10	+57	9	+34
Nephrotic	42	2.28	19	+32	12	+63	12	+38
			Na acetoacetate		Na butyrate		Alanine	
Control			8	+31	8	+38	11	+68
Nephrotic			11	+24	11	+21	19	+33

TABLE II. Effect of Glucose, Lactate and Alanine on Oxygen Uptake of Liver Slices from Control and Nephrotic Rats.

Rats	No substrate		Glucose Q_{O_2} % change	Na lactate Q_{O_2} % change	Alanine Q_{O_2} % change
	No.	Q_{O_2}			
Control	6	1.26	+4	+34	+12
Nephrotic	9	1.24	+4	+27	+ 5

lower average figure observed with butyrate was due to the fact that this substrate did not increase the oxygen uptake in 2 of 11 experiments in which nephrotic kidney slices were used.

Alanine stimulated oxygen uptake of the slices to a lesser degree in the nephrotic group. In the latter an increase in Q_{O_2} equal to or higher than the lowest observed in the controls (+45%) was noted in only 7 of 19 experiments.

Similar experiments using glucose, sodium lactate and alanine as substrates were undertaken with liver slices of 6 control and 9 nephrotic rats (Table II). The oxygen uptake in Krebs-Ringer solution without substrate was the same for liver tissue obtained from healthy controls or nephrotic rats. It was, as is well known, about one half that of renal cortical tissue. There was no appreciable difference in the increase in oxygen uptake after addition of glucose and lactate by liver slices obtained from control or nephrotic animals. The diminished effect of alanine on the uptake of oxygen of liver slices obtained from nephrotic rats was due to the fact that 3 of the 9 experiments yielded values well below those obtained in the controls, while in the remaining 6 the values were within normal range.

Discussion. The data reported indicate that in the absence of added substrate the respiration of kidney slices from nephrotic rats is essentially unaltered. The oxidation of glucose and fructose, however, appears to be impaired. The reduction in oxidative utilization of glucose and of fructose cannot be the result of a depression of the cytochrome system, since oxidation of succinate by these slices was not affected. Furthermore, the increase in Q_{O_2} produced by this substrate in both normal and nephrotic slices was greater than that noted after the addition of glucose to the control slices. Since oxidation of lactate, succinate, and of glutamate appeared to proceed at a normal rate, the inability of glucose and fructose to raise the oxygen uptake of nephrotic kidney slices cannot be ascribed to an inhibition of the enzyme systems involved in the tricarboxylic acid cycle. Therefore this metabolic lesion must affect one or several reactions preceding the conversion of hexoses to lactic or pyruvic acid. Possibly this lesion may be the result of impaired phosphorylating mechanisms.

The observation that these two hexoses frequently reduced the oxygen uptage of nephrotic kidney slices below the level obtained without any substrate may indicate the oxidation of a different substrate. The oxidation of this substrate might be inhibited by hexoses possibly by a process of substrate competition.

Since in almost every experiment on addition of acetoacetate or of butyrate the same increase in the oxygen uptake was observed in normal and nephrotic kidney slices, no evidence is available indicating that fatty acid oxidation is impaired in the latter. Further studies using l- and d-alanine separately will be necessary for a proper interpretation of the results obtained with the racemic amino acid.

It should be pointed out that this metabolic lesion was independent of the duration of the renal disease, of the degree of the proteinuria, of the degree of hypertension, and of the severity of the histological lesion. The inability of glucose or fructose to increase the oxygen uptake was noted as early as 24 hours after the injection of heteronephrotoxins. It was also observed in rats which had the renal disease for 14 months with a normal or a high systolic blood pressure.

It has been shown(8) that heteronephrotoxins are rapidly and quite specifically adsorbed in the kidneys. In accordance with this mechanism the metabolic lesion was found in renal tissue but not in liver slices. It also has been reported(9) that the metabolism of renal cortex differs from the metabolism of renal medulla. Accordingly it is planned to extend the studies to medullar tissue, since there is evidence to support the view that the nephrotic syndrome affects the entire nephron even though the glomerulus is a primary site of the disease process(10,11).

Summary. 1. The oxygen uptake of renal cortex slices of 29 control rats and 42 animals in which the nephrotic syndrome had been induced by the intravenous injection of nephrotoxic sera was studied. 2. In Krebs-Ringer solution, without added substrates, the average oxygen uptake of cortex slices of nephrotic rats did not differ from that of the controls. 3. Addition of glucose or of fructose resulted invariably in an increase in the oxygen uptake in the control slices. No consistent increase after addition of these two hexoses was noted when the kidney slices were obtained from nephrotic rats. 4. The increase in oxygen uptake of nephrotic slices produced by lactate, succinate, glutamate, acetoacetate and butyrate was equal to or only slightly less than in the corresponding control experiments. 5. Racemic alanine stimulated the oxygen uptake of the slices to a lesser degree in the nephrotic group. 6. Since oxidation of lactate, of succinate, and of glutamate proceeded at a normal rate, the inability of glucose and fructose to raise the oxygen uptake of nephrotic kidney slices cannot be ascribed to an inhibition of the cytochrome system or of the enzymes involved in the tricarboxylic acid cycle.

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