Chim. Biol., 1896, v48, 486.
4. Parhou, L., and Werner, G., *ibid.*, 1931, v107, 401.
5. Byers, S. O., and Friedman, M., *J. Physiol.*, 1952, v168, 297.
6. Wibler, C. G., *J. Mammalogy*, 1952, v33, 105.
7. Haslewood, G. A. D., and Wootton, V., *Biochem. J.*, 1950, v47, 584.
8. Sjövall, J., *Acta Chem. Scand.*, 1954, v8, 339.
9. Foldes, F. F., *J. Lab. and Clin. Med.*, 1942-1943, v28, 1889.
10. Foldes, F. F., and Wilson, B. C., *Anal. Chem.*, 1950, v20, 1210.
11. Irvin, J. D., Johnston, C. G., and Kopola, J., *J. Biol. Chem.*, 1944, v153, 439.
12. Isaksson, B., *Acta Chem. Scand.*, 1954, v8, 889.
13. Szalkowski, C. R., and Mader, W. J., *Anal. Chem.*, 1954, v24, 1602.
14. Carey, J. B., and Bloch, H. S., *J. Lab. and Clin. Med.*, 1954, v44, 486.
15. King, S. J., *Biochem. J.*, 1932, v26, 292.
16. Fieser, L. F., and Rajagopalan, S., *J. Am. Chem. Soc.*, 1949, v71, 3935.
17. ———, *ibid.*, 1950, v72, 5530.
18. Cortese, F., and Bauman, L., *ibid.*, 1937, v59, 2532.

Received May 28, 1957. P.S.E.B.M., 1957, v95.

Carbohydrate Metabolism in Experimental Nephrosis (23332)

N. KALANT, M. CLAMEN AND M. M. HOFFMAN
(Introduced by J. S. L. Browne)
Departments of Medicine and Investigative Medicine, McGill University, and Research Laboratory, Jewish General Hospital, Montreal

It has been suggested that the nephrotic syndrome may be considered as "protein diabetes," the primary defect being loss of protein in the urine, and other biochemical abnormalities occurring as a consequence of this (1). In support of this view, Drabkin and Marsh(2) reported decreased liver glycogen levels and marked hypoglycemia on fasting in experimental nephrosis, changes interpreted as evidence of decreased gluconeogenesis consequent on the proteinuria. However, these observations were made under conditions which make interpretation difficult: (a) food intake was not controlled, (b) the animals were acutely ill and would have died within a few days(3). To investigate these results, a study has been made of carbohydrate metabolism in experimentally nephrotic animals after the acute stage, while on a fixed food intake.

Materials and methods. Adult male Sprague-Dawley rats weighing 380-420 g were used. They were fed twice daily by gavage with a diet providing a daily allowance of 55 calories, and containing 57% carbohydrate, 24% protein and 15% fat. There was free access to water. Animals were housed in individual metabolism cages in a room maintained at 26 ± 1°C and 40 ± 2% relative humidity. Urine was collected under toluene. Post-absorptive blood glucose, 24-hour urine glucose, glucose tolerance and insulin sensitivity were measured before and at intervals after induction of nephrosis. Each animal thus served as its own control. Blood sugar measurements were made on 5 animals subjected to a 48-hour fast after 28 days of nephrosis. Since the animals had not been subjected to such a procedure during the control period, a new group of 6 normal rats of the same weight range were adapted to tube feeding and then fasted for 48 hours. Nephrosis was produced with anti-kidney serum by the method of Heymann and Lund(4). The daily protein excretion was 400-700 mg. Intravenous glucose tolerance tests were performed by injecting 1.5 g of glucose/kg body weight in 50% solution; tail tip blood was sampled 5, 10, 15, 20 and 30 minutes later. A plot of the log of blood sugar concentration against time gave a straight line from which the half-life of the injected glucose was obtained. To test insulin sensitivity 0.1 unit of commercial insulin/kg body weight was injected intravenously; blood sugar was measured at 0, 15, 30, 45, 60, 90 and 150 minutes after injection and the values plotted against time. Two

TABLE I. Urinary Excretion of Nitrogen and Glucose in Nephrosis. N intake—360 mg/24 hr.

		Days after NTS					
	Control	0–5	6–10	11–15	16–20	21–25	26–30
Nitrogen (mg/day)	341*	393*	385*	321	345	322	331
Glucose "	13*	15*	14*	11	10	9	9

* Mean values derived from 8 animals. Others are mean values derived from 5 animals.

criteria of insulin effect were used; (a) fall in blood sugar concentration at 15 minutes (maximum fall almost invariably occurred at this time), and (b) area contained between the curve and a baseline drawn at the level of initial concentration. Urinary nitrogen was measured daily in 3 animals for 10 days and in 5 animals for 30 days after the production of nephrosis. Chemical measurements were made according to the following methods: (a) glucose—Somogyi modification(5) of the Nelson Method(6); (b) blood urea nitrogen —Schales(7); (c) nitrogen—semi-micro Kjeldahl procedure; (d) proteins—biuret procedure using the reagent of Gornall *et al.*(8); (e) serum cholesterol—Zlatkis *et al.*(9).

Results. The results are shown in Tables I-IV. Some animals died or were sacrificed during the experiment; data obtained within 2 days of death have not been included. Table II contains data which confirm the presence of the characteristic hypoproteinemia and hypercholesterolemia with transient elevation of the blood urea concentration.

Urinary glucose excretion was not affected by the presence of nephrosis. Post-absorptive blood sugar concentrations showed a small but statistically significant fall ($p<.01$). The half-life of intravenously administered glucose rose during the early stage of nephrosis, but then fell to or below control levels. These changes were significant at the 5% level. Similarly the fall in blood sugar produced by insulin was increased in the early period of nephrosis ($p<.05$) and later returned to normal. The areas under the blood sugar-time curve after insulin showed the same trends, but the changes were not significant at the 5% level.

Nitrogen excretion was increased during the first 10 days of nephrosis but then returned to control levels.

The response of nephrotic animals to a 48-hour fast was essentially the same as that of control animals.

Discussion. Evidence indicating abnormal carbohydrate metabolism in nephrosis is of 2 types: (a) Drabkin and Marsh(2) concluded on the basis of low liver glycogen levels and fasting hypoglycemia, that there was a decrease in gluconeogenesis in experimental nephrosis of rats, and (b) Gottfried, Steinman and Kramer(10) found flat oral glucose tolerance curves in nephrotic children. The animal studies, however, were obtained during early, fulminating nephrosis not comparable to the chronic disease of humans. The results of Gottfried *et al.* in children might be taken to indicate an increased rate of glucose utilization, but alterations in rate of glucose ab-

TABLE II. Changes in Blood and Serum Constituents in Nephrosis.

		Days after NTS inj.			
Constituent	Control	0–10	11–20	21–30	31–40
Blood sugar (mg/100 ml)	116 ±1.7 (16)	103 ± 3.7 (16)	107 ± 5.2 (10)	102 ± 4.1 (5)	100 ± 5.8 (4)
Serum proteins (g/100 ml)	7.2± .19 (10)	5.2± .19 (10)	5.5± .22 (7)	5.6± .22 (4)	5.5 (2)
Serum cholesterol (mg/100 ml)	103 ±4.6 (10)	435 ±55 (9)	320 ±68 (7)	547 ±188 (4)	403 (2)
Blood urea nitrogen (mg/100 ml)	14.3± .5 (14)	34.6± 5.3 (13)	18.3± 2.5 (8)	14.2± 1.6 (4)	

Values are for mean and stand. error of mean.
Figures in parentheses indicate No. of animals from which values obtained.

TABLE III. Effect of Nephrosis on Tests of Carbohydrate Metabolism.

Criterion	Control	Days after NTS 6-12	26
Fall in blood sugar 15 min. after insulin (mg/100 ml)	38 ± 5.2 (11)	49 ± 6 (11)	38.4 ± 7.9 (5)
Area under blood sugar-time curve following insulin (arbitrary units)	828 ± 187 (11)	1054 ± 241 (11)	842 ± 313 (5)

	Control	5	14	29
Half-life of blood sugar following intrav. inj. of glucose (min.)	18.7 ± 1.2 (9)	25.6 ± 2.4 (7)	15 ± 1.5 (5)	13 ± 1.2 (5)

Values shown are mean ± stand. error of mean.
Figures in parentheses indicate No. of animals from which data derived.

sorption can as readily explain the facts. Furthermore, when hypertension and azotemia are present, oral glucose tests may show high prolonged curves similar to those of mild diabetes(11). Thus the evidence of a specific alteration in carbohydrate metabolism is not strong.

In our work, the animals had moderately severe nephrosis but were not moribund; they were studied early in the course of the disease, and again at a later date when their illness corresponded to chronic nephrosis without azotemia in humans.

In the first 4-7 days of nephrosis, Drabkin and Marsh observed a fall in blood sugar levels and in hepatic glycogen(2). At a comparable period in the present work, a decrease in post-absorptive glycemia was noted, together with an increased responsiveness to insulin and a decrease in glucose tolerance. None of these changes was marked and the latter 2 parameters soon returned to normal.

TABLE IV. Effects of Fasting in Experimental Nephrosis.

	Fast day 1		Fast day 2	
	Control	Nephrotic	Control	Nephrotic
Nitrogen excretion (mg/day)	160	172	148	132
Blood sugar* (mg/100 ml)	69	82	64	68
Protein excretion (% of mean excretion of preceding 5 days)		64		49
Wt loss (cumulative) (g)	10	8	21	18

* Blood taken on following morning.
Each figure represents mean value for 5 animals.

Hypoglycemia was not seen in the post-absorptive state at any time during the course of the disease or after a 48-hour fast carried out when the disease was "stabilized." This would indicate a normal capacity for gluconeogenesis.

The proteinuria in these animals, though high, accounted for only 2-3 calories/day, and after the first 5-10 days did not disturb the nitrogen balance. It is unlikely that such a small energy loss would disturb the homeostasis sufficiently to cause a significant decrease in gluconeogenesis or an increase in mobilization of depot fat great enough to produce hyperlipemia, as suggested by the concept of "protein diabetes."

Summary. Urinary glucose, blood glucose, insulin sensitivity and glucose tolerance were measured in experimentally nephrotic rats. In the early stage of nephrosis there was a slight fall in blood sugar concentration, a decrease in glucose tolerance and a decrease in insulin sensitivity. The latter 2 parameters later returned to normal. The response of nephrotic rats to fasting was essentially the same as that of normal rats. The grosser aspects of carbohydrate metabolism appear to be normal in experimental nephrosis.

1. Epstein, A. A., *J.A.M.A.*, 1917, v69, 444.
2. Drabkin, D. L., and Marsh, J. B., *J. Biol. Chem.*, 1955, v212, 623.
3. Drabkin, D. L., *Proc. 7th annual conf. on nephrotic syndrome*, 1955, p25.
4. Heymann, W., and Lund, H. Z., *Pediatrics*, 1951, v7, 691.
5. Somogyi, M., *J. Biol. Chem.*, 1952, v195, 19.
6. Nelson, N., *ibid.*, 1944, v153, 375.
7. Schales, O. in Reiner, M., ed., *Standard Methods*

of Clinical Chemistry, Vol. 1, N. Y., Academic Press, 1953, p118.

8. Gornall, A. G., Barawill, C. J., and David, M. M., *J. Biol. Chem.*, 1949, v177, 751.

9. Zlatkis, A., Zak, B., and Boyle, A. J., *J. Lab. and Clin. Med.*, 1953, v41, 486.

10. Gottfried, S. P., Steinman, J. F., and Kramer, B., *Am. J. Dis. Child.*, 1947, v74, 283.

11. Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry Interpretations*, Vol. 1, 2nd edi., Baltimore, Williams and Wilkins Co., 1946, p352.

Received May 29, 1957. P.S.E.B.M., 1957, v95.

Multiplication of Foot and Mouth Disease Virus in Adult Kidney, and Embryonic Lung and Heart Bovine Tissue Cultures. (23333)

L. MELÉNDEZ V., A. GAGGERO C., R. RODRIGUEZ T., AND M. NORAMBUENA G.
(Introduced by J. F. Enders)
Animal Virus Department, Instituto Bacteriologico de Chile, Santiago

The multiplication of foot and mouth disease virus in tissue culture has been recently described by Seller(1), Bachrach(2), and Mazzaracchio(3) by using cultures of both bovine and pig kidney cells. The relatively large scale of virus production obtained with these methods seems to prove that tissue culture technics are, presently, the most promising way for mass production of virus and a useful technic for the general assay of its activity. In our laboratory, we have succeeded in multiplying our 3 types of f. and m. virus, by using cultures of adult bovine kidney cells, and foetal bovine lung and heart tissues.

Methods. At the beginning we used the Dulbecco and Vogt method modified by Youngner(4), in slant tubes. After the work by Bodian(5) we started the setting up of Roux flasks of trypsinized cells with the advantage that its massive proliferation has also been used as source of cells for slant tubes. The cultures of bovine foetal lung and heart tissues have been made by planting minced tissue in plasma clot in Roux flasks. A good outgrowth of cells is obtained after 5 to 6 days in stationary incubation at 37°C. The nutrient medium for both types of cultures consisted in bicarbonated Hanks solution, with 0.5% lactalbumin hydrolysate, 20% horse serum and the usual antibiotics. Previously we had used the Enders' medium, but we changed due to our difficulties in obtaining regularly the cow amniotic fluid. The virus used has been our 3 types stock strains of f. and m. virus. For the first virus inoculation, suspensions of cattle epithelium of fresh bovine passages were inoculated in the tubes. After 2 hours standing at 37°C the fluid was removed, and fresh nutrient medium was added. Ten passages were made of the 3 types of virus by using as inoculum a 10^{-3} virus dilution, before performing a T.C. titration of its activity.

Results. Two of the types of virus used gave the characteristic cytopathogenic picture at first passage. One strain of the O type required 2 blind passages before any cytopathogenic activity was detected. Virus was harvested in all cases at 18-20 hours after inoculation, and stored at −25°C, in aliquot parts with 10% peptone saline.

The virus multiplied in bovine kidney cells was titrated at the 10th passage in slant tubes of the same origin. Titres obtained showed that a definite multiplication has occurred in the cultures, as shown by titres of $10^{-6.5}$; $10^{-6.5}$; $10^{-5.5}$ and $10^{-6.5}$ ID/ml, for the strains C, C_2, A and O respectively.

The kidney cell cultures are now being used for typing field strains of virus and for titrations of guinea pig and bovine antisera.

Infected fluid from tissue culture has been used for the hyperimmunization of rabbits according to the method described by Ramos Alvarez and Sabin(6) and Freund and Thompson(7).

In the bovine foetal lung and heart tissue culture the same types of viruses have been