to glycerol in this protective capacity but proved more toxic during storage at 22°C both before freezing and after thawing. An interaction of protective and toxic effects was noted in a comparison of 5, 10, 15 and 20% DMSO which suggested the 10% level as the most favorable. On the basis of findings, DMSO is not recommended as a substitute for glycerol in the preservation of human spermatozoa by freezing with the methods described.

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Liver UDPG-Transglucosylase and Phosphorylase in Fasted, Refed and Nephrotic Rats. (29553)

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Selective changes in the activity of enzymes concerned with liver glycogen metabolism occur following dietary and hormonal influences(1,2). Changes in the activity of transglucosylase (UDPG glucose: α-1,4 glucan α-4-glucosyl transferase) and phosphorylase (α-1,4-glucan phosphorylase) effected by fasting and refeeding were the subject of this study as a background for understanding the alterations in carbohydrate metabolism of nephrotic animals. Decreased liver glycogen levels reported by Drabkin and Marsh(3) in nephrotic animals were ascribed to shunting of glucose for the support of increased protein and fat synthesis, characteristic of the nephrotic syndrome, or possibly due to changes in food intake.

Experimental. Male albino rats weighing about 150 g were used. Aminonucleoside or Kidney Antiserum nephrosis was induced as described previously(4). All treated rats had severe proteinuria, hypoalbuminemia and hyperlipidemia. The rats were investigated after a period of fasting or refeeding and on sacrifice the fasted, refed or nephrotic animals were interspersed with normal controls and the enzyme assays performed within a short span of time, to minimize the chance of fluctuations in enzyme activity or in glycogen content. The activity of transglucosylase was measured by a modification of the procedure of Leloir and Goldemberg(5), in which the evolution of UDP from UDPG and the interaction of UDP with phosphoenolpyruvate and pyruvic kinase proceeded in the same reaction mixture(6). Phosphorylase was measured according to Sutherland(7). Units of enzyme activity refer to μmoles of the respective substrate utilized per mg of liver protein per hr. Liver glycogen was isolated according to Good *et al*(8) and determined by the anthrone reaction. Liver pro-

TABLE I. Effect of Fasting and Refeeding on Activity of Liver Transglucosylase and Phosphorylase of Normal Rats.

State and (No.) of animals	Transglucosylase (units/mg protein) With glucose-6-phosphate	Transglucosylase (units/mg protein) Without glucose-6-phosphate	Phosphorylase (units/mg protein)	Protein (mg/g wet wt)	Glycogen (mg/g wet wt)
Control (22)	1.94 ± .06	.91 ± .02	12.2 ± .7	108 ± 4	42 ± 5
Fasted 96 hr (12)	2.08 ± .10	1.12 ± .03	3.5 ± .3	123 ± 3	<1.0
Refed 4 hr (8)	1.96 ± .14	—	3.9 ± .4	112 ± 4	27 ± 5
" 6 hr (4)	1.71 ± .12	—	—	118 ± 3	68 ± 4
" 8 hr (8)	1.72 ± .13	—	5.7 ± .4	114 ± 5	66 ±11
" 12 hr (15)	1.82 ± .09	.73 ± .03	7.1 ± .3	90 ± 3	83 ± 5
" 24 hr (15)	2.30 ± .12	.92 ± .02	9.2 ± .4	93 ± 4	115 ± 8
" 48 hr (9)	2.26 ± .10	.97 ± .02	11.3 ± .3	91 ± 5	80 ± 7

Values are means ± S.E. for number of animals indicated in parentheses. Unit of enzyme activity corresponds to utilization of 1 μmole of respective substrate per hr.

tein was determined according to Lowry *et al* (9).

Results and discussion. Table I demonstrates the considerable differences in the course of changes of phosphorylase and transglucosylase activities on fasting and refeeding of normal rats. Phosphorylase declined to about ⅓ on fasting and rose slowly on refeeding, whereas no decline in activity of transglucosylase after 96 hours of fasting, and even some increase was noted. The loss in phosphorylase was even more pronounced when evaluated on the basis of total liver mass, since the latter is known to decrease by about ½ on prolonged fast(10). With regard to transglucosylase some decrease in total available activity had actually occurred due to shrinkage of liver size. These findings are in accord with those of Niemeyer *et al* (2).

During the first 12 hours of refeeding a small but significant reduction in the activity of transglucosylase was recorded. While the fluctuations in activity of transglucosylase generally appeared to follow the changes in liver protein, this drop suggested that recovery on refeeding lagged behind the renewal of protein. At 24 and 48 hours the size of the liver had returned to normal along with the activity of transglucosylase. Preferential depletion and restoration of other enzymes of carbohydrate metabolism upon fasting and refeeding has been described by various authors(1,2,10).

The pronounced alterations in liver glycogen were without apparent relationship to the small changes in transglucosylase activity, as measured in the presence of glucose-6-phosphate. The presence of this ester in the assay mixture was considered essential to bring about maximal enzyme activity(11). However, the glucose-6-phosphate-independent activity was also determined since the possibility of better correlation between the physiologic state of the tissue and glucose-6-phosphate-independent activity was hinted at by the results of Villar-Palasi and Larner (12) in isolated muscle and of Steiner *et al* (13) in the liver of diabetic rats. The results of Table I do not show, however, an appreciable deviation from the control values in the ratio of glucose-6-phosphate-independent to total transglucosylase activities. Moreover, to simulate the conditions of enhanced insulin release upon refeeding, long-acting insulin with glucose was administered to *ad libitum* fed rats, but failed to increase significantly the activity of transglucosylase when assayed with or without glucose-6-phosphate, although liver glycogen content rose considerably (unpublished observations). Similar results were obtained with regard to transglucosylase activity of rat adipose tissue(6).

Enzyme activities in nephrotic animals (Table II) were variable and were therefore followed at various periods after induction of the syndrome. As observed previously(4) the animals were in a state of partial inanition during the injections of Aminonucleoside

TABLE II. Levels of Transglucosylase and Phosphorylase in Nephrotic Rats.

State and (No.) of animals	Transglucosylase (units/mg protein)	Phosphorylase (units/mg protein)	Protein (mg/g wet wt)	Glycogen (mg/g wet wt)
Control (13)	2.10 ± .20	12.7 ± .4	103 ± 4	55 ± 3
Aminonucleoside nephrotic				
3 days (8)	2.82 ± .31	9.1 ± .6	98 ± 5	29 ± 2
5 " (6)	3.25 ± .34	9.3 ± .5	95 ± 3	31 ± 7
8 " (13)	1.46 ± .23	8.6 ± .6	102 ± 6	113 ± 6
12 " (10)	1.54 ± .32	9.7 ± .6	107 ± 5	68 ± 5
Kidney antiserum nephrotic (6)	2.03 ± .22	11.4 ± .5	105 ± 3	25 ± 3

Values are means ± S.E. for number of animals indicated in parentheses. Unit of enzyme activity corresponds to utilization of 1 μmole of respective substrate per hr.

and for a few days thereafter, so that the synthesis and breakdown of glycogen might have been affected by the low food intake, by the metabolic adjustments enforcing the support of increased protein and lipid synthesis in the nephrotic animal(14), and possibly by a specific glycogenolytic effect induced by Aminonucleoside(15). The activities of phosphorylase and transglucosylase were observed to change in a way generally resembling normal rats during refeeding, except that in nephrotic animals the alterations were protracted in time and probably associated with a slow recovery from undernutrition. At 2 and 5 days after cessation of injections, when food consumption had not yet reached the normal level, liver glycogen was somewhat low, as was the activity of phosphorylase, whereas the activity of transglucosylase exceeded that of normal controls. This pattern was similar to that of fasted animals in Table I, although in nephrotic animals at this stage neither a shrinkage in liver size nor a rise in protein concentration was observed, so that the total available activity of transglucosylase must have been elevated. During the second week of nephrosis, when food intake was normal, liver glycogen rose as in refeeding, phosphorylase activity remained lower than normal, whereas transglucosylase dropped to about 70% of its normal activity. This drop is difficult to explain in the light of absence of appreciable changes in liver size and protein content and there is only a remote chance of continued direct effect of Aminonucleoside at this time.

Table II includes a group of animals one week after induction of nephrosis by Kidney Antiserum. In these animals, whose food intake was unaffected, the enzyme activities did not deviate from normal controls. Since in this type of nephrosis the imposed necessity of replacement of urinary protein loss and of increased lipogenesis is no less severe than in Aminonucleoside treated animals, there is no evidence that the alterations in the enzymatic mechanism of glycogen synthesis and breakdown in the latter are connected with enhanced channeling of glucose into lipids and protein synthesis.

Summary. Activity of liver phosphorylase in normal rats decreased after 4 days of fasting by about two-thirds and recovered slowly on refeeding, whereas that of transglucosylase was not markedly affected. The glucose-6-phosphate-independent activity of transglucosylase was also only slightly influenced by fasting and feeding.

In Aminonucleoside nephrotic rats, the activities of phosphorylase and transglucosylase and the content of liver glycogen exhibited a pattern of variations probably caused by a prolonged recovery from undernutrition following the injections or due to a direct effect of the drug. Absence of similar changes in animals rendered nephrotic by kidney antiserum is not compatible with the theory that the metabolic adjustments of the nephrotic state are associated with changes in the activity of enzymes of glycogen metabolism.

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Detection of *Toxacara canis* Antibodies with the Fluorescent Antibody Technique.* (29554)

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Recent reports describe the use of the indirect fluorescent antibody (FA) technique in the serodiagnosis of schistosomiasis, filariasis, and trichinosis. Sadun(1) found that, while viable *Trichinella* larvae could not be stained with fluorescein-labeled antiserum, the cuticle of larvae fixed with formalin stains readily. He demonstrated that this is a specific antigen-antibody reaction.

This report describes an attempt to use formalin-fixed second stage *Toxacara canis* larvae in the indirect FA technique to detect *Toxacara* antibodies.

Materials and methods. Four groups of sera were tested. Group A consisted of sera from 4 rabbits and 2 monkeys infected with embryonated eggs of *T. canis* and 2 rabbits and 2 monkeys infected with embryonated eggs of *Ascaris suum* from swine. Embrynated eggs containing second stage larvae were administered by stomach tube.

Group B was made up of sera from 8 rabbits inoculated with "Quadrigen,"† a vaccine containing diphtheria and tetanus toxoids, pertussis vaccine and aluminum phosphate adsorbed and poliomyelitis vaccine, 2 pools of rabbit antisera representing 11 serotypes of enteropathogenic *Escherichia coli*, one rabbit inoculated with *Vibrio cholerae*, 3 lots of commercial normal rabbit serum, one rabbit superinfected with *Trichinella spiralis* larvae, and 5 monkeys infected with polio virus.

Group C included sera from 25 children, age 19-22 months, from a study of "Quadrigen" vaccine. Group D included 49 normal adult blood donors.

Specific anti-human, anti-rabbit, and anti-monkey globulins conjugated with fluorescein

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