to qualify as a substrate for the xylose carrier. Apparently the hexose and pentose structures can share the carrier. The aldehyde structure is not an absolute necessity as xylitol inhibits the xylose transport the same way as arabinose. It is also interesting that ketose sugars may partially share the transport mechanism with xylose. The present results can be more easily explained by assuming a multiple attachment between the substrate and the carrier sites rather than assuming a rigid structural requirement for the carrier transport. Such a flexible concept will allow the assumption of a partial attachment of various structural groups within a given molecule. This would result in a wide variety of "affinities" and would allow a broad spectrum in the rate of mutual inhibition.

Summary. The intestinal transport of D-Xylose was studied in the rat using 2 different methods of *in situ* perfusion. The absorption of xylose is inhibited by the presence in the perfusate of any of the following substances (listed in decreasing order of their inhibitory ability): phlorizin > glucose > 3-0-methylglucose > fructose > 3-0-methylfructose > xylitol > arabinose. The results can be explained by assuming a xylose carrier site of a rather complicated nature on the epithelial membrane.

1. Verzár, F., McDougall, E. J., Absorption from the Intestine, Longmans, Green & Co., London, 1936.
2. Salomon, L. L., Allums, J. A., Smith, D. E., Biochem. Biophys. Res. Commun., 1961, v4, 123.
3. Csáky, T. Z., Lassen, U. V., Biochem. Biophys. Acta, 1964, v82, 215.
4. Alvarado, F., Experientia, 1964, v20, 302.
5. Teller, J. D., 130th Meeting, A.C.S., 1956, 69c.
6. Nelson, N., J. Biol. Chem., 1944, v153, 375.

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Effect of Dietary Orotic Acid on Liver Glycogen.* (30549)

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Rats fed a purified diet containing 1% orotic acid develop a fatty liver with a periportal distribution of lipid(1,2). Addition of adenine to the diet prevents the fatty liver (2). The hepatic lipid accumulation is more severe in female than in male rats(3).

We have recently observed that in the orotic acid-fed rat, there is also an increase in liver glycogen as well as in liver lipid. A similar parallelism between increased lipid and glycogen in the liver has been described in human cases of kwashiorkor(4) and of glycogen storage disease(5,6) and also in experimental studies such as with amino acid or protein deficiency(7,8) and following administration of cortisone(9,10). This paper describes our data related to the increased liver glycogen in rats force-fed for 3 days a purified diet containing 1% orotic acid. The increased hepatic glycogen, unlike the increased hepatic lipid, is not prevented by adenine.

Materials and methods. Female rats of the Wistar (Carworth Farms) strain weighing 135 to 185 g were used. The animals were maintained with a commercial chow (Wayne Lab-Blox) before beginning the special diets. All animals had free access to water. In all experiments, several groups of 3 to 5 rats each of the same age and weight were used. The animals were housed in individual wire cages in an air-conditioned room maintained at 25.5°C.

The basal diet was the same as that used in earlier studies(3,11) and was composed of 16% vitamin free casein, 5% corn oil, 4% salts, 5% vitamin-sucrose mixture and 70% sucrose. In the force-feeding experiments rats were tube-fed according to the method of Shay and Gruenstein(12) using plastic tubes.

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The basal diet was force-fed to the control rats throughout and to all experimental animals for one day prior to beginning the orotic acid-containing diets. The experimental diets contained compounds added to the basal diet so that each one made up the following percentage of the diet: orotic acid, 1% and adenine sulfate, 0.25%. Each diet was blended with distilled water so that one milliliter of diet mixture contained 0.7 g of diet. The diet mixtures were tube-fed 3 times daily at 8:30 AM, 1 PM, and 5:30 PM for 3 days and each animal received an average daily feeding of 0.65 g of diet/10 g of initial body weight. In a few experiments, an aqueous solution of adenine sulfate (10 mg/ml) adjusted to pH 7.4 was injected intraperitoneally into animals at 11 AM and at 3 PM. Each animal received a daily dose of 1.6 mg/10 g body weight.

In 2 experiments, groups of animals were force-fed or fed *ad libitum* the basal or orotic acid diet and then killed after 1, 2, or 3 days. *Ad libitum* fed animals had free access to the dry diet mixture until the morning the animals were killed. In one experiment animals were force-fed the basal or orotic acid diet for 3 days, then on the morning of the fourth day were treated with one of the following: epinephrine (adrenalin chloride—Parke Davis & Co.)—0.05 mg/rat given intraperitoneally each hour for 3 doses and then killed 1 hour after the last dose; glycine—5 ml of a 20% solution (1 g) by stomach tube and killed 4 hours later; or sucrose—5 ml of a 50% solution (2.5 g) by stomach tube and killed 3½ hours later.

Rats were weighed at the beginning and at the termination of each experiment. The animals were killed by decapitation. The liver was rapidly removed and weighed. Equal pieces from the median and left lateral lobes, that weighed together approximately 1 g, were immediately placed in 30% KOH for glycogen determination, which was performed according to the method of Seifter *et al*(13). The same method was used also for determination of blood glucose, on tungstic acid filtrates of blood samples obtained from the abdominal aorta of ether anesthetized animals. Another liver sample was frozen and stored at —4°C for subsequent lipid analysis. After thawing, the liver sample was ground with anhydrous sodium sulfate to a dry powder. This powder was extracted with chloroform for 24 hours using a soxhlet extraction apparatus. Chloroform was then evaporated and the residue was extracted with petroleum ether. The lipid remaining on evaporation of this second solvent was weighed. Liver glucose-6-phosphatase activity was assayed by the method of Cori and Cori(14), and liver phosphorylase activity by the method of Sutherland and Wosilait(15) using 0.02 M glycerophosphate buffer as described by Madsen(16). Liver amylo-1,6-glucosidase activity was assayed according to the method of Hers(17).

Results. Effect of orotic acid on liver glycogen and lipid. Female rats force-fed for 3 days the purified basal diet containing 1% orotic acid developed a fatty liver and an increase in hepatic glycogen (Table I). The addition of 0.25% adenine sulfate to the orotic acid-containing diet prevented the increase in liver lipid but did not appreciably decrease the elevated liver glycogen. However, administration of adenine sulfate intraperitoneally to animals fed the orotic acid diet only partially protected against the increase in liver lipid and did not appreciably decrease the elevated liver glycogen. Liver weights were significantly increased in animals fed the orotic acid-containing diets. The average changes in body weight of the animals during the 3-day experiments on the different diets were as follows: Basal diet, + 3.4 g; orotic acid, + 4.8 g; orotic acid plus oral adenine sulfate, + 4.4 g; orotic acid plus intraperitoneal adenine sulfate, + 4.4 g; and adenine sulfate, + 4.0 g.

To determine how rapidly liver lipid and glycogen increased in animals fed the orotic acid diet, groups of animals were killed after 1, 2, or 3 days (Table II). In one experiment animals were force-fed and in another the animals were fed *ad libitum*. In the force-fed experiment liver lipid and glycogen began to increase after 2 days. In the *ad libitum* fed experiment liver glycogen increased after 1 day and liver lipid after 2 days. Liver glycogen was much greater in the *ad libitum*

TABLE I. Liver Weight, Lipid and Glycogen of Rats Force-Fed a Purified Basal Diet Containing 1% Orotic Acid for 3 Days.

Supplement to basal diet	No. of rats	Liver		
		Weight, g/100 g body wt	Lipid, mg/100 g body wt	Glycogen, mg/100 g body wt
None	23	4.06 ± .08†	308 ± 15†	78 ± 10†
1% Orotic acid	22	5.56 ± .11*	715 ± 52*	256 ± 18*
1% Orotic acid + .25% adenine sulfate	17	4.90 ± .17*	347 ± 44	196 ± 24*
1% Orotic acid + IP adenine sulfate‡	21	5.34 ± .13*	575 ± 66*	181 ± 21*
.25% Adenine sulfate	9	4.06 ± .15	320 ± 33	90 ± 16

* $P < .01$.
† Mean ± standard error of mean.
‡ An aqueous solution of adenine sulfate (10 mg/ml) injected intraperitoneally (IP) twice daily (1.6 mg/10 g body wt/day).

fed animals than in the force-fed animals because the former had free access to food up until time of killing while the force-fed animals received their last food approximately 17 hours before killing.

Effect of glycogenolytic agents and of sucrose administration. To determine whether increased liver glycogen of animals force-fed the orotic acid diet would respond to glycogenolytic agents, animals in one experiment were treated with epinephrine or glycine(18) on the fourth day before killing (Table III). Following epinephrine administration, the orotic acid-fed animals had a moderate (41%) drop in liver glycogen with a marked (119%) increase in blood glucose. Following glycine administration, the orotic acid-fed animals had a marked (97%) drop in liver glycogen with little (+10%) change in blood glucose. Following sucrose ingestion, the orotic acid-fed animals had a marked (172%) increase in liver glycogen and a moderate (81%) increase in blood glucose. The experimental and control animals responded to the 3 different agents in a similar manner. When aliquots of liver of animals force-fed the orotic acid-containing diet were left at room temperature for 2 hours, there was a 59% decrease in liver glycogen in comparison to aliquots measured at zero time.

Enzymatic activities. In 2 experiments liver phosphorylase was assayed. The mean values, expressed as mg P liberated/30 min at 30°C per liver/100 g body wt, were for 8 control animals—144 ± 5 and for 6 orotic acid fed animals—173 ± 11. In one experiment liver glucose-6-phosphatase was assayed and the mean values, expressed as mg P liberated/15 min at 30°C per liver/100 g body wt, were for 3 control animals—24.2 ± 1.1 and for 3 orotic acid fed animals—22.4 ± 0.5. In one experiment liver amylo-1,6-glucosidase activity was assayed. The values, expressed as cpm/liver/100 g body wt, were for a control animal—82.3×10^4 and for an orotic acid-fed animal—76.2×10^4. The data for the 3 enzymes indicate only small differences between the 2 groups.

Discussion. The results of this study indicate that rats fed a purified diet containing 1% orotic acid develop an increase in hepatic glycogen as well as fat. Addition of 0.25% adenine sulfate to the diet prevents the development of the fatty liver but does not significantly decrease the increased hepatic glycogen.

In an earlier study on the inhibitory effect of 6-azauracil on induction of fatty liver by dietary orotic acid Suva *et al*(19) reported values of liver glycogen for animals fed *ad libitum* for 2 weeks. Their results, although expressed as extinction values on a concentration basis without indicating liver weights, were consistent with our present results. Also the finding by Deamer *et al*(20) of an increase in the hepatic lipid-free residue of rats fed an orotic acid diet in comparison with rats fed a control diet strongly suggests that much of this increase was probably due to increased glycogen.

The protective effect of adenine on orotic acid-induced fatty liver is well documented (2,19). The results with adenine in our study show a similar protection against fatty

TABLE II. Liver Weight, Lipid and Glycogen of Rats Force-Fed or Fed *Ad libitum* a Purified Basal Diet Containing 1% Orotic Acid for 1, 2 or 3 Days.

Diet	Days on diet	No. of rats: Force-fed	No. of rats: *Ad libitum*	Liver: Weight, g/100 g body wt: Force-fed	Liver: Weight, g/100 g body wt: *Ad libitum*	Liver: Lipid, mg/100 g body wt: Force-fed	Liver: Lipid, mg/100 g body wt: *Ad libitum*	Liver: Glycogen, mg/100 g body wt: Force-fed	Liver: Glycogen, mg/100 g body wt: *Ad libitum*
Basal	1	3	3	3.58 ± .29‡	4.51 ± .46‡	234 ± 17‡	311 ± 43‡	17 ± 8‡	234 ± 87‡
1% Orotic acid	1	3	3	3.71 ± .15	4.72 ± .07	259 ± 21	252 ± 29	23 ± 11	348 ± 63
Basal	2	3	3	3.85 ± .06	4.07 ± .11	247 ± 30	277 ± 31	46 ± 4	178 ± 19
1% Orotic acid	2	3	3	4.66 ± .15†	4.85 ± .18*	383 ± 67	347 ± 45	109 ± 14*	468 ± 108*
Basal	3	4	4	4.23 ± .09	4.60 ± .26	327 ± 25	285 ± 29	64 ± 17	355 ± 36
1% Orotic acid	3	4	4	5.35 ± .25†	5.53 ± .23*	507 ± 104	634 ± 95*	226 ± 42*	687 ± 150

* $.05 > P > .01$. † $P < .01$ (basal *vs* orotic acid). ‡ Mean ± standard error of mean.

liver. However, adenine given intraperitoneally twice daily at the same dosage level did not prevent the fatty liver. These results are reminiscent of earlier findings(11) in which it was found that orotic acid must be incorporated in the diet, rather than given separately by stomach tube, to induce a fatty liver. Since orotic acid *per se* does not induce a fatty liver(11), and since the nature and quantity of the purified diet profoundly alter the response of the rat to the feeding of orotic acid(11), and further since adenine must be incorporated in the diet to be protective, it becomes apparent that the action of orotic acid, as well as the protection by adenine, is indeed complex and is probably due to some subtle metabolic imbalance at present not understood.

In addition to increased liver lipid and glycogen, increased hepatic protein(11,20,21) and ribonucleic acid(22) have been induced by dietary orotic acid. Although the increase in liver protein is reversed by adenine(21), the elevated liver ribonucleic acid is not. Actually the addition of adenine to the orotic acid diet produced a further elevation in hepatic ribonucleic acid(22). Thus it is apparent that many but not all of the hepatic alterations produced by orotic acid are protected or reversed by adenine. The failure of adenine to inhibit the increased hepatic glycogen suggests that the mechanism responsible for the increased glycogen is either not identical to that responsible for the other alterations or is, more probably, due to the action of orotic acid or its metabolites at some stage of hepatic glycogen metabolism where adenine is no longer protective.

The concomitant increase in liver lipid and glycogen in a variety of human(4-6) and experimental(7-10) conditions is of much interest. Whether these two pathologic manifestations which occur together in different conditions have a common or unrelated pathogenesis is at present unknown. The availability of a number of different experimental conditions which rapidly induce these hepatic changes offers useful models which may be of value in the ultimate understanding of the mechanisms involved.

Summary. Female Wistar strain rats were

TABLE III. Effect of Administration of Epinephrine, Glycine or Sucrose on Liver Glycogen and Blood Glucose of Rats Force-Fed a Purified Basal Diet Containing 1% Orotic Acid for 3 Days.

Diet	Treatment	No. of rats	Liver glycogen, mg/100 g body wt	Blood glucose, mg %
Basal	None	3	30 ± 20§	108 ± 9§
1% Orotic acid	"	3	172 ± 64	88 ± 1
Basal	Epinephrine*	3	14 ± 2	240 ±11
1% Orotic acid	"	3	101 ± 11	193 ±13
Basal	Glycine†	3	1 ± 0	91 ± 6
1% Orotic acid	"	2	5 ± 1	97 ± 1
Basal	Sucrose‡	3	263 ± 14	125 ± 7
1% Orotic acid	"	2	468 ± 6	159 ± 3

* Adrenalin chloride, .05 mg/rat given intraperitoneally each hr for 3 doses and killed 1 hr after the last dose.
† Glycine, 5 ml of a 20% solution (1 g) by stomach tube and killed 4 hr later.
‡ Sucrose, 5 ml of a 50% solution (2.5 g) by stomach tube and killed 3½ hr later.
§ Mean ± standard error of mean.

force-fed for 3 days a purified diet containing 1% orotic acid and liver lipid and glycogen were determined. Liver glycogen as well as liver lipid became significantly increased. Addition of 0.25% adenine sulfate to the orotic acid diet prevented the increase in hepatic lipid but did not appreciably affect the increased hepatic glycogen. Intraperitoneal administration of adenine did not affect the increased hepatic lipid and glycogen. Liver activities of glucose-6-phosphatase, phosphorylase and amylo-1,6-glucosidase were similar in orotic acid-fed and in basal diet-fed animals. Hepatic glycogen of orotic acid-fed animals responded to administration of glycogenolytic agents (epinephrine and glycine) and to sucrose.

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1. Standerfer, S. B., Handler, P., Proc. Soc. Exp. Biol. and Med., 1955, v90, 270.
2. Handschumacher, R. E., Creasey, W. A., Jaffe, J. J., Pasternak, C. A., Hankin, L., Proc. Nat. Acad. Sci., U.S.A., 1960, v46, 178.
3. Sidransky, H., Endocrinology, 1963, v72, 709.
4. Waterlow, J. C., Weisz, T., J. Clin. Invest., 1956, v35, 346.
5. Howell, R. R., Ashton, D. M., Wyngaarden, J. B., Pediatrics, 1962, v29, 553.
6. Holling, H. E., Ann. Int. Med., 1963, v58, 654.
7. Sidransky, H., Verney, E., Arch. Path., 1964, v78, 134.
8. Platt, B. S., Proc. Royal Soc. B, 1962, v156, 337.
9. Ingle, D. J., J. Clin. Endocrinol., 1950, v10, 1312.
10. Hill, R. B., Jr., Droke, W. A., Proc. Soc. Exp. Biol. and Med., 1963, v114, 766.
11. Sidransky, H., Verney, E., Lombardi, B., J. Nutrition, 1963, v81, 348.
12. Shay, H., Gruenstein, M., J. Lab. Clin. Med., 1946, v31, 1384.
13. Seifter, S., Dayton, S., Novic, B., Muntwyler, E., Arch. Biochem., 1950, v25, 191.
14. Cori, G. T., Cori, C. F., J. Biol. Chem., 1952, v199, 661.
15. Sutherland, E. W., Wosilait, W. D., ibid., 1956, v218, 459.
16. Madsen, N. B., Biochem. Biophys. Res. Comm., 1961, v6, 310.
17. Hers, H. G., Rev. Int. Hepat., 1959, v9, 35.
18. Munro, H. N., Clark, C. M., Goodlad, G. A. J., Biochem. J., 1961, v80, 453.
19. Suva, J., Cerhova, M., Habermannov, S., Habermann, V., Acta Vitaminologica, 1961, v3, 97.
20. Deamer, D. W., Kruger, F. A., Cornwell, D. G., Biochim. Biophys. Acta, 1965, v97, 149.
21. Hankin, L., J. Nutrition, 1963, v79, 519.
22. Caldarera, C. M., Puddu, P., Marchetti, M., Boll. Soc. Ital. Biol. Sper., 1962, v38, 1818.

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