4. T. L. Gleason and F. Friedberg, *Physiol. Zool.* **26**, 95 (1953).
5. L. W. Janssen, *Verhandel. Koninkl. Ned. Akad. Wetenschap., Afdel. Natuurk.*, Sect. II, **47** (3), 1 (1951).
6. We are indebted to H. Hirai for the Whatman filter paper.
7. H. Irisawa and Aya F. Irisawa, *J. Hiroshima Med. Sci.*, in press.
8. H. G. Kunkel and A. Tiselius, *J. Gen. Physiol.* **35**, 89 (1953).
9. T. Kusunoki, *J. Biochem (Japan)* **40**, 177 (1953).

24 May 1954.

Glucose-6-Phosphatase Studies in Fasting

George Weber* and Antonio Cantero

Montreal Cancer Institute, Research Laboratories, Notre Dame Hospital, Montreal, Quebec, Canada

Glucose-6-phosphatase, an enzyme, which hydrolyzes glucose-6-phosphate to glucose and inorganic phosphate, was first demonstrated by Fantl *et al.* (*1*) and has been studied by Swanson (*2*) and De Duve *et al.* (*3*). The enzyme has been demonstrated in different organs, but it is the most active in liver and kidney. It can be distinguished from other nonspecific phosphatases by its *p*H optimum (6.5 to 6.8), its thermal instability, its sensitivity to acids, and its substrate specificity on glucose-6-phosphate, with negligible or no activity toward glycerophosphate, glucose-1-phosphate, or fructose-6-phosphate. The liver enzyme is associated mainly with the microsome fraction. Chiquoine (*4*) demonstrated with histochemical method that the enzyme is more abundant in the peripheral third of the hepatic lobule than in the inner two-thirds. Within the hepatic cell glucose-6-phosphatase is concentrated about the nuclear membrane.

However, there is very little known about the physiological and pathological behavior of this enzyme. Cori and Cori (*5*) demonstrated its importance in the pathology of carbohydrate metabolism. They reported the almost complete absence of the enzyme from liver in human cases of glycogen storage disease.

A well-established fact is that liver is virtually the sole source of the blood sugar in the fasting animal (*6*). Since the blood sugar level is maintained throughout long periods of fasting, the glucose secreted by the liver into the blood, therefore, must be derived from stored carbohydrate or noncarbohydrate precursors. It seemed to be of interest to study the behavior of liver glucose-6-phosphatase in fasting (*7*).

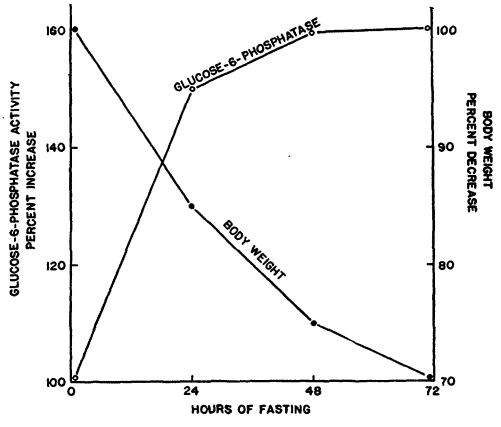

Fig. 1. Effect of fasting on body weight and liver glucose-6-phosphatase activity in mice.

Table 1. Effect of 48-hr fasting on the glucose-6-phosphatase activity in rat liver.

Rat	Body weight (g)	Liver glycogen (g percent)	Liver glucose-6-phosphatase activity*
Controls: fed chow			
1	210	4.80	292
2	210	4.96	292
3	210	2.91	185
4	210	4.26	321
5	210	3.00	250
6	210	4.20	281
Mean	210	4.02	270
Percentage	100	100	100
Fasted: for 48 hr			
7	180	0.12	415
8	185	.06	467
9	190	.69	395
10	190	.09	421
11	178	.01	410
12	180	.32	381
Mean	184	0.22	432
Percentage of control values	88	5.5	160

* Expressed in micrograms of phosphorus liberated in 15 min at 31°C per 100 mg liver wet weight.

Twenty-eight male albino mice (31 to 33 g) were divided into four groups, each containing seven mice. The three fasting groups received no Purina Fox Chow for 24, 48, and 72 hr. Water was given *ad libitum* to all groups. Animals were sacrificed by a blow on the head, decapitated, and bled. Livers were quickly excised and pooled on ice and 5-percent homogenate was prepared in 0.25*M* sucrose. The glucose-6-phosphatase was assayed according to the method of Cori and Cori (*5*). Incubation time was 30 min at 31°C. Figure 1 shows the increase in glucose-6-phosphatase activity as expressed in percentage increase when the control value is taken as 100 percent.

The increased liver glucose-6-phosphatase activity was also demonstrated in fasting rats. The following experiment is a representative example. Twelve male Wistar rats of weight 210 g were separated into two groups. The control group had a free access to Purina Fox Chow and water, while the fasting group received only water for 48 hr. Animals were sacrificed, and the enzyme was determined as described in the foregoing paragraph, except that 10-percent homogenates were prepared from each liver, and they were assayed in separated, instead of pooled, livers. Incubation time

was 15 min at 31°C. A small sample of tissue was rapidly snipped from the right lobe of the liver for glycogen estimation. This was determined by the method of Good, Kramer, and Somogyi (*8*), employing the Nelson's adaptation of the Somogyi method for glucose (*9*).

Table 1 shows that while the rats lose about 12 percent of body weight during the 48-hr fast and liver glycogen decreases to 5 percent of the original value, the glucose-6-phosphatase activity is increased by 60 percent.

These experimental data have been repeatedly confirmed in this laboratory in the course of other experiments involving various periods of fasting, and it was found that the difference between the glucose-6-phosphatase activity of normal and 48-hr fasted animals is significant also when expressed on nitrogen or liver weight-to-body weight ratio basis (*10*).

The afore-cited data demonstrate that the glucose-6-phosphatase activity increases in the liver of fasting mice and rats. A survey of the literature on the effect of fasting on liver enzymes shows that most enzymes decrease under the described conditions here. The increase of glucose-6-phosphatase activity during fasting can, therefore, be considered as a physiological adaptive change to the stress of fasting during which the glycogen stores of the liver are depleted.

References and Notes

* Senior fellow, Cancer Research Society, Montreal.

1. P. Fantl, M. N. Rome, and J. F. Nelson, *Australian J. Exptl. Biol. Med. Sci.* **20**, 121 (1942); P. Fantl and M. N. Rome, *ibid.* **23**, 21 (1945).
2. M. J. Swanson, *J. Biol. Chem.* **184**, 647 (1950).
3. C. De Duve *et al.*, *Bull. soc. chim. biol.* **31**, 1242 (1949); H. G. Hers and C. De Duve, *ibid* **32**, 20 (1950); H. G. Hers *et al.*, *ibid.* **33**, 21 (1951).
4. A. D. Chiquoine, *J. Histochemistry and Cytochemistry* **1**, 429 (1953).
5. G. T. and C. F. Cori, *J. Biol. Chem.* **199**, 661 (1952).
6. G. G Duncan, *Diseases of Metabolism*, (Saunders, Philadelphia, ed 3, 1952), p. 53.

7. This project has been supported by grants from the Cancer Research Society. The valuable assistance of Vilma Jansons is gratefully acknowledged.

8. C. A. Good, H. Kramer, and M. Somogyi, *J. Biol. Chem.* **100**, 485 (1953).
9. N. Nelson, *ibid.* **153**, 375 (1944).
10. Unpublished.

6 July 1954.

Communications

Inherited Jaundice in *Peromyscus*

This is to report (*1*) a mutation in *Peromyscus maniculatus* (*2*) that is typically associated with neonatal jaundice, chronic splenomegaly, anisocytosis, polychromatophilia, and reticulocytosis. Affected mice usually become jaundiced during their first postnatal 24 hr. The duration of the yellow color varies but it is not, as a rule, perceptible after the second postnatal day. The intensity of the color varies from deep to pale yellow, and in some individuals it may not be sufficiently marked to make identification possible upon first inspection.

The yellow color is succeeded by pallor, which may be very pronounced and may last until the fifth postnatal day when, in any event, it would be masked by the outgrowing pelage. All of more than 50 jaundiced young that were examined on successive days became pallid if they survived, and this symptom, because of its duration, is more useful than the neonatal jaundice in the identification of affected individuals.

All of 30 newborn mice recorded as jaundiced and/or pale had splenomegaly when, to test the point, they were posted at 3 mo of age or later. A much larger number of mice examined at an age when affected individuals would be jaundiced or pale, and recorded as not affected, had spleens of normal color and proportions. The normal spleen in the deer mouse is a slender leaflike organ that is pale red or dark red in color. Jaundiced mice develop a spleen that is very dark red or black in color and about double the size of the normal spleen in each dimension. There is variation in the size and color of both normal and hypertrophied spleens, but intergradation in size or color has not yet been observed.

The erythrocytes of adult splenomegalic mice vary in size and shape and this erythrocytic variability is an immediately obvious feature upon microscopic examination of either blood smears or the vascular areas of tissue sections. Blood smears of affected mice, stained by the Osgood-Wilhelm technique (*3*), have, in the 22 cases examined, exhibited a significantly higher proportion of reticulocytes than was found in the blood of normal individuals, of similar age, prepared at the same time. The reticulocyte counts (*4*) are shown in Table 1.

Table 1. Percentages of reticulocytes in smears made from jaundiced mice between 3 and 6 mo of age and of normal mice 3 mo old.

Percentage	20	18	16	14	12	10	8	6	4	2	n
Normal mice										7	7
Jaundiced mice	2		1		6	3	2	1			15

A chi-square test of the difference in the number of mice in the two classes produced by the mating of normal hybrid mice with jaundiced mice is given in Table 2. The phenotypes of all parent mice and all of their offspring were identified by more than one person during the first postnatal day and, if necessary, on a succeeding day. Since 15 of the 19 broods contained young of both classes, comparison assisted separation into two classes. The data fit the assumption that the characteristics of the syndrome described here are brought about by a single recessive gene effect.