Stimulation of Pentose Phosphate Pathway Dehydrogenase Enzyme Activities in Ethionine-Treated Mice

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1. Mice treated with ethionine (intraperitoneally, 5mg./day for 4 days or 10mg./day for 3 days) showed a profound loss of hepatic glycogen, a decrease of glycogen synthetase activity, a development of hypoglycaemia, a two- to five-fold increase in the activity of glucose 6-phosphate dehydrogenase but no change in 6-phosphogluconate dehydrogenase and an earlier manifestation of the solubilization of phosphorylase as compared with glycogen synthetase. The administration of ATP did not prevent these effects. 2. During the early post-injection period (2-3 days) there was a further enhancement of the activity of glucose 6-phosphate dehydrogenase (tenfold) in the liver and a clear elevation of 6-phosphogluconate dehydrogenase activity (twofold). Subsequently, the glycogen concentration was restored, followed by an earlier reassociation of glycogen particle with phosphorylase than with glycogen synthetase, along with a disappearance of ethionine effect at about the eighteenth day. 3. Glucose 6-phosphate dehydrogenase from both control and ethionine-treated animals showed a marked preference for glucose 6-phosphate as substrate rather than for galactose 6-phosphate, whose rate of oxidation was only 10% of that of the glucose 6-phosphate. 4. Since actinomycin D, puromycin, 5-fluorouracil and DL-*p*-fluorophenylalanine failed to block the ethionine-enhanced glucose 6-phosphate dehydrogenase activity, the possibility that new enzyme protein synthesis is responsible for the effect is doubtful.

Ethionine-fed rats show an increased activity of glucose 6-phosphate dehydrogenase (EC 1.1.1.49), an effect which was considered paradoxical (Sie & Hablani, 1965a) since enzymes such as pyruvate kinase (EC 2.7.1.40) exhibit a decrease in activity, as expected on the basis of the ethionine-mediated liver-ATP-depletion theory of Stekol, Mody, Bedrak, Keller & Perry (1960), and later confirmed by Shull (1962). Subsequently, in ethionine-fed mice, a similar finding of elevated liver glucose 6-phosphate dehydrogenase activity was obtained, in addition to a decrease in glycogen synthetase (EC 2.4.1.11) (Sie & Hablani, 1965b). Is the elevation of glucose 6-phosphate dehydrogenase a reflection of a more generalized stimulation of the pentose phosphate pathway, and, if so, is this increased enzyme activity still a reflection of the liver effects of ethionine on ATP concentrations? These are the questions that the present study seeks to answer.

Two circumstances indicated a stimulation of 6-phosphogluconate dehydrogenase (EC 1.1.1.44) as well as glucose 6-phosphate dehydrogenase: the introduction of the intraperitoneal route of ethionine administration (10mg./day for 4 days) and the

careful examination of the biochemical changes in the liver for a period of 19 days after the last ethionine injection. In the latter interval, in addition to a great enhancement of glucose 6-phosphate dehydrogenase activity and a clear elevation in 6-phosphogluconate dehydrogenase activity, there was a prolonged hypoglycaemia along with a decrease in liver glycogen and glycogen synthetase.

The administration of ample amounts of ATP could not prevent these changes, nor could the administration of inhibitors of protein synthesis such as puromycin, 5-fluorouracil and actinomycin D.

We therefore decided to look for the explanation of the enhanced dehydrogenase enzyme activity outside the boundaries of current interpretation of the liver effects of ethionine. A description of the pertinent experiments and the development of a new interpretation are given in the present paper.

MATERIALS AND METHODS

Chemicals and enzymes. AMP, ATP, UDP-glucose (90% pure), NADP⁺, glucose 1-phosphate (dipotassium salt), glucose 6-phosphate (disodium salt), 6-phosphogluconate

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(tricyclohexylammonium salt) and DL-ethionine were obtained from California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.; D-galactose 6-phosphate (disodium salt) was from Sigma Chemical Co., St Louis, Mo., U.S.A.; rabbit liver glycogen and puromycin were from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.; DL-p-fluorophenylalanine was obtained from Mann Research Laboratories, New York, N.Y., U.S.A.; 5-fluorouracil was a gift from Hoffmann-La Roche Inc., Nutley, N.J., U.S.A., and actinomycin D was a gift from Merck and Co., Rahway, N.J., U.S.A. All other chemicals were the best reagent grade available from commercial sources.

Animals. Three-month-old male A/Jax mice (25–30g.) were maintained on a water and Purina Chow diet *ad libitum*. To study the effect of ethionine in relation to its dosage, animals in groups of five or six were injected with 0.5 ml. of ethionine (5–24 mg.) in sterile 0.9% NaCl intra-peritoneally daily for 4 consecutive days. The animals were killed 24 hr. after the last injection.

To observe the effect produced during both the injection period of 10 mg. of ethionine/day for 4 days and the post-injection period, the animals in groups of six were killed 24 hr. after the last specified injection or at the designated interval. Organs were quickly removed and chilled on ice.

To determine whether ATP could influence the ethionine-induced increase of dehydrogenase, each animal was given 125 mg. of ATP 30 min. before daily injection of 10 mg. of ethionine for 4 days.

In the study of the inhibitors of protein synthesis, the dosages were as follows: DL-p-fluorophenylalanine, 20 mg., 30 min. before ethionine injection daily for 4 days; puromycin, 3 mg., 60 min. before ethionine injection daily for 4 days; 5-fluorouracil, 5 mg., 60 min. before the first three daily injections of ethionine, and actinomycin D, 1 µg. divided into two doses given 8 hr. apart; the first dose was given 30 min. before each injection of 5 mg. of ethionine for 4 days. The control animals were given only ethionine. Both test and control groups were killed 24 hr. after the last injection.

A pair-fed experiment was conducted with a lower dose of ethionine (5 mg./day) as follows: before setting up the experimental and control groups it was found that mice on ethionine ingested an average of 2.5 g. of ground Purina Chow and therefore this was the amount fed to the controls. Animals selected at random were housed in individual cages

and were provided with 2.5 g. of food daily at 5 p.m. until 9 a.m. the next day for 4–5 days. Those that weighed between 22 and 24 g. were then selected and injected intra-peritoneally with 5 mg. of ethionine (in 0.5 ml. of 0.9% NaCl) daily for 4 days. Groups of animals were killed on different days of treatment. The controls were maintained similarly and were injected with 0.9% NaCl.

Enzyme assay procedure. Tissue homogenate was prepared in 0.25 M-sucrose–0.1 M-NaF–1 mM-EDTA. The supernatant fractions were obtained after centrifugation of the homogenate at 100 000g for 30 min. at 0° in a refrigerated Spinco model L centrifuge. The enzyme activities of glycogen synthetase and phosphorylase were measured in both homogenate and supernatant preparations, whereas 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase were determined in the supernatant fractions. The individual enzyme activities and the references to the methods employed for their measurement were: phosphorylase (EC 2.4.1.1) (Cori, Illingworth & Keller, 1955; Leloir & Goldemberg, 1960), glycogen synthetase (Leloir & Goldemberg, 1960), 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase (Fitch, Hill & Chaikoff, 1959). When galactose 6-phosphate was used as substrate for glucose 6-phosphate dehydrogenase, the activity was determined by a method similar to that for 6-phosphogluconate dehydrogenase. The enzyme activities are expressed as µmoles of substrate utilized or of product liberated/mg. of protein. Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951), with bovine serum albumin as reference standard.

Other assays. Glycogen was extracted by hot KOH and precipitated by 95% (w/v) ethanol. It was estimated as glucose units by the cysteine-sulphuric acid reaction (Dische, 1949). Blood glucose was determined by the Nelson-Somogyi method (Nelson, 1944; Somogyi, 1945).

RESULTS

The effects of ethionine as a function of its daily dosage are indicated in Table 1. Given intra-peritoneally from a minimum of 5 mg. up to 24 mg. per day for 4 days, ethionine induces noticeable biochemical changes, namely a depletion of hepatic

Table 1. *Liver glycogen content, blood glucose, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase as functions of ethionine dosage for 4 days*

Results are presented as means $\pm$ S.E.M. Enzyme specific activities are expressed as µmoles of product formed/mg. of protein/hr. For assay procedures see the text. Numbers in parentheses indicate the number of animals in each group.

Treatment (mg. of ethionine/day/animal)	Glycogen (g./100g.)	Blood glucose (mg./100 ml.)	Sp. activity	
			6-Phosphogluconate dehydrogenase	Glucose 6-phosphate dehydrogenase
0 (8)	2.01 $\pm$ 0.60	154.80 $\pm$ 13.95	0.65 $\pm$ 0.11	0.34 $\pm$ 0.06
5 (5)	0.25 $\pm$ 0.33*	86.10 $\pm$ 23.70*	0.71 $\pm$ 0.15	1.28 $\pm$ 0.56*
10 (8)	0.10 $\pm$ 0.10*	70.30 $\pm$ 25.6*	0.76 $\pm$ 0.21	1.27 $\pm$ 0.39*
15 (5)	0.18 $\pm$ 0.16*	73.60 $\pm$ 18.7*	0.96 $\pm$ 0.11	1.46 $\pm$ 0.12*
24 (3)	0.03 $\pm$ 0.01*	73.50 $\pm$ 11.50	0.87 $\pm$ 0.20	1.64 $\pm$ 0.26*

* $P < 0.01$, statistically significant as compared with the group receiving no ethionine.

Table 2. *Effect of number of days of administration of 10mg. of ethionine/day on glycogen, blood glucose, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase*

Enzyme specific activities are expressed as μ moles of product formed/mg. of protein/hr. For assay procedures see the text. Results are presented as means $\pm$ s.e.m., and numbers in parentheses indicate the number of animals in each group.

Days of treatment	Glycogen (g./100g.)	Blood glucose (mg./100ml.)	Sp. activity	
			6-Phosphogluconate dehydrogenase	Glucose 6-phosphate dehydrogenase
0 (6)	2.01 $\pm$ 0.60	152.10 $\pm$ 24.20	0.64 $\pm$ 0.13	0.38 $\pm$ 0.04
1 (6)	3.14 $\pm$ 0.75	171.60 $\pm$ 46.30	0.60 $\pm$ 0.09	0.36 $\pm$ 0.13
2 (6)	1.19 $\pm$ 0.61	161.30 $\pm$ 36.20	0.58 $\pm$ 0.09	0.49 $\pm$ 0.10
3 (7)	0.20 $\pm$ 0.26*	99.40 $\pm$ 8.90*	0.72 $\pm$ 0.04	0.83 $\pm$ 0.13*
4 (7)	0.17 $\pm$ 0.26*	72.50 $\pm$ 22.30*	0.68 $\pm$ 0.56	1.50 $\pm$ 0.76*

* $P < 0.01$, statistically significant as compared with the group receiving no ethionine.

Fig. 1. Concentrations of liver glycogen (O) and blood glucose (●) in mice during and after 4 days of ethionine treatment. In this Figure and Figs. 2 and 3 the shaded area represents the 4-day interval of daily injections of 10mg. of ethionine/mouse.

glycogen, development of hypoglycaemia and an elevation in activity of glucose 6-phosphate dehydrogenase. The last-named effect is not a function of increased dosage of ethionine given.

The administration of three to four doses of ethionine (10mg.) are required to induce a low hepatic glycogen and a moderately but significantly decreased blood glucose. The activity of liver glucose 6-phosphate dehydrogenase is profoundly increased whereas no significant change has been

Fig. 2. Relation of the specific activity of liver (a) glycogen synthetase and (b) phosphorylase in mice during and after ethionine treatment: ■, enzyme in the sedimentable particulate fraction; ○, enzyme not sedimented at 100000g for 30min., Liver glycogen concentration, for reference.

found in 6-phosphogluconate dehydrogenase (Table 2) by 4 days.

Figs. 1, 2 and 3 demonstrate the effect of ethionine

Fig. 3. Specific activity of glucose 6-phosphate dehydrogenase (●) and 6-phosphogluconate dehydrogenase (○) in the liver of mice during and after 4 days of ethionine treatment.

as a function of dose for 4 days and on the post-injection interval of 18 days on the hepatic glycogen content, blood glucose concentration and the activities of the different enzyme systems. The glycogen concentration declines sharply (Fig. 1), almost disappearing by the third day of the injection period, and not until the sixth post-injection day is there a significant amount of deposition noticeable. The administration of ethionine produces a prolonged hypoglycaemia, which is in agreement with the findings of Combes & Schenker (1966).

The activity of glycogen synthetase shows an extensive loss in the particulate fraction as well as a much smaller increase in the supernatant fraction. The full association of glycogen synthetase with the glycogen particle is accomplished by the eighteenth day.

Ethionine first produces an apparent activation of phosphorylase followed by a great decrease of activity in the particulate fraction on the fourth day of injection, paralleled by an equivalent increase in the supernatant fraction. The restoration of a glycogen-associated phosphorylase occurs somewhat earlier than it does for glycogen synthetase (Fig. 2).

Table 3. Lack of effect of ATP on ethionine-induced liver changes

Enzyme specific activities are expressed as μ moles of product formed/mg. of protein/hr. Results are presented as means $\pm$ S.E.M., and six animals were used in each group. In the ethionine-treated group each animal received 10mg. of ethionine/day for 4 days. In the ethionine + ATP-treated group each animal received 125 mg. of ATP intraperitoneally 30 min. before ethionine administration.

	Ethionine	Ethionine + ATP
Glycogen content (g./100g. of liver)	0.02 $\pm$ 0.02	0.07 $\pm$ 0.02
Blood glucose (mg./100ml.)	70.3 $\pm$ 25.6	82.6 $\pm$ 30.2
	Sp. activity	
Glycogen synthetase		
Supernatant fraction	1.85 $\pm$ 0.67	1.27 $\pm$ 0.34
Particulate fraction	0.61 $\pm$ 0.70	0.54 $\pm$ 0.58
Phosphorylase		
Supernatant fraction	14.8 $\pm$ 5.02	16.1 $\pm$ 2.96
Particulate fraction	1.6 $\pm$ 2.96	1.83 $\pm$ 1.85
6-Phosphogluconate dehydrogenase	0.59 $\pm$ 0.42	0.65 $\pm$ 0.19
Glucose 6-phosphate dehydrogenase	0.91 $\pm$ 0.22	0.86 $\pm$ 0.25

Table 4. Effect of DL-p-fluorophenylalanine, puromycin and 5-fluorouracil on liver glucose 6-phosphate dehydrogenase of animals treated with ethionine (10mg./day) for 4 days

Enzyme specific activities are expressed as μ moles of product formed/mg. of protein/hr. Results are presented as means $\pm$ S.E.M., and number in parentheses indicate number of animals in each group.

Conditions	Sp. activity of glucose 6-phosphate dehydrogenase
Expt. 1	
Controls (6)	1.03 $\pm$ 0.35
20mg. of p-fluorophenylalanine/day for 4 days, 30 min. before ethionine injection (6)	1.23 $\pm$ 0.14
3mg. of puromycin/day for 4 days, 60 min. before ethionine injection (6)	1.21 $\pm$ 0.99
Expt. 2	
Controls (6)	0.61 $\pm$ 0.28
5mg. of 5-fluorouracil/day for 3 days, 60 min. before ethionine injection (8)	0.68 $\pm$ 0.16
Expt. 3	
1 μ g. of actinomycin D/day for 4 days, 30 min. before injection of 5mg. of ethionine (3)	1.33; 1.66; 2.04

Table 5. *Effect of ethionine on liver 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase in pair-fed animals*

Enzyme specific activities are expressed in μ moles of product formed/mg. of protein/hr. Results are presented as means $\pm$ S.E.M.

Days of treatment	No. of animals used	Sp. activity	
		6-Phosphogluconate dehydrogenase	Glucose 6-phosphate dehydrogenase
0	5	0.57 $\pm$ 0.06	0.33 $\pm$ 0.08
3	5	0.51 $\pm$ 0.04	0.83 $\pm$ 0.04*
4	5	0.64 $\pm$ 0.14	1.23 $\pm$ 0.25*
5	6	0.88 $\pm$ 0.12*	1.65 $\pm$ 0.34*
6	6	0.76 $\pm$ 0.11†	1.01 $\pm$ 0.32*

* $P < 0.01$, as compared with control group.

† $P = 0.05$.

Table 6. *Specific activity of liver glucose 6-phosphate dehydrogenase towards glucose 6-phosphate and galactose 6-phosphate in the control and ethionine-treated mice (5 mg./day for 4 days)*

Enzyme specific activities are expressed as μ moles of product formed/mg. of protein/hr. Results are presented as means $\pm$ S.E.M. and numbers in parentheses indicate the number of animals used.

	Sp. activity		
	Galactose 6-phosphate (a)	Glucose 6-phosphate (b)	(a)/(b) (%)
Control (7)	0.049 $\pm$ 0.012	0.48 $\pm$ 0.08	8.2 $\pm$ 2.5
Ethionine (8)	0.098 $\pm$ 0.016	1.13 $\pm$ 0.23*	9.1 $\pm$ 2.7

* $P < 0.01$, statistically significant as compared with the group receiving no ethionine.

Compared with the values in animals not receiving ethionine, there is (Fig. 3) a fivefold increase of glucose 6-phosphate dehydrogenase 24 hr. after the fourth administration of ethionine. This becomes tenfold 2 or 3 days later; thereafter the enzyme activity gradually drops back to that of the control.

The activity of 6-phosphogluconate dehydrogenase remains relatively constant during the 4 days of injection, but rises significantly 2 or 3 days later. This increase likewise declines to the normal value by the end of the experimental period (Fig. 3).

The inability of ATP to offer protection against the diversified effects of ethionine is demonstrated by the results of experiments shown in Table 3.

The ineffectiveness of inhibitors of protein synthesis, such as puromycin, 5-fluorouracil or actinomycin D, or the anti-metabolite DL-p-fluorophenylalanine, to prevent the ethionine-produced increase of glucose 6-phosphate dehydrogenase is shown in Table 4.

The results of the pair-fed ethionine experiment

(Table 5) show the stimulation of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, although this was less pronounced, as expected from the lower ethionine dose.

To test whether ethionine may induce the formation of one or other of two known glucose 6-phosphate dehydrogenase isoenzymes (Shaw, Koen & Barto, 1967), preparations from both control and experimental animals were tested according to their substrate specificity towards glucose 6-phosphate and galactose 6-phosphate. Table 6 indicates that, whether from control or ethionine-treated animals, liver glucose 6-phosphate dehydrogenase shows a marked preference for glucose 6-phosphate, the maximum rate for the oxidation of galactose 6-phosphate being about 10% of that for glucose 6-phosphate. In this respect, purified human erythrocyte glucose 6-phosphate dehydrogenase exhibited a corresponding figure of 14% (Kirkman, 1962).

DISCUSSION

Two circumstances have made it necessary to look for a role for ethionine different from that of an inhibitor of liver protein synthesis in explaining the stimulation of the pentose phosphate pathway enzymes: the unequivocal negative results obtained with ATP, puromycin and actinomycin D in an attempt to prevent the stimulation, and the unexpected post-ethionine effects, including a further elevation in glucose 6-phosphate dehydrogenase, an increase of 6-phosphogluconate dehydrogenase and the development of hypoglycaemia accompanied by a persistent deficit of glycogen synthetase.

The post-ethionine prolongation of hypoglycaemia indicated that ethionine might be producing hyperinsulinism.

One of the mechanisms for the regulation of hepatic 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase activity has been related to hormonal control. Glock & McLean

(1955) reported a decrease in the activities in these two dehydrogenases in diabetic rat liver. Fawaz, Fawaz & Dahl (1962-63) confirmed this result and showed that after the administration of insulin there was a tenfold increase in the activity of glucose 6-phosphate dehydrogenase and only a twofold increase of 6-phosphogluconate dehydrogenase. This greater stimulation of glucose 6-phosphate dehydrogenase as compared with that of 6-phosphogluconate dehydrogenase was also observed by Steiner & King (1964) and by Weber & Covery (1966). In addition, the latter authors noted that the same phenomenon in non-diabetic rats followed insulin injection, and this increase could be prevented by administration of actinomycin D. Similar elevation in glucose 6-phosphate dehydrogenase activity had been shown in rats fed with azo-dyes (Nodes & Reid, 1963) and in rats fed with ethionine for 2-4 weeks (McLean, 1966).

The best-known effect of ethionine on pancreas (Farber & Popper, 1950; Goldberg, Chaikoff & Dodge, 1950) is the one producing necrosis of acinar tissue. Less well-known is the neogenesis of the islets of Langerhans in the rat, observed by Duhart, Bur & Foglia (1966) in rats on a daily dose of 0.03 g. of ethionine/100 g. body wt. for 33 days. Others (Gorski, 1965) reported that the islets of Langerhans in mice killed 1-4 months after intrapancreatic administration of methylcholanthrene or 9,10-dimethyl-1,2-benz[*a*]anthracene were strongly resistant to the action of these carcinogens with new islets developing in the areas where sarcoma affected the connective tissue. Therefore a common mechanism of insulin production may operate in both cases.

There is a degree of organ specificity in the ethionine stimulation of glucose 6-phosphate dehydrogenase, since it occurs only in liver and not in brain, kidney, lung and haemolysate.

Ethionine administration induces other hepatic biochemical defects such as the diminution of glycogen synthetase in spite of the provision of excess of ATP. Here one may assume that ethionine is inhibiting the production of glycogen synthetase protein and, in this respect, our data are in agreement with those of Steiner & King (1964), who demonstrated that ethionine administration prevented a several-fold increase in hepatic glycogen synthetase activity of alloxan-diabetic rats injected with insulin.

The observed fluctuation in particulate and non-particulate activity of glycogen synthetase and phosphorylase contributed more information to the subject of glycogen biochemistry (Sie, Hablanian & Fishman, 1964; Tata, 1964). Thus phosphorylase appears before glycogen synthetase in the non-sedimentable fraction. Also, phosphorylase is restored before synthetase to the particulate

glycogen fraction during the post-ethionine period.

These observations fit our previous view about glycogenolysis, namely that phosphorylase is primarily associated with glycogen of smaller molecule weight, whereas glycogen synthetase is mainly associated with the larger molecules (Sie, Hablanian & Fishman, 1967). A similar finding was reported in rats by Barber, Orrell & Bueding (1967).

The pentose phosphate pathway has drawn much biochemical interest, especially in relation to oncology. References should be made to investigations in proliferating and malignant cells (Chayen *et al.* 1962), in lymphatic leukaemia and Ehrlich tumour (Kit, 1956), in Novikoff hepatoma (Weber & Cantero, 1957) and in human uterine cervix (Ayre & Goldberg, 1966). In transplanted hepatoma of varying degrees of deviation from normal livers, Weber & Morris (1963) noted enhancement most frequently of the activities of glucose 6-phosphate dehydrogenase followed by 6-phosphogluconate dehydrogenase. McLean & Brown (1966) showed increased glucose 6-phosphate dehydrogenase activity in ethionine-induced tumour. It is not possible yet to state the relevance of pancreatic insulin in these studies.

Finally, an important question, still unanswered, is whether or not the increase in glucose 6-phosphate dehydrogenase produced by ethionine is due to enzyme synthesis. Studies on glucocorticoid induction of various enzyme systems have been interpreted as enzyme protein synthesis, e.g. glucose 6-phosphatase (EC 3.1.3.9), fructose 1,6-diphosphatase (EC 3.1.3.11), tyrosine transaminase (EC 2.6.1.5) (Weber & Singhal, 1964), tryptophan pyrrolase (EC 1.13.1.12) (Feigelson & Greengard, 1962) and phosphoenolpyruvate carboxylase (EC 4.1.1.32) (Ray, Foster & Lardy, 1964), owing to the ability of actinomycin D to prevent enzyme enhancement. However, since actinomycin D cannot prevent the ethionine-stimulated increase of glucose 6-phosphate dehydrogenase, glucocorticoid induction would appear to be unrelated to the phenomenon and enzyme protein synthesis is unlikely.

Potter & Ono (1961) not only showed that puromycin could block the nutritionally induced increase of glucose 6-phosphate dehydrogenase but also indicated that cortisone was to be considered a controlling factor of the hepatic glucose 6-phosphate dehydrogenase activity in the re-fed adrenalectomized rat. These facts were interpreted to be a consequence of enzyme synthesis.

The possibility that one of the two isoenzymes of liver glucose 6-phosphate dehydrogenase may be preferentially increased by ethionine was ruled out in the present experiments.

Insulin has been shown to stimulate RNA synthesis and RNA polymerase activity in alloxan-

diabetic rat liver (Steiner & King, 1966) and formation of RNA and mammary protein *in vitro* (Mayne, Barry & Rivera, 1966). On the other hand, evidence for the action of insulin as a suppressor of biosynthesis of hepatic enzymes was also demonstrated, e.g. glucose 6-phosphatase and fructose 1,6-diphosphatase (Weber, Singhal & Srivastava, 1965). These authors indicated that the increased activities of the same two enzymes induced by glucocorticoid could be prevented by insulin administration, and it was postulated that the attacking point of the insulin is at the level of enzyme-forming system, affecting gene transcription into specific enzyme protein.

In the phenomena we are investigating therefore the primary effect of ethionine administration may be the stimulation of pancreatic production of insulin with the elevation of the oxidative pentose phosphate pathway and the maintenance of hypoglycaemia as secondary liver reflections. This line of thought is further substantiated by our latest observation (Sie & Fishman, 1967) that the extent of glucose 6-phosphate dehydrogenase elevation by ethionine in alloxan-diabetic rats was only 25% of that observed in normal rats given ethionine.

The mechanism of action of insulin in these experiments is yet to be established. Three possibilities seem relevant at this time. Insulin may stabilize the dehydrogenase molecule as tryptophan stabilizes tryptophan pyrrolase (Schimke, Sweeney & Berlin, 1965), it may preserve the enzyme template, as with serine dehydrase in the Morris hepatoma 5123 (Pitot, Peraino, Lamar & Kennan, 1965), or it may be responsible for the synthesis of the dehydrogenase stimulated in the ethionine-treated animals.

The inability of actinomycin D to prevent the stimulation of the pentose phosphate pathway by ethionine would on the surface militate against the third possibility. However, the interval of the actinomycin D effect (30 min. before ethionine) may not have been adequate for its inhibition to be evident. Moreover, the interaction of actinomycin D and ethionine in the protein-synthetic pathways would complicate the interpretation.

The recent finding of an increased formation of RNA and DNA in rat liver within 24 hr. of ethionine ingestion (Stekol, Bulba, Blicharska & Clash, 1967) warrants direct evidence that utilizes the incorporation of radioactive amino acids into glucose 6-phosphate dehydrogenase in livers of both the control and ethionine-treated animals, to settle the mechanism of stimulation of pentose phosphate pathway dehydrogenases.

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REFERENCES

- Ayre, H. A. & Goldberg, D. M. (1966). *Brit. J. Cancer*, **20**, 743.
- Barber, A. A., Orrell, S. A. & Bueding, E. (1967). *Fed. Proc.* **26**, 161.
- Chayen, J., Bitensky, L., Aves, E. K., Jones, G. R., Silcox, A. A. & Cunningham, G. J. (1962). *Nature, Lond.*, **203**, 714.
- Combes, B. & Schenker, S. (1966). *Nature, Lond.*, **209**, 911.
- Cori, G. T., Illingworth, B. & Keller, P. J. (1955). In *Methods in Enzymology*, vol. 1, p. 200. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Dische, Z. (1949). *J. biol. Chem.* **181**, 379.
- Duhart, J. E., Bur, G. E. & Foglia, V. C. (1966). *C.R. Soc. Biol., Paris*, **160**, 701.
- Farber, E. & Popper, H. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 838.
- Fawaz, E. N., Fawaz, G. & Dahl, K. V. (1962-63). *Enzymol. biol. clin.* **2**, 73.
- Feigelson, P. & Greengard, O. (1962). *J. biol. Chem.* **237**, 1190.
- Fitch, W. M., Hill, R. & Chaikoff, I. L. (1959). *J. biol. Chem.* **234**, 1048.
- Glock, G. E. & McLean, P. (1955). *Biochem. J.* **61**, 390.
- Goldberg, R. C., Chaikoff, I. L. & Dodge, A. H. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 869.
- Gorski, T. (1965). *Folia morph., Warsz.*, **16**, 87.
- Kirkman, H. N. (1962). *J. biol. Chem.* **237**, 2364.
- Kit, S. (1956). *Cancer Res.* **16**, 70.
- Leloir, L. F. & Goldemberg, S. H. (1960). *J. biol. Chem.* **235**, 919.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- McLean, P. (1966). *Biochem. J.* **99**, 776.
- McLean, P. & Brown, J. (1966). *Biochem. J.* **98**, 874.
- Mayne, R., Barry, J. M. & Rivera, E. M. (1966). *Biochem. J.* **99**, 688.
- Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
- Nodes, J. T. & Reid, E. (1963). *Brit. J. Cancer*, **17**, 745.
- Pitot, H. C., Peraino, C., Lamar, C., jun. & Kennan, A. L. (1965). *Proc. nat. Acad. Sci., Wash.*, **54**, 845.
- Potter, V. R. & Ono, T. (1961). *Cold Spr. Harb. Symp. quant. Biol.* **26**, 355.
- Ray, P. D., Foster, D. D. & Lardy, H. A. (1964). *J. biol. Chem.* **239**, 3396.
- Schimke, R. T., Sweeney, E. W. & Berlin, C. M. (1965). *J. biol. Chem.* **240**, 322.
- Shaw, C. R., Koen, A. L. & Barto, E. (1967). *Ann. N.Y. Acad. Sci.* (in the Press).
- Shull, K. H. (1962). *J. biol. Chem.* **237**, PC1734.
- Sie, H. G. & Fishman, W. H. (1967). *Abstr. 7th int. Congr. Biochem., Tokyo*, p. 847, G-42.
- Sie, H. G. & Hablani, A. (1965a). *Nature, Lond.*, **205**, 1317.
- Sie, H. G. & Hablani, A. (1965b). *Biochem. J.* **97**, 32.
- Sie, H. G., Hablani, A. & Fishman, W. H. (1964). *Nature, Lond.*, **201**, 393.
- Sie, H. G., Hablani, A. & Fishman, W. H. (1967). *Biochem. J.* **102**, 103.

- Somogyi, M. (1945). *J. biol. Chem.* **160**, 61.
- Steiner, D. F. & King, J. (1964). *J. biol. Chem.* **239**, 1292.
- Steiner, D. F. & King, J. (1966). *Biochim. biophys. Acta*, **119**, 510.
- Stekol, J. A., Bulba, S., Blicharska, J. & Clash, R. (1967). *Abstr. Pap. Amer. chem. Soc., 154th Meet., Chicago*, p. 214c.
- Stekol, J. A., Mody, U., Bedrak, E., Keller, S. & Perry, J. (1960). *Fed. Proc.* **19**, 37.
- Tata, J. R. (1964). *Biochem. J.* **90**, 284.
- Weber, G. & Cantero, A. (1957). *Cancer Res.* **17**, 995.
- Weber, G. & Covery, H. J. H. (1966). *Life Sci.*, **5**, 1139.
- Weber, G. & Morris, H. P. (1963). *Cancer. Res.* **23**, 987.
- Weber, G. & Singhal, R. L. (1964). *J. biol. Chem.* **239**, 521.
- Weber, G., Singhal, R. L. & Srivastava, S. K. (1965). *Proc. nat. Acad. Sci., Wash.*, **53**, 96.