

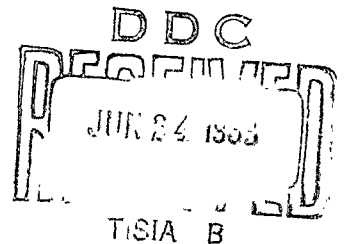
CATALOGED BY DDC
AS AD No. 406887

ARCTIC SURVIVAL RATIONS. X. DIURNAL VARIATIONS
OF SOME LIVER CONSTITUENTS IN RATS FED
PEMMICAN MEALS

D. A. Vaughan
R. L. Winders

TECHNICAL DOCUMENTARY REPORT AAL-TDR-62-54

March 1963



ARCTIC AEROMEDICAL LABORATORY

AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
FORT WAINWRIGHT, ALASKA

Project 8238-03

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related government procurement operation, the government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

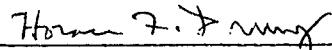
US Government agencies and other qualified ASTIA users may obtain copies of this report from the Armed Services Technical Information Agency, Documents Service Center, Arlington 12, Virginia.

This report has been released to the Office of Technical Services, U.S. Department of Commerce, Washington 25, D.C., for sale to the general public.

ABSTRACT

Diurnal variations in liver glycogen content and the activities of certain liver enzymes following 2-hour feeding periods were studied in trained-fed rats adapted to high-carbohydrate or carbohydrate-free diets. The results indicated that time after feeding a high-carbohydrate meal affected the activities of glucose-6-phosphatase and glutamic-pyruvic transaminase, but not glucose-6-phosphate dehydrogenase. These time-related changes were modified by meal-feeding the carbohydrate-free diet. Glycogen deposition following a carbohydrate-free meal indicated a rapid takeover of gluconeogenesis.

PUBLICATION REVIEW



HORACE F. DRURY
Director of Research

ARCTIC SURVIVAL RATIONS. X. DIURNAL VARIATIONS OF SOME LIVER CONSTITUENTS IN RATS FED PEMMICAN MEALS

SECTION 1. INTRODUCTION

In studies of pemmican as a survival ration our interest has been directed toward the maintenance of blood glucose levels (Drury et al, 1959) and glycogen storage in the liver (Vaughan et al, 1958). We, as well as others (Thorn and Scheitza, 1961), have shown that animals on carbohydrate-free diets can maintain appreciable levels of liver glycogen. It is a well known fact, however, that these entities fluctuate widely as a result of ingestion of food. Changes in enzyme activity related to time after feeding also have been reported (Nielson and Klitgaard, 1961). Consequently, in comparing some of the reactions which may be involved in the regulation of gluconeogenesis during various dietary regimens, it appeared to us that more valid information could be obtained by training animals to eat meals than by allowing them to nibble freely. In the experiments reported here we studied the activities of glutamic-pyruvic transaminase (GPT), glucose-6-phosphatase (G-6-Pase) and glucose-6-phosphate dehydrogenase (G-6-PDH) — as well as levels of liver glycogen — as related to time after ingestion of high-carbohydrate and carbohydrate-free (pemmican) meals in rats either adapted or not adapted to the pemmican diet.

SECTION 2. SUMMARY

The sequential effect on certain liver enzymes and on liver glycogen of high-carbohydrate or carbohydrate-free meals was studied in rats either adapted to high-carbohydrate or carbohydrate-free diets. The pattern of glycogen deposition and repletion on the various treatments was indicative of a rapid takeover of gluconeogenic reactions to maintain appreciable glycogen stores during a carbohydrate-free dietary regimen. This ability was lost following a single high-carbohydrate meal. The activity of GPT varied significantly with respect to time after feeding. This effect was somewhat modified by the carbohydrate-free, high-fat regimen. G-6-Pase activity showed some evidence of substrate induction 24 hours after high-carbohydrate meals, but this also disappeared during carbohydrate-free feeding. G-6-PDH exhibited no definite 24-hour pattern, but its activity was depressed considerably during the carbohydrate-free

regimen. At 24 hours after a meal of the high-carbohydrate diet, however, G-6-PDH activity was significantly increased over previous values obtained during carbohydrate-free feeding. These results were interpreted to mean that diurnal patterns in the activity of these enzymes may be appreciably influenced by dietary composition, especially if fat content is increased, and that this effect, as well as others discussed, may be related to the rate at which the respective substrates reach the liver.

SECTION 3. METHODS

Male, Sprague-Dawley rats were trained to eat their daily ration within a 2-hour period. The high-carbohydrate diet contained 18% casein, 68% sucrose, 10% vegetable oil, 4% U.S.P. salt mixture no. 2, and all required vitamins. The carbohydrate-free diet consisted of Quartermaster Meat Food Bar (hereafter called pemmican) supplemented with bone meal to give levels of 46% protein, 46% fat and 8% salts. Rats adapted to pemmican were maintained for at least six weeks on this diet; the high-carbohydrate rats were maintained for at least three weeks on their diet. When sacrificed, body weights were 250 to 300 grams.

Animals were killed 4, 8, 16 and 24 hours after the final meal. Methods used for the assay of liver G-6-Pase, G-6-PDH and glycogen have been previously described (Vaughan et al, 1958; Hannon and Vaughan, 1960). GPT was assayed by the method of Wróblewski and LaDue (1956). Enzyme activities are expressed as μ M of substrate converted per minute per 100 grams of body weight, since this mode of expression seems to us to be more relevant to dietary adaptation where changes in liver to body weight ratios may be quite marked (see Table I). Liver to body weight ratios were calculated from body weights obtained just before the respective meals were offered.

SECTION 4. RESULTS AND DISCUSSION

Results are summarized in Table I. They are arranged so as to present a reasonable sequence of events, reading from top to bottom. First, diurnal events in the liver during the feeding of high-carbohydrate meals were measured. Then, enzyme and glycogen responses to the first 24 hours of adjustment to a carbohydrate-free meal were observed. After

TABLE I. Response of Various Liver Constituents to Experimental Treatments

Adapted to	Meal	Time after meal (Hours)	Glycogen per cent of liver	Liver Weight/100 g body wt.	GPT $\mu\text{M}/100 \text{ g body wt.}$	G-6- Pase $\mu\text{M}/100 \text{ g body wt.}$	G-6-PDH $\mu\text{M}/100 \text{ g body wt.}$
High Carbo-hydrate	High Carbo-hydrate	4	4.65 $\pm$ 0.34*	3.88 $\pm$ 0.13	147 $\pm$ 13	46.0 $\pm$ 3.2	24.8 $\pm$ 4.1
		8	8.09 $\pm$ 0.38	4.54 $\pm$ 0.12	174 $\pm$ 7 (8)	45.3 $\pm$ 2.8	29.1 $\pm$ 2.8
		16	4.58 $\pm$ 0.65	3.92 $\pm$ 0.22	203 $\pm$ 10 (8)	41.3 $\pm$ 1.5	34.3 $\pm$ 3.3
		24	1.05 $\pm$ 0.15	3.45 $\pm$ 0.17	160 $\pm$ 17 (8)	47.6 $\pm$ 1.3	33.9 $\pm$ 6.5
	Carbo-hydrate free (pemmican)	4	0.43 $\pm$ 0.21	3.24 $\pm$ 0.10	171 $\pm$ 6	43.7 $\pm$ 2.9	23.5 $\pm$ 3.0
		8	0.90 $\pm$ 0.15	3.63 $\pm$ 0.08	179 $\pm$ 11 (8)	46.6 $\pm$ 2.9	28.7 $\pm$ 4.5
		16	2.39 $\pm$ 0.30	3.70 $\pm$ 0.03	220 $\pm$ 11 (8)	44.7 $\pm$ 1.4	33.3 $\pm$ 5.6
		24	1.75 $\pm$ 0.19	3.61 $\pm$ 0.11	191 $\pm$ 11 (8)	48.3 $\pm$ 3.3	26.8 $\pm$ 3.9
Carbo-hydrate free (pemmican)	Carbo-hydrate free (pemmican)	4	1.96 $\pm$ 0.32	4.03 $\pm$ 0.13	179 $\pm$ 10	40.1 $\pm$ 2.4	4.8 $\pm$ 1.5
		8	2.79 $\pm$ 0.22	4.32 $\pm$ 0.03	188 $\pm$ 14 (8)	43.6 $\pm$ 0.9	4.4 $\pm$ 0.5
		16	2.91 $\pm$ 0.09	4.05 $\pm$ 0.05	207 $\pm$ 6 (7)	45.8 $\pm$ 1.3	3.0 $\pm$ 0.3
		24	2.15 $\pm$ 0.26	3.59 $\pm$ 0.08	176 $\pm$ 6 (8)	40.1 $\pm$ 2.2	3.7 $\pm$ 0.4
	High Carbo-hydrate	4	4.13 $\pm$ 0.36	4.13 $\pm$ 0.24	154 $\pm$ 24	43.3 $\pm$ 5.7	4.9 $\pm$ 0.5
		8	7.09 $\pm$ 0.40	4.29 $\pm$ 0.11	156 $\pm$ 9 (8)	40.6 $\pm$ 2.6	4.7 $\pm$ 0.4
		16	5.60 $\pm$ 0.32	4.02 $\pm$ 0.14	214 $\pm$ 3 (6)	41.0 $\pm$ 1.7	3.4 $\pm$ 0.6
		24	1.09 $\pm$ 0.23	3.31 $\pm$ 0.11	183 $\pm$ 11 (8)	46.9 $\pm$ 2.9	9.6 $\pm$ 1.1

* Standard error of the mean.

** Animals per group for this assay. Means not marked represent 5 animals.

adaptation for six weeks to the carbohydrate-free meal, the diurnal responses to a pemmican meal were measured. Finally, the first stages of de-adaptation from pemmican were studied by feeding a high-carbohydrate meal.

After feeding a high-carbohydrate meal to carbohydrate-adapted rats, there was an initial rapid deposition of liver glycogen and later removal until quite low levels were reached. Upon receiving a carbohydrate-free meal, these levels fell even further for a while, but by 16 hours synthesis of glycogen was evidently proceeding, probably from protein. At 24 hours, 1.75 per cent remained. After six weeks on the carbohydrate-free diet, liver glycogen, following a carbohydrate-free meal, did not peak appreciably at 8 hours, while at 24 hours, over 2 per cent still remained in the liver. Finally, upon receiving one meal of the high-carbohydrate diet, rats which were adapted to pemmican exhibited the same pattern of glycogen deposition as the rats not adapted.

It is evident, thus, that rats receiving this type of carbohydrate-free diet maintain appreciable levels of liver glycogen during the 24 hours following a meal. They appear to be less dependent on glycogen for glycolytic reactions and/or blood sugar formation, for their glycogen levels do not fall as precipitately as with rats on a high-carbohydrate diet. This ability to maintain glycogen levels is evidently lost immediately upon receiving only one high-carbohydrate meal, for glycogen content again fell to the low 24-hour level observed before. These observations are in line with results reported by Samuels et al (1942) and Pagé and Babineau (1954) who have shown that animals fed high-fat rations are able to maintain higher liver glycogen levels when fasted.

It might be expected, then, that during pemmican feeding gluconeogenesis would be increased and that this increase would be reflected in elevated levels of G-6-Pase and GPT (Freedland and Harper, 1958; Rosen et al, 1959). As the results show, however, the activity of GPT was not elevated significantly as a result of the ingestion of pemmican if the activities at identical times after feeding are compared. Another point of interest is the rather wide variation in activity at different times after feeding. Especially in animals fed a high-carbohydrate meal, the highest values (16 hours) were 39% higher than the lowest (4 hours). This pattern was modified somewhat when pemmican was fed, in particular following adaptation to this diet when the peak elevation was only 16% higher than the lowest value observed.

The failure of GPT activity to increase as a result of the increased protein intake from pemmican was somewhat unexpected, since Rosen et al (1959) and Muramatsu and Ashida (1962) have shown that GPT activity

increases with increased protein intake. We have also observed (to be published) that the cold-induced increase in GPT activity is apparently a simple response to increased protein intake. It is possible that, in the present experiments, the simultaneously increased fat content nullified the effect of increased protein as well as modified the pattern of GPT activity following a meal.

The only noticeable response in G-6-Pase activity occurred at 24 hours versus 16 hours when the animals had been fed a high-carbohydrate meal. It is our opinion that this slight rise could be a temporary response to substrate (glucose-6-phosphate) arising from glycogen mobilization. That it is not a stress response to blood glucose depletion would seem to be indicated by its failure to increase further after the first meal of pemmican, when no sugar was ingested and glycogen fell to even lower levels. In this connection it should be noted that no difficulty was encountered in persuading rats to eat the unfamiliar pemmican, so that as far as the animal as a whole was concerned, the fasting period did not extend beyond 24 hours.

Glucose-6-phosphate dehydrogenase activity fell to virtually minimum levels after six weeks of the pemmican diet. We have observed previously that no depression in its activity occurred when a carbohydrate-free diet of 90% protein was fed (Vaughan et al, 1960). In the present experiment, however, although the fat content was only 46%, activity fell to levels comparable to those previously observed on 70% fat diets containing much less protein (Vaughan et al, 1960). The retarding influence of dietary fat on absorption may be involved here, in that fat may prevent flooding of certain systems with consequent substrate induction. The reduced activity of this enzyme (Tepperman and Tepperman, 1958a), as well as "malic enzyme" (Fitch and Chaikoff, 1961), reported during stock diet feeding may possibly be related to the slower rate of release of starch-derived glucose.

When fed a high-carbohydrate meal, these pemmican-adapted rats exhibited a significant rise in G-6-PDH by the end of the 24-hour period. These results are similar to those observed by Tepperman and Tepperman (1958b) with refeeding experiments. The activity of this enzyme during high-carbohydrate meal feeding also fell in the ranges reported by these authors and by Hollifield and Parson (1962) as a result of meal feeding, and is likewise appreciably higher than reported by us (Vaughan et al, 1960) during ad libitum feeding of high-carbohydrate diets. It is furthermore evident that, once a level characteristic of a particular dietary treatment is attained, little, if any, effect of time after feeding is noticeable on the activity of G-6-PDH.

We did not study lipogenesis during these experimental treatments. It seems likely, however, that it would have been depressed, considering the fact that others (Tepperman and Tepperman, 1958b; 1961) have shown a correlation, if not a causal effect, between shunt activity and lipogenesis. Still others (Hausberger and Milstein, 1955) have demonstrated lipogenic depression when high fat diets are fed. In view of the modifications arising from increased fat intake shown here, it would seem pertinent to study the effect on lipogenesis and fat deposition of meal feeding diets higher in fat than are commonly fed rats, especially with regard to hypotheses proposed by some (Hollifield and Parson, 1962; Cohn and Joseph, 1960) concerning the effect of eating patterns on human obesity.

REFERENCES

1. Cohn, C. and D. Joseph. Role of ingestion of diet on regulation of intermediary metabolism. *Metabolism* 9:492, 1960.
2. Drury, H. F., D. A. Vaughan and J. P. Hannon. Some metabolic effects of a high-fat, high-protein diet during semistarvation under winter field conditions. *J. Nutrition* 67:85, 1959.
3. Fitch, W. M. and I. L. Chaikoff. Effect of previous diet on response of hepatic enzyme activities to a 24-hour fast. *Arch. Biochem. Biophys.* 94:380, 1961.
4. Freedland, R. A. and A. E. Harper. Metabolic adaptations in higher animals. II. Changes with time in the adaptive response of glucose-6-phosphatase. *J. Biol. Chem.* 230:833, 1958.
5. Hannon, J. P. and D. A. Vaughan. Effect of prolonged cold exposure on the glycolytic enzymes of liver and muscle. *Am. J. Physiol.* 198:375, 1960.
6. Hausberger, F. X. and S. W. Milstein. Dietary effects on lipogenesis in adipose tissue. *J. Biol. Chem.* 214:483, 1955.
7. Hollifield, G. and W. Parson. Metabolic adaptations to a "stuff and starve" feeding program. II. Obesity and the persistence of adaptive changes in adipose tissue and liver occurring in rats limited to a short daily feeding period. *J. Clin. Invest.* 41:250, 1962.
8. Muramatsu, K. and K. Ashida. Effect of dietary protein level on growth and liver enzyme activities of rats. *J. Nutrition* 76:143, 1962.
9. Nielson, R. R. and H. M. Klitgaard. Changes in succinic dehydrogenase activity related to feeding. *Am. J. Physiol.* 201:37, 1961.
10. Page, E. and L. M. Babineau. Tissue glycogen and glucose absorption in rats adapted to cold. *Can. J. Biochem. Physiol.* 32:395, 1954.

11. Rosen, F., N.R. Roberts and C.A. Nichol. Glucocorticosteroids and transaminase activity. I. Increased activity of glutamic-pyruvic transaminase in four conditions associated with gluconeogenesis. *J. Biol. Chem.* 234:476, 1959.
12. Samuels, L.T., R.M. Reinecke and H.A. Ball. Liver fats and glycogen of hypophysectomized rats on high carbohydrate and high fat diets. *Proc. Soc. Exp. Biol. Med.* 49:456, 1942.
13. Tepperman, J. and H.M. Tepperman. Effects of antecedent food intake pattern on hepatic lipogenesis. *Am. J. Physiol.* 193:55, 1958a.
14. Tepperman, H.M. and J. Tepperman. The hexosemonophosphate shunt and adaptive lipogenesis. *Diabetes* 7:478, 1958b.
15. Tepperman, J. and H.M. Tepperman. Metabolism of glucose-1- C^{14} and glucose-6- C^{14} by liver slices of refed rats. *Am. J. Physiol.* 200:1069, 1961.
16. Thorn, W. and H. Scheitza. Glucosebildung und Glykogeneinlagerung in Gehirn, Leber, Niere, Herz und Skelettmuskel bei kohlenhydratfreier Ernährung. *Pflugers Arch. ges Physiol.* 273:18, 1961.
17. Vaughan, D.A., J.P. Hannon and L.N. Vaughan. Associated effects of diet, environmental temperature and duration of exposure on the major constituents of the livers of rats. *Am. J. Physiol.* 194:441, 1958.
18. Vaughan, D.A., J.P. Hannon and L.N. Vaughan. Effects of diet on selected glycolytic enzymes of the rat. *Am. J. Physiol.* 199:1041, 1960.
19. Wróblewski, F. and J.S. LaDue. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. *Proc. Soc. Exp. Biol. Med.* 91:569, 1956.

Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash. Rpt. AAL-TDR-62-54. ARCTIC SURVIVAL RATIONS. X. DIURNAL VARIATIONS OF SOME LIVER CONSTITUENTS IN RATS FED PEMMICAN MEALS. March 1963. 8 p. incl. table, 19 refs. Unclassified Report Diurnal variations in liver glycogen content and the activities of certain liver enzymes following 2-hour feeding periods were studied in trained-fed rats adapted to high-carbohydrate or carbohydrate-free diets. The results indicated that time after feeding a high-carbohydrate meal affected the activities of glucose-6-phosphatase and glutamic-pyruvic transaminase, but not glucose-6-phosphate dehydrogenase. These time-related changes were modified by meal-feeding the carbohydrate-free diet. Glycogen deposition following a carbohydrate-free meal indicated a rapid takeover of gluconeogenesis.	1. Survival 2. Military Rations -- 3. Liver 4. Enzymes 5. Carbohydrates 6. Rats I. Project 8238-03 II. Vaughan, D. A. and R. L. Winders III. Available from OTS IV. In ASTIA collection
--	--

Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash. Rpt. AAL-TDR-62-54. ARCTIC SURVIVAL RATIONS. X. DIURNAL VARIATIONS OF SOME LIVER CONSTITUENTS IN RATS FED PEMMICAN MEALS. March 1963. 8 p. incl. table, 19 refs. Unclassified Report Diurnal variations in liver glycogen content and the activities of certain liver enzymes following 2-hour feeding periods were studied in trained-fed rats adapted to high-carbohydrate or carbohydrate-free diets. The results indicated that time after feeding a high-carbohydrate meal affected the activities of glucose-6-phosphatase and glutamic-pyruvic transaminase, but not glucose-6-phosphate dehydrogenase. These time-related changes were modified by meal-feeding the carbohydrate-free diet. Glycogen deposition following a carbohydrate-free meal indicated a rapid takeover of gluconeogenesis.	1. Survival 2. Military Rations -- 3. Liver 4. Enzymes 5. Carbohydrates 6. Rats I. Project 8238-03 II. Vaughan, D. A. and R. L. Winders III. Available from OTS IV. In ASTIA collection
--	--