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Lipogenic activity of some hepatic enzymes in rat : effects of dietary protein level, insulin and cortisol

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Summary. The influence of high protein diets and endocrine factors (insulin or cortisol) on the activity of hepatic glucose-6-phosphate dehydrogenase (G6P-DH), malic enzyme (ME), acetyl CoA carboxylase (Ac CoAcx) and fatty acid synthetase (FAS) was studied in adult rats. ME, AC CoACx and FAS activities decreased as the dietary protein level augmented, reaching a minimum 8 days after the beginning of high protein intake. This low lipogenic enzyme activity level remained steady up to the end of the experiment (40 days). In rats fed 10 p. 100 protein, insulin administration enhanced all enzyme activities involved in lipogenesis, but cortisol had no effect. In rats adapted to a high protein diet (24 days), neither insulin nor cortisol modified the low ME, Ac CoACx and FAS activities.

Introduction.

Since Mackay *et al.* (1941) showed that rats fed high protein diets have a low body fat deposition, several investigations have reported that increasing the level of dietary proteins depresses fatty acid synthesis in the liver (Masoro *et al.*, 1950 ; Cohen and Teitelbaum, 1966) and in the adipose tissue (Cohen and Teitelbaum, 1966 ; Leveille, 1967). Despite these observations, the effects of dietary protein on lipogenic enzyme activity have not been extensively studied (Romsos and Leveille, 1974). Although the lipogenesis-stimulating effect of insulin has been demonstrated (Goodridge, 1975), the influence of glucocorticoids on insulin-dependent lipogenic enzymes has been only recently investigated (Diamant and Shafrir, 1975) in spite of the fact that glucocorticoid administration is accompanied by hyperlipidemia (Reaven *et al.*, 1974).

The experiments reported here were designed to study the hepatic activities of glucose-6-phosphate dehydrogenase, malic enzyme, acetyl CoA carboxylase and fatty acid synthetase in rats following changes in the dietary protein level, with or without insulin and cortisol administration.

Material and methods.

Animals and experimental procedures. — Three different experiments were carried out using 140 8-week old Wistar CF rats weighing between 180 and 200 g. On arrival, they were housed in the laboratory under controlled temperature (22 ± 2 °C) and

lighting (light from 07 : 00 to 19 : 00 hrs) conditions and were fed a diet containing 10 p. 100 casein for 10 days to accustom them to these conditions.

Diets. — The composition of the experimental diets is shown in table 1. The variations in protein content (casein, 83 p. 100 protein) depended mainly on carbohydrate content (starch and sugar), but the diets had practically equal energy content.

TABLE 1

Composition of diet (1)
(g/100 g)

	Experiment 1			Experiments 2 et 3	
Casein	19.9	64.3	90.7	12.7	61.1
Peanut oil	8.0	8.0	2.3	8.0	8.0
Sucrose	10.0	10.0	—	10.0	10.0
Starch	55.1	10.7	—	63.3	14.9
Protein (N × 6.25)	16,5	53.4	75.3	10.5	50.7

(1) All diets contained (g/100 g) : mineral salts (*Hubbel et al.*, 1937), 4.0 ; Vitamin mixture (*Peret et al.*, 1973), 1.0 ; powdered cellulose, 1.0.

Experiment 1. Eighty-four animals were divided into 3 groups of 28 animals each and were fed diets containing either 15, 50 or 75 p. 100 casein protein. Seven animals in each group were killed by decapitation between 09 : 00 and 10 : 00 hrs after 1, 4, 8 and 40 days, and the livers removed, weighed and then stored at — 80 °C.

Experiment 2. Twenty animals were divided into 2 groups of 10 each and fed diets containing either 10 or 50 p. 100 casein protein for 24 days. At the end of the experimental period, they were killed by decapitation between 09 : 00 and 10 : 00 hrs.

Experiment 3. Forty-two rats were divided into 2 groups and fed diets containing either 10 or 50 p. 100 casein protein for 24 days. Each of the 2 groups was then subdivided into 3 lots, the first acting as a control ; in the last 5 days of the experiment, the 2 others received either cortisol intraperitoneally (5 mg/day/animal) or protamine-zinc-insulin intramuscularly (4 I.U./day/animal). At the end of the experimental period, they were killed by decapitation.

Methods. — The activities of glucose-6-phosphate dehydrogenase (G6P-DH) (EC 1.1.1.49) and malic enzyme (ME) (EC 1.1.1.40) were determined by the method of Fitch *et al.* (1959) and Hsu and Lardy (1969), respectively. NADPH release was measured at 37 °C and 340 nm using a Gilford 300 T spectrophotometer. The activities of acetyl CoA carboxylase (Ac CoACx) (EC 6.4.1.2) and fatty acid synthetase (FAS) * were measured using the methods of Inoue and Lowenstein (1975) and Maeda *et al.* (1975), respectively. The ^{14}C incorporated was measured using a Packard Tricarb

* An EC number is not given for FAS because it is a complex of enzymes.

liquid scintillation counter. All activities were measured in the supernatant at 50,000 × g and the protein content was determined by the Kjeldahl method (N × 6.25). They were expressed as nanomoles of substrate per minute and per mg protein at 37 °C. The statistical analysis of results was carried out as described by Snedecor and Cochran (1967).

Results.

Experiments 1 and 2. — Lipogenic enzyme activity after dietary shift from adequate to high dietary protein.

Figure 1 represents the evolution of the hepatic G6P-DH, ME and AcCoACx activities of rats fed for 1, 4, 8 and 40 days with either 15, 50 or 75 p. 100 protein. Opposite changes in the activity levels of NADPH-liberating enzymes were observed. The G6P-DH activity increased with the dietary protein content, and after 8 days of feeding high protein diet tended to stabilize until the end of the experiment. However, the increase was preceded by a transitory decrease the first day after the dietary shift. Malic enzyme activity, on the other hand, decreased progressively with dietary protein content, and after 40 days of high protein diet its activity represented 30 p. 100 of that of the controls. Furthermore, it seems that beyond a certain threshold of protein intake, this decrease in

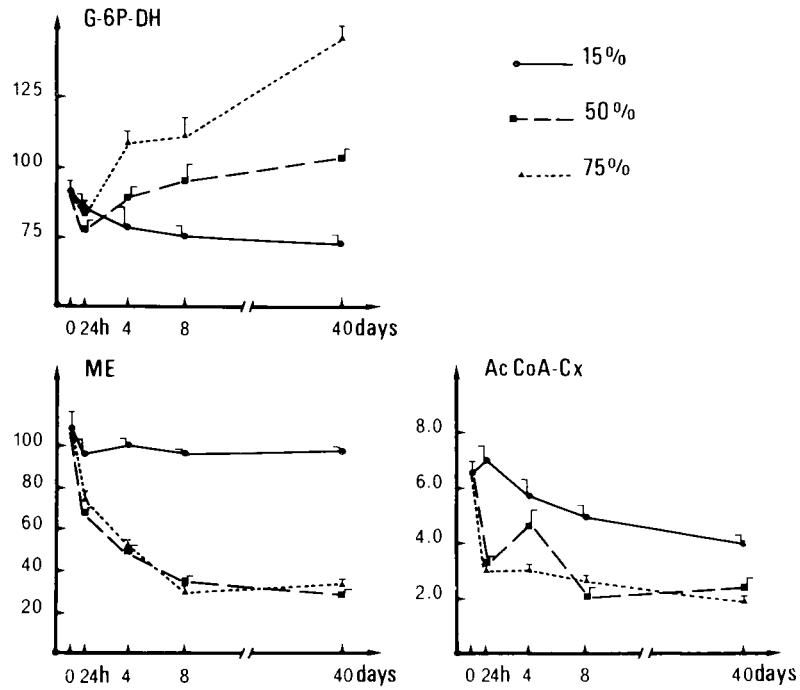

FIG. 1. — *Hepatic glucose 6 phosphate dehydrogenase, malic enzyme and acetyl CoA carboxylase activities in rats fed 40 days with diets of different protein levels.* Enzyme activites are expressed in nanomoles of substrate converted at 37 °C per minute and per mg of protein. Each point represents a mean (7 animals) ± SEM.

enzyme activity is maximal since there were no differences found between rats fed 50 p. 100 protein and those receiving 75 p. 100.

Twenty-four hours after the beginning of the experiment, acetyl-CoA-carboxylase activity fell sharply ($P < 0.05$) in animals fed high protein diets. From days 8 to 40, its activity was low and represented 50 p. 100 of that of animals fed an adequate dietary protein level. There were no differences between subjects fed 50 or 75 p. 100 protein.

In rats receiving a 50 p. 100 protein regimen for 24 days (fig. 2), the variations in G6P-DH, ME and AcCoACx activities were similar to those observed in the first experiment after 8 or 40 days of feeding the same diet. Furthermore, FAS activity fell, and it represented only 40 to 50 p. 100 of that of the rats fed a diet containing 10 p. 100 protein.

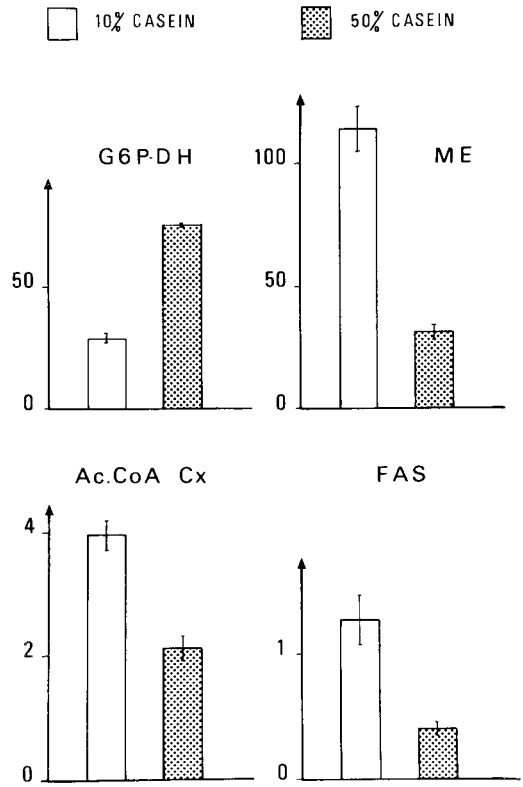

FIG. 2. — *Hepatic glucose 6 phosphate dehydrogenase, malic enzyme, acetyl CoA carboxylase and fatty acid synthetase activities in rats fed 24 days with 10 and 50 p. 100 protein.* Enzyme activities are expressed in nanomoles of substrate converted at 37 °C per minute and per mg of protein. Height of the bar depicts the mean of 10 animals. The vertical line represents SEM.

Experiment 3. — Hormonal factors.

As is shown in table 1, insulin enhanced the activities of all the enzymes involved directly (AcCoACx and FAS) or indirectly (G6P-DH and ME) in the lipogenesis of rats fed a 10 p. 100 casein protein diet for 24 days, whereas cortisol did not show any marked effects on these activities. Moreover, the effects of the 50 p. 100 casein protein diet

(reduction of ME, AcCoACx and FAS activities) were not influenced by any hormone treatment.

TABLE 2

Effect of high protein diet, cortisol and insulin on the activity (1) of lipogenic enzymes in the liver

Enzyme activity treatment	G6PDH	ME	Ac CoA carbox.	FAS
10 p. 100 (7) (2) protein	(3) $31.6^{a} \pm 2.15$	$120.3^{ab} \pm 11.83$	$3.79^{b} \pm 0.26$	$1.39^{b} \pm 0.27$
10 p. 100 (7) protein insulin	$56.2^{bc} \pm 4.66$	$139.4^{a} \pm 12.00$	$5.22^{a} \pm 0.53$	$2.07^{a} \pm 0.12$
10 p. 100 (7) protein cortisol	$46.1^{c} \pm 2.93$	$106.7^{b} \pm 5.92$	$3.43^{b} \pm 0.36$	$1.94^{ab} \pm 0.17$
50 p. 100 (7) protein	$75.9^{a} \pm 3.15$	$30.5^{c} \pm 4.25$	$2.18^{c} \pm 0.25$	$0.42^{c} \pm 0.07$
50 p. 100 (7) protein insulin	$82.5^{a}: \pm 7.58$	$28.1^{c} \pm 2.50$	$1.37^{d} \pm 0.19$	$0.42^{c} \pm 0.07$
50 p. 100 (7) protein cortisol	$69.7^{ab} \pm 8.08$	$23.8^{c} \pm 2.56$	$1.48^{d} \pm 0,05$	$0.27^{c} \pm 0.08$

(1) Enzymes activities are expressed in nanomoles of substrate converted at 37 °C per minute and per mg of protein.
(2) Number of animals.
(3) Mean ± SEM. Means not followed by the same letter are significantly different ($P < 0.05$; Hartley, Tukey and Keuls test) ; all comparisons are vertical ($a < b < c <$ etc...).

Discussion.

Our results on the variations of NADPH-liberating enzymes are similar to those in the literature. G6P-DH activity increases with the protein content (Schimke, 1962 ; Vaughan and Winders, 1964 ; Szepesi and Freedland, 1967 ; Peret *et al.*, 1975), whereas that of ME decreases (Vaughan and Winders, 1964 ; Cohen and Teitelbaum, 1966 ; Szepesi and Freedland, 1967, 1968 ; Yeh and Leveille, 1969 ; Peret *et al.*, 1975). The increase in G6P-DH activity with the dietary protein level seems to be related to the use of casein in the diet. The origin of this phenomenon is unknown. It does not occur anymore when the dietary casein is replaced by its amino acid pattern (Taketa *et al.*, 1972-1973). Moreover, when rats are fed with a high egg yolk protein diet, the enzyme activity decreases (Peret *et al.*, 1975). The first day, the decrease in the G6P-DH activity is probably due to the sharp but transitory reduction of food intake observed when rats are fed high protein diets (Harper, 1965 ; Anderson *et al.*, 1968). The decrease in the ME activity with increasing protein intake might either be explained by the reduction of glucose intake, a specific inducer of ME (Romsos and Leveille, 1974), or by a glucagon effect since glucagon blocks malic enzyme activity in hepatocytes in culture (Goodridge, 1975). This latter aspect will be discussed further.

Likewise, the fall of AcCOACx and FAS activities seems to be related to the large reduction in glucose intake. When animals have a high glucose intake (10 or 15 p. 100

protein diet), pyruvate obtained by glycolysis may be metabolized in the mitochondria, under pyruvate dehydrogenase action, into acetyl CoA which is either oxidized (tricarboxylic cycle) or used for fatty acid synthesis. In the latter case, acetyl CoA is used as a substrate in two successive reactions : in the presence of ATP the first is catalyzed by acetyl CoA carboxylase, and in the presence of malonyl CoA and NADPH the second is catalyzed by the fatty acid synthetase complex. When the carbohydrate intake is low, as with a high protein diet, pyruvate is mainly produced by transamination. Since the animal may be in a relative state of energy (glucose) shortage, pyruvate may be primarily used for glucose synthesis when converted into oxaloacetate and phosphoenolpyruvate under the action of pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEP-CK). When gluconeogenesis is predominant, the ATP necessary for glucose synthesis is obtained by oxidation of fatty acids (Krebs, 1966). In addition, fatty acids also supply the NADH and acetyl-CoA which are activators of PC and PEP-CK (Scrutton and Utter, 1968) ; NADH is also an inhibitor of pyruvate dehydrogenase (Garland and Randle, 1964 ; Portenhauser and Wieland, 1972). Furthermore, in these conditions there is an activation of oxaloacetate transfer as malate or aspartate (Williamson *et al.*, 1969) and a relative inhibition of acetyl-CoA transfer as citrate (Halperin *et al.*, 1972, 1975) from the mitochondria to the cytosol. Thus, the decreased activity of acetyl CoA carboxylase, the enzyme controlling the conversion of acetyl CoA into fatty acids, may be explained by the unavailability of both the acetyl CoA (substrate) and the ATP (energy) without excluding other factors related to the allosteric nature of acetyl CoA carboxylase (Gregolin *et al.*, 1966, 1968*a*, 1968*b*). Glucagon, on the other hand, seems to play an important role in the regulation of lipogenic enzyme activities ; the hormone inhibits fatty acid synthesis in isolated tissues (Goodridge, 1973*a*, 1973*b* ; Volpe and Marasa, 1975) and the blockade of AcCoACx activity stimulation accompanying refeeding the glucose to starved rats (Klain, 1977 ; Lakshmanan *et al.*, 1972). Since rats fed high protein diets had a high level of plasma glucagon (Eisenstein *et al.*, 1974), it can be assumed that glucagon may be involved in the decrease of ME, AcCoACx and FAS activities observed in our experiments. All these explanations support the large decrease in fatty acid synthesis found in chicks or rats fed high protein diets (Nishida *et al.*, 1960 ; Cohen and Teitelbaum, 1966 ; Leveille, 1967 ; Yeh and Leveille, 1969).

In rats fed 10 p. 100 protein, we found the well-known insulin-stimulating effect on all the enzymes involved in lipogenesis (Romsos and Leveille, 1974 ; Goodridge, 1975). With the exception of G6P-DH, high casein protein lowered these activities and insulin seemed unable to induce lipogenic enzymes when there was a dietary shortage of carbohydrates. On the other hand, no increase was observed in ME, AcCoACx and FAS activities after 5 days of cortisol administration in rats fed a low level of protein. These results agree with those of Volpe and Marasa (1975), but not with the data of Diamant and Shafrir (1975), who used triamcinolone and dexamethasone instead of cortisol. The differences in drug potency might explain the discrepancies.

In conclusion, our results show that :

— ME, AcCoACx and FAS activities decreased when the dietary protein level increased, reaching a minimum 4 to 8 days after the beginning of high protein diet intake. Moreover, this low level of lipogenic enzyme activity remained steady until the end of the experiment (40 days) ;

— in rats fed 10 p. 100 protein, insulin administration enhanced all enzyme activities involved in lipogenesis, but cortisol had no effect;

— in rats adapted to a high protein diet, neither insulin nor cortisol modified the low ME, AcCoACx and FAS activities.

Résumé. On a étudié sur des rats mâles âgés de 2 mois l'influence des régimes riches en protéines et des facteurs hormonaux (insuline et cortisol) sur les activités enzymatiques hépatiques suivantes : glucose-6-phosphate déshydrogénase (G6P-DH), enzyme malique (EM), acétyl CoA carboxylase (AcCoACx) et la synthétase des acides gras (FAS). Les activités de l'EM, l'AcCoACx et de la FAS diminuent avec l'augmentation du taux protéique du régime, en atteignant le minimum 8 jours après le début de l'ingestion des régimes hyperprotéiques. Par la suite, leurs activités basses se maintiennent constantes jusqu'à la fin de l'expérience (40 jours). Chez les animaux nourris avec un régime à 10 p. 100 de protéines, l'administration de l'insuline augmente l'activité de toutes les enzymes impliquées dans la lipogenèse ; par contre, l'administration du cortisol n'a aucun effet. Chez les rats adaptés à un régime à 50 p. 100 de protéines (24 jours) ni l'insuline, ni le cortisol n'ont modifié les activités faibles de l'EM, l'AcCoACx et de la FAS.

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References

ANDERSON H. L., BENEVENGA N. J., HARPER A. E., 1968. Associations among food and protein intake, serine dehydratase, and plasma aminoacids. *Amer. J. Physiol.*, **214,** 1008-1013.

COHEN A. M., TEITELBAUM A., 1966. Effects of different levels of protein in sucrose and starch diets on lipid synthesis in the rat. *Isr. J. Med. Sci.*, **2,** 727-732.

DIAMANT S., SHAFRIR E., 1975. Modulation of the activity of insulin dependent enzymes of lipogenesis by glucocorticoids. *Eur. J. Biochem.*, **53,** 541-546.

EISENSTEIN A. B., STRACK I., STEINER A., 1974. Glucagon stimulation of hepatic gluconeogenesis in rats fed a high protein, carbohydrate-free diet. *Metabolism*, **23,** 15-23.

FITCH W. M., HILL R., CHAIKOFF I. L., 1959. The effect of fructose feeding on glycolytic enzyme activities of the normal rat liver. *J. biol. Chem.*, **234,** 1048-1051.

GARLAND P. B., RANDLE P. J., 1964. Control of pyruvate dehydrogenase in the perfused rat heart by the intracellular concentration of acetyl coenzyme A. *Biochem. J.*, **91,** 6C-7C.

GOODRIDGE A. G., 1973*a*. Regulation of fatty acid synthesis in isolated hepatocytes prepared from the livers of neonatal ducks. *J. biol. Chem.*, **248,** 1924-1931.

GOODRIDGE A. G., 1973*b*. Regulation of fatty acid synthesis in isolated hepatocytes. Evidence for a physiological role for long chain fatty acyl coenzyme A and citrate. *J. biol. Chem.*, **248,** 4318-4326.

GOODRIDGE A. G., 1975. Hormonal regulation of the activity of the fatty acid synthesizing system and of the malic enzyme concentration in liver cells. *Fed. Proc.*, **34,** 117-123.

GREGOLIN C., RYDER E., KLEINSCHMIDT A. K., WARNER R. C., LANE M. D., 1966. Molecular characteristics of liver acetyl CoA carboxylase. *Proc. nat. Acad. Sci. US.*, **56,** 148-156.

GREGOLIN C., RYDER E., LANE M. D., 1968*a*. Liver acetyl coenzyme A carboxylase. I. Isolation and catalytic properties. *J. biol. Chem.*, **243,** 4227-4235.

GREGOLIN C., RYDER E., WARNER R. C., KLEINSCHMIDT A. K., CHANG A. C., LANE M. D., 1968*b*. Liver acetyl coenzyme A carboxylase. II. Further molecular characterization. *J. biol. Chem.* **243,** 4236-4245.

HALPERIN M. L., ROBINSON B. H., FRITZ I. B., 1972. Effects of palmitoyl-CoA on citrate and malat transport by rat liver mitochondria. *Proc. nat. Acad. Sci. US.*, **69,** 1003-1007.

HALPERIN M. L., CHEEMA-DHADLI S., TAYLOR W. M., FRITZ I. B., 1975. Role of the citrate transporter in the control of fatty acid synthesis. *Adv. Enzyme Regul.*, **13,** 435-445.

HARPER A. E., 1965. Effect of variation in protein intake on enzymes of amino acids metabolism. *Canad. J. Biochem.*, **43,** 1589-1608.

HUBBEL R. B., MENDEL L. B., WAKEMAN A. J., 1937. A new salt in mixture for use in experimental diets. *J. Nutr.*, **14,** 273-275.

HSU R. Y., LARDY H. A., 1969. Malic enzyme, 230-235. In LOWENSTEIN J. M., *Methods in enzymology*, vol. **17**. Acad. Press. New York, London.

INOUE H., LOWENSTEIN J. M., 1975. Acetyl coenzyme A carboxylase from rat liver, 3-11. In LOWENSTEIN J. M., *Methods in enzymology*, vol. **35**, part B. Acad. Press, N. Y., San Francisco, London.

KLAIN G. J., 1977. *In vivo* effects of glucagon on fatty acid synthesis in fasted and refed rats. *J. Nutr.*, **107**, 942-948.

KREBS H. A., 1966. The regulation of the release of ketone bodies by the liver. *Adv. Enzyme Regul.*, **4**, 339-353.

LAKSHMANAN M. R., NEPOKROEFF C. M., PORTER J. W., 1972. Control of the synthesis of fatty-acid synthetase in rat liver by insulin, glucagon and adenosine 3′ : 5′ cyclic monophosphate. *Proc. nat. Acad. Sci. US.*, **69**, 3516-3519.

LEVEILLE G. A., 1967. Influence of dietary fat and protein on metabolic and enzymatic activities in adipose tissue of meal fed rats. *J. Nutr.*, **91**, 25-34.

MACKAY E. M., BARNES R. H., CARNE H. O., 1941. The influence of a diet with high protein content upon the appetite and deposition of fat. *Am. J. Physiol.*, **135**, 193-201.

MAEDA H., INEDA I., SUGANO M., 1975. Behaviour of liver key lipogenesis enzymes in rats fed threonine reebalanced diet. *Nutr. Rep. Int.*, **12**, 61-67.

MASORO E. J., CHAIKOFF I. L., CHERNICK S. S., FELTS J. M., 1950. Previous nutritional state and glucose conversion to fatty acids in liver slices. *J. biol. Chem.*, **185**, 845-856.

NISHIDA T., VENO A., KUMMEROW F. A., 1960. Effect of dietary protein on metabolism of sodium acetate 1-^{14}C in chicks. *J. Nutr.* **71**, 379-386.

PERET J., MACAIRE I., CHANEZ M., 1973. Schedule of protein ingestion nitrogen and energy utilization and circadian rhythm of hepatic glycogen, plasma corticosterone and insulin in rats. *J. Nutr.*, **103**, 866-874.

PERET J., CHANEZ M., COTA J., MACAIRE I., 1975. Effects of quantity and quality of dietary protein and variation in certain enzyme activities on glucose metabolism in the rat. *J. Nutr.*, **105**, 1525-1534.

PORTENHAUSER R., WIELAND O., 1972. Regulation of pyruvate dehydrogenase in mitochondria of rat liver. *Eur. J. Biochem.*, **31**, 308-314.

REAVEN E. P., KOLTERMAN D. G., REAVEN G. M., 1974. Ultrastructural and physiological evidence for corticosteroid-induced alterations in hepatic production of very low density lipoprotein particles. *J. Lipid Res.*, **15**, 74-83.

ROMSOS D. R., LEVEILLE G. A., 1974. Effect of diet on activity of enzymes involved in fatty acid and cholesterol synthesis. *Adv. Lipid Res.*, **12**, 97-146.

SCHIMKE R. T., 1962. Adaptive characteristics of urea cycle enzyme in the rat. *J. biol. Chem.*, **237**, 459-468.

SCRUTTON M. C., UTTER M. F., 1968. Regulation of glycolysis and gluconeogenesis in animal tissues. *Annu. Rev. Biochem.*, **37**, 249-302.

SNEDECOR G. W., COCHRAN W. G., 1967. *Statistical methods*. Ed. 6. Iowa State Univ. Press, Ames, Iowa.

SZEPESI B., FREEDLAND R. A., 1967. Alterations on the activities of several rat liver enzymes at various times after initiation of a high protein regimen. *J. Nutr.*, **93**, 301-306.

SZEPESI B., FREEDLAND R. A., 1968. Time-course of changes in rat liver enzyme activities after initiation of a high protein regimen. *J. Nutr.*, **94**, 463-468.

TAKETA K., HANESHIGE Y., KOSAKA K., 1972-1973. Dual control of glucose 6-phosphate dehydrogenase induction in rat liver by dietary glucose and amino acids. *Enzyme*, **14**, 105-116.

VAUGHAN D. A., WINDERS R. L., 1964. Effects of diet on HMP dehydrogenase and malic TPN dehydrogenase in the rat. *Amer. J. Physiol.*, **206**, 1081-1084.

VOLPE J. J., MARASA J. C., 1975. Hormonal regulation of fatty acid synthetase, acetyl-CoA carboxylase and fatty acid synthesis in mammalian adipose tissue and liver. *Bioch. biophys. Acta.*, **380**, 454-472.

WILLIAMSON J. R., BROWNING E. T., SCHOLZ R., 1969. Control mechanisms of gluconogenesis and ketogenesis I. Effects of oleate on gluconeogenesis in perfused rat liver. *J. biol. Chem.*, **244**, 4607-4616.

YEH YU-YAN, LEVEILLE G. A., 1969. Effect of dietary protein on hepatic lipogenesis in the growing chick. *J. Nutr.*, **98**, 356-366.