medium containing hormones (Table). The spinal cord is morphologically, biochemically, and functionally mature in chicks by the 16th day of incubation[14]. It is suggested, therefore, that mature neural tissue is not influenced by these hormones.

AChE activity was significantly higher in cerebellar explants cultured on basal medium containing estradiol or cortisol than without hormone and did not differ from that in non-cultured 16-day-old cerebellar tissue (Table). The cerebellum is immature in 16-day-old chick embryos and AChE does not reach peak activity in chicks until 90 days after hatching[15]. Developing elements of the cerebellum may, therefore, be hormone-dependent for their growth and maintenance. Studies in this and other laboratories have given evidence that CNS growth may be significantly influenced by the presence of specific hormones[16]. Hormonal dependence for biochemical maintenance of neural explants cannot directly explain hormonal dependence of in vivo developing systems. Studies in vitro, however, may elucidate some of the underlying factors involved in neural hormonal sensitivity during development[17].

Résumé. L'activité acétylcholinestérasique et la teneur globale en protéines de fragments de cervelet et de moelle d'embryon de poulet de 16 jours diminue lorsqu'ils sont maintenus en culture organotypique dans le milieu standard de EAGLE. Après addition de cortisol ou d'oestradiol au milieu, les caractéristiques des tissus ne sont pas alterées par la mise en culture.

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[14] A. VERNADAKIS and A. BURKHALTER, Proc. Soc. exp. Biol. Med. *119*, 512 (1965); Nature, in press.

[15] J. J. PETERS and A. R. VONDERAHE, Electroenceph. clin. Neurophysiol. *13*, 146 (1961).

[16] S. GEEL and P. S. TIMIRAS, Brain Res., Osaka *4*, 135 (1967); S. GEEL, T. VALCANA and P. S. TIMIRAS, Brain Res., Osaka *4*, 143 (1967).

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Enhanced Hypoglycaemic Effect of Exogenous Insulin Associated with an Increased Response of Adipose Tissue and a Diminished Response of the Diaphragm in 'Meal Fed' Rats

Periodic hyperphagia evoked by infrequent feeding (e.g. training rats to consume their daily ration within 2 h or force feeding them twice a day by stomach tube) leads to enhanced lipogenesis[1-5] and may result in an increased amount of body fat – 'obesity without overweight'[6-9]. As MAYER[10] has pointed out, obesities of varying etiology differ greatly in many features, including the response to hormone administration. Thus the insulin sensitivity or goldthioglucose-treated obese mice was found to be normal, whereas mice with the hereditary obese hyperglycaemic syndrome are extremely resistant even to large doses of insulin[11,12]. This work was undertaken to assess the influence of feeding periodicity on the sensitivity of rats to exogenous insulin.

Female Wistar rats weighing 150–250 g and fed a standard laboratory diet[13] were used. The animals had either free access to food (controls) or were allowed to eat only for 2 h each day (from 07.00–09.00); these are referred to as 'meal fed' rats. All animals had water ad libitum. The hypoglycaemic effect of insulin was estimated after at least 5 weeks of experimental feeding. Furthermore, the glycogen content and in vitro $^{14}CO_2$ production by parametrial adipose tissue and diaphragm and incorporation of ^{14}C-U-glucose into total lipids of adipose tissue was measured in order to throw some light on the

Table I. The effect of crystalline insulin on blood glucose levels of control and 'meal fed' rats

Experimental group	Doses of insulin U/kg body weight	Blood glucose initial level mg/100 ml	Blood glucose changes after insulin injection (% of initial level)			
			30 [a]	60 [a]	120 [a]	240 [a]
Control (5)	0.1	82.0 ± 3.9	101.4 ± 6.9 [b]	88.2 ± 5.6	79.0 ± 3.8	88.3 ± 5.6
'Meal fed' (6)		72.0 ± 4.6	92.1 ± 8.8	60.2 ± 6.9 [d]	74.9 ± 7.2	81.1 ± 6.2
Control (10)	0.2	91.3 ± 7.36	76.3 ± 6.5	55.4 ± 5.6	47.1 ± 3.7	70.9 ± 3.8
'Meal fed' (11)		83.7 ± 1.45	55.8 ± 7.7 [c]	38.0 ± 2.4 [e]	41.7 ± 3.5	64.5 ± 3.1
Control (5)	0.4	85.6 ± 3.13	55.9 ± 3.9	56.0 ± 3.6	57.9 ± 4.1	81.9 ± 4.8
'Meal fed' (5)		92.6 ± 2.50	48.4 ± 4.6	43.0 ± 1.9 [d]	47.4 ± 3.9	78.6 ± 3.1

The figures in parentheses indicate the number of rats in each group. [a] Min after insulin injection. [b] Mean values ± standard error of mean. Symbols for statistical significance of differences between compared group averages: [c] ($P < 0.05$); [d] ($P < 0.02$); [e] ($P < 0.01$).

possible mechanism involved. Crystalline insulin (SPOFA) was given i.p. to rats fasted 22 h. The blood glucose level was estimated by a modification of the SOMOGYI-NELSON method[14], tissue glycogen by the anthrone method[15] after extraction with 30% KOH. Radioactivity was measured in a Tracer lab. scintillation spectrometer.

From Table I it is apparent that in the 'meal fed' rats insulin given at 0.1, 0.2 and 0.4 U/kg body weight induced a greater drop in the blood sugar level than in the controls. This difference was particularly marked 60 min after insulin injection, when the decrease in the blood sugar level is greatest. This result indicates that the overall effect of insulin on carbohydrate metabolism is enhanced by 'meal feeding'. Nevertheless, we have found substantial differences in the response of individual organs. At least one of them is the increased sensitivity of adipose tissue in the 'meal fed' rats to insulin, as evidenced by increased glycogen and lipid synthesis (Tables II and III), as well as a higher rate of $^{14}CO_2$ production from uniformly labelled glucose (Table III). On the other hand, the response of the diaphragm of 'meal fed' rats has been found to be diminished as indicated by a smaller increase in the glycogen content after insulin administration (Table IV). This is probably due to a decreased synthesis of glycogen rather than to its enhanced breakdown and oxidation, since we did not find significant differences in the in vitro formation of $^{14}CO_2$ from labelled glucose by diaphragms removed from rats of the 2 groups 45 min after insulin injection (Table IV).

The enhanced hypoglycaemic effect of insulin is probably not due to a decreased glucose output by the liver; in the course of insulin hypoglycaemia liver glycogen decreased in both groups in proportion to the fall in the blood glucose level.

An increased sensitivity of adipose tissue, and a decreased sensitivity of diaphragm to exogenous insulin,

Table II. Effect of crystalline insulin on in vitro glycogen synthesis in adipose tissue of control and 'meal fed' rats

Experimental group	Glycogen content of adipose tissue		
	Without hormone μg/g wet weight	Insulin μg/g wet weight	% of means of groups without hormone
Experiment A			
Control	47.7 ± 2.6[a]	60.1 ± 7.0	126
'Meal fed'	75.0 ± 10.4[b]	117.0 ± 21.6[b]	156
Experiment B			
Control	90.9 ± 12.7	76.5 ± 10.9	84
'Meal fed'	103.3 ± 16.8	147.3 ± 15.7[c]	142

In experiment (A) adipose tissue was removed from rats 60 min after injection of saline or insulin (0.2 U/kg body weight) and incubated for 60 min in 5 ml KRB buffer, pH 7.4, containing 25 μmoles glucose (no insulin added). In experiment (B) adipose tissue was incubated for 120 min in 5 ml KRB buffer, pH 7.4, containing 25 μmoles glucose and 12.5 mg bovine albumin without hormone or with 1000 μU insulin per ml. [a] Mean values ± standard error of mean from 6 animals per group. Symbols for statistical significance of differences between compared group averages: [b] ($P < 0.05$); [c] ($P < 0.01$).

[1] J. R. BEATON, V. FELEKI, A. J. SZLAVKO and J. A. STEVENSON, Can. J. Biochem. Physiol. *42*, 665 (1964).
[2] G. HOLLIFIELD and W. PARSON, J. clin. Invest. *41*, 245 (1962).
[3] G. A. LEVEILLE and R. W. HANSON, J. Lipid Res. *7*, 46 (1965).
[4] J. TEPPERMAN, J. R. BROBECK and C. N. H. LONG, Yale J. Biol. Med. *15*, 855 (1943).
[5] J. TEPPERMAN and H. M. TEPPERMAN, Am. J. Physiol. *193*, 55 (1958).
[6] C. COHN, D. JOSEPH and E. SHRAGO, Metabolism 6, 381 (1957).
[7] P. FÁBRY, T. BRAUN, R. PETRÁSEK, E. HORÁKOVÁ and E. KONOPÁSEK, Physiologia bohemoslov. *13*, 333 (1964).
[8] A. KEKWICK and G. L. S. PAWAN, Metabolism *15*, 173 (1966).
[9] L. M. VAN PUTTEN, D. W. VAN BEKKUM and A. QUERIDO, Metabolism *4*, 68 (1955).
[10] J. MAYER, *The Annie B. Cunning Lectures on Nutrition No. 13*, (The Royal Australian College of Physicians, Sydney, 1964).
[11] J. MAYER, R. E. RUSSELL, M. W. BATES and M. M. DICKIE, Metabolism *2*, 9 (1953).
[12] J. MAYER and C. Y. ZIGHERA, Science *119*, 96 (1954).
[13] P. FÁBRY, Čslká Fysiol. *8*, 529 (1959).
[14] H. FRANK and E. KIRBERGER, Biochem. Z. *320*, 359 (1950).
[15] N. V. CARROLL, R. W. LONGLEY and J. H. ROE, J. biol. Chem. *220*, 583 (1965).

Table III. Effect of crystalline insulin on lipogenesis and $^{14}CO_2$ production from ^{14}C-U-glucose by adipose tissue of control and 'meal fed' rats

Experimental group	Lipogenesis from ^{14}C-U-glucose[a] in vivo[b]			Lipogenesis from ^{14}C-U-glucose in vitro[c]			$^{14}CO_2$ production from ^{14}C-U-glucose in vitro[c]		
	Without hormone counts/min mg protein	Insulin counts/min mg protein	% of mean of groups without hormone	Without hormone counts/min mg protein	Insulin counts/min mg protein	% of mean of groups without hormone	Without hormone counts/min mg protein	Insulin counts/min mg protein	% of mean of groups without hormone
Control	104.3 ± 23.7[d] (6)	480.4 ± 85.4 (6)	461	638.0 ± 198.0 (6)	1580.0 ± 287.0 (6)	231	248.5 ± 22.9 (6)	452.2 ± 25.2 (6)	182
'Meal fed'	133.3 ± 27.0 (6)	1823.0[e] ± 464.0 (6)	1368	430.0 ± 86.4 (6)	4450.0[f] ± 769.0 (5)	1035	280.3 ± 34.6 (6)	543.2[e] ± 20.7 (6)	193

[a] ^{14}C-U-glucose (4 μC/100 g) administered i.p. simultaneously with saline or insulin. [b] Adipose tissue removed 45 min after injection of saline or insulin (0.2 U/kg). [c] Animals treated as above adipose tissue incubated for 60 min in 5 ml KRP buffer, pH 7.4 containing 25 μmoles glucose and 0.25/μC ^{14}C-U-glucose (no insulin added). [d] Mean values ± standard error of mean; number of rats in parentheses. Symbols for statistical differences between compared group averages: [e] ($P = 0.02$); [f] ($P < 0.01$).

Table IV. Effect of crystalline insulin on glycogen content of diaphragm and $^{14}CO_2$ production from ^{14}C-U-glucose by diaphragms of control and 'meal fed' rats

Experimental group	Glycogen content of diaphragm [b]			$^{14}CO_2$ production from ^{14}C-U-glucose in vitro [c]		
	Without hormone mg/100 g wet weight	Insulin		Without hormone counts/min 100 mg wet weight	Insulin	
		mg/100 g wet weight	% of mean of groups without hormone		counts/min 100 mg wet weight	% of mean of groups without hormone
Control	274 ± 9.8 [a] (5)	745 ± 16.5 (5)	272	948.8 ± 91.4 (6)	1991.8 ± 177.6 (6)	210
'Meal fed'	298 ± 19.6 (6)	617 ± 32.3 [e] (6)	207	1241.7 ± 58.2 [d] (6)	1959.9 ± 151.0 (6)	158

[a] Mean values ± standard error of mean; number of rats in parentheses. [b] Diaphragms removed 45 min after injection of saline or insulin (0.2 U/kg). [c] Animals treated as above, diaphragms incubated for 60 min in 5 ml KRP buffer, pH 7.4, containing 25 μmoles glucose and 0.25 μC ^{14}C-U-glucose (no insulin added). Symbols for statistical differences between compared group averages: [d] ($P = 0.02$); [e] ($P < 0.01$).

has similarly been reported recently for hereditary obese[16], but not for goldthioglucose-treated obese mice[17]. It therefore appears that in the 'meal fed' rats a nutritionally induced regulatory mechanism is brought into play which influences the sensitivity of target organs to insulin and directs the hormone's metabolic effect towards adipose tissue. The nature of this regulatory mechanism is as yet unknown.

The increased sensitivity of adipose tissue to insulin may be an important factor in the mechanism of the greater synthetic capacity found in adipose tissue of meal fed' rats[18,19].

Zusammenfassung. Intraperitoneale Verabreichung von Insulin führte bei Ratten, die täglich 2 h gefüttert wurden, im Vergleich mit ad libitum gefütterten Kontrolltieren, zu einer grösseren Herabsetzung des Blutzuckerspiegels, einer höheren Fett- und Glykogensynthese im Fettgewebe, jedoch zu einer verminderten Glykogensynthese im Zwerchfell.

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Physiology Department of the Institute of Human Nutrition, Prague-Krč (Czechoslovakia), 25th November 1966.

[16] W. STAUFFACHER, O. B. CROFFORD, B. JEANRENAUD and A. E. RENOLD, Ann. N.Y. Acad. Sci. *131*, 528 (1965).
[17] W. STAUFFACHER and A. E. RENOLD, Schweiz. med. Wschr. *96*, 735 (1966).
[18] T. BRAUN, P. FÁBRY, L. KAZDOVÁ, Proc. VIIth Int. Congr. Nutr. Hamburg (1966).
[19] G. A. LEVEILLE and R. W. HANSON, Can. J. Biochem. Physiol. *43*, 857 (1965).

The Effect of Endotoxin on the Lactic Acid Production in Pregnant and Non-Pregnant Rats

The effect of endotoxin on aerobic glycolysis has been examined in certain tumours[1,2], leucocytes[3–5], peritoneal macrophages, spleen[6], brain[2], kidney, mucosa of small intestine, thymocytes[7] and placenta[8,9]. Generally the action was found to be biphasic[5,10].

After an initial enhancement of aerobic glycolysis, a transitory notable inhibition set in, followed again by a hyperfunction. The course of these events is remarkably dose-dependent. The K-2 carcinoma[6] thymocytes, kidney, mucosa of small intestine[7], however, were endotoxin insensitive. Finally we found in placentas nothing but a permanent inhibition of aerobic glycolysis[8,9].

This report concerns a comparative study of lactic acid production of some tissues of pregnant and non-pregnant rats after endotoxin treatment.

Method. Rats of mixed breed (National Institute of Public Health) in day $17^1/_2$ to day $18^1/_2$ of pregnancy were used throughout. The endotoxin was extracted from *Serratia marcescens* by the method of BOIVIN and MESROBEANU[11]. In preliminary titrations in non-pregnant rats, the preparation was adjusted to contain one LD_{10}/ml. This dose was inoculated i.p. into pregnant and non-pregnant rats. Similarly pregnant and non-pregnant rats were given 1 ml saline by the same route for control purposes. The animals were killed by decapitation 24 h later. Leucocytes were obtained from peritoneal exudates produced by the i.p. injection of 20 ml sterile broth (bouillon) into each rat 6 h prior to decapitation. The peritoneal exudates contained $8–12 \cdot 10^7$ cells/ml. Bone

[1] M. W. WOODS, M. LANDY and M. J. SHEAR, Fedn Proc. Fedn Am. Socs exp. Biol. *18*, 355 (1959).
[2] K. GIGER, Proc. Am. Ass. Cancer Res. *3*, 227 (1961).
[3] S. P. MARTIN, G. R. MCKINNEY and R. GREEN, Ann. N.Y. Ac. Sci. *29*, 996 (1955).
[4] Z. M. COHN and S. I. MORSE, J. exp. Med. *111*, 667 (1960).
[5] A. YUNIS and W. J. HARRINGTON, J. Lab. clin. Med. *55*, 584 (1960).
[6] M. W. WOODS, M. LANDY, J. L. WHITBY and D. BURK, Bact. Rev. *25*, 447 (1961).
[7] M.-NÉ SAJGÓ and P. KERTAI, Acta Microbiol. hung. in press.
[8] P. KERTAI, M.-NÉ SAJGÓ, E. CZEIZEL, I. KOVÁCS and M. KOVÁCS, Magy. Nöorvos. Lap. *28*, 286 (1965).
[9] E. CZEIZEL, M.-NÉ SAJGÓ, GY. TARJÁN and P. KERTAI, Am. J. Obstet. Gynec. in press.
[10] M. W. WOODS, M. LANDY, D. BURK and T. HOWARD, in *Bacterial Endotoxins* (Ed. M. LANDY and W. BRAUN; Institute of Microbiology, New Brunswick, N.J. 1964) p. 160.
[11] A. BOIVIN and L. MESROBEANU, Revue Immunol. Thér. Antimicrob. *1*, 553 (1935).