fluorescence pattern of the 'sweet-sensitive' protein then is quite dissimilar to that of glucose. While the greater magnitude of change noted with sucrose versus glucose might be due to the greater sweetness of sucrose in the dog[9], the enhancement by sucrose and the quenching by glucose is not so easily explained. One readily apparent possibility is that there are separate sites for interaction by monosaccharides (glucose) and disaccharides (sucrose), and that these interactions result in quenching and exhaltation, respectively. Studies involving different monosaccharides and disaccharides are in progress.

To ascertain the ability of the sugars to non-specifically affect the quantum yield values of a protein, a control was examined. Bovine γ-globulin of similar molecular weight and aromatic amino acid content[10], as the dog (unpublished amino acid analysis), was subjected to glucose and sucrose titration under identical conditions. The results of the sucrose study are presented in Figure 4. The protein titrated with either sucrose, glucose, or buffer gave similar fluorescence quantum yields. No quenching or exhaltation was observed.

The interactions of sucrose and glucose with the dog 'sweet-sensitive' protein appear to be specific. The fluorescence technique would seem to be ideally suited for study of those interactions because of the high sensitivity of emission of the aromatic chromophores[11].

Zusammenfassung. Komplexe, welche das «süssigkeitsempfindliche» Protein mit Glukose und Rohrzucker bilden, wurden mit Hilfe der Fluoreszenz-Spektroskopie untersucht.

F. R. DASTOLI[12]

U.S. Natick Laboratories, Pioneering Research Laboratory, Natick (Massachusetts 01760, USA), 25 October 1971.

[9] H. T. ANDERSON, M. FUNAKOSHI and Y. ZOTTERMAN, in *Olfaction and Taste* (Ed. Y. ZOTTERMAN; Macmillan, New York 1963), p. 177.

[10] F. W. J. TEALE, Biochem. J. *76*, 381 (1960).

[11] Acknowledgment. The advice, suggestions and technical assistance of Mr. LEO G. HOLMES are greatly appreciated. The study was supported by an Associateship from the National Academy of Sciences-National Research Council and a grant from the Army Research Office.

[12] Current Address: MIRLIN™ Corporation, 8 Kane Industrial Drive, Hudson, Massachusetts, 01749 U.S.A.

In vitro and in vivo Catabolism of Cholesterol in Meal-Fed Rats

A decrease in feeding frequency in chickens[1,2] and rabbits[3] potentiates distinctly the effect of atherogenic cholesterol diet. In meal-fed monkeys, the level of serum cholesterol is higher also when these are fed a diet without cholesterol[4]. Similar experiments with rats did not give explicit results: only in females fed a cholesterol diet the meal-feeding regimen did increase cholestrolemia[5]. On the contrary, we have found in our experiments that the application of a long term meal-feeding regimen in rats has caused a cholesterol accumulation in some organs and in the carcass of the experimental animals also when a diet without cholesterol was used. Therefore in this work we have followed the extent of cholesterol catabolism to bile acids as one of the possibilities through which the meal-feeding regimen interferes in the regulation of cholesterol metabolism. There are no experimental data on this problem up to now.

Experimental. In all experiments male Wistar rats initially weighing about 220 g, fed a standard diet[6] were used. The control animals were fed ad libitum; access to food of the animals from the experimental group was gradually shortened in the course of 8 weeks down to 2 h daily (in the first 2 weeks 8 h daily, in the 3rd and 4th week 6 h, in the 5th and 6th week 4 h, in the 7th and 8th week 3 h daily). During the following 35 weeks, the meal-fed group was fed 2 h daily between 08.00–10.00 o'clock. All animals had free access to water. In all cases the animals were decapitated at the end of the experiment after 22 h of starvation. In extracts[7] of blood serum, some tissues and carcass[8], total cholesterol was determined[9].

In vitro experiment. At the end of the experiment, immediately after decapitation, liver was quickly excized

[1] C. COHN, R. PICK and L. N. KATZ, Circulation *20*, 969 (1959).

[2] C. COHN, R. PICK and L. N. KATZ, Circul. Res. *9*, 139 (1961).

[3] W. W. WELLS, R. QUAN-MA, C. R. COOK and S. C. ANDERSON, J. Nutr. *76*, 41 (1962).

[4] C. GOPALAN, S. G. SRIKANTIA, S. N. JAGANNATHAN and K. S. RAMANATHAN, Am. J. clin. Nutr. *10*, 322 (1962).

[5] R. OKEY, G. SCHEIER and R. REID, J. Am. Diet. Ass. *36*, 441 (1960).

[6] T. BRAUN, M. KOHOUT and P. FÁBRY, Can. J. Biochem. *45*, 1470 (1967).

[7] J. FOLCH, M. LEES and G. H. S. STANLEY, J. biol. Chem. *226*, 497 (1957).

[8] G. R. JANSEN, M. E. ZANETTI and C. F. HUTCHISON, Biochem. J. *101*, 811 (1966).

[9] R. P. COOK, *Cholesterol. Chemistry, Biochemistry and Pathology* (Academic Press, New York 1958).

Table I. Cholesterol level in blood serum and organs of ad libitum and meal-fed rats

Group	Serum	Liver	Small intestine	Adrenals	Epididymal fat	Brain	Carcass
No. of animals	10/15[a]	10/15	10/15	9/13	10/14	10/15	10/8
Ad libitum	55 ± 5[b]	273 ± 9	208 ± 11	2096 ± 183	35 ± 4	1355 ± 88	112 ± 8
Meal-fed	57 ± 4	325 ± 27	288 ± 17	3260 ± 277	103 ± 8	1371 ± 45	147 ± 12
Statistical significance (P)	—	—	< 0.002	< 0.01	< 0.001	—	< 0.05

[a] Number of animals in ad libitum fed group/in meal-fed group. [b] Mean values ± S.E. The values are expressed in terms of mg per 100 ml of blood serum or 100 g of wet wt. of tissue, in the case of carcass per 100 g of dead weight of the animal without gastrointestinal tract.

Table II. Catabolism of cholesterol-4-^{14}C in liver slices of ad libitum and meal-fed rats

Group	No. of animals	% activity recovered as Neutral sterols	Bile acids
Ad libitum	11	84.8±1.1[a]	11.3±0.6
Meal-fed	11	85.6±0.7	10.9±0.8

[a] Mean values ± S.E. Liver slices were incubated in an atmosphere consisting of 95% O_2 and 5% CO_2 at a temperature of 37°C for 3 h in Krebs-Ringer phosphate buffer, pH 7.4, containing about 1.13 μC cholesterol-4-^{14}C (Amersham, Great Britain). Dosage of radioactivity was controlled by weighing cholesterol-4-^{14}C solution, pipetted into an empty incubation flask.

Table III. Excretion of neutral sterols and bile acids through faeces in ad libitum and meal-fed rats during 96 h after i.p. administered cholesterol-4-^{14}C

Group	No. of animals	% of activity recovered as Neutral sterols	Bile acids
Ad libitum	7	1.8±0.16[a]	11.2±1.3
Meal-fed	7	1.9±0.17	11.1±0.8

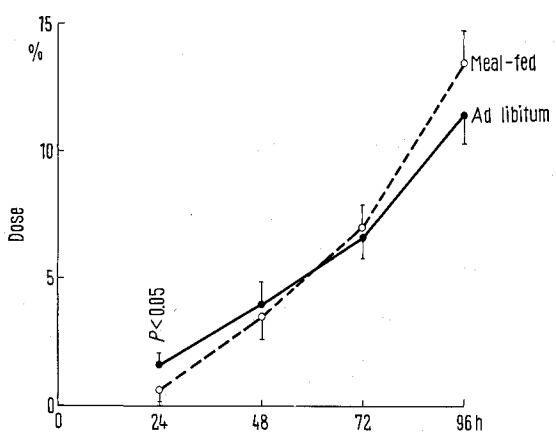
[a] Mean values ± S.E. Individually collected faeces was dried to a constant weight; faeces collected for 96 h was then combined and homogenized and from an aliquot neutral sterols and bile acids were isolated by extraction[7].

and placed in cold saline (details see Table II). After an incubation with cholesterol-4-^{14}C, activity in liver slices was determined in a fraction of neutral sterols and bile acids[10].

In vivo experiment. At the end of the experiment the animals were given i.p. 3 μC of cholesterol-4-^{14}C in physiological solution with Tween 80. The feeding regimen was kept unchanged. In four 24 hour-intervals, the total ^{14}C activity excreted in feces was examined[11] and a portion of neutral sterols and bile acids in the total excreted ^{14}C activity was determined for the whole 96 h period. ^{14}C activity was measured by scintillation spectrometry on Mark 1 (Nuclear Chicago) using external standardization. All results were statistically evaluated by Student's *t*-test.

Results and discussion. Food consumption in rats fed 2 h daily was significantly lower during the whole experiment when compared with controls (controls: 5.7 ± 0.2, meal-fed group: 4.6 ± 0.3; $P < 0.01$). The body weight of meal-fed rats at the end of the experiment was lower (controls: 403.3 ± 12.1 g, meal-fed group: 362.2 ± 12.0 g; $P < 0.05$). These findings are in agreement with those by other authors using Wistar rats and the same model of meal-feeding regimen[12,13]. Relative and absolute weight of liver was not significantly affected by the meal-feeding regimen.

The meal-feeding regimen significantly increased cholesterol levels in some tissues and carcass (Table I). The results presented in Table II show that the ability of liver slices to catabolize cholesterol to bile acids was not affected by a long adaptation to meal-feeding regimen. From the point of view of cholesterol degradation in an organism, this finding becomes important with regard to the decisive role of liver tissue in this process[14]. Results of a 4-days balance study show (Table III) that, also in the intact organism, the process of cholesterol degradation and excertion of cholesterol catabolite in the faeces were not affected by meal-feeding regimen. The ratio of neutral sterols and bile acids in the total excreted activity was identical in both groups. The Figure proves that, during the first 4 days after cholesterol-4-^{14}C administration, the elimination of fecal ^{14}C remained practically unchanged in both groups. The results presented show that at least during the first 96 h the catabolism of cholesterol-4-^{14}C administered, as well as the excretion of its end products in the faeces were not affected by the meal-feeding regimen. As there are more authors who reported an increased synthesis of cholesterol in liver of meal-fed rats[15,16], our results lead to the assumption that increased cholesterol levels are due to accelerated anabolic process and not to the changes in cholesterol catabolism. It is also possible to consider changes in distribution of endogenous cholesterol in organs morphologically changed during the long adaptation to meal-feeding regimen[12].



Total activity excreted through faeces during 24, 48, 72 and 96 h after administration of cholesterol-4-^{14}C in ad libitum (solid line) and meal-fed (broken line) animals. Aliquot of each 24 h faeces dried to a constant weight were extracted into chloroform-methanol 2:1[8].

Zusammenfassung. Bei männlichen Wistar-Ratten wurde der Einfluss des «meal-feeding»-Regimes nach täglicher 2stündlicher Fütterung an Leberschnitten auf den Katabolismus von Cholesterol-4-^{14}C geprüft und normal befunden. Im In-vivo-Versuch unter denselben experimentellen Bedingungen zeigte weder der Katabolismus noch die Exkretion des i.p. applizierten Cholesterol-4-^{14}C eine Veränderung.

P. BOBEK, E. GINTER and J. CERVEN

Institute of Human Nutrition Research, Nam 4. aprila 7, Bratislava (Czechoslovakia), 3 March 1971.

[10] M. D. SIPERSTEIN, M. E. JYKO, I. L. CHAIKOFF and W. G. DAUBEN, Proc. Soc. exp. Biol. Med. *81*, 720 (1952).
[11] J. D. WILSON, J. Lipid. Res. *5*, 409 (1954).
[12] P. FÁBRY, in *Feeding Pattern and Nutritional Adaptations* (Academia, Prague 1969).
[13] J. A. F. STEVENSON, V. FELEKI, A. SZLAVKO and J. R. BEATON, Proc. Soc. exper. Biol. Med. *116*, 178 (1964).
[14] J. R. MEIER, M. D. SIPERSTEIN and I. L. CHAIKOFF, J. biol. Chem. *198*, 105 (1952).
[15] G. A. LEVEILLE and R. W. HANSON, Can. J. Physiol. Pharmac. *43*, 857 (1965).
[16] R. PETRÁSEK, R. POLEDNE and M. VAVREČKA, Čslká Fysiol. *15*, 81 (1966).