

### The Effect of Fasting, Refeeding and Meal Eating on the Ribonucleic Acid Content of Adipose Tissue in the Rat

The feeding pattern influences in a marked way the metabolic activity and composition of adipose tissue. In the present work it was revealed that also the amount and concentration of RNA and the ratio of RNA:DNA in adipose tissue changes in relation to the nutritional status.

The experiments were carried out on Wistar rats of both sexes, fed a standard laboratory diet<sup>1</sup>. In the first series, the mesenteric, epididymal and retroperitoneal adipose tissues of fed animals, animals fasting for 48 or 72 h and those refed with a high-carbohydrate diet<sup>1</sup> for 24, 48, 72 or 120 h after previous 48 or 72 h fasting, were analysed. In the second series, the mesenteric and retroperitoneal adipose tissues of controls fed ad libitum and animals adapted for a period of 7–15 weeks to eating their daily ration within 2 h (0700–0900) were compared. During the last 48 h before the animals were sacrificed, both groups were given measured amounts of food (10 g per 100 g body weight per day) which they could ingest

freely without any time limitation. All animals had free access to water. The RNA and DNA in the previously defatted adipose tissue<sup>2</sup> was assessed colorimetrically according to SCHNEIDER<sup>3</sup>.

From Table I it is apparent that fasting for 48 and 72 h respectively led to a decline of RNA and a marked reduction of the RNA:DNA ratio in adipose tissue. In the course of feeding, the RNA content rose to initial levels or exceeded them (experiments on males). The ratio of RNA:DNA increased as a result of refeeding significantly above initial levels in males as well as in females; the maximum increase was recorded after 48–72 h refeeding. Similar changes of the RNA content and the ratio of RNA:DNA, as listed in Table I for epididymal

<sup>1</sup> P. FÁBRY, Čslk. Fysiol. 8, 529 (1959).

<sup>2</sup> V. J. STEELE, N. OKAMURA, and H. BUSCH, Biochem. biophys. Acta 87, 490 (1964).

<sup>3</sup> W. C. SCHNEIDER, in S. P. COLOWICK and N. O. KAPLAN, *Methods in Enzymology*, vol. III (Academic Press Inc., New York 1957).

Table I. RNA content in adipose tissue of rats fed, fasting and realimented with a high-carbohydrate diet<sup>a</sup> (average  $\pm$  S.E.; six animals in each group)

Group <sup>b</sup>	Body weight (g)	Weight of fat body (g)	RNA content		RNA:DNA
			mg/fat body	mg/100 g fresh weight	
Males: Epididymal fat body					
C	277 ± 3.5	3.60 ± 0.20	0.65 ± 0.02	18.68 ± 1.34	1.05 ± 0.02
S 72 h	246 ± 4.2	3.97 ± 0.37	0.51 ± 0.07	12.35 ± 1.04 <sup>d</sup>	0.91 ± 0.02 <sup>d</sup>
R 24 h	256 ± 4.6	2.71 ± 0.25	0.64 ± 0.04	24.43 ± 2.17 <sup>e</sup>	1.09 ± 0.02
R 48 h	266 ± 3.3	2.92 ± 0.28	0.73 ± 0.05	27.75 ± 2.29 <sup>d</sup>	1.37 ± 0.07 <sup>d</sup>
R 72 h	270 ± 3.1	3.39 ± 0.27	0.72 ± 0.01 <sup>d</sup>	21.95 ± 1.44	1.17 ± 0.05
R 120 h	279 ± 3.3	3.13 ± 0.43	0.76 ± 0.10	24.95 ± 2.85	1.17 ± 0.06
Females: Mesenterial fat body					
C	174 ± 2.16	3.81 ± 0.37	0.96 ± 0.11	25.10 ± 1.26	1.21 ± 0.07
S 48 h	148 ± 1.58	1.73 ± 0.24	0.40 ± 0.04 <sup>d</sup>	23.20 ± 4.11	0.74 ± 0.03 <sup>e</sup>
R 24 h	162 ± 1.73	1.94 ± 0.45	0.60 ± 0.09	32.40 ± 2.71 <sup>c</sup>	1.13 ± 0.03
R 48 h	163 ± 2.27	2.03 ± 0.32	0.70 ± 0.05	36.90 ± 3.57 <sup>d</sup>	1.24 ± 0.09
R 72 h	164 ± 2.27	2.18 ± 0.42	0.87 ± 0.10	42.40 ± 3.15 <sup>e</sup>	1.47 ± 0.02 <sup>d</sup>

<sup>a</sup> Composition: caloric volume in %: 76% carbohydrate, 14% protein, and 10% fat. <sup>b</sup> C = fed controls; S = fasting; R = realimentation.

<sup>c</sup> Difference as compared with control group statistically significant  $P < 0.05$ . <sup>d</sup>  $P < 0.01$ . <sup>e</sup>  $P < 0.001$ .

Table II. RNA content of adipose tissue of female rats fed ad libitum and of rats adapted to feeding for 2 h per day (average  $\pm$  S.E.; 14 animals in each group)

Group	Body weight (g)	Weight of fat body (g)	RNA content		RNA:DNA
			mg/fat body	mg/100 g fresh weight	
Mesenterial fat body					
Fed ad libitum	228 ± 7.0	5.80 ± 0.30	1.06 ± 0.06	18.41 ± 0.85	1.27 ± 0.04
Fed for 2 h per day	226 ± 5.1	5.83 ± 0.33	1.36 ± 0.07 <sup>b</sup>	23.25 ± 0.73 <sup>e</sup>	1.39 ± 0.04 <sup>a</sup>
Retroperitoneal fat body					
Fed ad libitum	—	1.65 ± 0.08	0.36 ± 0.02	21.87 ± 1.10	1.26 ± 0.05
Fed for 2 h per day	—	1.72 ± 0.02	0.48 ± 0.003 <sup>e</sup>	28.24 ± 0.64 <sup>e</sup>	1.65 ± 0.08 <sup>e</sup>

<sup>a</sup> Difference as compared with ad libitum fed controls statistically significant  $P < 0.05$ . <sup>b</sup>  $P < 0.01$ . <sup>e</sup>  $P < 0.001$ .

and mesenterial adipose tissue, were found also in retroperitoneal adipose tissue.

In animals adapted to ingesting their daily ration within 2 h, a significant increase of the absolute and relative amount of RNA and the RNA:DNA ratio in the mesenterial and retroperitoneal adipose tissue was found (Table II).

From other work, it is known that as a result of starvation the amount of RNA in the liver, kidneys and spleen<sup>4-6</sup> declines and that after refeeding the RNA:DNA ratio increases<sup>6</sup>. Our results indicate that the same applies to adipose tissue. It can be assumed that during starvation synthetic processes are inhibited not only for lack of energy but also due to a loss of cellular RNA. In the course of refeeding, when synthetic processes are renewed, RNA values in adipose tissue not only return to normal levels, but increase above values of normally fed controls, in particular when calculated per DNA. The raised RNA values in adipose tissue of rats adapted to food intake for 2 h per day, where short-term hyperphagia alternates with fasting, is probably connected with increased synthetic activity of adipose tissue during the refeeding stage,

including enhanced formation of enzymes concerned with glycogen and fat synthesis.

*Zusammenfassung.* Durch experimentellen Hunger (48 bis 72 h) kommt es bei Ratten zu einer RNS-Minderung im mesenterialen, epididymalen und retroperitonealen Fettgewebe. Normale Fütterung oder Adaptation an beschränkte Fütterungsdauer (2 h pro Tag) führen zu einer Erhöhung des RNS-Gehaltes über die Normalwerte.

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<sup>4</sup> H. W. KOSTERLITZ, J. Physiol. 106, 194 (1947).

<sup>5</sup> J. A. D. COOPER, J. biol. Chem. 200, 155 (1952).

<sup>6</sup> J. D. SUMMERS and H. FISHER, J. Nutr. 76, 187 (1962).

## Biochemical Modifications of the Crystalline Lens in the Experimental Hypercholesteremia of Rabbits

In the field of experimental hypercholesteremia, regarding the eyeball, we found cholesterol depositions in the iris, the choroid and the ciliary body, as well as in the ciliary process. Similar observations were made by other authors: DONEGAN<sup>1</sup>, JANES<sup>2</sup>, VERSÉ<sup>3</sup>. Nevertheless, only few data are available in connection with the crystalline lens. This made it necessary to examine biochemically the crystalline lens in experimental hypercholesteremia.

*Materials and methods.* Our experiments were made on 10 mature rabbits, having the same weight (1900–2200 g) and age. The animals were fed with a basic diet. The

rabbits received a daily dose of 1 g cholesterol mixed with corn flour. A daily dose of 1 g cholesterol was given during 5½ months to 7 grey rabbits, and during 4 months to 3 albino rabbits. This group was supplemented with 12 control animals.

*Biochemical examination of the blood.* According to Bloor's method, the serum cholesterol values varied between 100–750 mg%. The cholesterol levels in normal rabbits were 44–79 mg%.

*Biochemical examination of the crystalline lens.* Using the right crystalline lens, we determined its cholesterol content in 12 control rabbits, as well as in 10 cholesterol-treated animals. These determinations were made according to the method of KINGSLEY and SCHAFFERT<sup>4</sup>.

The results are illustrated in the Table. As compared with the controls, we established a 24.6% increase in the crystalline lens of the cholesterol-treated animals, the difference being significant ( $p < 0.001$ ).

*Résumé.* Les auteurs ont examiné par des méthodes biochimiques, en particulier celle de KINGSLEY et SCHAFFERT, le cristallin des lapins souffrant d'hypercholestérolémie expérimentale. Comparés aux contrôles, les animaux traités, ont présenté une augmentation de cholestérol de 24,6%, cette différence est significative ( $p < 0,001$ ).

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<sup>1</sup> J. M. DONEGAN, Trans. Am. ophthalm. Soc. 51, 475 (1954).

<sup>2</sup> R. G. JANES, Am. J. Ophthalm. 58, 819 (1964).

<sup>3</sup> M. VERSÉ, Virchows Arch. Path. Anat. 250, 252 (1924).

<sup>4</sup> G. R. KINGSLEY and R. R. SCHAFFERT, J. biol. Chem. 180, 315 (1949).

Crystalline lens

Rabbit No.	Duration of diet (months)	Dry substance (mg)	Cholesterol content (mg) in 100 g dry substance	Dry substance (mg)	Cholesterol content (mg) in 100 g dry substance
Cholesterol-treated animals			Controls		
1	5.5	221	713	186	616
2	5.5	214	672	189	532
3	5.5	194	654	232	597
4	5.5	207	702	196	549
5	5.5	206	727	215	602
6	5.5	229	851	198	517
7	4	194	691	178	491
8	4	188	678	209	651
9	4	211	756	201	638
10	5.5	223	849	225	626
11	—	—	—	191	527
12	—	—	—	227	673
Media:		208.7	729.3 ± 22.3	203.9	584.9 ± 17.1