Tab. II. Body-weight and carcass composition of rats after 11 weeks of experimental feeding (mean ± S.E., body-weight as the nearest whole number)

| Group | No. of rats | Average food intake (g/rat/day) | Body-weight (g) initial | Body-weight (g) final | Body-fat % | Body-fat change[a] % | Body-protein % | Body-protein change[a] % |
|---|---|---|---|---|---|---|---|---|
| Controls | 17 | 27.6 | 163 ± 4.2 | 291 ± 9.3 | 10.53 ± 0.21 | + 99.0 | 20.00 ± 0.35 | + 78.0 |
| Intermittent starvation | 18 | 11.6 | 164 ± 3.3 | 156 ± 3.5 | 11.74 ± 0.25[b] | + 10.0 | 17.00 ± 0.16[b] | − 21.3 |
| Continuous underfeeding (pair-fed) | 17 | 11.6 | 164 ± 3.6 | 155 ± 3.1 | 9.93 ± 0.22 | − 6.8 | 19.14 ± 0.24 | − 12.0 |

[a] Based on the amount of fat and protein in control animals, comparable as to body-weight, killed at beginning of the feeding period.
[b] Difference, as compared with both remaining groups, is significant for $P < 0.01$.

The results are in keeping with our previous work where we assumed enhanced lipogenesis in intermittently starving rats in view of their raised respiratory quotient[6]. Periodic concentration of food ingestion into brief periods thus leads to markedly accentuated lipogenesis not only when the caloric intake is nearly adequate[7] but also when the supply of calories is substantially reduced. This fact could possibly interfere with adipose tissue reduction in some obese patients whose self-imposed dietary habits sometimes resemble the dietary pattern of intermittent starvation.

*Zusammenfassung.* Albinoratten, bei denen zwischen die Fütterungstage 1–2tägige Hungerperioden eingesetzt wurden, zeigten nach 3 Wochen eine wesentlich erhöhte Inkorporation von 1-$C^{14}$-Acetat in die Fettsäuren der Leberschnitte. Selbst bei Herabsetzung der Kalorienzufuhr um ca. 50% stieg hier, im Gegensatz zu kontinuierlich unterernährten Tieren, der prozentuale Körperfettgehalt. Die Resultate sprechen für eine Steigerung der Lipogenese.

P. FÁBRY, R. PETRÁSEK, T. BRAUN,
M. BEDNÁREK, E. HORÁKOVÁ, and E. KONOPÁSEK

*Physiological Department of the Institute of Human Nutrition, Prague (Czechoslovakia), August 9, 1962.*

[6] R. PETRÁSEK and P. FÁBRY, Čs. gastroenterol. výž. *12*, 309 (1958).
[7] J. TEPPERMAN, J. R. BROBECK, and C. N. H. LONG, Yale J. Biol. Med. *15*, 855 (1942). – J. TEPPERMAN and H. M. TEPPERMAN, Amer. J. Physiol. *193*, 55 (1958). – C. COHN and D. JOSEPH, Metabolism *9*, 492 (1960).

## Antigen-Distribution in Rat Liver Mitochondria

It has been shown that particulate components, sedimented from homogenates of rat liver by means of differential centrifugation and cell sap itself, have immunological properties[1].

These findings suggest the possibility that a study of the antigenic properties of cellular components might yield information as to the pattern of distribution of intracellular proteins.

Results on the immunological properties of rat liver mitochondria and their deoxycholate sub-fractions will be reported here.

The perfused livers were homogenized in 0.44 *M* sucrose in water and the mitochondria isolated by centrifugation at 10000 *g* for 10 min, after removal of cell debris and nuclei at 1000 *g* for 15 min. The microsomal fraction was obtained by centrifugation for 1 h at 105000 *g* of mitochondria supernatant, previously, however, centrifuged at 30000 *g* for 30 min. Both the mitochondrial and microsomal fractions were washed twice with sucrose. By treatment of the particulate fractions with sodium deoxycholate 3% at pH 7.8 (0.3% final concentration), deoxycholate soluble (dc-sol) and deoxycholate-insoluble (dc-ins) fractions were obtained. The dc-ins fraction from mitochondria will be referred to as mitochondria membranes[2]. Cell sap was obtained by centrifugation at 105000 *g* for 1 h of the supernatant of microsome preparations.

To obtain the antisera, the samples were injected into rabbits using adjuvant technique[3]. 6 rabbits were injected with each antigen preparation. For the immunological experiments the double diffusion agar technique was used[4] and the reactions were allowed to proceed at 20°C. After completion, the agar plates were washed and recorded photographically.

Mitochondria were tested with sera produced against mitochondria themselves, dc-sol mitochondria fraction, mitochondria membranes, microsomes, and cell sap.

As shown in Figure 1, mitochondria preparations tested with a homologous antiserum gave rise to several precipitation lines. When the same mitochondria suspension was allowed to react with an antimembrane serum, only two groups of antigens were demonstrated by precipitation lines joining with identical lines present in the reaction with mitochondria antiserum. One of these has further proved to be identical with one of the several lines present in the reaction between mitochondria and anti-dc-sol mitochondria fraction, while the other one crossed all of them.

[1] J. FURTH and E. KABAT, J. exp. Med. *74*, 247 (1941). – W. HENLE, L. CHAMBERS, and V. GROUPE, J. exp. Med. *74*, 495 (1941). – P. PERLMANN and V. D'AMELIO, Nature *181*, 491 (1958). – P. VOGT, Nature *182*, 1807 (1958). – P. PERLMANN, T. HULTIN, V. D'AMELIO, and W. S. MORGAN, Exp. Cell Res., Suppl. *7*, 279 (1959). – V. D'AMELIO and P. PERLMANN, Exp. Cell Res. *19*, 383 (1960). – P. K. VOGT, Z. Naturforsch. *15b*, 213, 218, 221 (1960).
[2] M. L. WATSON and P. SIEKEVITZ, J. Biophysic. Biochem. Cytol. *2*, 639 (1956).
[3] M. COHN, in *Methods in Medical Research* (A. CORCORAN, Ed., The Year Book Publishers, Inc., Chicago 1952), vol. 5, p. 271.
[4] Ö. OUCHTERLONY, Acta pathol. microbiol. scand. *26*, 507 (1949).

The same pattern of reaction noted between mitochondria and antimembrane serum was obtained when mitochondria membranes were used as test antigen. It is to be noted that two out of the six antimembrane sera used gave rise to only one precipitation line with mitochondria membranes, and that no reaction at all occurred with dc-sol mitochondria fraction.

When the membranes were allowed to react with an antiserum against dc-sol mitochondria fraction, one or two precipitation lines (depending on the antiserum used in the reaction) appeared; these lines merged with one or two of the several lines obtained in the reaction between the same antiserum and the homologous antigen. In the reaction between the dc-sol mitochondria fraction and its homologous antiserum, these latter two lines were located very close to the well containing the antigen (Figure 2). Since our serological tests were always carried out with antigens at the same protein concentration, the different position of these lines facing the antigen well suggests that the concentration of the antigens in the dc-sol mitochondria fraction producing such a reaction is lower than in the mitochondria membranes.

The latter experiments indicate that mitochondria membranes and dc-sol mitochondria fraction have at least two antigens in common.

The mitochondrial membranes did not react with sera against the microsomal fractions and cell sap (Figure 3).

The dc-ins microsomal fraction sometimes produced a reaction with the antimembrane serum identical to the one described for the membranes. This, however, seems to be due to a contamination of the microsomal fraction with mitochondria membranes. This interpretation is supported by the following observations: (a) Higher protein concentration of microsomal antigens than mitochondria membranes was necessary to obtain such a reaction. (b) When microsomal antigens were tested at the same RNA concentration of mitochondrial membranes, no reaction appeared (RNA in the mitochondria membranes was considered as an indication of microsomal contamination). (c) Our dc-ins microsomal fractions at that protein concentration which reacts with antimembrane serum, appeared to contain some cytochromoxydase activity; we consider this as an indication of contamination with mitochondria membranes.

No rat-serum-like antigens were demonstrated in the typical membrane antigens.

Many precipitation lines were obtained in the reaction between the dc-sol mitochondria fraction and homologus antiserum (Figure 2).

Sometimes, when such antigen was allowed to react with sera produced against the dc-sol microsomal fraction, two precipitation lines appeared (Figure 4). This is presumably due to contamination with microsomes in our mitochondria preparation, as these two lines proved to be identical with two lines produced by microsomes. The dc-sol mitochondrial fraction, when tested against anti-dc-ins microsomal sera, does not show any reaction.

Our results thus show that rat liver mitochondria contain at least three groups of antigens, one belonging to the membranes, one to deoxycholate soluble fraction and one present in both.

There is, however, the possibility that the latter may actually belong to the membrane, although being slowly extracted in 0.3% deoxycholate[5].

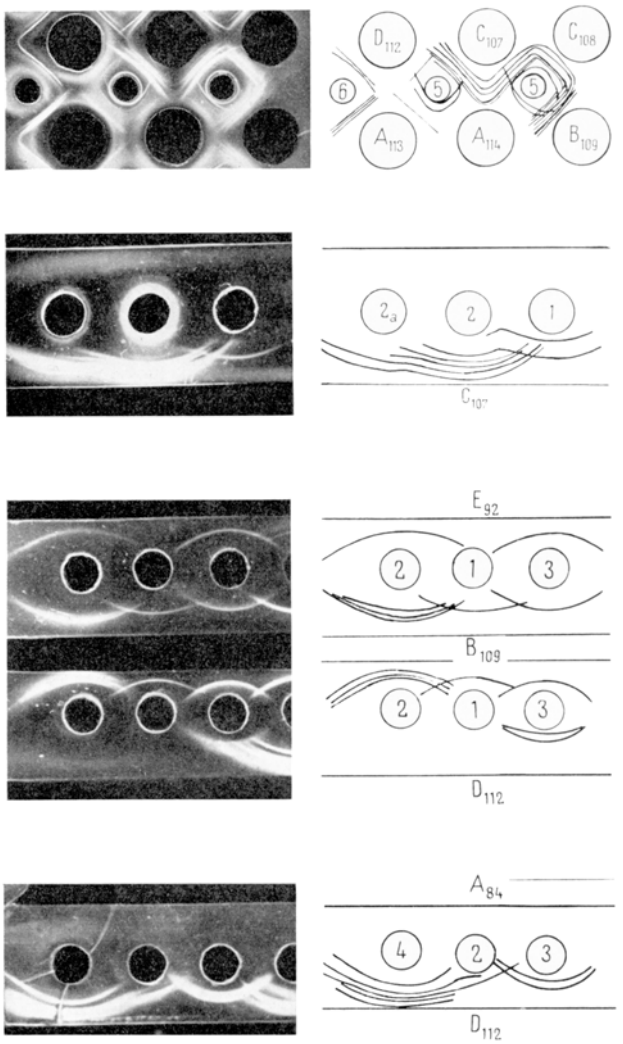


Fig. 1–4. Agar double diffusion precipiting reaction. On the left side the dark field photographs, on the right a diagramatic redrawn of the same. The horizontal lines, marked with capital letters, indicate the position of the trough containing the anti-sera. Antigens were put in the round wells (marked with numbers in the diagrams). A = mitochondrial membranes anti-serum; B = mitochondria anti-serum; C = dc-sol mitochondria fraction anti-serum; D = microsome anti-serum; E = cell sap anti-serum; 1 = mitochondrial membranes; 2 = dc-sol mitochondria; 3 = dc-ins microsomes; 4 = dc-sol microsomes; 5 = mitochondria; 6 = microsomes.
The antigens contained 6 mg protein/ml (in sub-a and sub-b 3 mg and 1.5 mg protein/ml respectively).

*Riassunto.* I mitocondri di fegato di ratto possiedono almeno tre gruppi di antigeni, dei quali uno è esclusivamente legato alle membrane mitocondriali, uno è presente nella frazione solubile in 0,3% desossicolato di sodio ed un altro, che, sebbene principalmente localizzato nelle membrane, è anche dimostrabile nella frazione desossicolato solubile.

V. Mutolo and V. D'Amelio

*Biological Laboratory of the Centro Tumori, Palermo, and Laboratory of Comparative Anatomy, University of Palermo (Italy), July 9, 1962.*

[5] This investigation has been supported by a grant from the Consiglio Nazionale delle Ricerche and the National Institute of Health, U.S. Public Health Service (RG-6211) to the Laboratory of Comparative Anatomy.