the acetoacetate formation by liver slices from suckling rats[7] and since the utilization of acetoacetate by the brain is highest in the suckling period[8]. It appears that brown adipose tissue, of all the organs examined, assumes a special position which could well be related to its heat production, which requires both fatty acids and a sparker, evidently obtained by glycolysis[9].

Zusammenfassung. Die Pyruvatkinaseaktivität wurde im Gehirn und in den Muskeln von Ratten während der Entwicklung bestimmt. Die Aktivität war in Feten höher als in säugenden Jungen und stieg dann wieder an. Kortisonacetat (5 mg · 100 g/Tag, 3 Tage) erhöht die Pyruvatkinaseaktivität am 10. Tag im braunen Fettgewebe und erniedrigt sie in der Leber. Eine hohe Fettdiät, vom 14. Tag an gefüttert, hat denselben Effekt am 30. Tag. Das Muskel- und Gehirnenzym kann man durch hohe Fettdiät oder Kortison nicht verändern.

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Differences Between the Soluble Mitochondrial Proteins in Various Organs of the Rat

Only the liver's soluble mitochondrial proteins have already been studied by numerous workers, using several techniques such as: ultracentrifugation[1], moving boundary electrophoresis[2], paper electrophoresis[3], microelectrophoresis on agar gel[4] and immunoelectrophoresis[5]. But little is known about the soluble proteins of mitochondria isolated from other organs.

The present paper describes the electrophoretic patterns obtained from the extractible proteins of liver, heart and kidney mitochondria. In order to exclude the interference of soluble proteins from other cytoplasmic particles, special care was taken to obtain mitochondrial fractions as pure as possible. To eliminate lysosomes, Triton WR-1339 was administered to the rat[6,7] and microsomal contamination was almost suppressed by washing the mitochondrial pellet and recentrifuging at 5000 *g*.

Male adult rats, of the Wister strain, received an i.v. injection of Triton WR-1339 (Rohm & Haas Co. Philadelphia, Pa.) at a dosage of 200 mg, 4 days before decapitation. Organs from several animals were pooled for all the experiments.

We used differential centrifugation for isolation of the intracellular particles, according to SCHNEIDER and HOGEBOOM[8], with some modifications in order to obtain a better isolation of the mitochondria. The livers were homogenized in 0.25 *M* sucrose and fractionated at 4 °C. As the initial mitochondrial pellet is more or less contaminated with microsomes, this pellet was washed twice by resuspension and sedimentation at 5000 *g* for 20 min[9].

Homogenates of kidney and heart were prepared in the same way as for the liver, only the cardiac muscle required more prolonged homogenization.

In order to liberate the soluble proteins, the mitochondria were placed for 30 min in a 0.1% solution of Triton X-100, which does not affect the structural proteins. After centrifugation at 9000 *g*, the supernatant was collected and the soluble mitochondrial proteins were determined by the biuret method[10].

In order to evaluate the purity of the fraction, enzymic criteria[11] were employed. The fractions were analysed for the total activity of various enzymes. With regard to the microsomal contamination (as determined by glucose-6-phosphatase activity), the mitochondria contained respectively: 0.5, 1.5 and 1.5% for the heart, the liver and the kidney preparations. Without the specific step to eliminate the lysosomal fraction, 20–25% of lysosomal activity (as determined by acid phosphatase activity) was present in the mitochondrial preparation from liver and kidney (data for the heart are not available). For liver and kidney, this contamination falls to approximately 10% after injection of Triton WR-1339 and to 2% for the heart. Inclusion of this small amounts of non-mitochondrial material was considered not to affect significantly the basic values of the electrophoretic pattern of normal mitochondria.

The electrophoretic runs were performed according to the procedure of ORNSTEIN and DAVIS[12] with a *Tris*-HCl buffer (0.01 *M*; pH 8.3). About 20 µl of the sample (0.3–0.4 mg of protein) were analysed and stained either with amidoblack or with coomassie blue[13].

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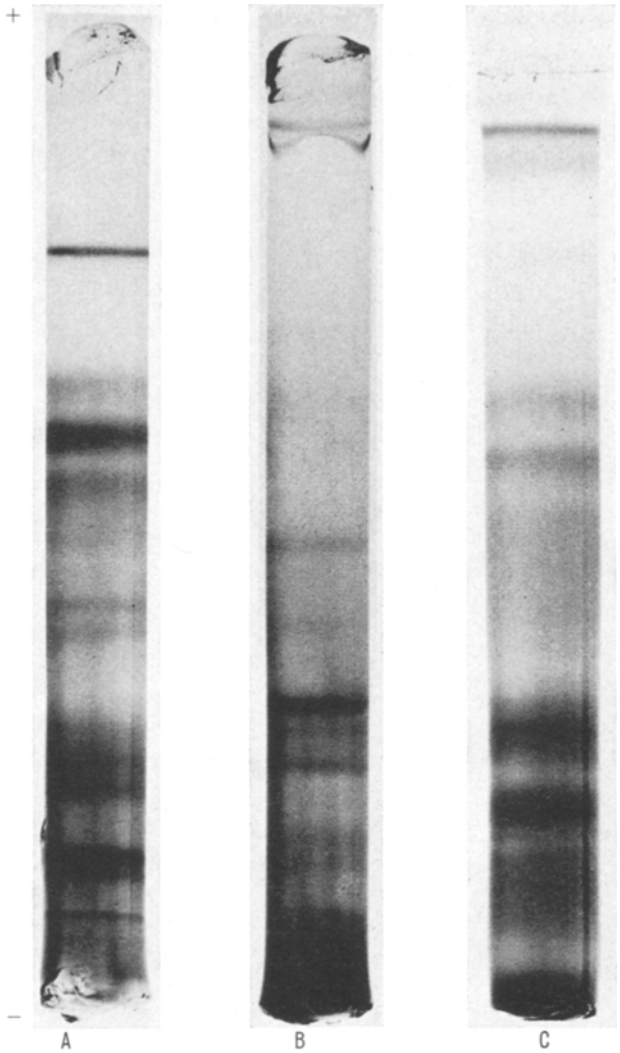

Fig. 1. Disc electrophoresis of the soluble proteins isolated from the mitochondrial fraction of (A) kidney, (B) liver and (c) heart. Several minor bands are too weak to be seen in the photograph.

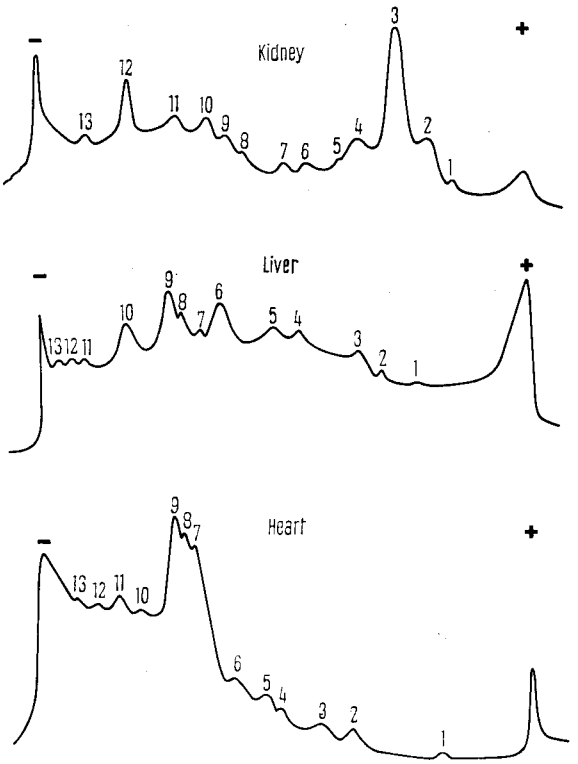

Fig. 2. Densitometric curves of mitochondrial pherograms from kidney, liver and heart. All the patterns have been drawn to the same scale.

The densitometric measurements were performed by means of the photoelectric scanner Chromoscan (Joyce and Loebl, Great Britain).

Figure 1 shows the electrophoretic patterns of soluble mitochondrial proteins of kidney, liver and heart. Diagrams of the densitometric curves are shown in Figure 2. These diagrams have been calculated and drawn to the same distance of migration in order to facilitate their comparison. The main fractions seem to be characteristic for each organ, since the experiments have been repeated several times and the patterns are quite reproducible, if fresh extracts are used. Freeze-dried extracts show a certain denaturation.

Figure 2 shows the soluble mitochondrial proteins of the kidney, which are spread over 13 fractions. Their distribution is more homogenous than that of the liver and the heart. Fraction 3 is the most important and absolutely characteristic for this tissue. At the cathodic end, there is a group of up to 6 distinct bands, from which fraction 12 is the most important.

As for the kidney, the liver also contains 13 bands (Figure 2). Most of the dense fractions are located at the cathodic end, and the number of fractions decreases progressively to the anodic end. Fraction 1 is very faint, as well as fractions 11, 12 and 13. These latter, situated very near the cathodic end, are not always well separated in all the electrophoretic runs.

The soluble proteins of heart mitochondria (Figure 2) are characterised by 3 sharply resolved bands 7, 8 and 9 migrating very close to each other.

These findings indicate a heterogeneity of the mitochondrial proteins. It is known that the quantitative distribution of enzymes varies in different organs[14]. Even in a single tissue, mitochondria show differences in their structure and enzymic activity[15]. It remains to be established whether the heterogeneity observed in the soluble mitochondrial proteins of the 3 tissues also corresponds to different biochemical activities[16].

Résumé. Les phérogrammes obtenus après électrophorèse en acrylamide des protéines mitochondriales solubles du rein, du foie et du cœur, montrent des différences dans ces 3 organes. Le profil électrophorétique est reproductible et caractéristique pour la fraction mitochondriale de ces organes.

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