observed in DNP-treated rat tissues. These processes were inhibited by glucose. Rat mast cells seem to be capable of considerable aerobic glycolytic activity[8]: by placing a heavy demand on ATP required for its transport into the cell, exogenous glucose could prevent ATP, resulting from DNP-stimulated processes, from reaching effective concentration levels at selected mast cell sites important for granule extrusion and histamine release.

Resumen. El 2-4-dinitrofenol es capaz de producir degranulación rápida aunque parcial de mastocitos y liberación de histamina de los tejidos de la rata in vitro. Estos efectos solamente pueden ser demostrados en tejidos mantenidos a baja temperatura antes del tratamiento; son inhibidos por glucosa. La estimulación del consumo de oxígeno no parece ser la causa de los efectos del dinitrofenol.

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Pyruvate Kinase Activity in Brain, Liver and Brown Adipose Tissue and the Effect of Cortisone in Suckling Rats

Pyruvate kinase (E.C. 2.7.1.40) is one of the rate limiting enzymes of glycolysis and hence it is probable that changes in its activity reflect changes in the formation of acetyl CoA from different precursors. In the rat liver activity decreases at birth and increases again when the high fat milk diet is replaced by the solid high carbohydrate laboratory diet[1,2]. In brown adipose tissue postnatal changes are just the opposite to those found in the liver[1] and it is hence difficult to correlate changes found in this tissue with changes in the composition of the diet. It was therefore of interest to examine development of PK activity in other tissues and to ascertain the effect of cortisone administration, which in general speeds up development of the adult enzyme pattern[3] and also inhibits PK activity in the liver of adult rats.

Rats aged 7 days were injected i.m. with 5 mg/100 g body weight cortisone (SPOFA) for 3 days and were sacrificed on the tenth day of life. Pyruvate kinase activity was determined as described previously[1].

It is apparent from the Table that the activity of this enzyme in both brain and muscle changes in a way similar to that found in liver. Activity falls at birth, is low during the suckling period and increases again after weaning. If a high fat diet is fed from the time of weaning (day 14) activity in the liver remains decreased while it is elevated in brown fat, but that in the brain is not affected. For liver this has been reported[2].

Administration of cortisone has an equivocal effect on brain pyruvate kinase, decreases activity in the liver and increases it in brown adipose tissue (Table). Adrenalectomy on day 14 postnatally has no effect on enzyme activity 4 days later in any of the tissues examined.

These results indicate that either cortisone does not directly affect the metabolic patterns of all the tissues examined or else that pyruvate kinase activity in the different organs is represented by different enzymes (isozymes have been demonstrated[4]). We incline towards the first alternative for the following reasons: Gluconeogenesis is accentuated in the suckling period[5] and after cortisone administration[6]. This is the case in the liver and apparently cortisone given to suckling rats further accentuates this process in this organ. Obviously this results in increased glucose formation which in turn could stimulate glycolysis in brown adipose tissue. It can, of course, be argued that a similar change should occur in the brain. Why this is not so is not clear, particularly since cortisone is known to decrease the rate

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Pyruvate kinase activity in muscle (gastrocnemius), brain (cortex), brown adipose tissue and liver of developing rats (μmoles/mg protein per min ± S.E.)

Organ	Fetus	1 day	10 days		30 days	
			0	cortisone	ND[a]	HF[b]
Muscle	15 ± 0.5	9 ± 0.5	15 ± 0.8	15 ± 0.7	30 ± 1.5	
Brain	12 ± 1.1	4 ± 0.3	4.5 ± 0.4	4 ± 0.7	11 ± 0.8	10.5 ± 1.1
Brown fat			11.5 ± 0.6	16.4 ± 0.7	3.4 ± 0.03	5.7 ± 0.4
Liver			3.6 ± 0.2	2.3 ± 0.1	4.2 ± 0.12	3.0 ± 0.1

[a] ND, normal pellet diet. [b] HF, high fat diet (60% margarine, 30% casein, no carbohydrate) fed from day 14.

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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