

mechanism of protein synthesis, a question that might be posed is which ribosomal constituents are responsible for the variation in activity of ribosomes. It has also been observed that the nucleotide composition of the ribosomal RNA does not change significantly throughout the incubation phase, but a consistent variation in the RNA/protein ratio of the ribosomal pellet can be found. The change in the rate of synthesis of mRNA suggests that the messenger RNA content of the ribosome may differ. Such a condition could also lead to a variation of the polysome content of the cell, and these questions are now being investigated¹⁵.

Zusammenfassung. Unter dem Einfluss der Indolessigsäure ist die Aminoacyl-SRNA-Synthese nicht verändert,

dagegen zeigen die Ribosomen eine gesteigerte Fähigkeit von Aminosäure-Inkorporation.

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The Postnatal Development of Hormone Sensitive Lipase in Brown and White Adipose Tissue of the Rat

It has been shown in previous work that in suckling rats the blood level of free fatty acids (FFA) is very high and decreases during starvation (NOVÁK et al.¹). Administration of adrenalin or noradrenalin to such animals does not lead to the rise in the blood FFA level observed in adult rats (SKÁLA et al.²) nor does the level of blood glucose change. Since FFA are mobilized from adipose tissue, and since hormone sensitive lipase seems to be responsible, at least in part, for this mobilization (VAUGHAN et al.³), the activity of this enzyme was determined in rat adipose tissue during postnatal development.

Lipase activity was determined according to VAUGHAN et al.³ in brown and white adipose tissue after homogenization with 10 times its volume of 0.25M KCl, and after incubation of 0.1 ml of this homogenate in 0.9 ml Krebs-Ringer phosphate solution (pH 7.4) with 3% bovine serum albumin (fraction V) for 5–30 min at 37°C. The FFA content at the beginning and end of incubation was determined colorimetrically according to NOVÁK¹. The rate of FFA production was linear for at least 20 min, and hence only values for the 20 min incubation period are given. Brown adipose tissue was collected from between the shoulder blades and samples from two animals were usually pooled. White adipose tissue came from the subcutaneous layer above the knee joint. In new-born rats there is hardly any white adipose tissue present and hence only brown tissue was studied.

It is apparent from Table I that lipase activity is low in both brown and white adipose tissue in the suckling period and rises between day 10 and 18, and that this is not due to changes in fat or fat free dry matter content, since these occur mostly between day 1 and 10 (Table I). Thus at a time when the level of FFA in the blood of fed infant rats decreases and is raised during starvation, lipase activity is seen to increase. In white adipose tissue, lipase activity in adult rats is much lower than in brown adipose tissue.

In view of the fact that the rate of acetoacetate production by rat liver slices has been shown to decrease between day 10 and 18 postnatally, and that the administration of corticosterone before day 10 leads to a premature fall in this rate (HAHN et al.⁴), the effect of corticosterone was also tested. 1 mg corticosterone/100 g body weight/day was injected into rats for 3 days between day 7 and 10 and lipase activity was again examined. An increase in activity was noted in both white and brown adipose tissue (Table II). It was not possible, however, to delay the rise in activity by adrenalectomy performed on day 14 or by feeding a high fat diet from day 14.

¹ M. NOVÁK, P. HAHN, O. KOLDOVSKÝ, and V. MELICHAR, *Physiol. bohemoslov.* 14, 38 (1965).

² J. SKÁLA, P. HAHN, K. VIZEK, O. KOLDOVSKÝ, V. MELICHAR, and M. NOVÁK, *Čs. fysiolog.* 12, 336 (1963).

³ M. VAUGHAN, J. E. BERGER, and D. STEINBERG, *J. biol. Chem.* 239, 401 (1964).

⁴ P. HAHN, Z. DRAHOTA, and O. KOLDOVSKÝ, *Exper.* 20, 625 (1964).

Table I. Development of hormone sensitive lipase activity and tissue composition of white and brown adipose tissue

Age (days)	Fat content (% wet weight) ^a		Fat free dry substance (% wet weight) ^a		Lipase activity (μEq/g/20 min)	
	white	brown	white	brown	white	brown
1	0	14 ± 2.3	7 ± 1.2	18 ± 2.0	—	2.3 ± 0.70
10	65 ± 3.1	40 ± 4.0	8 ± 1.3	11 ± 2.1	3.5 ± 0.68	3.0 ± 0.10
21	62 ± 3.6	40 ± 3.5	9 ± 2.0	14 ± 2.3	8.3 ± 0.14	8.6 ± 0.20
Adult males	48 ± 4.5	55 ± 3.2	16 ± 2.1	14 ± 2.6	5.8 ± 0.25	8.0 ± 0.21

^a Determined by differential weighing after drying to constant weight and fat extraction with petroleum ether. 6 to 10 animals for each group.

Finally, the effect of adrenalin and noradrenalin was examined. Both hormones increased lipolytic activity of white adipose tissue at all ages but were without effect in brown tissue (Table II).

The results presented here show that the greater utilization of fat in the suckling period is not related to greater breakdown of triglycerides in adipose tissue, but that, on the contrary, exogenous fat seems to be the main source of energy and of fat laid down in the body. They also show that, as far as hormone sensitive lipase is concerned, differences between white and brown adipose tissue are apparent only in adult animals and that adrenalin and

noradrenalin are without effect in the latter, which is surprising in view of the finding that brown adipose tissue in new-born animals has been reported to be very responsive to noradrenalin and to be the main 'stove' of the body (DAWKINS⁵). Nevertheless, there are some indications that in new-born rats brown adipose tissue is of special significance. First, it is the only adipose tissue present, and, secondly, lipase activity, if calculated per unit body weight, is greater on day 1 than on day 10, since brown adipose tissue hardly grows after birth. Thirdly, corticosterone is more effective in brown than white immature tissue.

The ubiquitous effect of corticosterone on enzyme activity has again been demonstrated (see HAHN et al⁴). It may be suspected that there is some common factor that starts off the many changes described after steroid administration to infant mammals, though its nature is still a mystery.

Table II. The effect of corticosterone (1 mg/100 g/day for 3 days) in vivo and adrenalin and noradrenalin in vitro on lipase activity in white and brown adipose tissue

Age in days	Treatment	Lipase activity in % of non-treated tissue	
		white	brown
10	corticosterone	155*	360*
10	saline	100	100
10	noradrenalin ^b	144*	136
10	adrenalin	266*	90
21	noradrenalin	366*	100
21	adrenalin	250*	100

* Significant difference against control value of same age ($p < 0.01$ to 0.02). ^b Adipose tissue was incubated for 110 min in Krebs Ringer bicarbonate solution with 3% albumin and a further 10 min with 10γ adrenalin or noradrenalin (see ³). Lipase activity was then determined as described in the text. 6 to 10 animals in each group.

Zusammenfassung. Die Aktivität hormonsensitiver Lipasen im weissen und braunen Fettgewebe der Ratte steigt zwischen dem 10. und 18. Tag nach der Geburt. Corticosteronzufuhr verursacht bei 10tägigen Ratten einen Aktivitätsanstieg. Adrenalin und Noradrenalin haben im braunen Fettgewebe keinen Effekt.

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Über DPNH-Diaphorase und alkalische Phosphatase bei Präimplantationsstadien des Goldhamsters (*Mesocricetus auratus* Waterh.)

Über die Aktivität von verschiedenen Fermenten bei frühen Furchungsstadien von Nichtsäugern, insbesondere von Seeigeln, liegen mehrere Arbeiten vor¹. Dagegen finden sich in der Literatur nur wenige Arbeiten über die histochemisch nachweisbaren Fermentaktivitäten bei Präimplantationsstadien von Säugern²⁻⁴. Wir haben uns daher die Aufgabe gestellt, die Befruchtungs- und Segmentationsstadien bei kleinen Säugern histochemisch zu untersuchen. In der vorliegenden Arbeit berichten wir über die ersten Ergebnisse an den Eiern des Goldhamsters (*Mesocricetus auratus* Waterh.).

Material und Technik. Histochemisch haben wir die Aktivität der DPNH-Diaphorase an 20 Eiern und diejenige der alkalischen Phosphatase an 5 Eiern von insgesamt sechs Goldhamstern untersucht. Nach der Gewinnung der Eier durch Tubenspülung mit Tyrodescher Lösung wurden diese mit einer Pipette in einen speziell konstruierten Färbetrichter⁵ gebracht, in welchem die Reaktion ohne Vorfixierung durchgeführt wurde. Für den Nachweis der DPNH-Diaphorase verwendeten wir als Diazosalz das MTT, für denjenigen der alkalischen Phosphatase das Echtblausalz BB. Nach Beendigung der

Reaktion wurden die Eier mit Formol-Dextran fixiert und auf einen Objektträger übertragen, auf welchem sie mit Glycerin-Gelatine eingeschlossen wurden. Anschliessend wurde der Gehalt der Eier an Reaktionsprodukten unter Verwendung geeigneter Interferenzfilter histophotometrisch untersucht. In jeder Eizelle, respektive Blastomere wurden an 5 bis 9 Stellen die Absorptionswerte bestimmt.

Ergebnisse. (a) DPNH-Diaphorase: In allen 7 untersuchten befruchteten 1-Zellstadien fällt die Reaktion deutlich positiv aus. Der durchschnittliche Absorptionswert ist bedeutend höher als bei den untersuchten 2- und 4-Zellstadien. Wir haben bei sämtlichen Eizellen eine auffallend polare Verteilung des Reaktionsproduktes im

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