gives, therefore, a sensitive measure of the hydrolytic reaction: at 283 nm the extinction change is maximum, and this wavelength was chosen for a quantitative optical test of acylphosphatase activity with benzoylphosphate as substrate. It must be pointed out that this wavelength corresponds to a steep descending zone of the absorption curve: a careful calibration of the spectrophotometer is therefore needed in order to minimize errors. It is however recommended, before starting a series of measurements, to check directly the working conditions with standard solutions of benzoylphosphate and benzoic acid.

Optimal conditions for the test were investigated with the muscle enzyme at 283 nm and 25 °C: the effects of substrate concentration and pH upon enzyme activity are shown in Figure 2. On the basis of these data the test composition for a standard assay of acylphosphatase in a 'split beam' recording spectrophotometer can be the following:

	Volume	Final concentration
Sample cell (light path 0.5 cm)	ml	m*M*
200 m*M* acetate buffer, pH 5.3	0.750	100
15 m*M* Li benzoylphosphate	0.500	5
Enzyme solution	0.010 or more	
Water to	1.500	
Reference cell (light path 0.5 cm)		
200 m*M* acetate buffer, pH 5.3	0.750	100
15 m*M* Li benzoylphosphate	0.300	3
Water to	1.500	

The chart of an assay carried out in this way with 2 amounts of enzyme is shown in Figure 3: for a reaction time of 4 min, corresponding to a hydrolysis extent of 7 and 14%, there is good agreement with apparent zero-order kinetics and a good proportionality between the amount of enzyme and the rate of reaction.

According to the measured extinction change coefficient of 0.630 mM^{-1} cm^{-1} (see Figure 1), in the above test conditions the μmoles of substrate split per minute are obtained by multiplying the ΔE/min by 4.8.

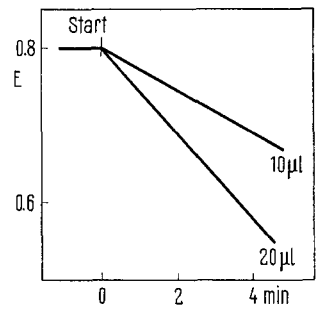

Fig. 3. Continuous record of an optical assay of acylphosphatase activity at 25 °C. The assay conditions are indicated in the text. The final preparation of purified enzyme was opportunely diluted before addition in the spectrophotometer cell: assay starts with enzyme, 10 and 20 μl, as indicated in the figure. 283 nm, 0.5 cm cell, final volume 1.5 ml, chart speed 12.5 mm/min.

Discussion. By the use of an aromatic acylphosphate as substrate the well-known advantages of the continuous optical tests are made available for acylphosphatase estimation.

The described optical test has shown itself accurate, simple and rapid: it is reliable even with crude tissue extracts, provided that not too strong an interference is caused by the presence of materials, like proteins or phenols, absorbing around 280 nm[9].

Riassunto. Viene descritto un metodo ottico continuo per la determinazione dell'acilfosfatasi, usando come substrato il benzoilfosfato di litio. Il metodo si basa sulla differenza di estinzione tra benzoilfosfato e benzoato.

G. RAMPONI, C. TREVES, and A. GUERRITORE

Istituto di Chimica biologica dell'Università di Firenze (Italy), April 20, 1966.

[9] We are indebted to Mr. G. CAMICI for his skilful technical assistance.

The Activities of Citrate Cleavage Enzyme, Acetyl-CoA Synthetase and Lipoprotein Lipase in White and Brown Adipose Tissue and the Liver of the Rat during Development

The activity of citrate cleavage enzyme (E.C. 4.1.3.6), and to a lesser extent also that of acetyl-CoA synthetase (E.C. 6.2.1.1), correlates well with the nutritional state of the animal and available evidence suggests that particularly the former participates in fatty acid (FA) synthesis[1].

In suckling rats FA synthesis in the liver is low[2,3] and indirect evidence indicates that the same applies to adipose tissue[4], which shows low activity of hormone sensitive lipase, thought to be responsible for FA mobilization. It has been suggested that the high fat diet (milk) consumed by infant rats may be responsible for many of these metabolic conditions[5]. If that is true then one would expect low activities of citrate cleavage enzyme and acetyl-CoA synthetase and high activity of lipoprotein lipase, at least in adipose tissue, since this latter enzyme is said to be active in the transfer of lipids into adipose tissue[6].

Citrate cleavage enzyme and acetyl-CoA synthetase activities were assayed in high-speed supernatants (57,000 *g*, MSE centrifuge) of liver and white and brown

[1] M. S. KORNACKER and J. M. LOWENSTEIN, Biochem. J. *94*, 209 (1965).
[2] C. A. VILLEE, in *Physiology of Prematurity* (Ed. J. T. LANMAN, 1957), p. 26, Transactions of 2nd Conference, Josiah Macy Fndtn, New York.
[3] K. K. CARROL, Can. J. Biochem. *42*, 79 (1964).
[4] P. HAHN, Experientia *21*, 634 (1965).
[5] P. HAHN and O. KOLDOVSKÝ, Physiologia bohemoslov. *9*, 172 (1960).
[6] J. PÁV and J. WENKEOVÁ, Nature *185*, 926 (1960).

Table I. Activities of citrate cleavage enzyme (EC 4.1.3.6.) and acetyl-CoA synthetase (E.C. 6.2.1.1) in liver and white and brown adipose tissue of rats of different ages (μM/10 mg protein/40 min)

	Fetus	1st day	2nd day	9th to 18th day	35th day	39th day	3 months old
Citrate cleavage enzyme							
Liver	2.0 ± 0.09	2.1 ± 0.05	0.36 ± 0.09	0.85 ± 0.04	3.66 ± 0.1	5.2 ± 0.1	4.5 ± 0.07
White adipose tissue		1.2 ± 0.06	0.65 ± 0.03	0.41 ± 0.07		0.8 ± 0.04	
Brown adipose tissue	2.2 ± 0.10	2.45 ± 0.08	0.71 ± 0.06	0.65 ± 0.08	1.3 ± 0.06[a]	2.14 ± 0.04	
Acetate thiokinase							
Liver		0.65 ± 0.05	0.45 ± 0.03	0.40 ± 0.02	1.1 ± 0.05[a]	1.7 ± 0.08	
White adipose tissue		0.77 ± 0.04		0.41 ± 0.03	0.4 ± 0.05[a]	0.8 ± 0.03	
Brown adipose tissue		1.22 ± 0.06		0.65 ± 0.04	1.3 ± 0.06[a]	1.58 ± 0.02	

[a] Age: 31 days. 3-8 rats per age group. Fetuses and 1- and 2-day-old rats were pooled but at least 3 samples from different rats were examined.

Table II. Lipoprotein lipase activity in white and brown adipose tissue (μEq/10 mg protein)

	Fetus	Post-natal days			
		2	9	19	32
Brown adipose tissue	13.0 ± 1.0	12.1 ± 1.2	9.9 ± 0.8	6.3 ± 0.9	10.0 ± 1.3
Brown adipose tissue + cortisone[a]			10.2 ± 1.3		
White adipose tissue		16.5 ± 0.8	10.1 ± 0.6	5.8 ± 0.8	9.8 ± 1.4
White adipose tissue + cortisone[a]			11.0 ± 1.2		

[a] Cortisone (1 mg/100 g/day) for 3 days before the experiment. 4–6 samples from 1–8 rats in each group.

adipose tissue according to KORNACKER and LOWENSTEIN[1], and lipoprotein lipase activity of both adipose tissues according to SCHOTZ and GARNFINKEL[7]. Proteins were determined according to LOWRY et al.[8].

Citrate cleavage enzyme activity was found to decrease after birth from higher pre-natal values in both brown adipose tissue and liver. In white adipose tissue (not developed at birth and in fetuses) activity was higher at the end of the 1st post-natal day than later in the suckling period. With weaning (starting on day 15–18) activity increased slowly, attaining adult values after the 30th day, i.e. at the end of weaning (Table I).

Per unit, protein activity was highest in the liver and lowest in white adipose tissue at all ages.

The development of acetyl-CoA synthetase was similar, but changes were less pronounced and activity was much smaller particularly later in life (Table I).

The course of development of lipoprotein lipase activity in brown adipose tissue is exactly the opposite to that described for the above 2 enzymes and a similar picture is also seen for white adipose tissue, except that no determinations could be made pre-natally (Table II). Activity is high at birth and lowest on about day 20, when solid food is started to be consumed[9] and when fat is most easily mobilized on food deprivation[5].

Thus, in the fetus before birth, enzymes of FA synthesis and lipoprotein lipase show relatively high activities, probably because FA synthesis from the main fuel (carbohydrate) is high[2] and because additional fat is laid down in brown adipose tissue. Changes after birth seem to be due to the high fat diet. A surplus of fat is supplied to all tissues and is rapidly laid down in both types of adipose tissue. FA synthesis is suppressed and so is the release of FA from adipose tissue into the circulation, as indicated by the low activity of hormone sensitive lipase[4]. Lipid uptake, on the other hand, is high, since lipoprotein lipase activity is high. In addition FA are evidently directly oxidized in brown adipose tissue to produce heat[10,11].

It may also be pointed out that the fact that activities of hormone sensitive lipase and lipoprotein lipase develop in the opposite directions, and that the former but not the latter can be affected by cortisone administration (Table II), is a further indication that these two enzymes really represent different entities.

Zusammenfassung. Während der Rattenontogenese wurde die Aktivität des Citrat-Cleavage-Enzyms und der Acetyl-CoA-Synthetase in der Leber und im braunen und weissen Fettgewebe bestimmt. In allen Geweben wird die Aktivität vor der Geburt höher als nach der Geburt, wobei die Adultwerte nach dem 30. postnatalen Tag erreicht werden. Die Aktivität der Lipoproteinlipase ist im Säuglingsalter gross und sinkt nach der Entwöhnung ab.

P. HAHN and Z. DRAHOTA

Institute of Physiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia), April 25, 1966.

[7] M. C. SCHOTZ and A. S. GARNFINKEL, Biochim. biophys. Acta *106*, 202 (1965).

[8] O. H. LOWRY, N. J. ROSEBRAUGH, A. L. FARR, and H. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

[9] J. KŘEČEK, J. KŘEČKOVÁ, and H. DLOUHÁ, Physiologia bohemoslov. *9*, 172 (1960).

[10] P. HAHN, J. SKÁLA, K. VÍZEK, and M. NOVÁK, Physiologia bohemoslov. *14*, 546 (1965).

[11] M. J. R. DAWKINS and D. HULL, J. Physiol., Lond. *172*, 216 (1964).