The Effect of the Adrenal Cortex and Diet Composition on the Rate of Acetoacetate Formation by Liver Slices from Infant and Adult Rats

It has been shown previously (DRAHOTA et al.[1]) that during postnatal development of the rat the rate of acetoacetate formation (AA) by rat liver slices increases rapidly after birth and decreases again at the time of weaning, when solid food with a lower fat content than that of milk is first consumed. It is well known that in the rat the rate of AA formation is affected by the adrenal cortex (SCOW and CHERNICK[2]) and by the composition of the diet (e.g. WIELAND and LÖFFLER[3]). It was asked how far these two factors were responsible for the changes observed during postnatal development.

First, infant rats aged 14 days, together with the mother rat, were fed a high-fat isocaloric diet (60% Cal. margarine, FÁBRY[4]) up to the 30th day, and on that day the rate of AA formation by their livers was compared with that of normally fed rats of the same age and of adult rats fed the same high fat diet for 15 days. Slices were incubated as described previously (DRAHOTA et al.[1]) and AA was determined according to WALKER[5]. Animals were killed in the fed state. It is apparent from Table I that feeding the high fat diet to infant rats for 15 days leads to a rate of AA formation that is much greater than feeding the same diet to adult animals. Hence it appears that (a) the change-over from the milk diet to the adult type of diet is in part responsible for the fall in the rate of AA formation at weaning, and (b) that some other factor must also be active, since otherwise it should have been possible to raise the rate of AA formation to the same extent in adult animals.

Corticosterone was applied intramuscularly in a dose of 2 mg/100 g/day for 4 days between the 7th and 10th postnatal day and for the same period of time to adult animals. It is apparent from Table I that application of this hormone considerably depresses the high rate of AA formation in infant rats but has no effect in adult animals, regardless of whether they have been fed or starved prior to the determination of AA. Thus it appears that in infant rats the rate of adrenal cortical hormone production is smaller than later in life, as has been suggested previously (HAHN and KOLDOVSKÝ[6], KOLDOVSKÝ et al.[7]).

Table I. The effect of feeding a high-fat diet for 15 days to rats aged 15 days and to adult animals, and the effect of corticosterone

Treatment	Age	No. of rats	Acetoacetate formed μM/g liver slices	Remarks
Normal diet	30 days	8	2.51 ± 0.09[a]	
High fat	30 days	8	14.04 ± 1.52	
Normal diet	3 months	8	3.5 ± 0.90	
High fat	3 months	8	8.2 ± 1.10	
Saline i.m.	10 days	7	8.44 ± 1.39	
Corticosterone	10 days	7	4.52 ± 0.38	
Saline	6 months	6	12.1 ± 0.82	24 h starvation
Corticosterone	6 months	6	12.2 ± 1.2	24 h starvation
Saline	6 months	6	3.1 ± 0.1	Fed
Corticosterone	6 months	6	3.09 ± 0.02	Fed

Control and experimental animals of the same age were always from two litters equally mixed on the 2nd day after birth. [a] = S.E.

Rats were adrenalectomized on the 15th postnatal day and the rate of AA formation by their livers was studied 4 days later. Adult animals were studied in the same fashion. A 0.9 NaCl solution was offered instead of water. Table II shows that the rate of AA formation by the livers of 19-day old adrenalectomized rats was considerably greater than that of control animals of the same age and the state of satiety had little effect. In adult animals adrenalectomy decreased the rate of AA formation in rats starved for 24 h but raised it in fed animals, in both cases the changes being much smaller than in infancy.

It has been postulated (DRAHOTA et al.[1]) that the high-fat milk diet is responsible for the high rate of AA formation by liver slices from suckling rats. This is suggested by the immediate and rapid postnatal rise in AA formation and the gradual decrease at the time of weaning between the 15th and 30th days, when a low-fat high-carbohydrate diet is first consumed. This is confirmed by the fact that continued feeding of a high fat diet from the 14th day maintains this very high rate of AA formation, while in adult animals the rate is also increased, but to a lesser extent. Hence other factors must play a role; and apparently the adrenal glands are involved, since adrenalectomy on the 15th day has the same effect as feeding a high fat diet, while cortisone depresses the high rate of AA formation in suckling rats.

It therefore appears probable that the particular metabolic situation of the suckling rat (high fat utiliza-

Table II. The effect of adrenalectomy performed 4 days previously on the rate of acetoacetate formation by liver slices

Treatment	Age	No. of rats	Acetoacetate formed μM/g liver/h	Remarks
Sham operated	19 days	5	4.8 ± 1.47[a]	Fed
Sham operated	19 days	5	6.4 ± 1.18	24 h starvation
Adrenalectomized	19 days	6	18.1 ± 0.60	Fed
Adrenalectomized	19 days	15	17.0 ± 0.95	24 h starvation
Sham operated	19 days	13	9.7 ± 1.26	24 h starvation
Sham operated	8 months	6	12.1 ± 0.82	24 h starvation
Adrenalectomized	8 months	6	4.75 ± 0.27	24 h starvation
Sham operated	8 months	6	3.10 ± 0.09	Fed
Adrenalectomized	8 months	6	9.36 ± 1.55	Fed

[a] = S.E.

[1] Z. DRAHOTA, P. HAHN, A. KLEINZELLER, and A. KOSTOLÁNSKÁ, Biochem. J., in press (1964).
[2] R. O. SCOW and S. S. CHERNICK, Recent Progr. Hormone Res. *16*, 497 (1960).
[3] A. WIELAND and G. LÖFFLER, Biochem. Z. *339*, 204 (1963).
[4] P. FÁBRY, Čs. fysiol. *8*, 529 (1959).
[5] P. G. WALKER, Biochem. J. *58*, 699 (1954).
[6] P. HAHN and O. KOLDOVSKÝ, Nature *181*, 847 (1958).
[7] O. KOLDOVSKÝ, F. CHYTIL, and H. MUZYČENKOVA, Exper. *20*, 87 (1964).

tion) and the developing pattern of enzyme activities and endocrine functions are all responsible for the pronounced difference between adult and infant rats.

Finally, it is worth pointing out that cortisone administration to infant rats appears always to speed up development (e.g. pancreatic and intestinal functions, ROKOS et al.[6]) while adrenalectomy slows down development (e.g. β-galactosidase activity in the gut, KOLDOVSKÝ et al.[7]), maintaining high enzyme activity if such is the usual state in the suckling period, or low enzyme activity if that is the normal condition in infancy. A similar phenomenon has been shown in this paper.

Zusammenfassung. Hoher Acetoacetatanteil in Leber junger Ratten wird durch Kortikosteronverabreichung *in vivo* am 10. Tag nach Geburt erniedrigt. Bei erwachsenen Ratten kein Hormoneinfluss. Adrenalektomie (14. Tag nach Geburt) führt zu hoher Produktion (19. Tag). Nur bei erwachsenen, nicht hungernden Ratten ist dies weniger ausgeprägt.

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[6] J. ROKOS, P. HAHN, O. KOLDOVSKÝ, and P. PROCHÁZKA, Physiol. Bohemoslov. *12*, 213 (1963).

Effect of Sulphates on the Intestinal Absorption of Sr-85 in Rats

In our previous experiments, barium sulphate was shown to be an effective means for diminishing the absorption of radioactive strontium from the intestine shortly after exposure in rats and man[1-3]. In further work, the effect of various sulphates and their combinations on the metabolism of orally administered Sr-85 in rats was compared. Several factors which might influence the effectiveness of the treatment were also studied. The details of this work will be published elsewhere.

When different sulphates were given orally in equimolar amounts 10 min after Sr-85 administration, 40 to 60% less of Sr-85 was retained in the femurs of rats receiving sodium, magnesium, calcium and barium sulphates, whereas strontium sulphate reduced the skeletal retention of Sr-85 only by 30% compared with controls.

The effect of individual sulphates and their combinations was compared under the same experimental conditions (Table). There was a significant difference in the retention of Sr-85 between the groups receiving calcium and barium sulphates only, or calcium sulphate with sodium and magnesium sulphates, and those receiving barium and sodium or magnesium sulphates. The addition of excess SO_4^{2-} ions markedly increased the effectiveness of barium sulphate, so that nearly 80% less of Sr-85 was retained in the bones of treated animals in comparison with controls.

Several barium sulphate preparations showed different affinity to Sr-85 *in vitro* as well as *in vivo*. However, when excess SO_4^{2-} ions were added, the proportion of adsorbed Sr-85 increased substantially and all preparations exerted similar effect.

When up to 100 μMoles of carrier strontium were added either to Sr-85 solution or to the suspension of sulphates, only slight differences in the effectiveness of sodium and barium sulphates were observed. The sulphates seem to be equally effective in younger and in older animals.

Barium sulphate alone, given to starved or fed animals, decreased the retention of Sr-85 only in starved rats, while in the fed group it was ineffective. However, following the administration of barium sulphate combined with sodium sulphate, the skeletal retention of Sr-85 was reduced by 90 and 50% in the starved and fed animals respectively.

In other experiments, the effectiveness of various amounts of sulphates given orally in a single dose 10 min after Sr-85 contamination was investigated. The Figure presents such data on a semi-logarithmic plot. The relative decrease of skeletal Sr-85 with the increasing dose of barium sulphate follows a straight line through the whole dose range investigated. Similar dose dependence can be demonstrated in the case of sodium sulphate or its combinations with barium sulphate, but only when increasing the doses up to a certain level. Best results were obtained when 1.6 mMoles of barium and sodium sulphates were given, i.e. 93 and 94% reduction in the skeletal content of Sr-85 in treated rats as compared with controls.

When treatment was delayed, its effect decreased with time. Even the best acting agents, given 80 min after Sr-85 administration to starved rats, lost their effectiveness. However, one single dose given early after contamination was found to be sufficient to decrease substan-

Retention of Sr-85 2 days after oral administration in rats treated with various sulphates and their combinations

No. of rats	Agents[a]	% of dose in femur[b]
12	Controls	1.12 ± 0.34
6	$CaSO_4$	0.79 ± 0.32
6	$BaSo_4$	0.53 ± 0.16
6	$CaSO_4 + Na_2SO_4$	0.47 ± 0.17
6	$CaSO_4 + MgSO_4$	0.53 ± 0.18
6	$BaSO_4 + Na_2SO_4$	0.25 ± 0.13
6	$BaSO_4 + MgSO_4$	0.27 ± 0.18

[a] 0.8 mMol of each sulphate was given orally 10 min after Sr-85 administration to fasted animals. [b] Mean ± standard error of the mean multiplied by t – value for 95% confidence level.

[1] V. VOLF, Nature *184*, 1401 (1959).
[2] V. VOLF, Phys. Med. Biol. *6*, 287 (1961).
[3] V. VOLF, in *Diagnosis and Treatment of Radioactive Poisoning* (International Atomic Energy Agency, Vienna 1963), p. 131.