HEPATIC KETOGENESIS AND KETOLYSIS IN DIFFERENT SPECIES

BY

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Introduction

It is generally agreed that in the animal body the liver is the chief site of ketogenesis (1) (2) (3) (4). Ketolysis, on the other hand, has been claimed to be chiefly an extra hepatic function (3) (4) (5) (6) (7) (8) (9) (10).

Jowett and Quastel (4) reported that rat liver slices were capable of destroying ketone bodies under aerobic conditions, an effect which they found was inhibited by malonic acid. This finding has been confirmed by Edson and Leloir (8) and Stark and Cohen (11).

The fact that rat liver slices are capable of destroying acetoacetic acid raised the question of this effect in other species. The fact that certain species differ in their ability to produce or destroy ketone bodies led us to study the rates of hepatic ketogenesis and ketolysis in the well-fed and starved rat, rabbit, guinea pig and monkey.

Methods

Liver slices from healthy well-fed and starved male white rats, rabbits, guinea pigs and Macaccus Rhesus monkeys were used in a Barcroft-Warburg apparatus. The starvation period was twenty-four hours except in the case of rabbits which were starved for thirty-six hours. The liver slices, 10-20 mgms. of dry weight, were immersed in a Ringer-phosphate buffer solution of pH 7.4, to which was added the substrate previously neutralized to the same pH. The flasks were filled with pure oxygen and shaken for one hour at 38°C.

Sodium butyrate made up to give .01M concentration was used as a substrate for determining the rate of ketogenesis. Sodium acetoacetate, prepared according to Ljungren (12), and freed of methyl alcohol, was used as substrate in determining the rate of ketolysis. Acetoacetic and beta hydroxy butyric acids were determined at the end of the experimental period.

Edson's (13) method of filtrate preparation and acetoacetic and beta hydroxy butyric acid determinations was used with slight modifications. Control experiments, to which no substrate was added, were run in all cases and treated similarly to the non-control experiments. Sodium acetoacetate solutions were freshly prepared on the day previous and the strength of the solution determined in quadruplicate on the day it was used as substrate. The concentration of acetoacetic acid used as substrate was between 0.006-0.008 molar in the different experiments. Liver glycogen determinations, using the combined methods of Good, Kramer and Somogyi (15) and Somogyi(16) were made in order to check the nutritional state of the animals.

Procedure

Four differential manometers and eight flasks were set up to permit measuring the rate of ketogenesis, ketolysis, spontaneous ketogenesis (no substrate added) and oxygen consumption, using liver slices from a single animal. In measuring the rate of ketolysis quadruplicate experiments were employed. Spontaneous ketogenesis and ketogenesis from butyric acid were run in duplicate.

At the end of one hour of shaking at 38°C. the flasks

were removed from the water bath and placed in an ice bath for 20 minutes to insure condensation of volatile products. The liver slices were then removed and washed thoroughly with fine streams of distilled water. The washings and the flask contents were combined and treated with $Ca(OH)_2$ and $CuSO_4$ to give a protein and carbohydrate-free filtrate. Acetoacetic and beta hydroxy butyric acids were determined on these filtrates.

Units

ΔQO_2 = μl of oxygen consumed per mgm. of tissue (dry weight) per hour in the experimental flask (substrate present) over an equal weight of tissue in the control flask (no substrate present).

QAcOAc = μl of CO_2 equivalent to acetoacetic acid formed per mgm. of tissue (dry weight) per hour.

QBOH = μl of CO_2 equivalent to beta hydroxy butyric acid formed per mgm. of tissue (dry weight) per hour.

Qket = Sum of QAcOAc and QBOH

QAcOAc (theor.) = μl of CO_2 equivalent to acetoacetic acid added initially per mgm. of tissue (dry weight).

$-$QAcOAc = differentce between QAcOAc (theor.) and Qketone at end of one hour.

Results

The rate of spontaneous ketogenesis (no substrate added) in starved and well-fed animals is seen in Table I. It is apparent that starvation results in a considerable increase in ketone body formation.

in all four species.

The rate of ketogenesis from butyric acid in starved and well-fed animals is seen in Table II. In all four species there was an apparent higher rate of ketogenesis in the case of the starved animals, although the increase is marked only in the case of the rabbit. This may be related to the fact that the rabbits were starved for 36 hours, whereas the other species were starved 24 hours.

Edson (13) has reported increases in QAcOAc of approximately double the normal with liver slices from starved rats in the presence of butyric acid. Jowett and Quastel (17) on the other hand have reported slight but significant increases in QAcOAc in the case of liver slices from starved guinea pigs in the presence of butyric acid.

It is to be noted that the QBOH values in all instances are approximately equal to the QAcOAc values. This is in confirmation of Jowett and Quastel (18) and emphasizes the need for determining both QBOH and QAcOAc where a measure of ketogenesis is desired.

The QO_2 values for rat and guinea pig liver in the presence of butyrate are in agreement with those previously reported by Cohen (14), Edson (13), and Jowett and Quastel (17) (18).

The rate of hepatic ketolysis in starved and well-fed animals is seen in Table III. It is seen from these data that the well-fed rat, rabbit, and guinea pig have a considerable capacity for destroying acetoacetate other than by conversion to beta hydroxy butyric acid. The percentage destruction in these instances is of the order of 14, 25, and 20, respectively, for the above three species. These values are in reasonable agreement with those of Snapper and Grünbaum (6) for perfused dog liver, which showed about 15% destruction. The -QAcOAc values for rat and guinea pig are

considerably higher than those reported by Edson and Leloir (8) and Quastel and Wheatley (9).

The most striking thing in these data is the inability of liver slices from a well-fed monkey to appreciably destroy acetoacetic acid.

In the case of liver slices from starved animals, there is a marked decrease in ketolytic activity with the rat, rabbit, and guinea pig, while monkey liver shows but a slight decrease. It should be noted, however, that liver slices from starved rats, rabbits, and guinea pigs show a slightly greater capacity for ketolysis in all cases than liver slices from a well-fed monkey.

The ΔQO_2 values for liver slices in the presence of acetoacetate are very low and in two instances are actually positive. The $+\Delta QO_2$ values mean that the rate of oxygen consumption by liver slices in the presence of acetoacetate is less than that of an equal weight of liver slices without any added substrate. Stöhr (19) has reported a decrease in QO_2 values in rat liver and kidney slices in the presence of acetoacetate. Earlier work by Wigglesworth (20) showed an increase in oxygen consumption by liver slices in the presence of acetoacetate. He interpreted this to mean an oxidative destruction of acetoacetate. While Stöhr (19) could not establish a significant difference between the oxygen consumption of liver slices from well-fed and starved rats in the presence of acetoacetate, it would appear from Table III that there is a small increase in ΔQO_2 with liver slices from the starved animals as compared with the normals. The increase appears to be marked only in the case of the starved rat.

Table IV shows the effect of starvation on ketogenesis and ketolysis. The values are the differences between the normal and starved animals. It appears from this table that in the case of the rat and guinea pig there is a greater increase in ketogenesis on starvation in the absence of substrate than in the presence of butyrate. This would imply that liver slices from starved rats and guinea pigs oxidiz e less butyric acid than those of normal animals. The lowered ΔQO_2 (Table II) values in these instances are in keeping with this. It is possible that the rapid production of ketone bodies by spontaneous ketogenesis in these starved animals has an inhibitory effect on butyric acid oxidation. It is to be noted that the liver slices from the starved rabbit on the other hand oxidize more butyric acid than the normal. The ΔQO_2 is also increased in this case (Table II). The difference in the ability of liver slices from starved rabbits, as compared to starved rats and guinea pigs, to oxidize butyric acid may be due to the longer starvation period in the case of the rabbit. It is conceivable that the liver of the rabbit starved for 36 hours has used up a larger portion of its metabolites including fat. This is supported by the lower level of spontaneous ketogenesis of the starved rabbit as compared with the starved rat and guinea pig. (Table I). It follows that the addition of butyric acid to liver slices from the starved rabbit will be more readily oxidized both because of the depletion of other substrates and the smaller concentration of ketone bodies to act as an inhibitor of the butyrat oxidation.

In the case of the monkey, the rate of ketogenesis in the presence of butyrate is of about the same order as the rate of spontaneous ketogenesis in the starved animal. Thus in this

case butyrate is oxidized at about the same rate. The ΔQO_2 values are in support of this (Table II).

The decrease in ketolytic power on starvation is seen in column three of Table IV. It is apparent that with exception of the monkey, the decrease in ketolytic capacity on starvation is of such a magnitude as to more than account for the increase in ketogenesis. It is of interest to note that the greatest decrease in rate of ketolysis with starvation (rabbit) is associated with the greatest increase in ketogenesis from butyric acid. In the case of the monkey, the decrease of ketolysis with starvation is slight as would be expected from the fact that the normal liver has but a very limited ketolytic capacity.

Table V gives glycogen values for the well-fed and starved rat and monkey. It is to be noted that at the time of introduction to the flasks, of the liver slices from the well-fed monkey, the glycogen value is quite high (3.20%). Thus the failure of normal monkey liver to break down acetoacetate cannot be due to a low liver glycogen.

Discussion

Quastel and Wheatley (9) and Edson and Leloir (8) using liver slices from normal animals have reported much lower rates of hepatic ketolysis than those reported here. The rates reported by these workers in the case of normal rat and guinea pig are of the order reported here for starved animals. A possible explanation for the differences resides in the fact that these workers employed a bicarbonate-buffer system in their experiments whereas a phosphate-Ringer buffer system was employed by us. Ciaranfi (21) studying the

metabolism of butyric acid and ketone bodies in guinea pig liver slices reported that in the presence of phosphate-Ringer there is a considerably greater oxidation of butyric acid and breakdown of acetoacetate than in the presence of bicarbonate-Ringer solution. On this basis one might speculate that a phosphorylation mechanism may be involved in hepatic ketolysis.

The failure of liver slices from a well-fed monkey to destroy ketone bodies to any appreciable extent, as compared with the other three species, is difficult to explain on any other basis than a species difference. It is well known that there is a marked difference between primates and the lower species in their susceptibility to a ketosis (22((23) (24). Whether or not this difference in hepatic ketolysis is part of the explanation for the difference in susceptibility to a ketosis between the species is difficult to say. The large amount of work with various techniques showing the extra hepatic tissues, especially the muscles and kidneys (3) (4) (5) (6) (7) (8) (9) (10), to be much more active ketolytically than the liver is very convincing. However, it is conceivable that hepatic ketolysis can keep pace with hepatic ketogenesis, within broad limits in the case of the rat, rabbit, and guinea pig, and very narrow limits in the case of the monkey, and so serve to keep the level of ketonemia low. The lowering of liver glycogen, as by starvation, would result early in a ketonemia in the caseof the monkey, and only late in the case of the other three species, because of the differences in their capacity for hepatic ketolysis.

The relationship between starvation and hepatic ketolysis would seem to implicate liver slycogen as the most important factor. From the data presented here, it would appear that the increase in

hepatic ketogenesis (spontaneous) with starvation is more than accounted for by a failure in hepatic ketolysis. The metabolic relationship between hepatic glycogen and ketolysis, however, is s still obscure. The mechanism of ketolysis, expecially in the kidney, has been studied by Edson and Leloir (8) and Quastel and Wheatley(9). The possible role of the succinate system in the mechanism has been considered by both groups of the above workers but there is no basis as yet for stressing the importance of this system in explaining the ketolytic mechanism. The observed action of malonate on inhibiting the breakdown of acetoacetate (4((8((11) is probably not entirely due to an inhibition of the succinate system. It is to be hoped that further investigations on the intermediate stages of the ketolytic mechanisms will bring out the relationship between glycogen and ketolysis more clearly.

Summary

1. Liver slices from a well-fed monkey have a very low rate of ketolysis as compared with liver slices from well-fed rats, rabbits, and guinea pigs.

2. Liver slices from starved rats, rabbits, and guinea pigs have very low rates of ketolysis, but higher than liver slices from a well-fed monkey. Starvation in the case of the monkey results in a slightly lower rate of ketolysis.

3. The difference between the rates of hepatic ketolysis of the monkey and the other three species is discussed with relation to the differences in susceptibility to ketosis between the primates and the lower species.

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Table I

Spontaneous Ketogenesis (No Substrate)

	QAcOAc	QBOH	Qket
Normal Rat	0.88	1.52	2.40
Starved Rat	1.83	2.19	4.02
Normal Rabbit	0.76	0.62	1.38
Starved Rabbit	1.51	1.02	2.53
Normal Guinea Pig	0.74	0.58	1.28
Starved Guinea Pig	1.43	1.32	2.75
Normal Monkey	1.17	1.05	2.22
Starved Monkey	1.00	2.13	3.13

Table II

Ketogenesis from Butyric Acid (.01M)

	ΔQO_2	QAcOAc	QBOH	Qket
Normal Rat	-4.66	4.10	4.27	8.37
Starved Rat	-3.07	5.10	4.07	9.17
Normal Rabbit	-2.61	2.78	2.81	5.59
Starved Rabbit	-3.76	4.54	3.45	7.99
Normal Guinea Pig	-2.35	1.99	1.82	3.81
Starved Guinea Pig	-1.91	2.14	2.26	4.40
Normal Monkey	-4.57	2.83	3.55	6.38
Starved Monkey	-4.45	3.04	4.40	7.44

Table III

Ketolysis of Acetoacetic Acid (0.0060-0.0080 M)

	$-\Delta Q_{O_2}$	QBOH	-QAcOAc
Normal Rat	+ 0.46	4.01	3.15
Starved Rat	-1.11	7.28	0.80
Normal Rabbit	-0.43	3.88	3.92
Starved Rabbit	-0.65	3.27	0.65
Normal Guinea Pig	+ 0.19	3.65	2.30
Starved Guinea Pig	-0.28	3.75	0.63
Normal Monkey	-0.10	3.07	0.45
Starved Monkey	-0.40	5.43	0.10

Table IV

Effect of Starvation on Ketogenesis and Ketolysis

	Δ Qket (no substrate)	Δ Qket (butyrate)	− QAcOAc
Rat	1.62	0.80	-2.35
Rabbit	1.15	2.40	-3.27
Guinea Pig	1.47	0.59	-1.67
Monkey	0.91	1.06	-0.35

Table V

Glycogen Content of Rat and Monkey Liver

	Normal Rat	Normal Monkey	Starved Rat	Starved Monkey
Per Cent Glycogen:				
immediately*	4.09	5.09	0.55	0.37
later**	1.97	3.20	0.43	0.33

* Immediately on death of animal.

** At time of introduction of slices to flasks--usually 45-60 minutes after death of animal.

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