CCXXXII. RESPIRATION AND KETOGENESIS IN THE "CHOLESTEROL" FATTY LIVER.

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THE respiration of tissue slices cut from normal and fatty livers has been investigated by Welch *et al.* [1935], who found that oxygen consumption decreased regularly with increasing fat content. The fatty livers were produced by feeding on a diet low in choline; and these workers were concerned with the effect of choline on the oxygen consumption. Best *et al.* [1936] consider that the diminished oxygen uptake cannot be attributed wholly to dilution of active protoplasm with fat.

We have studied respiration and ketogenesis in the livers of a small number of rats and guinea-pigs which were fed on various diets adequate with respect to choline, but containing 2% of cholesterol. Since some of the livers showed extensive fatty infiltration, it was thought that this change would be reflected in the respiratory and fatty acid metabolism.

METHODS.

The composition of the diets, the methods of fat estimation, the effects on growth and the pathological appearances are described in the preceding paper [Cook, 1936]. Respiration and ketone-body formation were investigated by means of the tissue slice technique.

Conical Warburg cups with one side-bulb were used. The liver slices (about 20 mg. dry weight) were immersed in 3.0 ml. phosphate saline [Krebs, 1933], pH 7.4, and shaken under an oxygen atmosphere for 2 hours at 37.5°, during which the oxygen consumption was measured. After removal of the slices and the caustic alkali acetoacetic (β -ketonic) acid was determined manometrically by the aniline citrate method [Edson, 1935].

The metabolism is expressed by the usual quotients, Q_{O_2} and Q_{Acac} , calculated from dry weights of tissue.

EXPERIMENTAL RESULTS.

The data concerning the animals, the diets, liver weights and percentages of ether-extractable lipins (liver "fat") are given in Table I. Respiration and ketogenesis of control and of cholesterol-fed animals were examined both in the absence of added substrate and in the presence of sodium butyrate, the initial concentration of which was 0.01 *M* (Table II). The serial numbers of experiments in Table II correspond to those in Table I.

DISCUSSION.

The results show the following facts.

1. Rats which received the stock diet (Exp. 1) and the fat-free diet failed to develop fatty infiltration in spite of cholesterol feeding. The livers of these animals gave a normal respiration in absence of added substrate and the usual increase

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Table I. *Liver changes in rats and guinea-pigs fed on diets containing 2% of cholesterol.*

No. of exp.	Diet	Days on diet	Wt. of animal g.	Sex	Wt. of liver g.	Naked-eye appearance of liver
A. Rats.						
1	Stock diet	20	378	♂	17.6	Normal
	Stock diet + cholesterol		382		18.3	”
2	Fat-free	131	284	♂	12	Normal
	Fat-free + cholesterol		265		11.3	”
3	Normal synthetic	41	190	♂	9.7	Normal
	Normal synthetic + cholesterol		132		7.8	Fatty
4	Normal synthetic	107	302	♀	12.4	Normal
	Normal synthetic + cholesterol		354		12.8	Fatty
5	Normal synthetic	117	274	♂	11.5	Normal
	Normal synthetic + cholesterol		237		12.6	Fatty
B. Guinea-pigs.						
6	Stock diet + cholesterol	15	265	♂	13	Normal
7	Stock diet	40	445	♂	16.5	Normal
	Stock diet + cholesterol		409		20.6	Fatty
8	Stock diet	57	367	♂	14.7	Normal
	Stock diet + cholesterol		262		20.7	Fatty

Table II. *Respiration and ketogenesis in liver slices of the same animals.*

No. of exp.	Substrate	Controls			Cholesterol-fed animals		
		Q_{O_2}	Q_{Acac}	Liver "fat" %	Q_{O_2}	Q_{Acac}	Liver "fat" %
A. Rats.							
1	Nil	-10.1	0.97	2.50	-10.2	0.77	2.63
	Butyrate	-14.9	4.88	—	-14.8	3.65	—
2	(a) Nil	-12.3	0.23	1.41	-13.7	0.57	1.33
	Butyrate	-16.3	2.13	—	-16.4	2.75	—
	(b) Nil	-14.2	0.46	0.23	-13.2	0.44	0.54
	Butyrate	-17.5	3.82	—	-16.2	2.62	—
3	Nil	-12.3	1.23	4.43	-12.4	1.01	8.25
	Butyrate	-14.3	4.54	—	-13.2	4.72	—
	Glucose, 0.01 <i>M</i>	-12.9	1.26	—	-12.3	0.98	—
4	Nil	-12.5	0.87	1.11	-9.5	1.07	12.5
	Butyrate	-14.8	3.01	—	-10.6	2.89	—
5	Nil	-13.4	0.72	3.6	-9.4	1.31	12.7
	Butyrate	-15.5	3.93	—	-10.7	3.18	—
B. Guinea-pigs.							
6	Nil	—	—	—	-6.4	0.46	2.39
		—	—	—	-6.8	0.43	—
	Butyrate	—	—	—	-9.6	2.21	—
		—	—	—	-9.8	2.12	—
7	Nil	-5.6	0.08	2.46	-5.3	0.47	12.6
		-5.8	0.11	—	-5.5	0.47	—
	Butyrate	-9.8	2.48	—	-8.8	1.95	—
		-9.6	2.26	—	-8.5	1.78	—
8	Nil	-7.1	0.49	2.92	-7.0	0.07	6.70
	Butyrate	-10.1	1.96	—	-9.9	1.03	—

of oxygen uptake and ketone-body formation in presence of butyrate. The guinea-pigs which were killed after 15 days on the stock diet plus cholesterol also presented a normal metabolism (Exp. 6).

2. Guinea-pigs kept on the cholesterol-containing diet for a longer period exhibited no significant change in respiration, but the ketone-body formation in presence of butyrate was less than that of the controls (Exps. 7 and 8).

3. The liver tissue of rats fed on the normal synthetic diet plus cholesterol for a long period (Exps. 4 and 5) had a significantly lower respiration than the controls both in presence and absence of added butyrate. The values of Q_{Acac} show no significant differences except in Exp. 5. In Exp. 3 where the time was shorter and the fat deposition less the diminished oxygen uptake was not observed.

4. The "spontaneous" ketogenesis (i.e. in absence of added fatty acid) found in the cholesterol fatty liver is not of such a high order as that which occurs in livers of rats starved 24 hours. In such animals the value of Q_{Acac} is 2 to 2.5 or even higher.

The metabolic quotients were recalculated on the basis of "fat-free" dry weights, but the figures were not sufficiently numerous to permit an analysis that would prove or disprove the view that the lowering of respiration is caused by dilution of active tissue with fat. In general it may be stated, however, that there are no great changes in respiration or ketone-body formation in the fatty livers which we have examined.

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