of virus in the liver, pathologic lesions in other organs as well as in the pancreas, and a uniformly fatal outcome. 2. A lethal infection was prevented by neutralization of the virus or passive immunization of adult mice with specific antiserum. 3. The LD_{50} for adult mice at 4°C was approximately ten times that for suckling mice at 25°C. 4. It was demonstrated that to initiate a fatal infection exposure to cold had to be continuous and begun within a few days after virus inoculation.

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Effects of Short Chain Fatty Acids on Hepatic Acetate Metabolism in Cold Exposure and Fasting.* (22731)

E. J. MASORO AND SYLVIA S. PANAGOS†

Department of Physiology, Tufts University School of Medicine, Boston, Mass.

We reported that liver slices prepared from rats fasted for a period of 24 hours at an environmental temperature of 0-2°C ("cold-fasted" rats) showed severe depression in oxidation of acetate-1-C^{14} to $C^{14}O_2$(1). This defect in acetate oxidation could be restored to normal by addition of glucose(1) or pyruvate(2) to the incubation medium. The evidence suggested that this effect of glucose and pyruvate must be related to a metabolic event prior to entry into the Krebs cycle because succinate was much less effective than pyruvate and glucose in reversing this metabolic defect while citrate and α-ketoglutarate were without effect at all.

Since phases of carbohydrate metabolism other than the Krebs cycle are involved, it is important to know whether acetate oxidation is specifically linked to carbohydrate metabolism or whether other substances can serve as energy sources capable of reversing the block in acetate oxidation imposed by cold exposure and fasting. The latter possibility was tested by adding short chain fatty acids to the incubation medium. The results of these experiments are reported below.

Methods. Adult male rats of Wistar strain were allowed to eat, *ad libitum* for 7 to 10 days, a diet composed of 25 g casein, 10 g fat, 51 g dextrose, 3 g salt mix(3), 5 g cellulose, 6 g Brewer's yeast, 0.04 g cod liver oil concentrate and 0.01 g α-tocopherol. Following dietary stabilization the rats were either given the same diet, *ad libitum*, at 25°C or were fasted for 24 hours at 0-2°C. The animals were killed by blow on head. The liver was rapidly excised and placed in a beaker containing ice-cold Krebs-Henseleit bicarbonate buffer solution(4). Tissue slices were prepared free-hand with razor blade and these slices were collected in petri dish containing ice-cold buffer. The liver slices were blotted free of excess solution on filter paper. Approximately 250 mg of tissue were weighed out and then transferred to specially designed

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† Predoctoral Fellow of National Science Foundation.

incubation flasks(5). All incubations were carried on in duplicate flasks. The main compartment of the incubation flasks contained 250 mg of liver slices, 4.5 ml of Krebs-Henseleit bicarbonate buffer and 0.5 ml of the solution containing the acetate-1-C^{14} as substrate, at concentration in all cases of 1 x 10^{-3} M. When unlabeled substrates were also present, they were neutralized to pH 7.4 with sodium hydroxide and dissolved in the buffer. The system was incubated for 3 hours at 37.5°C with continuous shaking. Total lipids (fatty acids and unsaponifiable materials) were extracted from liver slices by the method of Baruch and Chaikoff(5). An aliquot of the chloroform extract (containing the total lipids) was used for determination of cholesterol digitonide using the method of Sperry and Webb(6). The total C^{14} content of fatty acids was calculated by subtracting the C^{14} content of the cholesterol digitonide from the C^{14} content of the total lipid fraction. The C^{14} content of the lipids and the cholesterol digitonide was determined by methods already described(7). The $C^{14}O_2$ formed during the incubation period was collected by the method of Chernick *et al.*(8) and counted in the form of $BaC^{14}O_3$ by the method of Entenman *et al.*(9). The acetate-C^{14} content was determined by methods described in an earlier paper(7). The unlabeled short chain fatty acids were purchased from commercial sources and were of the highest purity obtainable. Each was further purified by fractional distillation; a Widmer fractionating column was used in these distillations.

Results. The term "control" rat designates rats allowed to eat *ad libitum* at 25°C while "cold-fasted" rat describes rats fasted at 0-2°C for 24 hours.

Liver slices from control rats oxidized 34 ± 0.7% of the acetate-1-C^{14} to CO_2, converted 6.7 ± 0.84% to fatty acids and 0.5 ± 0.07% to cholesterol (Table I). In the case of experiments with control rats, the liver slices were prepared from the same group of rats that were used for the "cold-fasted" experiments and the slices were incubated at the same time and under identical conditions as those of the "cold-fasted" rats to be described below.

TABLE I. Acetate Metabolism by Liver Slices from Control Rats.

No. of rats	% conversion* of acetate-1-C^{14} to CO_2	Fatty acids	Cholesterol
15	34 ± .7†	6.7 ± .84	.5 ± .07

* % of acetate-1-C^{14} converted to CO_2, fatty acids and cholesterol is calculated for a system consisting of 500 mg of liver slices in 10 ml of buffer solution containing 1 × 10^{-3}M sodium acetate-1-C^{14}; the incubation flasks were shaken in water bath at 37.5°C for 3 hr.

† Stand. dev. of mean.

The effects that certain short chain fatty acids have on hepatic acetate metabolism of the "cold-fasted" rats are recorded in Table II. Liver slices from the same rat were incubated with and without the further addition of unlabeled substrate in concentrations noted in this table.

Propionate was added to the incubation medium at 4 x 10^{-3} or 8 x 10^{-3} M concentrations; at neither concentration did it restore the capacity of these slices to oxidize acetate-1-C^{14} to $C^{14}O_2$ but rather further reduced the extent of this conversion. Propionate had little or no stimulating effect on the lipogenic and cholesterogenic activity of these tissues.

Addition of unlabeled butyrate also further depressed acetate oxidation of the "cold-fasted" livers rather than stimulating it, and as with propionate there was no effect on the ability to convert acetate to lipids.

Since caproate is an even-numbered short chain fatty acid, it might be expected to have an action similar to butyrate. Such was found to be the case.

It was shown by Atchley(10) that isobutyrate can be converted to propionate by mammalian mitochondrial preparations; we therefore might expect that isobutyrate would behave in a manner similar to propionate. This proved to be true.

Acetoacetate at 4 x 10^{-3} M and 8 x 10^{-3} M concentrations not only did not stimulate acetate lipogenesis and cholesterogenesis, but, unlike the short-chain fatty acids, had no effect on $C^{14}O_2$ production as well.

Discussion. The fact that butyrate and caproate failed to reverse the block in acetate

TABLE II. Acetate Metabolism by Liver Slices from "Cold-Fasted" Rats.

No. of rats	Unlabeled substrate: Compound	Conc. × 10^{-3}M	% conversion* of acetate-1-C^{14} to: CO_2	Fatty acids	Cholesterol
4	None	0	8 ± 1.7†	.1 ± .00	.1 ± .04
	Propionate	4	2 ± .6	.1 ± .00	.0 ± .03
4	None	0	8 ± 1.7	.1 ± .00	.1 ± .04
	Propionate	8	1 ± .4	.1 ± .03	.0 ± .03
2	None	0	14 ± 1.0	.1 ± .02	.0 ± .03
	Butyrate	4	4 ± .5	.1 ± .02	.0 ± .02
9	None	0	9 ± .7	.1 ± .00	.0 ± .00
	Isobutyrate	4	3 ± .7	.1 ± .00	.0 ± .00
4	None	0	12 ± 2.9	.1 ± .00	.1 ± .00
	Caproate	4	5 ± .7	.1 ± .07	.1 ± .07
2	None	0	16 ± 4.5	.1 ± .00	.1 ± .07
	Acetoacetate	4	16 ± 4.2	.1 ± .00	.0 ± .00
2	None	0	16 ± 4.5	.1 ± .00	.1 ± .07
	Acetoacetate	8	13 ± 2.6	.1 ± .00	.0 ± .00

* Refer to footnote, Table I. † Stand. dev. of mean.

oxidation by the "cold-fasted" liver strongly suggests that fatty acid metabolism is not linked with acetate oxidation in the way that glucose and pyruvate metabolism are. Since it was found that butyrate is oxidized by the "cold-fasted" liver at high rates (unpublished), it is apparent that merely the lack of an adequate energy source is not the factor in the "cold-fasted" liver responsible for the low rate of acetate oxidation.

We found it particularly surprising that propionate failed to stimulate acetate oxidation in "cold-fasted" livers since it has long been known that propionate is a glucogenic compound capable of forming considerable amounts of glucose and pyruvate. However, it has recently been reported that propionate does not directly form a carbohydrate intermediate but rather that it enters the carbohydrate schema via the Krebs cycle (by forming succinate) (11). We have already shown that Krebs cycle intermediates (2) are not efficient in reversing the block in acetate oxidation of hepatic tissue from "cold-fasted" rats. We have also demonstrated (2) that relatively large amounts of pyruvate and glucose must be metabolized to reverse this block in acetate oxidation; it is therefore probable that neither Krebs cycle intermediates nor propionate are capable of forming sufficient quantities of carbohydrates and carbohydrate intermediates to effect acetate metabolism appreciably.

In each case there was a reduction in $C^{14}O_2$ formation in the presence of short chain fatty acids; a fact which suggested that those compounds may have an inhibitory action on acetate metabolism. This point has been the subject of an extensive investigation and will be considered elsewhere.

Summary and conclusions. Butyrate, isobutyrate, propionate, caproate, and acetoacetate failed to reverse the block in acetate-1-C^{14} oxidation found in liver slices from rats fasted at 0-2°C for 24 hours. It appears that the ability of glucose and pyruvate to increase the acetate oxidation activity of these liver slices is related to a specific step in carbohydrate metabolism rather than solely to the fact that carbohydrates are excellent sources of energy.

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Blood Levels of 17-Hydroxycorticosteroids in Hyperthermic Dogs.* (22732)

GEORGE BARLOW, HELMER P. AGERSBORG, JR., AND HARMON E. KEYS
(Introduced by Richard R. Overman)

Clinical Physiology Laboratories, University of Tennessee College of Medicine, Memphis

That stress produces an elevation in the level of 17-hydroxycorticosteroids has been indicated by a number of publications(1-5). These observations have ranged from noting the decreased quantity of cholesterol present in the adrenal glands of animals subjected to hemorrhage, to direct measurements of plasma 17-hydroxycorticosteroids in animals and man subjected to surgical, bacterial pyrogen, E.C.T., and whole body radiation procedures. Recently Eik-Nes has reported by abstract, that dogs given an intravenous pyrogenic dose of bacterial mucopolysaccharides exhibited prolonged elevations in plasma 17-hydroxycorticosteroids. The adrenal cortical hormone(s) are known to exert considerable influence not only on the bodily economy of fluids and electrolytes, but also on the partition of these substances. Overman *et al.*(6) have shown that in man and the monkey, marked alterations in Na and K distribution result from prolonged exposure to a febrile condition having its origin in the malarious state. It was suggested that this is indicative of an increased level of certain adrenal cortical hormones in the body.

Since little of a correlative nature is known concerning the stressful hyperpyrexic state, efforts have been made in this laboratory to understand the aberrant physiology of fever. The report of one facet of that investigation —concerning alterations in plasma concentrations of 17-hydroxycorticosteroids, of Na and of K—is the principal concern of this communication.

Materials and methods. Twenty-four healthy mongrel dogs of both sexes and weighing between 10 and 22 kg were subjected to exogenous hyperthermia. Before the animals were placed in the hypertherm cabinet but *after* the administration of intraperitoneal pentobarbital sodium (1 ml of 7% solution/5 lbs. body weight) and after placing of the arterial cannulae, control determinations were made of plasma and R.B.C. electrolytes, rectal temperature and plasma steroid concentrations. Na and K were determined by flame photometry. Rectal temperatures were taken 4 inches proximal to the anus using a thermistor probe. Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels(7). The dogs were exposed to an ambient temperature of 50°C, generated by shielded infrared heat lamps, for periods sufficient to provoke a rise in rectal temperature to 42.5°C. This temperature was maintained for one hour. All blood samples were obtained from a cannulated carotid artery and were heparinized. Ten dogs, treated in a manner similar to that of the fever experiments with regard to anesthesia, minor surgery and elapsed experimental time, were utilized as controls. The survival of experimental animals was found to be less than 10% 24 hours after heating.

Results. The values obtained from the analysis of plasma for 17-hydroxycorticosteroids are presented in Table I. It will be noted

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