Ethanol-1-C^{14} and Acetate-1-C^{14} Incorporation into Lipid Fractions in the Mouse. (25808)

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There is general agreement that the metabolism of ethyl alcohol proceeds through acetaldehyde to enter the acetate pool. From this point there is participation in all reactions involving the remaining 2-carbon fragment, such as fatty acid and cholesterol synthesis and oxidation *via* the tri-carboxylic acid cycle. Schulman *et al.*(1) have found that the protein, glycogen, fatty acids and cholesterol from rats given a single dose of radioactive alcohol contained 2 to 3 times the labelled carbon in these same constituents from rats receiving C^{14} labelled acetate. When report appeared, we had begun similar experiments though of somewhat different design. Since our results differed somewhat from those of Schulman, we considered it worthwhile to pursue our studies further. We found that tissue fatty acids, non-saponifiable fraction, and phospholipids take up almost equal amounts of C^{14} after either radioactive alcohol or acetate administration.

Methods. In one set of experiments, pairs of male C-57 B mice were matched in body weight and placed in separate cages where drinking water contained approximately one μc/ml of ethanol-1-C^{14} or of acetate-1-C^{14}. When solutions prepared from commercially available ethanol-1-C^{14} and acetate-1-C^{14} were not of equal activity, as determined by Packard Tri-Carb scintillation counter, appropriate dilutions were made until the same activity level was measured in each. Non-radioactive acetate or ethanol was added so that final concentration of additives was 0.2 M. The animals could not tolerate a higher concentration of acetate without loss of weight. To ethanol-water solution was also added 0.2 M NaCl to match the sodium concentration in the acetate solution as closely as possible. Mice remained on this regime with *ad lib.* feeding of Purina Rat Chow for 5 days, then, 12 hours before autopsy, the remaining water containing radioactive constituents was measured and replaced by fresh water. This time delay before autopsy was chosen to allow complete incorporation of imbibed alcohol and acetate into the tissues. When different amounts of water were drunk by the 2 mice, appropriate correction factor was applied to final result. In other experiments, radioactive solutions prepared in same manner as drinking water were injected in amounts of one ml, 3 times daily. These mice were provided with fresh drinking water and laboratory chow *ad lib.* Injections were carried on for 5 days, the last injection occurring 12 hours before autopsy. At autopsy, the gut, liver, brain, and a piece of genital and kidney fat were removed from each animal and each homogenized separately in a 2:1 chloroform-methanol mixture (1 g tissue/20 ml solvent). Homogenized mixtures were allowed to stand overnight, then centrifuged to remove cellular debris. After evaporation of supernatant solution, the dried material was again taken up in chloroform-methanol, centrifuged again, and evaporated to dryness. The residue was dissolved in 3 ml of petroleum ether and phospholipids were precipitated according to the method of Sinclair and Dolan(2). After overnight in refrigerator, the supernatant was decanted and saved for further treatment, while the precipitate was redissolved in petroleum ether and again reprecipitated. The precipitate was again dissolved, plated on tared planchets, and specific activity was determined. The supernatant from the first precipitate was evaporated to a small volume and saponified in 30% alcoholic potassium hydroxide. The saponified mixture was extracted with petroleum ether, and the extracts pooled, evaporated to dryness, redissolved in petroleum ether, and plated as the non-saponifiable fraction. To the residue after extraction, was added a small amount of water and the mixture was acidi-

* Deceased Sept. 19, 1959.

TABLE I. Specific Activity of Lipid Fractions from Various Organs from a Pair of Mice Ingesting Ethanol-1-C^{14} or Acetate-1-C^{14} in Drinking Water for 5 Days.

	Ethanol-1-C^{14}	Acetate-1-C^{14}
	cpm/mg	
Phospholipids		
Liver	1136	1003
Gut	1183	1048
Brain	450	474
Non-saponifiable fraction		
Liver	1041	1462
Gut	1031	861
Brain	117	93
Fatty acids		
Liver	1296	1531
Gut	719	618
Brain	253	245
Fat	154	201

fied. Precipitated fatty acids were collected on filter paper, washed, dried, dissolved in acetone, and plated. All plating was done on aluminum or copper planchets, and radioactivity was measured in Nuclear-Chicago gas flow counter, Model 183B. Appropriate correction for self absorption was made by means of previously determined curve. In all, 7 pairs of animals were used, 5 pairs receiving the radioactive compounds in drinking water, and 2 pairs by injection.

Results. The labelling pattern of a typical experiment is shown in Table I. As may be expected, liver and gut showed a high degree of labelling in fractions isolated, while brain and fat fractions were not as active. In general, there was no difference in ratio of carbon uptake from these 2 compounds whether radioactive agents were administered by mouth or by injection. A difference in absolute counts/minute/mg was observed, however, with fractions in injected animals showing greater specific activity. A great range in values of ethanol/acetate uptake ratios was observed as might be expected in this type of experiment. The extremes varied from 0.32 to 6.28. When ratios in individual tissues were averaged, however, values approaching unity were obtained with one exception (Table II). The non-saponifiable fraction from the gut showed ethanol/acetate uptake ratios of greatest variation, the average value being nearly 3. In all other cases, ethanol/acetate uptake ratios fell closer to 1 than to values of 2-3 obtained by Schulman *et al.*(1). Dissimilar experimental plans such as species differences (rat *vs.* mice) and the effect of one injection *vs.* chronic administration may account for the difference in results.

Discussion. Bernhard(3) was the first to show that ethanol is an effective acetylating agent and therefore enters the acetate pool in the organism. Bloch and Rittenberg(4) further demonstrated that deutero-ethanol, when administered in diet of rats, gives rise to deutero-cholesterol. Since that time, there has been little work on the nature of entry of ethanol into the acetate pool, although the definition of "active acetate" has changed a great deal during the intervening period. At present, there is incomplete knowledge of the specific enzyme by which acetaldehyde is oxidized to acetate, although most recent evidence points to the diphosphopyridine nucleotide enzyme, aldehyde dehydrogenase as the chief agent(5).

If ethanol and acetate are to label equally their metabolic products, it is necessary that they enter the metabolic pool at approximately equal rates, as seems to be the case here. It is also necessary that there be no major accessory metabolic pathways which selectively exclude either compound, *e.g.*, a branching pathway at the acetaldehyde stage of ethanol oxidation to yield such compounds as acetoin or 5-carbon sugars. Since tagged alcohol and acetate in our experiments seemed equally able to label various lipid compounds,

TABLE II. Average Ratios of Specific Activities of Lipid Fractions from Pairs of Mice to Which Ethanol-1-C^{14} or Acetate-1-C^{14} Had Been Administered. Figures indicate avg of 7 ratios of ethanol-mouse fractions/acetate-mouse fractions, while figures in parentheses denote range.

	Phospholipid	Non-saponifiable fraction	Fatty acids
	Eth/Ac		
Liver	1.39 (1.03-1.91)	1.34 (.71-2.33)	1.11 (.66-1.94)
Gut	1.13 (.86-1.59)	2.97 (.78-6.28)	1.18 (.43-1.94)
Brain	.94 (.64-1.31)	1.14 (.71-2.10)	.76 (.53-1.03)
Fat			1.15 (.32-2.00)

accessory and selective pathways, if existing at all, would seem to be of minor significance in relation to the amount metabolized along the conventional pathways.

Summary. Pairs of mice were administered either by mouth or by injection equal amounts of ethanol-1-C^{14} or acetate-1-C^{14}. The phospholipid, non-saponifiable fraction, and fatty acid fractions of liver, gut, brain, and fat were isolated and specific activities of these fractions were compared. Ethanol-1-C^{14} and acetate-1-C^{14} contributed approximately equal labelling to tissues investigated.

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Inhibition of Neutrophil Mobilization by Colchicine.* (25809)

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Colchicine is a powerful mitotic poison reported to cause extensive bone marrow damage, arresting division of both developing leukocytes and immature erythrocytes(1). In contrast to the depressive effects upon bone marrow, colchicine injection provokes a short-lived leukopenia followed by a marked increase in numbers of circulating granulocytes in the blood of dogs, rabbits, and mice(2,3). The relationship between bone marrow inhibition and peripheral blood leukocytosis is a puzzling one and remains to be elucidated. Therefore, it was felt that it would be of interest to investigate another parameter of this problem, namely ability of the animal to mobilize neutrophils following colchicine treatment.

Methods. Male rats of the Sprague-Dawley strain and weighing 200-240 g were used. All experimental rats received a single, subcutaneous injection of 0.2 mg colchicine (Lot 920 LPA, S. B. Penick Co.) dissolved in 0.2 ml of 0.9% saline; control animals received 0.2 ml of 0.9% saline. Immediately following subcutaneous injection and at 24, 48, 72 and 96 hours thereafter, groups of 6 animals were challenged by an intraperitoneal injection of 1 μg Piromen,† a Pseudomonas polysaccharide complex (Baxter Lab.) dissolved in 10 ml 0.9% sterile, nonpyrogenic saline. These injections were administered to animals while they were under light ether anesthesia. The rats were sacrificed 5 hours after intraperitoneal injection of bacterial endotoxin. Leukocytes in the peritoneal fluid were harvested and their numbers and types determined from hemacytometer counts and from Wright-Giemsa-stained smears. Previous work(4,5) had established the efficacy of this treatment in evoking a marked neutrophilia in peritoneal fluid of normal, untreated rats.

Results. Fig. 1 summarizes the effects of colchicine upon the pattern of neutrophil mobilization at various times following its injection. Rats challenged by an intraperitoneal injection immediately following injection of colchicine revealed a drastic reduction in numbers of neutrophils harvested from the peritoneal fluid 5 hours later. Such fluid contained less than 8 million neutrophils in contrast to over 59 million neutrophils that were obtained from non-colchicinized, control animals. Twenty-four hours after colchicine injection, challenged rats mobilized neutrophils

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