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Incorporation of C^{14} of Acetate-1-C^{14} and Pyruvate-2-C^{14} into Brain Cholesterol in the Intact Rat. (23593)

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Srere *et al.*(1) found that slices prepared from newborn rat brain were able to convert acetate to cholesterol. With slices from adult animals, however, no formation of cholesterol from acetate could be detected. In the present work, the incorporation of labeled acetate and pyruvate into brain cholesterol was studied in the intact rat by injecting the labeled compounds into the cisterna magna. Under these conditions, appreciable incorporation of both acetate and pyruvate was observed in rats up to 1 year of age.

Methods. Male Sprague-Dawley rats were injected with sodium acetate-1-C^{14} (Tracerlab) and sodium acetate-1-C^{14} (Nuclear Instrument and Chemical Corp.) in isotonic saline by a technic previously described(2). Both compounds had a specific activity of 1 mc per millimole. Unless otherwise indicated, all injections were made under Nembutal anesthesia. A stock laboratory diet was provided *ad libitum* throughout the experiments. At stated intervals after injection, the animals were killed by decapitation. The brains were removed and saponified in alcoholic KOH for 4 hours. The saponifiable and unsaponifiable fractions were separated by extracting the mixture with petroleum ether, evaporating the extract to dryness, and then extracting the residue with hot ethanol, the procedure being similar to that described by Van Bruggen *et al.*(3). Each ethanol extract was made to a volume of 25 ml, and aliquots were used for the determination and isolation of cholesterol. The cholesterol was precipitated from the ethanol extract as the dibromide, regenerated by debromination with zinc dust, and recrystallized from cold methanol, as described by Schwenk and Werthessen(4). Suspensions of the purified product in a 4:1 methanol-water mixture were then deposited for counting on 5 sq. cm aluminum disks by centrifuging the suspensions in a special plating assembly(5). Ordinarily a deposit of about 5 to 10 mg was obtained. Some loss occurred in the plating process, owing to the small but appreciable solubility of cholesterol in the methanol-water mixture and to the tendency of some of the solid to cling to the surface of the supernatant fluid. The actual amount of cholesterol plated was determined in each case by weighing. All determinations of radioactivity were carried out in a windowless gas flow counter. Corrections for the self-absorption of cholesterol were made according to the data for wax(6). The cholesterol content of the ethanol extracts was found by the method of Sperry and Webb(7). Most of the results are expressed as the % incorporation of C^{14} into cholesterol, calculated as the total activity of the cholesterol found in the ethanol extract divided by 1% of the administered activity. Since some degradation of brain cholesterol is known to occur during saponification(8), the % incorporation figures thus obtained must be somewhat lower than the actual values.

Results. Comparison of intracisternal and intraperitoneal injections of acetate-1-C^{14}. After preliminary experiments had indicated that adult brain cholesterol could be labeled *in vivo* with acetate-1-C^{14}, an experiment was performed in which different routes of

TABLE I. Incorporation of C^{14} of Acetate-1-C^{14} into Brain Cholesterol following Intracisternal and Intraperitoneal Injections into Rats.*

Injection	Acetate inj./100 g body wt (μc)	Specific activity of cholesterol§ (c.p.m./mg)	% incorporation§
Intracisternal†	6.7	190	.05
Intraper.†	6.7	28	.002
" ‡	40	40	.006

* 200 to 250 g rats.
† Single inj.
‡ Given in 3 equal inj. at 3 hr intervals without anesthesia.
§ Each result represents avg of 2 animals.

administration were compared. As was to be expected, intracisternal injection proved much more effective than intraperitoneal injection in labeling brain cholesterol (Table I). It was found, however, that the rates of incorporation into liver cholesterol were substantially the same for the two routes of administration.

Effect of age on incorporation. The incorporation of the C^{14} of both the acetate and the pyruvate into brain cholesterol was much greater in young rats than in older animals (Fig. 1 and 2). It will also be noted that the C^{14} of the acetate was incorporated to a greater extent than that of the pyruvate. With 200 to 250 g rats (no curve shown), the % incorporation values of the C^{14} of labeled acetate fell about midway between the values for the weanling and the 1-year-old animals.

Effect of acetate on incorporation of C^{14} of pyruvate-2-C^{14}. The rats used in this experiment were divided into 4 groups and injected intracisternally with a fixed dose of labeled pyruvate together with graded amounts of unlabeled acetate. The results (Table II) indicate that acetate inhibits incorporation of the C^{14} of pyruvate-2-C^{14} into brain cholesterol.

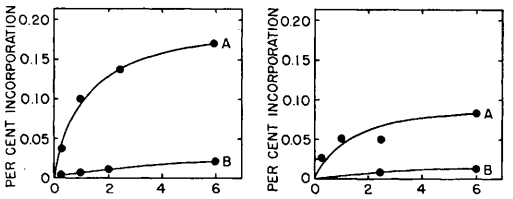

FIG. 1. (left). Effect of age on incorporation of the C^{14} of acetate-1-C^{14} into brain cholesterol. Each rat was injected intracisternally at zero time with 1.7 μc per 100 g of body weight. Each point represents the pooled brains of 2 animals. Curve A, weanling rats; curve B, 1-year-old rats (450-550 g).

FIG. 2 (right). Effect of age on incorporation of the C^{14} of pyruvate-2-C^{14}. Conditions are the same as those for acetate in Fig. 1.

TABLE II. Effect of Acetate on Incorporation of C^{14} of Pyruvate-2-C^{14} into Brain Cholesterol.

Rats weighing between 200 and 225 g were inj. intracisternally with 3.8 μc of labeled pyruvate plus the indicated amt of unlabeled acetate in .015 ml of solution. Animals were sacrificed 6 hr after inj.

No. of animals	Acetate inj. (μmoles)	Specific activity of cholesterol* (c.p.m./mg)
3	.0	73
1	.56	53
2	11.3	40
2	56.3	21

* Brains from animals in each group were pooled.

Time-course of labeled cholesterol. In this experiment, the specific activity of the brain cholesterol was followed for 72 days. After the initial rise, the activity remained fairly constant throughout the experimental period, as can be seen in Table III. No decline in activity was observed.

Discussion. The results indicate that the C^{14} of acetate-1-C^{14} and pyruvate-2-C^{14} is incorporated into the brain cholesterol of adult rats. That acetate is utilized directly by the brain for cholesterol formation is suggested by the fact that intracisternal injection produces much greater incorporation than intraperitoneal injection. Intracisternally administered acetate was also incorporated rapidly into liver cholesterol, but it seems unlikely that such cholesterol could have contributed

TABLE III. Time-Course of Labeled Brain Cholesterol.

Rats weighing between 200 and 250 g were inj. intracisternally with 6.7 μc of labeled acetate/100 g of body wt.

Time after inj. (days)	Specific activity of cholesterol* (c.p.m./mg)
2	420
14	540
28	520
49	515
70	560
72	550

* Each value represents avg of 3 rats.

appreciably to the labeled product found in the brain, since plasma cholesterol does not appear to be capable of penetrating the brain cells(9). The failure of labeled cholesterol to show a decline in activity during the time-course experiment (72 days) suggests the absence of turnover in the adult rat. However, the possibility of an appreciable re-utilization of C^{14} or a prolonged formation of cholesterol from a reservoir of labeled precursors cannot be excluded.

Summary. Incorporation of C^{14} into brain cholesterol following the intracisternal injection of acetate-1-C^{14} and pyruvate-2-C^{14} has been investigated. Incorporation was observed in both young and adult rats, but the amount of incorporation was much greater in the younger animals. The incorporation with pyruvate was less than that with acetate. Simultaneous injection of pyruvate-2-C^{14} and unlabeled acetate resulted in reduced incorporation of the C^{14} of the pyruvate. No decline in activity of cholesterol was observed during a 72 day period following the labeling of the compound by injecting labeled acetate.

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Effect of Native Dextran on Granulation Tissue Formation.* (23594)

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Lattes *et al.*(1) have suggested that acid mucopolysaccharides may be involved in stimulating the growth of granulation tissue in wounds. Wolman and Wolman(2), on the other hand, have reported that the administration of the neutral polysaccharides levan and dextran inhibited the formation of granulation tissue. We report here experiments designed to re-examine this question. Instead of using skin wounds or intraperitoneal talcum, as used by Wolman *et al.*(2), we have used the more constantly reproducible granuloma pouch(3).

Methods. Rats weighing 230 to 250 g each were used. 25 cc of air were injected subcutaneously into the backs of anesthetized animals. Into this space, 0.5 ml of a 1% solution of croton oil was immediately introduced. Two preparations of dextran were available. The high-polymer preparation (Lot No. 378, supplied by Commercial Solvents Corp.) had an average M.W. greater than 10^7. This was dissolved in 0.85% saline solution at a concentration of 40 mg/ml. The lower polymer preparation (Lot No. 359897, Commercial Solvents Corp.) had an average M.W. of 4×10^5; it was dissolved in saline solution at 60 mg/ml.

Results. In the first experiment, a daily intraperitoneal injection of 1 ml of the high-polymer dextran was administered to 9 rats and 1 ml daily of the lower-polymer solution to 10 rats. Nine control rats were not injected. On the 10th day, the animals were killed and the granuloma pouches removed.

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