Sterols in Brain and Liver of Young Rats Fed 20,25-Diaza-Cholesterol. (29868)

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(Introduced by M. Womack)
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Counsell *et al*(1) have synthesized a hypocholesteremic agent, 20,25-diaza-cholesterol (diazasterol), which is 15 times as potent as triparanol (Mer-29). Diazasterol when fed to rats in this laboratory at a level of 10 mg/kg body weight per day was found to cause the accumulation of large amounts of 24-dehydrocholesterol (desmosterol) in serum, liver, and carcass(2). An examination of the brains of rats fed diazasterol for 15 weeks revealed that 60% of the brain sterol was desmosterol, whereas only cholesterol could be detected in the brain sterol of rats not fed diazasterol.

This report will present the results of a study designed to investigate the possible origin of desmosterol in brain sterol of diazasterol-fed rats.

Methods. Weanling female BHE rats(3) were divided into 4 groups as shown in Table I. Rats of Group 1 (initial controls) were injected intraperitoneally with a tracer dose of Na-1-C^{14}-acetate, anesthetized with sodium amytal 20 minutes later and livers and brains excised. Groups 2, 3, and 4 were fed essentially cholesterol-free diets* either without cholesterol inhibitor (controls) or with the addition of 0.03% diazasterol.† After 3 weeks of feeding, Group 2 was injected with C^{14}-acetate, after 6 weeks Group 3 was injected with C^{14}-acetate, and Group 4 was injected with uniformly labeled C^{14}-glucose. Samples were obtained after 20 minutes as for Group 1. The short period of time allowed for exposure to the tracers was intended to show whether highly labeled precursors to cholesterol were present in the brain.

Lipids were extracted from liver and brain by the Folch procedure(4). Samples of the extracts were evaporated to remove solvents, saponified with 2% KOH in ethanol and extracted with petroleum ether. Samples of the non-saponifiable material were (a) plated and counted, (b) analyzed by gas-liquid chromatography (GLC)(5), and (c) treated with digitonin to precipitate the total sterol (6) which was then plated on filter paper, weighed and counted.‡ The non-saponifiable fractions of the livers from all rats receiving diazasterol and C^{14}-acetate were combined for separation of cholesterol and desmosterol, but only desmosterol could be secured in large enough quantity for purification and counting(2).

The carcasses of all rats receiving diazasterol and C^{14}-acetate were hydrolyzed in a concentrated solution of sodium hydroxide and non-saponifiable fractions were extracted and pooled. Cholesterol and desmosterol were separated from the pooled extract and both sterols were purified and counted(2).

Results. Accumulation of desmosterol in the brains of diazasterol-fed rats is shown in Table I. The brain sterol of the 21-day-old initial control rats and the control rats after 3 and 6 weeks of feeding contained no detectable desmosterol, whereas the brain sterol of diazasterol-fed animals contained 32% desmosterol in 3 weeks and 50% in 6 weeks. The liver sterol of these rats contained 88% desmosterol in 3 weeks and 93% in 6 weeks.

Synthesis of liver and brain sterol as shown by acetate incorporation was more rapid for initial controls than for animals fed the puri-

* Composition of cholesterol-free diet in g/100 g was: wheat gluten, 30; lysine-HCl, 0.62; non-nutritive cellulose, 2; vitamin mix, 2; Jones and Foster salt mixture, 4; $NaHCO_3$, 0.29; corn oil, 5; and the remainder cornstarch.

† Kindly supplied by Dr. R. E. Ranney of G. D. Searle Co., Chicago, Ill.

‡ Carbon-14 activity was accurately determined by counting for long periods on a Sharp "Wide-beta" geiger system (Beckman Instrument Co.) having a background of 0.5 counts per minute.

TABLE I. Influence of 20,25-Diaza-Cholesterol Upon Incorporation of C^{14}-Acetate and C^{14}-Glucose into and Composition of Brain and Liver Sterols of Young Rats.

Group	Diaza* in diet (%)	No. rats	Time on diet (weeks)	Tracer	Brain sterols				Liver sterols		
					Total (mg)	C^{14} inc† (cpm/mg§)	(C^{14} inc† % of non-sap)	Desmos‡ (% of sterol)	Total (mg)	C^{14} inc† (cpm/mg§)	Desmos‡ (% of sterol)
1	0	3	0	1-C^{14}-acetate	16.3	56	80	0	4.6	5082	0
2	0	2	3	"	26.4	12	48	0	14.2	768	0
	.03	3		"	25.4	7	32	32	9.7	1436	88
3	0	1	6	"	26.3	6	23	0	10.8	1413	0
	.03	3		"	24.0	2	24	50	12.4	1844	93
4	0	2	6	U-C^{14}-glucose	26.4	1	16	0	13.0	19	0
	.03	3		"	24.1	2	9	50	10.8	7	93

* 20,25-diaza-cholesterol.
† C^{14} incorporation adjusted for dose and body weight.
‡ Desmosterol (24-dehydrocholesterol).
§ Counts per minute per mg of cholesterol (specific activity).

fied diets either with or without diazasterol for 3 and 6 weeks. Acetate incorporation into liver sterol was more rapid for diazasterol-fed rats than for controls. Acetate incorporation into brain sterol was very small in comparison to that in liver sterol.

The per cent of labeled non-saponifiable material in brain total sterol declined with age indicating that cyclization of the sterol nucleus declined more than synthesis of long-chain precursors. Apparently some acetate derivatives other than preformed sterol reached the brain or labeled acetate reached the brain and a part of the sterol synthetic process occurred in that organ.

Under the conditions of this experiment C^{14}-glucose was incorporated into liver sterol in very small amounts. About the same amounts of C^{14}-glucose and C^{14}-acetate were incorporated into brain sterols.

The total digitonin precipitable sterols of the pooled livers of all diazasterol-fed rats injected with C^{14}-acetate had a specific activity of 1200 cpm/mg and desmosterol from this total sterol had a specific activity of 800 cpm/mg, both figures being unadjusted for dose and body weight. Analysis of the pooled liver sample by GLC showed that approximately 90% of the total sterol was desmosterol. Thus, a relatively small fraction of digitonin precipitable sterols caused the specific activity of the liver total sterol to be 1½ times the specific activity of the relatively large proportion of desmosterol, indicating a comparatively higher radioactivity for precursors present in quantities undetectable by GLC. In the pooled carcasses of these rats, cholesterol had a specific activity of only 1 cpm/mg compared to 15 cpm/mg for desmosterol.

Discussion. Fumagalli and Paoletti(7) reported that after 12 days of age desmosterol is not normally more than a small fraction of rat sterol although fetal and neonatal rats have as much as 40% desmosterol in brain sterol. The high level of desmosterol in the brains of 6- and 9-week-old rats fed diazasterol in the present study is abnormal for rats of this age and was accompanied by debility. The control rats had no detectable desmosterol. Fumagalli and Niemiro(8) re-

ported 8% desmosterol in brains of adult rats fed diazasterol for 10 days.

Avigan and others(9) have reported that there may be a turnover of 0.1% a day in brain cholesterol. They considered most of this turnover to be due to exchange between cholesterol in brain and serum. Morris and Chaikoff(10) reported a very small exchange of preformed cholesterol between serum and brain in suckling rats. The rats in the present report had a change of proportion between brain cholesterol and desmosterol of about 1.5% a day for the first 3 weeks of diazasterol feeding and almost 1% a day for the next 3 weeks; the latter corresponds to the data of Fumagalli and Niemiro for adult rats(8).

Interpretation of tracer data for brain sterols has varied considerably. Initial assumptions have determined what conclusions were possible. If it is assumed that there is transfer between serum and brain sterols, the serum sterols highly labeled by acetate-C^{14} could account for the brain sterol label; but the brain sterol label derived from glucose could not be accounted for by such a transfer. If it is assumed that brain sterol label arises only from synthesis within the brain the indicated rate of synthesis from acetate could be no more than 1/100 of the rate of synthesis in liver. If the difference in size of the cholesterol pools in brain and liver is taken into consideration, biosynthesis of sterol from acetate in the brain was approximately 1/50 that in liver.

A 2.5-day half-life of serum cholesterol and an 8.93-day for liver cholesterol of 200 g rats were reported by Wong and Van Bruggen(11). The specific activity of total sterol in liver of the rats in the present experiment decreased by a factor of about 4 from weanling (51 g) to 9 weeks of age (188 g). The amount of cholesterol approximately doubled so the net decrease in activity was associated with a decrease in turnover rate. On this basis it may be assumed that half-life of serum sterols of the weanling rats in this experiment was shorter than 2.5 days; half-life of liver sterols was shorter than 9 days. Replacement of 50% of the brain cholesterol by desmosterol occurred in 42 days. The C^{14} incorporation data may be reconciled with the rapid accumulation of desmosterol if the half-life of sterols of brain as well as of other organs was considerably shorter at 3 weeks of age than later.

Of the two possibilities for the mode of accumulation of desmosterol in brain it appears more likely that it was synthesized within the brain than transferred from serum. To explain fully the origin of the brain desmosterol will require clarification of normal brain sterol metabolism.

Summary. Feeding 0.03% diazasterol in essentially cholesterol-free diets to weanling rats for 3 or 6 weeks caused a large concentration of desmosterol in the brain and liver sterols. Studies using C^{14} showed that small amounts of acetate and glucose were incorporated into brain sterol of both control and inhibitor-fed rats and a mode by which this incorporation may account for the rate of desmosterol accumulation is postulated.

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Received October 16, 1964. P.S.E.B.M., 1965, v118.