on a population sample of apparently healthy persons and the sample size is considered statistically adequate. 2) There exists a definite trend towards a decrease in average weight of successive 5 year age groups after age of 65. There were 2 reasons for this: 1) individual weight loss accompanying aging due to loss of body substance, 2) high mortality rate among obese which favors survival of a larger proportion of underweight people. 3) Height and survival were not related.

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New Inhibitors of *in vitro* Conversion of Acetate and Mevalonate to Cholesterol. (25252)

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The *in vitro* conversion of labeled acetate (1) and labeled mevalonate(2) into cholesterol by rat liver homogenates have been reported, as well as the effect of known hypocholesteremic agents upon these syntheses(3,4). The present studies are concerned with the effect of several new compounds upon the enzymatic synthesis by rat liver homogenates of cholesterol from both acetate and mevalonate. The compounds are Δ1-testololactone, Δ4-androstene 17-*α*-ol-3-one-17*β*-oic acid, and *β*-hydroxy - *β* - fluoromethyl - δ - valerolactone. (fluoromevalonic acid).* The effects of fluoride ions, fluoroacetate, and zinc ions upon these conversions have also been investigated.

Methods. Livers were obtained from 100 g Sprague Dawley rats maintained on Purina Lab Chow. The animals were killed by decapitation and their livers quickly removed and placed on ice. Homogenization was carried out in the manner reported by Bucher(1), in 2.5 volumes of .1 M phosphate buffer of pH 7.0.† Five ml of the supernatant solution after refrigerated centrifugation at 700 x G for 10 minutes were used as the source of enzyme for each determination. To use the identical enzyme preparation for all levels of compounds studied in each test, several livers were pooled and 5 ml aliquots used for each level and for controls. To each aliquot was added .5 mg of DPN and .5 mg of ATP in .1 ml volumes. Twenty-five *μ*g of 2-C^{14} acetate (24.4 *μ*c/mg) contained in 1 mg of K acetate in .1 ml was the acetate substrate. Forty *μ*g of 2-C^{14} mevalonic acid (1.6 *μ*c/mg as the dibenzylethylenediammonium salt) in .1 ml volume was the mevalonate substrate. The mevalonic acid was liberated from its salt by treatment with alkali and extraction with ether(5). Compounds under test were added either as a fine suspension or as a solution in a .2 ml volume of 5% propylene glycol. The enzymatic synthesis was carried out in 50 ml Erlenmeyer flasks placed in a Dubnoff metabolic shaker under an atmosphere of 95% oxygen-5% CO_2. The samples were incubated for 2 hours at 34°C. After this period .5 mg of cholesterol was added to each flask and the entire contents saponified with alcoholic KOH in a

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† | ml | |
|---|---|
| 4 | 1M KH_2PO_4 |
| 6 | 1M K_2HPO_4 |
| 5 | .1M $MgCl_2$ |
| 3 | 1M Nicotinamide |
| 82 | deionized water |

TABLE I. Effect of Steroids on Acetate and Mevalonate Conversion to Cholesterol.

Compound	mg	Acetate conversion						Mevalonate conversion			
		Test 1		Test 2		Test 3		Test 1		Test 2	
		cpm/plate	% inhib.	cpm/plate	% inhib.	cpm/plate	% inhib.	cpm/plate	% inhib.	cpm/plate	% inhib.
Δ4-Androstene-17α-ol-3-one-17β-oic acid	2.0	153	97			0	100	5,976	73		
	1.0					3	100	14,836	32		
	.5					427	57				
	.25							24,140	11*		
	.067							21,485	2		
Δ1-Testololactone	2.0			857	92	125	87			26,755	27
	1.0					772	23			34,753	6
	.25									36,422	1
	.067									39,075	6*
Control		4,744		10,274		990		21,979		36,848	

The radioactivity added was 650,000 cpm for acetate and 130,000 cpm for mevalonate. * Values in excess of control are expressed as acceleration.

boiling water bath under a stream of nitrogen. When the alcohol had evaporated, the saponification was considered complete(6). After acidification, the solution was extracted with ether and the cholesterol was isolated as the digitonide from the ether extract. The digitonide was dissolved in 1 ml of absolute alcohol, and evaporated on an aluminum planchet under an infra-red lamp. Counts were made on a gas-flow proportional counter (Tracerlab-Deciscaler), corrected for self-absorption, and expressed as counts/minute/plate. In these studies the radioactive digitonide precipitate was considered as a precipitate of cholesterol only, based on the report of Frantz and Bucher(7) that the digitonin precipitable material produced by cell free homogenates is approximately 97% cholesterol.

The results of several studies where Δ4-androstene-17α-ol-3-one-17β-oic acid and Δ1-testololactone have been added to the liver homogenate system are shown in Table I. Both of these compounds are effective in inhibiting the conversion of acetate to cholesterol. When mevalonic acid was used as the substrate, Δ4-androstene-17α-ol-3-one-17β-oic acid showed marked inhibition at the 2 mg level, while Δ1-testololactone was much less effective at this concentration.

The best inhibition of both systems was obtained with fluoromevalonic acid. The results of this study are summarized in Table II. Fluoromevalonic acid appears to be 5-10 times more effective in inhibiting acetate conversion than mevalonate conversion. A 40% inhibition of the acetate system is obtained with .004 mg of fluoromevalonic acid while a 27% inhibition of the mevalonate system is obtained with .031 mg.

To rule out the possibility of fluoromevalonic acid being metabolized to either fluoroacetate or fluoride ion, both of these substances were tested in the range of concentration that would result if such metabolism did occur. The results are presented in Table III. Neither substance had any effect on either acetate or mevalonate conversions.

It is of interest to note that addition of .5 mg of unlabeled mevalonic acid to the acetate system caused only a 7% reduction in counts while .5 mg of fluoromevalonic acid caused a

TABLE II. Effect of Fluoromevalonic Acid on Acetate and Mevalonate Conversion to Cholesterol.

Compound	mg	Acetate conversion Test 1 cpm/plate	% inhib.	Mevalonate conversion Test 1 cpm/plate	% inhib.	Mevalonate conversion Test 2* cpm/plate	% inhib.
Fluoromevalonic acid	2.0			881	97		
	1.0	215	95	1,392	96		
	.5					11,720	85
	.25	333	92			11,473	79
	.125					29,030	66
	.063	495	89			39,872	51
	.031					59,526	27
	.015	1,580	66				
	.0039	3,064	40				
	.0009	4,834	3†				
Control		4,764		32,224		81,301	

Radioactivity added was 650,000 cpm for acetate and 130,000 cpm for mevalonate.
* 80 μg of labeled mevalonic acid used in this study with an activity of 260,000 cpm.
† Values in excess of control are expressed as acceleration.

TABLE III. Effect of Fluoride, Sodium Fluoroacetate, and Zinc on Acetate and Mevalonate Conversion to Cholesterol.

Compound	mg	Acetate conversion cpm/plate	% inhib.	Mevalonate conversion cpm/plate	% inhib.
Fluoride (as NaF)	.25			22,985	0
	.063			22,492	0
Control				22,993	
Sodium fluoroacetate	.1	4,527	2	37,132	1
	.01			34,882	7
	.001			37,942	1*
Control		4,632		37,444	
Zinc (as $ZnCl_2$)	.04	10	99	33,831	9
	.01	382	70	38,101	3*
	.0025	1,368	8*	39,063	5*
Control		1,272		37,045	

Radioactivity added was 650,000 cpm for acetate and 130,000 cpm for mevalonate.
* Values in excess of control are expressed as acceleration.

90% reduction. Addition of 1 mg of unlabeled mevalonic acid gave a 61% reduction in counts in the mevalonate system while 1 mg of fluoromevalonic acid brought about a 97% reduction.

In examining compounds for inhibitory properties, a striking effect of zinc ions was noted. Curran and Clute(8) had found no effect of .0001 M $ZnCl_2$ upon their liver cell cluster conversion of acetate to cholesterol. In our system, a definite inhibition upon acetate conversion was observed while no effect was seen upon mevalonate conversion with .01-.04 mg (.00003M to .00011M) concentrations of zinc ions. These data are also shown in Table III.

Summary. The effects of Δ1-testololactone, Δ4-androstene 17α-ol-3-one-17-β-oic acid, and fluoromevalonic acid upon *in vitro* conversion of labeled acetate and labeled mevalonate into cholesterol were studied. All of these compounds inhibited conversion of acetate, while only the latter 2 inhibited conversion of mevalonate. Fluoromevalonic acid was the most potent inhibitor studied. Fluoroacetate, fluoride ions, and zinc ions had no effect on the mevalonate system. Of these substances only zinc ions inhibited conversion of acetate to cholesterol.

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Biochemical Changes Occurring in Mouse Liver Cells with Ageing.* (25253)

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A number of workers have investigated biochemical changes occurring in tissues as animals age, but little information is available concerning such changes in the subcellular fractions of tissues. An opportunity arose in this laboratory, in connection with other studies, to measure nitrogen and phosphorus contents of large and small granules and cytoplasmic supernatant in liver cells from mice ranging in age from 1½ to 21 months. The data obtained demonstrate that as animals age changes occur in relative amounts of these constituents in all 3 subcellular fractions examined.

Method. Inbred virgin female mice of the C3H/Sp strain, reared on laboratory chow and water *ad lib* in air conditioned animal rooms, were employed. They were sacrificed by exsanguination via jugular vein and the livers removed without prior perfusion. Large granule, small granule, and cytoplasmic supernatant fractions were obtained by differential centrifugation employing a sucrose medium, essentially as described earlier(1). Nuclear counts were made(2) on tissue suspensions prior to preparation of the cell fractions. Nitrogen(3) and phosphorus(4) were determined on each fraction prepared, and were calculated on a per nucleus basis.

Results are shown in Table I. There was an increase with age in nitrogen content of all cell fractions examined. Increases were seen in the small granule and cytoplasmic supernatant fractions by 6-7 months, and in the large granule fractions by 10-11 months of age. Similar changes were seen in phosphorus contents of these fractions, but they generally were of smaller magnitude. In the oldest animals studied chemical compositions of the fractions had altered, as evidenced by lowered P/N ratios in all 3 fractions. There are probably no significant differences in numbers of binucleate cells present in livers of animals studied regardless of age(5). It therefore seems safe to conclude from the nitrogen data that, as the animal ages, the amounts of large and small granules and cytoplasmic supernatant increase within the cell. This appears to confirm the report of Franzini *et al.*(6) that with age there is a gradual increase of cytoplasm-nucleus ratio in livers of mice.

There were fewer cells/g of liver tissue, as indicated by decreasing numbers of nuclei, with increasing age but this appears to be largely compensated for by an increase in liver mass (Table I). There is probably less mitotic activity in livers of the older than in those of younger animals(5), which might suggest a decreasing vigor of the cells. However, total content of the 3 fractions examined in liver cells increased with age. That this process may be simply a form of compensatory hypertrophy is suggested by the observations of Weinbach and Garbus(7), who reported a lower absolute level of substrate oxidation by liver mitochondria from aged

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