O_2 Content, vol.%: M.V.B.† = 10.0 — art. blood = 14.0 — A—V difference = 4.0.

R.Q. from blood: $\frac{CO_2 \text{ A—V difference}}{O_2 \text{ A—V difference}} = .875.$

Cardiac Output, lit./min. from

CO_2 A—V difference $= \frac{192}{3.5} = 5.49.$

O_2 A—V difference $= \frac{225}{4.0} = 5.63.$

Cardiac Index = 3.35 lit./sq.m. B.S. area.
Heart rate per minute = 70.
Stroke Volume = 79.5 cc.

*Arterial blood sampling starting and ending approximately 15 seconds after mixed venous blood sampling. Total duration of sampling of both bloods 35 seconds. Expired air collected during blood sampling.

†M.V.B. (mixed venous blood) refers to blood drawn directly from the right auricle.

The protocol of one cardiac output determination by simultaneous collection of blood from the right auricle and femoral artery is included herewith. The number of simultaneous determinations of stroke volume measured by this method and compared with estimations from ballistocardiograph tracings is not large enough to warrant a statement concerning the validity of the ballistic method at this time.

12030

Middle and Old Age in Cholesterol-Fed Rats.*

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Most of the pathological conditions involving cholesterol deposition in tissues are characteristic of middle and old age rather than youth. Previous studies on the effect of cholesterol intake on tissue cholesterol in the rat have been made with comparatively young animals and for comparatively short periods of time.

Cholesterol-fed rats have appeared to be little the worse for their high sterol intake except in that they have developed fatty livers which have contained at least 20 times the normal percentage of

* Assistance of the Work Projects Administration in care of the animals used for this study is gratefully acknowledged. (Project 10-11578 A24.)

1 Okey, R., Gillum, H. L., and Yokela, E., *J. Biol. Chem.*, 1934, **107**, 207.

cholesterol.[1] Moreover, these rats have withstood the strain imposed by poorly balanced and vitamin deficient[2] diets only a little less successfully than their controls. Pregnancy and lactation[3] have been possible in this species and a second generation has been brought to maturity on the cholesterol-rich diet.

The present investigation was designed to show whether or not rats fed cholesterol from the time of weaning would continue to show the same powers of adaptation to the high sterol diet throughout middle and old age.

Diets and routine for care of the animals were essentially identical with those previously described.[3] Series "A" consisted of 16 male and 16 female rats fed cholesterol and a like number fed the control diet for a period of about 1½ years. Series "B" consisted of 15 control and 6 cholesterol-fed females which ate their respective diets for well over 2 years. Strictly speaking only the latter group can be considered to have attained more than middle age.

Growth in both control and cholesterol-fed rats was slightly slower than that of our stock rats, but adult size compared favorably with that of stock animals. Average weights attained by cholesterol-fed males at one year of age was 393 g, by control males, 395 g. Cholesterol-fed females at the same age averaged 261 g, controls 241 g.

Health records compared, with the exceptions noted below, quite favorably with those of stock animals.

Four female controls and 4 cholesterol-fed females were taken from series "A", bred and permitted to go through *pregnancy* and *lactation* on their respective diets with the slight modifications noted elsewhere.[3] They were then returned to the regular regime used for this experiment. They remained in excellent condition until they were killed at 450 days of age.

On the other hand, in series "A" 3 control and 4 cholesterol-fed females which had never been bred and had been kept in individual cages from the time of weaning showed evidence of severe vaginal hemorrhage when they were about 15 months of age. Autopsy of 2 animals made at the time hemorrhage was noted showed uteri much enlarged and distended with unclotted blood. In one case each horn of the uterus measured three-eighths inches in diameter. Rats which recovered spontaneously from these hemorrhages had enlarged and fibrous, and, in 2 cases, edematous uteri, but we were able to find pus in only one rat.

The 21 females of series "B", kept under as nearly the same

[2] Gillum, H. L., and Okey, R., *J. Nutr.*, 1936, **11**, 303, 309.

[3] Okey, R., Godfrey, L. S., and Gillum, F., *J. Biol. Chem.*, 1938, **124**, 489.

conditions as possible but 2 years later, added to the list only one control and one cholesterol-fed rat with uterine hemorrhage. These animals were kept until about 27 months of age. We have, therefore, no reason to assume that cholesterol in the diet was responsible for this condition. The fact that we have never observed it in stock animals is probably not significant, since females are not routinely kept in the laboratory after they cease to be useful for breeding, *i. e.*, after they are more than a year of age.

Rats of series "B" after they were 18 months or more of age, had a high incidence of tumors, some of which were apparently malignant. The small number of cholesterol-fed rats in this group makes it impossible to draw conclusions, but we may at least note that 4 out of 6 of the cholesterol-fed animals over 700 days old developed tumors of considerable size while only 3 of the 15 control animals had tumors. Two of these appeared to be subcutaneous lipomas.† Data on spontaneous occurrence of tumors in rats of this age is lacking but the incidence of tumors in our stock animals is very low.

Tissue lipids. Our technic for preparation and analyses of lipid extracts of rat tissues has been described previously.[1, 3]

The data, summarized in Table I, represent averages for animals killed while apparently healthy. It will be noted that in no tissues other than liver did the cholesterol-fed animals show more than a very slight increase in total cholesterol. Differences in free cholesterol content of tissues between cholesterol-fed and control animals were insignificant. This meant that any extra cholesterol which reached these tissues was promptly esterified. A very large proportion of the cholesterol retained in the rat's body was always to be found as liver cholesterol ester. It should be noted, however, that the actual percentage of fat and cholesterol ester in the livers of these older rats was no greater than that previously noted for younger animals on the same diet.

Discussion. Throughout these studies we were greatly impressed with the extraordinary capacity of the cholesterol-fed rats to remain in apparent good health for long periods of time. Grossly enlarged and fatty livers were present throughout the life span but histological examination of these livers showed fatty infiltration rather than degeneration of functioning tissue.

† Failure of the thermostatic control in our paraffin imbedding oven resulted in overheating and consequent loss of a large proportion of the tissues from series "B" while they were being prepared for histological examination. Consequently we are unable to report the exact nature of the tumors.

TABLE I.
Average Value for Tissue Lipids.

Series and tissue		No. rats	No. samples*	Avg age rats, days	Avg wt rats, g	Moisture, %	Fatty acid	Total Chol-esterol	Free Chol-esterol	Ester Chol-esterol	Lecithin
							—— % of moist wt ——				
Livers											
A. CF	♂	10	10	447	369	56.8	14.8	8.7	.43	8.3	
A. N	♂	11	11	445	391	69.5	5.6	.33	.23	.10	
A. CF	♀	11	11	445	256	57.9	11.8	7.3	.33	6.9	
A. N	♀	9	9	467	273	67.8	5.9	.34	.22	.12	
B. CF	♀	3	2	830	284	60.2	10.2	8.1	.39	7.7	2.5
B. N	♀	6	1	833	303	69.2	5.5	.41	.27	.14	2.7
Lungs											
A. CF	♂	12	5	447	369	78.9	3.4	.52	.38	.14	
A. N	♂	11	4	445	391	77.8	3.8	.40	.36	.04	
A. CF	♀	11	5	445	256	78.0	3.7	.53	.40	.13	
A. N	♀	9	3	467	273	76.8	4.1	.40	.36	.04	
B. CF	♀	3	2	830	284	80.8	2.4	.67	.49	.18	2.2
B. N	♀	6	2	833	303	79.4	3.4	.51	.42	.09	1.8
Hearts											
A. CF	♂	12	5	447	369	74.3	4.3	.22	.16	.06	
A. N	♂	11	3	445	391	71.4	6.5	.17	.14	.03	
A. CF	♀	11	3	445	256	74.6	5.3	.18	.15	.03	
A. N	♀	9	3	467	273	75.6	3.6	.17	.16	.01	
B. N	♀	6	1	833	303	75.7	4.8	.22	.15	.07	1.9
Spleens											
B. CF	♀	2	1	830	284	77.0	.92	.29	.28	.01	1.3
B. N	♀	1	1	833	303	75.0	2.7	.44	.38	.06	1.7
Blood											
B. CF	♀	2	1	830	284		.285	.112	.105	.007	.283
B. N	♀	3	1	833	303		.236	.095	.059	.036	.219

CF—Cholesterol-fed animals.
N—Controls.
*Analyses were sometimes made on pooled tissue samples from several animals. Averages are weighted accordingly.

A second interesting observation was the rapidity with which cholesterol disappeared from the liver when the animals developed infections or became ill. Infections with pus-forming organisms, even when they had not progressed to the stage that the animals appeared sick, were invariably associated with lowered ester cholesterol in the liver. Four male rats with weight losses of about 50 g and lung abscesses of moderate size and apparently benign nature had total liver cholesterol values averaging 1.6%, against an average value of 8.7% for total liver cholesterol in the whole group. Two of the cholesterol-fed females which had had severe uterine hemorrhages only a week before killing had total liver cholesterol values of 0.80 and 1.6% respectively.

It is very difficult to decide whether the clearing out of stored

liver cholesterol is the result of poor absorption, of mobilization of the sterol to meet some demand imposed by the illness, or of a combination of causes. Gillum in this laboratory has shown[4] that it takes a healthy rat 6 to 9 weeks to clear out its cholesterol ester stores when changed from a cholesterol-rich to a cholesterol-poor diet. This is certainly a very much slower process than that which takes place in the sick animal. Further study of cholesterol-fed animals with infections of known type and virulence seems indicated.

Summary. Rats have been fed diets containing 1% cholesterol from weaning throughout their life span. Their growth, health and time of survival have not differed significantly from those of control animals on the same basic diet without the cholesterol.

12031

Oxidation of Some Substituted Alcohols by Rat Liver.

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The alcohol oxidase of liver can oxidize a number of lower primary alcohols.[1] The effect of substitutions in the alcohol molecule has not been studied. The following is a report on the oxidizability by rat liver suspensions of a number of such substituted alcohols.

Experimental. The liver preparation was made by chopping with scissors, grinding with sand and M/20 phosphate buffer pH 7.8, and squeezing through muslin. The alcohols were obtained from the Eastman Kodak Co. They included ethanolamine, diethanolamine, β-β'-dihydroxyethyl ether, β-β'-dihydroxyethylsulfide, ethylene bromohydrin, tribromoethanol (avertin), glycerol, ethylene chlorohydrin, ethylene glycol, and β-hydroxypropionitrile. The last 3 were the only ones oxidized by the liver preparation. Figure 1 shows the oxidation of various concentrations of these 3 compounds compared with the oxididation of ethyl alcohol. The Schiff test

[4] H. L. Gillum, unpublished data.

* One of us (P. H.) is indebted to the John and Mary R. Markle Foundation for its support during this investigation.

[1] Lutwak-Mann, C., *Biochem. J.*, 1938, **32**, 1364.