TABLE 1

BLOOD SUGAR LEVELS

Obese mice				Nonobese mice			
Normal	Fasting	400 IU/kg insulin	20 IU/kg insulin	Normal	Fasting	400 IU/kg insulin	20 IU/kg insulin
197	148	231	273	79	125	Lethal	Lethal
	88	224	318	141	112	"	"
291	119		298	88			
155				127			
217							

method necessitate individual blood samples of 0.2 or 0.4 ml for duplicate determinations (*2, 3*). Such amounts can be taken safely from mice only once every three weeks, which seriously limits experimental possibilities. Urine collections, although presenting no special difficulties per se, are made more delicate by the small size of the samples.

A study was made of blood glucose levels of obese and nonobese animals under various experimental conditions, as well as of normal urine glucose levels. The animals used were housed in individual, screen-bottomed cages and fed water and pellets *ad lib*. The results are given in Table 1 on an individual basis because of the limited number of animals used. The figures demonstrate that fed obese animals have a high blood sugar level (generally above 200 mg%) whereas nonobese animals presented a blood sugar level of the order of 110 mg. The effect of fasting was next studied—a 4-hr period was used. It is evident from the results that the blood sugar level of obese animals is much more sensitive to fasting than that of the nonobese animals, as it drops 50–60%, whereas that of the nonobese animals does not change appreciably during this interval. This result, incidentally, is in agreement with the "glucostatic" scheme of the regulation of food intake, which sees food intake conditioned by the *variations* in the blood glucose level (*4*). More remarkable, it was found that all obese animals belonging to this strain were insulin-resistant. High (20 IU/kg) and massive (400 IU/kg) doses of insulin ("Iletin," Lilly) were administered to the obese animals. No convulsions or deaths were observed with those doses. The blood sugar levels 1 hr after injection are recorded in Table 1. The obese, diabetic mice were found to be as a rule totally unaffected, and conserved extremely high blood sugar on doses that would kill normal animals in a short time. On the other hand, the nonobese mice present a normal insulin sensitivity. Obese and nonobese animals seem to be quite responsive to the administration of epinephrine. Blood sugar values 1 hr after administration of 1 mg/kg of adrenaline were of the order of 500 mg%.

Obese animals normally present a glucosuria of the order of 3 g%.

These observations lead to the following important conclusions: First, for the first time the existence of hereditary diabetes, clearly independent of environmental influences, has been established. Second, an obvious link between pathology of carbohydrate regulation and obesity is evidenced in these animals. They thus furnished a striking illustration to the glucostatic theory of the mechanism of the regulation of food intake. Finally, these animals are useful subjects for the elucidation of insulin-resistant diabetes. Metabolic and biochemical studies are being continued.

Histological and pathological data concerning obese and nonobese animals will be published in the near future (*5*). Three results may be stated already: first, both obese and nonobese animals generally present enlarged islets of Langerhans; second, as compared to other strains, the liver of obese animals is characterized by an abnormally low glycogen content, and the liver of nonobese animals presents a definitely higher glycogen content; finally, obese mice frequently present ulcerative lesions not unlike the decubitus ulcers sometimes seen in obese diabetic humans.

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Significance of Endogenous Cholesterol in Arteriosclerosis: Synthesis in Arterial Tissue[1]

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Recent concepts of the pathogenesis of arteriosclerosis have stressed the importance of dietary cholesterol in the development of this lesion. Evidence that this sterol is synthesized by many tissues in the animal body is accumulating, however. The early balance experiments of Schoenheimer and Breusch (*1*) on mice fed cholesterol-free diets left no doubt that animals can synthesize cholesterol, and this conclusion has been amply confirmed in the isotopic studies of Bloch and his associates (*2, 3*). The latter demonstrated that surviving liver slices can convert acetate to cholesterol and, according to Srere *et al.* (*4, 5*), this conversion can also be carried out by the adrenal cortex, kidney, testes, small intestine, and skin. It thus becomes of some importance to evaluate the significance of this endogenously synthesized cholesterol in the development of atheromata. Experiments designed to do so are in progress in this laboratory. This paper reports the ability of the artery to synthesize cholesterol.

The chickens used were mature male White Leg-

[1] Aided by grants from the U. S. Public Health Service and the Life Insurance Medical Research Fund.

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horns and were obtained from the Poultry Division flock of this university. The rabbits were obtained on the open market and included both males and females.

The animals were sacrificed by cervical fracture. Following removal, the aortas were placed in a cold, oxygenated Krebs-bicarbonate buffer, and while there were rapidly freed of all adherent fat and connective tissue. They were then cut into sections approximately ½ cm long. These were blotted on filter paper, weighed, and incubated in 5 ml of Krebs-bicarbonate buffer containing 1 mg of doubly C^{14}-labeled acetate prepared by the method of Barker *et al.* The tissues were incubated for 3 hr at 37.5°, as described by Chernick *et al.* (*6*).

The tissue was removed from the flask and hydrolyzed on a steam bath with 2 ml of 90% KOH and 5 ml of 95% alcohol, for 6–12 hr. The fatty acids and cholesterol were precipitated by bringing the pH of the solution below 3 with 6*N* HCl. Precipitation was completed by allowing the flask to remain in an ice-box for several hours. The entire contents were then filtered (No. 1 Whatman paper), and the residue was washed with water acidified with HCl. Four additional acid-water washings were employed to remove most of the acetate from the lipids.

The residue on the filter paper was dissolved first with acetone, next with a mixture of acetone and ether (1:1), and finally with ethyl ether. The dissolved

TABLE 1

CONVERSION OF C^{14}-ACETATE TO CHOLESTEROL AND CO_2 BY ARTERIAL TISSUE

Expt. No.	Flask No.	Tissue		C^{14}-acetate added to flask (cpm)	C^{14} recovered as CO_2/g wet tissue (%)	Specific activity of isolated cholesterol*
		Type	Wet wt (mg)			
	1	Aorta	430	1.0×10^6	2.8	32
	2	"	330	1.0×10^6	3.8	34
	3	"	525	1.0×10^6	1.7	19
1	4	"	200	1.0×10^6	2.8	15
(Rabbit)	5	"	415	1.0×10^6	1.9	76
	6	"	555	1.0×10^6	1.6	36
	7	"	348	1.0×10^6	4.3	43
	8	Liver	515	1.0×10^6	13.0	1.3×10^4
	1	Aorta	684	1.5×10^6	3.1	28
2	2	"	495	1.5×10^6	7.1	23
(Rabbit)	3	Liver	535	1.5×10^6	15	1.3×10^4
	4	"	535	1.5×10^6	12	1.9×10^4
	1	Aorta	545	$.92 \times 10^6$	3.7	160
	2	"	563	$.92 \times 10^6$	5.2	84
	3	"	590	$.92 \times 10^6$	5.3	110
	4	"	477	$.92 \times 10^6$	2.4	54
3	5	"	630	$.92 \times 10^6$	7.2	420
(Chicken)	6	"	213	$.92 \times 10^6$	3.8	27
	7	Liver	504	$.18 \times 10^6$	37	1.5×10^3
	8	"	504	$.18 \times 10^6$	38	1.6×10^3
	9	Boiled aorta	467	$.92 \times 10^6$	—	1
	10	" "	510	$.92 \times 10^6$	—	3
	1	Aorta	676	$.45 \times 10^6$	3.8	60
	2	"	678	$.45 \times 10^6$	3.7	110
4	3	Liver	501	$.45 \times 10^6$	51	5.3×10^3
(Chicken)	4	"	497	$.45 \times 10^6$	52	5.4×10^3
	5	Boiled liver	488	$.45 \times 10^6$	0.10	0
	6	" "	488	$.45 \times 10^6$	0.00	0
	1	Aorta	543	1.0×10^6	2.9	69
	2	"	501	1.0×10^6	3.6	37
5	3	"	479	1.0×10^6	6.7	71
(Chicken)	4	Liver	503	1.0×10^6	41	2.6×10^3
	5	"	504	1.0×10^6	39	2.5×10^3
	6	Boiled liver	501	$.50 \times 10^6$	0.05	4

* Specific activity refers to cpm/mg cholesterol isolated, corrected for an acetate activity of 10^6 counts. Each flask contained 1 mg sodium acetate.

As a rule, arterial tissue obtained from a single animal (200–680 mg) was incubated in each flask. In the case of each experiment, a run was also made with liver slices. At the end of the 3-hr incubation period, the CO_2 evolved was trapped in KOH, and its C^{14} content was determined as described by Chernick *et al.* (*6*).

fatty acids and cholesterol were collected in a 250-cc Erlenmeyer flask equipped with a closed side arm. The solvents were evaporated on the steam bath, 3–5 ml of acidified water was then added, and the lipids in the resulting suspension were extracted three times with boiling petroleum ether. The petroleum ether solutions were transferred to a 50-cc Erlenmeyer flask, the side

arm on the 250-cc Erlenmeyer flask serving to trap the water during these transfers. After the petroleum ether was boiled off, the lipids were dissolved in 5 ml of a 1:1 mixture of acetone and alcohol, and to this was added 2 ml of a 0.5% digitonin solution in 50% alcohol. The cholesterol digitonide was allowed to precipitate overnight at room temperature and was then filtered through No. 50 Whatman filter paper. The fatty acids and other contaminants were washed off the digitonide, first with a 1:1 acetone-ether mixture and then three times with ethyl ether. While some ether still remained in the digitonide boiling distilled water was poured over it. This caused the platelike precipitate to break up, and the hot water dissolved any digitonin precipitated by the first acetone-ether wash. An acetone-ether (1:1) wash followed by an ethyl ether wash served both to dry the digitonide and to force it to the bottom of the filter paper. The funnel containing the filter paper was next placed in a vacuum oven at 50° C for 30 min. The dried digitonide was carefully transferred to a Van Slyke microcombustion apparatus (*7*), oxidized, and its C^{14} counted as $BaCO_3$. The specific activity was calculated as cpm/mg cholesterol.

Conversion of acetate to CO_2. It is apparent from the results shown in Table 1 that rabbit and chicken arteries can convert considerable quantities of acetate to CO_2. The amounts of arterial tissue added to each bath were not kept constant, and for this reason the percentages of the added C^{14} converted to CO_2 are recorded for 1 g wet tissue. The capacity of arterial tissues to oxidize acetate to CO_2 ranged from 6 to 60% of that found for corresponding livers.

Incorporation of acetate-C^{14} into cholesterol. The aortas of rabbits and chickens show a small but consistent ability to synthesize cholesterol. The specific activities of the cholesterol isolated after a 3-hr period of incubation ranged from 15 to 420 cpm/mg cholesterol. Since it is not possible to prepare uniform sheets of whole arterial tissue, a comparison of the results of the various runs is perhaps not indicated. It should be noted, however, that the highest specific activity of cholesterol obtained with arterial tissues was about one fourth that observed with liver slices.

Liver slices and arterial tissue that had been immersed in boiling water for 5 min were incubated in the presence of C^{14}-labeled acetate for 3 hr. The results of these experiments are also recorded in Table 1. The recoveries of the C^{14} as CO_2 and cholesterol in these experiments were negligible. This indicates that the values obtained with surviving tissues were not the result of gross bacterial contamination of the incubation mixture nor of contamination of the isolated cholesterol with the labeled acetate.

Until recently the view was widely held that changes in chemical composition of arterial tissue are, in large part, a reflection of its plasma environment, and this belief has been carried over to nearly all concepts of atherogenesis. However, it has been pointed out elsewhere (*8*), on the basis of Q_{O_2} measurements, that the artery cannot be considered totally passive. This conclusion is further emphasized by the findings reported here. It is demonstrated that the aorta has the capacity for oxidizing significant amounts of acetate to CO_2. Indeed, its capacity to do so under our experimental conditions reached 60% of that of the liver.

But metabolism in arterial tissue is not restricted to catabolic processes. That it also engages in synthetic reactions was shown earlier for phospholipides and fatty acids (*9*) and is again demonstrated here in the case of the conversion of acetate to cholesterol.

Cholesterol synthesis by the artery, it should be noted, is but one phase of a rather widespread reaction, which probably occurs throughout the animal body. The importance of this extradietary cholesterol in the pathogenesis of atherosclerosis in man remains to be evaluated.

That dietary cholesterol is involved in pathogenesis of arteriosclerosis is no longer doubted. The conclusion, however, that endogenous cholesterol *can* also be important in arterial degeneration is forced upon us by the demonstration that the chicken injected with stilbestrol develops hypercholesterolemia promptly, and atherosclerosis in about a year, even when the diet fed during this entire period is practically devoid of cholesterol (*10, 11*).

Cholesterol-bearing lipoproteins have recently been shown by Gofman and his associates to be correlated with excessive atherosclerosis in man (*12*). This fraction can be made to appear in plasma of man by the feeding of high-fat, high-cholesterol diets. It is of interest to report that this fraction also appears in the serum of stilbestrol-treated birds fed a low-cholesterol diet.[3] It is thus clear that synthesized cholesterol *can* be made to play a decisive role in the development of experimental atherosclerosis.

Our finding that the artery is capable of synthesizing cholesterol, coupled with the observations on the stilbestrol-treated bird, indicates that endogenous cholesterol, originating within the artery itself and elsewhere in the body, may be of greater importance in the pathogenesis of spontaneous arteriosclerosis than has hitherto been supposed.

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