of almost pure rods have been seen and one wandering cell, presumably a macrophage, showed a large preponderance of spherical forms, but the vast majority of infected cells show the co-existence of the two shapes.

In some favorable areas it was possible to see gradations between filaments and spheres. Fig. 3 shows rods with segmentation varying in period, the largest being approximately 100 mμ or the same as the spherical particles. Some seemingly "degenerate" filaments are shown in Fig. 4 with spheres spaced along them which suggest that segmented filaments may break down releasing spherical forms.

It is important to note that the number of segmented or transitional forms seen is much higher in these tissue cultures than in influenza virus preparations from allantoic fluid. Neither spherical nor rod-like forms have been seen in the controls.

Discussion. There is good evidence that the elongated filaments seen in influenza virus infections of the chick embryo are intimately connected with the development of the virus for 4 reasons. First, the rods have been seen in 6 old strains of influenza virus and in several newly isolated ones(7,8), all strains examined having shown them. Normal allantoic fluid from embryos infected with other viruses have not shown them; second, other investigators(7) have shown that the filaments adsorb and elute from red cells in the same way that the spherical virus particles do, an observation which we have confirmed; third, we have shown in this article that on direct examination of tissue culture epithelial cells bearing the infection, filaments and spheres appear in close association with each other; fourth, segmented or beaded forms in various stages suggesting a transition from filaments to spheres have been demonstrated.

Summary. 1. Epithelium from influenza A infected chick chorio-allantoic membranes were grown in tissue culture and examined with the electron microscope.

2. The infected cells showed spherical virus particles in close association with elongated rod-like structures like those seen in ultracentrifuged allantoic fluid from influenza infected eggs.

3. Various gradations between unsegmented rods and beaded chains were seen in certain areas strongly suggesting a link between the two forms.

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Thyroid Hormone and Tissue Cholesterol Distribution.* (17757)

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A number of investigators have demonstrated, both in humans and in experimental animals, that there is an inverse relationship between the activity of the thyroid gland and the plasma cholesterol level(1). The influence of the thyroid hormone on the cholesterol concentration of other tissues, however, has been studied only to a very limited extent(2). In view of the importance of this problem for

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1. Reviewed in: Peters, J. P., and Van Slyke, D. D., *Quant. Clin. Chemistry, Interpretations,* I, Baltimore, 1946, 497.

2. (a) Forbes, J. C., *Endocrinol.,* 1944, v35, 126; (b) May, L. G., Moseley, R. W., and Forbes, J. C., *Endocrinol.,* 1946, v38, 147; (c) Fleischmann, W., and Shumacker, H. B., *Bull. Johns Hopkins Hosp.,* 1942, v71, 175.

TABLE I.
Experimental Groups.

Group	Dietary supplements: Endocrine component	Dietary supplements: Cholesterol and Bile Salt	Feeding period, wk	No. of rats
I	—	—	—	17
II	—	+	26-38	9
III	Thyroid	—	6	5
IV	"	+	6-8	11
V	Thiouracil	—	30	7
VI	"	+	32	8

the etiology of arteriosclerosis, the effects of experimental hyper- and hypothyroidism on the cholesterol distribution in blood, liver, and aorta were investigated in rats fed a high cholesterol diet. The results are summarized in the present report.

Methods. Young adult rats (University of Southern California strain) were fed this laboratory's stock diet, slightly modified to contain more vitamin B complex(3). The experimental diets were supplemented either with desiccated thyroid, or with 0.25% thiouracil, each either alone, or in combination with 1% cholesterol and 0.5% bile salt. The hyperthyroid groups include animals treated with two different thyroid preparations; the first series received desiccated hog thyroid, containing 0.65% total I and 0.31% thyroxine, at a level of 0.4% of the diet; the food of the second group was supplemented with 0.6% of thyroid powder U.S.P. (Armour). The cholesterol was added to the diet dissolved in warm cottonseed oil, as reported previously(3). The experimental conditions are summarized in Table I. For purpose of comparison, data for control rats, and for rats fed cholesterol and bile salt for 26-38 weeks, are included (Groups I and II). These data were taken from a recent publication(4). Following feeding periods of 6-8 weeks for the animals fed thyroid (Groups III and IV), and of 30-32 weeks for the groups treated with thiouracil (Groups V and VI), the animals were autopsied (nembutal anaesthesia). Blood and liver were taken for chemical determinations of total cholesterol, and the aorta for histological examination. The techniques employed for cholesterol extraction and determination, and for histological examination of the aorta were recently described(4). Löwenthal(5) stressed the importance of making histological studies for cholesterol as soon as the tissues are fixed, to avoid loss during storage in formalin. Our own observations confirm this point. Unfortunately, it was not possible, in the present work, to process in time all the aortas of the thyroid-fed rats (Groups III and IV); therefore, only the structural findings will be reported when examination was delayed.

Results. The experimental results are summarized in Table II. In all groups, the aorta appeared macroscopically normal. The doses of thyroid given were toxic and produced the expected symptoms, such as loss in weight, nervousness, rapid breathing, shivering and a shaggy coat. The mortality was high in the groups fed thyroid alone (III); in the first series of Group III, in particular, all rats died within a period of 5 weeks.† In the animals treated with the same doses of thyroid, but in addition with cholesterol and bile salt (Group IV), the symptoms appeared to be somewhat milder, and the mortality was significantly lower(3).

Thyroid hormone alone (Group III) caused a slight decrease in plasma cholesterol, but it had no significant effect on the sterol content of the liver. In the animals fed thyroid

3. Marx, W., Meserve, E. R., and Deuel, H. J., Jr., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 385.

4. Marx, W., Marx, L., Lipsett, M., and Deuel, H. J., Jr., to be published.

5. Löwenthal, K., *Frankf. Z. Path.*, 1926, v34, 145.

† Rats which died during the experimental period are not included in the data given in Table II.

TABLE II.
Experimental Results.

Group	Cholesterol content: Plasma, mg/100 ml	Cholesterol content: Liver, %	Liver wt, % of body wt	Histologic changes of aorta: No. of rats Examined	Showing microscopic changes: Cholesterol deposits	Showing microscopic changes: Intimal lesions
I	60 ± 3	.21 ± .02	3.26 ± .12	11	0	0
II	138 ± 12	2.03 ± .35	4.03 ± .19	9	2	2
III	34 ± 3	.27 ± .01	4.28 ± .30	4*	—	0
IV	45 ± 4	1.69†	4.96 ± .12	11‡	2	3
V	118 ± 18	.25 ± .01	3.92 ± .18	7	0	2
VI	251 ± 46	1.46 ± .25	5.97 ± .20	8	1	1

* Examined only on paraffin sections.
† Samples pooled.
‡ Four of these 11 specimens examined only on paraffin sections.

in combination with cholesterol and bile salt (Group IV), the plasma cholesterol was slightly higher than in Group III, yet still somewhat below normal. In spite of this low plasma level, a significant accumulation of the sterol occurred in the liver comparable in extent to the liver cholesterol content of the rats given cholesterol and bile salt without the addition of thyroid (Group II).

Microscopic examination of the aortas revealed that in group IV‡ five of seven rats had fine cholesterol needles scattered through the aorta, mostly in the intimal coat; in 2 of these animals the crystals were especially numerous. The aortas of the 2 remaining rats were merely loaded heavily with stainable fat.

The endothelium was thickened in 2 of 4 rats of Group III, and in all specimens of Group IV; in 3 of 11 animals of the latter group it was considered atheromatous.

Thiouracil alone (Group V) caused a significant increase in the cholesterol content of the plasma, without modifying the hepatic concentration. By far the highest plasma cholesterol values were observed in the animals fed both thiouracil and cholesterol and bile salt (Group VI). It is interesting, however, that in this group the cholesterol accumulation in the liver was not higher but, if anything slightly lower than in Group IV, in spite of an appreciably longer feeding period.

Upon microscopic examination of the aortas in Group V, only one rat, a female with large fibroadenoma of the mamma, was found to have traces of cholesterol. The other animals of this group showed only moderate to large amounts of stainable fat in the intimal coat, and traces in the media.

In Group VI, localized birefringence was seen in the endothelium of 4 rats, marked only in one of these animals. In 3 of these rats, the media also contained birefringent crystals.

Spots of intimal thickening were observed in 2 of 7 specimens of Group V, and in one rat of Group VI. In the latter the hypertrophy was pronounced enough to be called a fatty plaque; underneath the plaque, the media carried stainable fat, and films covered the elastic lamellae. Two other rats of this group showed only minor intimal thickening. More severe vascular changes than those seen in the aorta were found in sections of lung tissue of Group VI.

The liver weight was increased as a consequence of cholesterol as well as of thyroid supplementation. A combination of the two agents produced an approximately additive effect (Table II, IV). Thiouracil alone caused only a moderate increase in liver weight; by far the largest livers were observed in the group fed both thiouracil and cholesterol and bile salt (VI).

Discussion. The experimental results pre-

‡ The specimens of group III were examined on paraffin sections only.

sented here indicate that the plasma cholesterol level depends on the concentration of circulating thyroid hormone, in agreement with earlier reports(1). The cholesterol content of the diet, on the other hand, does not seem to influence plasma cholesterol significantly in the hyperthyroid rat. The liver cholesterol content was practically normal in all groups fed the low cholesterol diet, and it was strikingly increased by the high cholesterol intake. In both cases, the liver sterol values were practically independent of the concentration of circulating thyroid hormone. These observations confirm results obtained by May and others on animals treated with thiouracil(2b). Forbes, however, reported that thyroxin tended to decrease the cholesterol content of the liver, as long as the choline intake was adequate(2a). In connection with the unexpected cholesterol distribution found in the hyperthyroid rats fed the high cholesterol diet, it is interesting that Fleischman and Shumacker reported that treatment with thyroxine of rats fed a low cholesterol diet caused a decrease of serum cholesterol, but an increase in the sterol content of the entire carcass(2c).

The finding that no gross changes were produced in the aorta, even in the cholesterol-fed hypothyroid rats—the animals showing the highest plasma cholesterol levels—is in agreement with a report by Horlick and Havel(6). These results confirm again that rats are highly resistant to cholesterol atheromatosis. It might be mentioned, in this connection, that in another resistant species, *i.e.*, the dog, Steiner and Kendall recently obtained grossly visible atheromata as a consequence of cholesterol and thiouracil feeding(7). A quantitative comparison between the results of the dog and rat experiments, however, is not possible because the experimental conditions were different. Microscopic cholesterol deposition in the aorta was seen in a few experimental animals. This deposition occurred in two forms, indicating two types of structural alteration of the aortic wall. Hyperthyroid rats showed widely scattered cholesterol crystals, while thiouracil-fed animals possessed focal cholesterol accumulations. The appearance of cholesterol in the aortas of the hyperthyroid rats was unexpected, but it is in agreement with findings of Bruger and Fitz who performed experiments along similar lines, treating rabbits with pituitary thyrotrophic hormone(8). Other investigators, however, reported that thyroid hormone prevented or counteracted aortic cholesterol deposition and atheroma formation in rabbits and chickens fed a high cholesterol diet(9).

A comparison of the tissue cholesterol distributions shows that neither plasma and liver cholesterol concentrations, nor plasma cholesterol level and aortic cholesterol deposition ran parallel in the different experimental groups. On the other hand, hepatic and aortic cholesterol depositions followed a similar pattern.

Summary. The effects of high and low concentrations of circulating thyroid hormone on the total cholesterol contents of plasma and liver, and on aortic cholesterol deposition were studied in rats fed a diet either low or high in cholesterol.

In the hyperthyroid groups, the plasma cholesterol values were normal or slightly lower, and to some extent independent of the cholesterol content of the diet; in the thiouracil-treated series, the plasma cholesterol levels were significantly increased, reaching the highest values in the rats fed the high cholesterol diet.

The liver cholesterol concentrations were normal in the animals not supplemented with dietary cholesterol, and strikingly increased in all groups fed the high cholesterol diet, to a great extent independently of the concentrations of circulating thyroid hormone.

The aorta appeared macroscopically normal

6. Horlick, L., and Havel, L., *J. Lab. Clin. Med.*, 1948, v33, 1029.

7. Steiner, A., and Kendall, F. E., *Arch. Path.*, 1946, v42, 433.

8. Bruger, M., and Fitz, F., *Arch. Path.*, 1942, v25, 637.

9. (a) Murata, M., and Kataoka, S., *Verhandl. Jap. Path. Gesellsch.*, 1917, v7, 27; 1918, v8, 221; (b) Turner, K. B., *J. Exp. Med.*, 1933, v58, 115; (c) Dauber, D., Horlick, L., and Katz, L. N., *Am. Heart J.*, 1949, v38, 25.

in all groups; microscopic examination revealed, in the cholesterol-fed animals, a moderate cholesterol deposition, diffuse in the hyperthyroid rats, and focal in the thiouracil fed animals. Isolated cases of early atheromata and widespread fatty infiltration were observed in the rats fed thyroid and cholesterol, and in both groups treated with thiouracil.

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A Skin Test for Infectious Hepatitis.* (17758)

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Evidence has been obtained that the virus of infectious hepatitis (IH) can be propagated in minced chick embryo tissue culture and in the embryonated hen's egg(1,2). Virus thus cultivated induced mild hepatitis without jaundice in human volunteers but no laboratory test for the presence of virus has evolved as yet. However, as will be shown in this paper, amniotic fluid of chick embryos infected with IH virus may serve as skin test antigen with apparently specific results.

The Akiba strain of IH virus was passed 10 times through chick embryo tissue culture and then transferred in 0.2 ml amounts to the amniotic cavity of 8 day old chick embryos. After incubation at 37° for 7 days the whole contents of the eggs minus the albumen were emulsified in an approximately equal volume of saline by means of a Waring blendor and passed amniotically to fresh embryos. After 3 such passages, when slight liver damage became apparent in some of the embryos, only amniotic fluids were harvested and passed in further transfers and the incubation period was reduced to 5 days. Amniotic fluids of the 6th and 7th consecutive passage were used for IH skin test antigens. Control materials were prepared under identical conditions except for the fact that the amniotic series was initiated with buffered saline instead of the Akiba tissue culture material. The specific and control antigens were irradiated with ultraviolet light for a period 5 times that required to inactivate influenza virus. The materials were cultured aerobically and anaerobically, and tested for safety by intraperitoneal and intracerebral inoculation of mice and by intradermal injection of human volunteers with no history of IH. The IH and control antigens were stored at 4°C until used. They were administered in 0.1 ml amounts on the right and left forearm, respectively.

Results of 161 skin tests are shown in the Table. They were performed (a) in human volunteers in corrective institution No. 1 who, 3 months to 2 years previously had submitted to infection with IH virus as contained in stools and serum taken during the acute stage of IH (Akiba or NL Strain) or in tissue culture; (b) on volunteers in institution No. 2 injected 5 months to 5 years previously with the virus of serum hepatitis (SH); (c) on 62 random subjects of institution No. 1; (d) on 9 young children 2½ to 4 years old; and, finally, (e) on 10 cases of spontaneous IH 12 days to 6 months after onset of illness. Positive skin tests to IH were characterized by the development within 24 hours of an area of erythema of a somewhat brownish red tinge and of induration which as a rule exceeded 10 mm in diameter (4 to 45 mm) and which persisted, although decreasing in intensity, some-

* This investigation was conducted under the Commission on Liver Diseases, Armed Forces Epidemiological Board, Preventive Medicine Service, Office of the Surgeon General, U.S. Army.

1. Henle, W., Harris, S., Henle, G., Harris, T. N., Drake, M., and Stokes, J., Jr., in press.

2. Drake, M., Kitts, A., Blanchard, M., Farquhar, J., Stokes, J., Jr., and Henle, W., in press.