circulation *via* the thoracic duct lymph does not mix or exchange to any appreciable extent with the cholesterol of serum or of other organs before being taken up by the liver. Labeled cholesterol given off from the liver is then distributed over the body.

This course of the metabolism of newly absorbed cholesterol derived from experiments on the whole animal is consistent with the finding of Biggs(2) that labeled cholesterol of thoracic duct lymph when intravenously injected into the rat is selectively taken up by the liver, and with the suggestion of Friedman *et al.*(7,8) that the liver Kupffer cells are responsible for removal of newly arrived cholesterol. On the other hand it has been reported that the cholesterol of chylomicrons from dog lymph exchanges with the cholesterol of serum high density lipoproteins during incubation *in vitro* and also on injection into dogs(9).

From the results obtained it is obvious that the late blood cholesterol activity maximum in the rat is caused by the hold up of the newly absorbed cholesterol first in the intestinal wall and later in the liver before its reappearance in the circulation. By increasing the dose of cholesterol fed to 10 mg, the maximum in the blood activity is further delayed and occurs during the second day in accord with the finding of Gould *et al.*(4). The shape of the blood activity curve thus is dependent on amount of cholesterol absorbed.

Summary. 1) A trace dose of cholesterol-4-C^{14} incorporated into a well-balanced test meal has been fed to rats. The labeled cholesterol is rapidly absorbed *i.e.* 50 to 70%. The newly absorbed cholesterol is then held up by the cells of the small intestinal mucosa, the halflife of the newly absorbed cholesterol in these cells being approximately 12 hours. From the intestinal wall the cholesterol enters the chyle, the peak specific activity of which is reached about 9 hours after the feeding. 2) The newly absorbed cholesterol of the thoracic duct lymph in the intact animal is selectively taken up by the liver and is only subsequently mixed in a metabolic sense with the cholesterol of blood and other tissues. 3) As a consequence of the delay of the absorbed cholesterol in the intestinal wall and the liver, blood cholesterol activity time curve reaches its maximum about 16 hours after the feeding. This delay in blood activity peak is prolonged to the second day if amount of cholesterol fed is increased to 10 mg.

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Serum Lipids in Hypercholesterolemic Rabbits. (24353)

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Kellner, Correll and Ladd(1) showed that intravenous injection of the surface active agent Triton A20 into rabbits fed a high cholesterol diet reduced incidence and severity of the resulting atherosclerotic lesions in spite of the fact that the serum cholesterol levels were, in most instances, higher than the levels found in the controls on the cholesterol diet itself.

Byers and Friedman(2) reported that serum of rats rendered hypercholesterolemic by Triton injections was very lipemic but could be clarified by centrifugation at 25,000 to 40,000 g. We have shown that practically all serum cholesterol of rabbits rendered hypercholesterolemic by cholesterol feeding was extracted when lyophilized serum was shaken with cold chloroform(3). Similar results were obtained with serum from Triton-injected animals. Since the degree of atherosclerosis which develops in these 2 conditions is so markedly different in spite of the fact that cholesterol extractability by cold chloroform was comparable in both, it was decided to investigate further the physical separation of various lipids by centrifugation at 20,000 g.

Methods. Rabbits weighing 4 to 5 lb. were fed a diet of Rockland Rabbit chow to which was added 5 or 10 g of cholesterol plus 50 g of cotton seed oil/950 g of chow. The cholesterol was dissolved in chloroform, added to cotton seed oil and the whole added to the chow. Chloroform was allowed to evaporate before the diet was fed. Some animals were given intravenous injections of 5 ml of a 20% solution of Triton WR1339 containing 0.6% sodium chloride. Others were given intravenous Triton while on normal diet. Blood samples were collected at intervals. The original serum and the subnatant following centrifugation at 20,000 g for at least 2 hours were analyzed for lipids. If the subnatant was still lipemic after high-speed centrifugation it was recentrifuged. In some of the Triton-injected animals 4 centrifugations were necessary in order to obtain a reasonably clear subnatant fluid. Once centrifugation produces a clear subnatant further centrifugation results in insignificant changes. Total cholesterol was determined by previously described procedure(4) modified for use in photoelectric colorimeter and with careful control of temperature during color development. Lipid phosphorus was determined by the procedure of Outhouse and Forbes(5). Modification of a method recently published by Van Handel and Zilversmit(6) was employed for direct determination of neutral fat. The complete procedure will be published elsewhere.

Results are shown in Table I. Triton injections, whether accompanied by high cholesterol diet or not, caused a marked rise in all lipids under study. Most of this increase was found in the fraction which rose to the surface during centrifugation at 20,000 g. Usually, at least two-thirds of total serum cholesterol was present in this fraction following 2 or more injections of the surface active agent. A considerably smaller percentage of total serum cholesterol was present in this chylomicron fraction when hypercholesterolemia was produced by cholesterol feeding alone. In spite of the fact that administration of Triton to cholesterol-fed animals caused a marked increase in total cholesterol the amount remaining in the subnatant following centrifugation at 20,000 g invariably showed a distinct drop following 2 or more Triton injections. Triton injected animals showed a tremendous increase in serum neutral fat following injections, most of which was present in the chylomicron fraction.

Discussion. Bragdon(7) showed that when serum of rabbits which were rendered hypercholesterolemic by cholesterol feeding was injected into normal animals, lesions resembling atherosclerosis were produced. If the serum was centrifuged at 42,000 rpm and the upper fraction injected, no lesions resulted. Lesions were produced when the subnatant fraction was injected. Both fractions were shown to contain cholesterol. When our results are considered with those of Bragdon a reasonable explanation for the low atherogenicity of Triton-induced hypercholesterolemia as well as for the protective action of Triton injections against atherogenicity of a high cholesterol diet seems evident. If only the material in the subnatant is atherogenic, then in the Triton-injected animals, only about 30% of total serum cholesterol would be atherogenic. For example, in Exp. 2 after several Triton injections, even though total cholesterol averaged over 2%, less than 0.4% remained in the subnatant. These same animals, after 50 days of cholesterol feeding alone, showed, out of a total cholesterol of 1.57%, 0.93% remaining in the subnatant. In Exp. 4, where no cholesterol was fed, only about 0.3% remained in the subnatant out of an average total cholesterol of about 1%.

TABLE I. Effect of Intravenous Triton on Physical State of Serum Lipids of Rabbits.*

Exp.	No. of rabbits	Avg serum lipid (range) Original serum T. chol., %	P.L., mg %	N.F., %	Subnatant T. chol., %	% drop	P.L., mg %	N.F., %	Remarks
1	5	.26 (.20– .31)			.24 (.15– .30)	8			After 17 days on .5% cholesterol diet
		.30 (.13– .51)			.27 (.10– .51)	10			After 54 days on diet
		1.64 (1.2 –2.5)			.94 (.32–1.2)	43			After 21 additional days on 1% cholesterol diet
		3.08 (1.4 –4.5)			.98 (.29–1.4)	68			3 days after 2 Triton inj. 3 days apart
2	5	.82 (.37–1.3)			.62 (.34–1.1)	24			After 32 days on 1% cholesterol diet
		1.26 (.91–1.6)			.93 (.77–1.0)	26			After 50 days on diet
		2.68 (1.8 –3.1)	53		.28 (.16– .39)	89	28		After several Triton inj.
		2.25 (1.2 –2.9)	57		.38 (.33– .43)	83	18		After several more inj.
3	2	1.83 (.56–3.1)	18	.38	.95 (.41–1.5)	48	12	.12	After 5 wk on 1% cholesterol diet
		1.88 (1.2 –2.6)	21	.34	1.22 (.93–1.5)	35	19	.20	After 2 additional wk on diet
		2.50 (2.0 –3.0)	54	3.0	1.22 (.93–1.5)	51	36	1.2	3 days after 1 Triton inj.
		3.10 (1.6 –4.6)	53	4.1	.80 (.30–1.3)	74	24	.81	3 days after 2 more inj. 3 days apart
4	4	.70 (.67– .75)	43	4.4	.30 (.27– .34)	57	19	1.4	Normal diet, 3 days after 1 Triton inj.
		.97 (.84–1.1)	48	4.9	.34 (.25– .51)	65	24	1.3	4 days after 2nd Triton inj.
		1.22 (1.1 –1.3)	71	8.4	.30 (.26– .35)	75	24	1.8	3 days after 2 more inj. 3 days apart

* Except where otherwise stated Triton inj. were given 6 or 7 days apart.
P.L. = Phospholipid phosphorus. N.F. = Neutral fat.

A similar explanation may account for the fact that we have encountered elevated total cholesterol and "readily extractable"* cholesterol in some patients without any clinical evidence of arteriosclerotic heart disease. Previous work (8) indicated that this "readily extractable" cholesterol represents cholesterol which is associated primarily with chylomicrons and lipomicrons.

Summary. Intravenous injections of the surface active agent Triton WR1339 into rabbits caused a marked increase in serum neutral fat as well as in cholesterol and phospholipids. A large percentage of this increase was found in the lipid fraction which rose to the surface when serum was centrifuged at 20,000 g for 2 hours or more. Administration of Triton to rabbits rendered hypercholesterolemic by cholesterol feeding decreased the percentage and absolute value of total cholesterol remaining in the subnatant fraction after high-speed centrifugation. After several injections, concentration of cholesterol in the subnatant dropped well below the pre-injection value in spite of increase in total serum cholesterol concentration. It is suggested that an increase in percentage of the cholesterol associated with chylomicrons together with a probable decrease in the fraction associated with lipomicrons might be factors in Triton-induced inhibition of development of atherosclerosis in cholesterol-fed rabbits.

* Cholesterol which is extracted by cold chloroform from lyophilized sera.

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Antigenic Variation of Asian Influenza Virus.* (24354)

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During the fall of 1957, there was an outbreak of Asian influenza at the State University of Iowa. Numerous paired human sera were collected and 20 virus isolations made. All isolates were identified as Asian or "A" virus. Many of patients' paired sera gave diagnostic rises in titer by complement fixation test (CF), but were negative by the hemagglutination inhibition test (HI) when A/Jap/305/57 virus was used as antigen. To ascertain whether a more satisfactory HI antigen was available, several early isolates were tested. There was little cross reaction by HI among these viruses against the same patients' sera and many sera previously negative by HI were positive. These results suggested that "A" viruses isolated were antigenically different, and that cross neutralization by HI should show this difference in antigenicity. This paper summarizes results of cross neutralization by HI of 19 of the "A" virus isolates, and the A/Jap/305/57 virus with 20 paired human sera.

Materials and methods. Viruses. "A" viruses were recovered from students at the State University of Iowa by use of embryonated eggs. Twenty strains were recovered and designated by abbreviation of patients' names. One strain A/Jap/305/57 was supplied by the Communicable Disease Center. *Hemagglutinating antigens.* The antigens were allantoic fluids from embryonated eggs infected with "A" viruses. The allantoic fluid antigens after 4 to 6 passages had titers ranging from 1:80 to 1:2560 against chicken red blood cells. Allantoic fluid virus from the same egg passage was used as antigen throughout the tests. Each antigen was bacteriologically sterile. *Paired sera from influenza patients.* All paired human sera, designated by abbreviation patients' names from whom they were drawn, were inactivated at 56°C for 30 min. and used without further treatment. *Serological procedures.* Hemagglutination inhibition (HI) titrations were done with chicken red blood cells using the Salk pattern test(1), with one modification. Instead of 0.5% chicken red blood cell suspension, a suspension of 0.25% cells was used. Titers for the HI test are reported in terms of initial serum dilution (1:10) and all titers adjusted for 4 hemagglutinating doses. A standard complement fixation test (CF) was used(1). All paired sera tested, except PET, showed at least a 4-fold rise in titer. PET sera showed a 2-fold rise.

Results. Twenty "A" viruses were tested against 20 paired human sera by HI. From results obtained, the viruses were classified as 2 main groups: (1) those against which both acute and convalescent sera had high HI titers and which were considered inhibitor susceptible, (2) those against which the acute

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