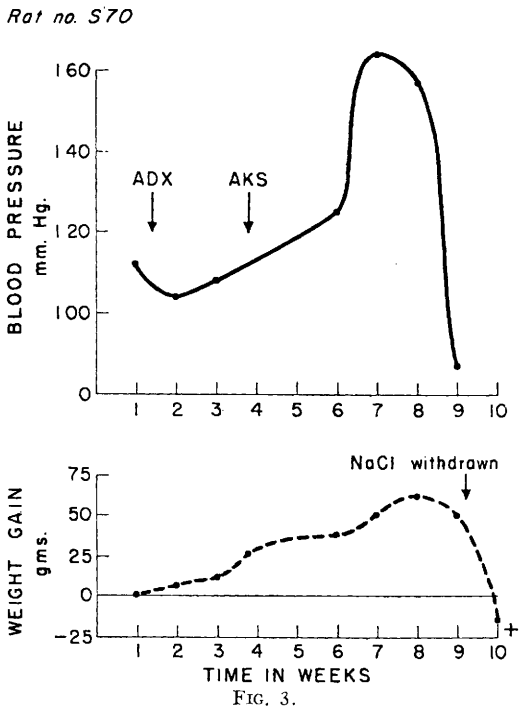

FIG. 3.

Chart of Rat No. S70—blood pressure readings and weights during experimental period.
ADX = Bilateral adrenalectomy
AKS = Rabbit anti-rat kidney serum.

Table III. From this it may be seen that the body weights of the adrenalectomized animals averaged less than that of the intact groups with the exception of the hypertensive adrenalectomized animals of Group 6. The combination of high NaCl intake and anti-kidney serum resulted in renal enlargement in the intact rats of Group 5. A similar degree of renal enlargement occurred among the hypertensive animals of Group 6. The non-hypertensive animals in this group had smaller kidneys, but since their body weights are so much less than that of Group 5, it is difficult to interpret these findings. Also included in this table is the number of rats showing histological evidence of renal damage. Renal lesions were, if anything, less frequent in the adrenalectomized rats of Group 6 than in the similarly treated intact Group 5 animals.

Summary. In response to cytotoxic serum nephritis, bilaterally adrenalectomized rats develop hypertension as frequently as do animals with intact adrenals. In order for hypertension to appear among such adrenalectomized rats, it is necessary that a satisfactory state of nutrition be maintained over a period of weeks. In our laboratory, the difficulties encountered in fulfilling this provision have been considerable in spite of the employment of a high NaCl regimen. This experience has led us to wonder whether the conflicting results in the literature relating to the question of hypertension in adrenalectomized animals may not be due, in part, to similar difficulties.

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Conjugated Linoleic Acid in Rat Tissue Lipids after Ingestion as Free Acid and as Triglyceride.*† (18009)

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The present study was conducted to test the use of conjugated linoleic acid for proposed investigations of the metabolism of glycerides by the use of carbon 14 labeled glycerol. The progression of the conjugated acid through the various tissues at successive times after its ingestion is intended as a guide for later studies with the labeled glycerol esterified with tagged acids. Advantage was taken of the experiment to test the observations of Frazer that fatty acids after ingestion go to the liver but that unhydro-

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lyzed glycerides go to the fat depots(1). The use of conjugated fatty acids as an indicator of the pathway of absorption and deposition of fat is not new. Miller and Burr(2) fed eleostearin to rats and determined its concentration in tissues at succeeding intervals. Their results were inconclusive because a new diene acid was apparently formed from the triene and the two acids were found in different concentrations. Later(3) the same laboratory reported that 5 minutes after feeding methyl or glycerol esters of conjugated linoleic acid the tagged acid constituted 26.7% of the total mucosa fatty acids. The same authors also reported(4) that after ingestion the conjugated fatty acids of corn oil were found in the acetone soluble fraction of the mucosa lipids rather than in the insoluble fraction.

Preparation of conjugated linoleic acid and its triglyceride. The fatty acids of cottonseed oil (Wesson Oil) were subjected to fractionation by the urea method(5). The diene fraction was then isomerized by the method of Bradley and Richardson(6) and the washed and dried conjugated acid esterified with glycerol by the use of the Twichell reagent at high vacuum and a temperature of 135°C. The contents of conjugated acid in the preparation were determined according to Brice *et al.*(7).

Experimental procedure. The test meals of either the conjugated acid or the triglyceride were prepared by emulsifying the preparation with tween and water so that 5 ml contained 500 mg of the conjugated acid. The material was fed by stomach tube without previous fasting at a level of 500 mg of conjugated acid per 250 g of body weight. Each experiment was performed with 2 rats in order to obtain adequate quantities of tissues for analyses. At 10, 16, 24 and 48 hours afterwards the animals were sacrificed and the blood, liver, pooled heart, lungs and kidneys and the mesenteric, perirenal and subcutaneous depot fats analyzed for their content of conjugated linoleic acid. The 10 and 16 hour experiments were performed twice; the figures in the tables being the average values.

All the tissues except blood were extracted in a Waring blendor with 10 volumes of chloroform. The mixtures were centrifuged and the partially cleared chloroform solution filtered. The chloroform was removed by vacuum distillation and approximately 100 mg samples of the lipids accurately weighed, dissolved in 100 ml of iso-octane and the density at 233 mμ determined in the Beckman spectrophotometer. Untreated animals were used as controls, the density used for final evaluation being the differences between the control and experimental tissues. Blood was analyzed by precipitation in 5 volumes of 3:1 alcohol-ether mixture. Fifteen to 20 ml of blood were obtained from a pair of rats. The solution was filtered through a small sintered glass funnel and the coagulum washed 3 times with ethyl ether. The combined extract and the washing were evaporated to dryness with reduced pressure. The impure lipids were dissolved in iso-octane. Some anhydrous Na_2SO_4 was added to assure dryness. The dry solution was filtered and made up to 100 ml. Fifty or 75 ml of the solution were used for the determination of its fat content and the spectral density determined with the remainder.

Findings. The analytical results are presented in Tables I and II. The results indicate that there is a difference in the rate and possibly in the extent of absorption, but that the pathways of absorption are probably the same. Thus, after glyceride ingestion, the maximum level of conjugated acid appears in the liver, blood and pooled organs at 16 hours while after free acid ingestion the maximum

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TABLE I.
Percentages of Conjugated Linoleic Acid in the Tissue Lipids of Rats Fed Conjugated Linoleic Acid or Its Triglyceride.

Tissue		Hrs after ingestion 10	16	24	48
		%	%	%	%
Liver	After Fatty Acid	2.02	2.75	3.05	.88
	" Glyceride	3.80	5.60	3.35	.81
Blood	After Fatty Acid	1.69	1.53	2.78	.00
	" Glyceride	2.00	2.58	1.38	.00
Organs	After Fatty Acid	0.27	0.69	0.78	.27
	" Glyceride	1.44	1.28	1.12	.65
Depot Fat	After Fatty Acid	0.12	0.13	0.28	.30
	" Glyceride	0.18	0.25	0.45	.30

TABLE II.
Percentages of Conjugated Linoleic Acid in the Depot Fats of Rats Fed Conjugated Linoleic Acid or Its Triglyceride.

Depot Fat		Hrs after ingestion 10	16	24	48
		%	%	%	%
Mesentery	After Fatty Acid	.19	.14	.39	.48
	" Glyceride	.22	.36	.52	.32
Perirenal	After Fatty Acid	.08	.18	.27	.22
	" Glyceride	.17	.22	.47	.32
Subcutaneous	After Fatty Acid	.09	.08	.17	.19
	" Glyceride	.15	.17	.36	.27

occurs at 24 hours. The maximum levels are also higher after glyceride ingestion, which would also indicate more rapid absorption. That glycerides may be absorbed more completely than fatty acids has been reported previously. Thus Lyman found that 95 to 96% of glycerol palmitate but only 80% of palmitic acid was absorbed by rats(8). The comparatively poor absorption of free fatty acid has also been reported by Perretti(9).

In Table II is given the percentage of conjugated linoleic acid in various depot fats. The average of these figures is given in Table I but the individual results are given here to show that some variations exist between the 3 depots. Labeled fat appeared more rapidly and to a greater extent in the mesentery fat, then in the perirenal and finally in the subcutaneous. It may be seen that the labeled acid increased in the depot fat for 24 hours and then leveled off, remaining at about the same concentration after 48 hours. The evidence presented here does not support the theory that fatty acids are absorbed by way of the portal blood to the liver and glycerides by way of the lymph directly to the fat depots. The higher content of conjugated acid in the fat depots after glyceride feeding is matched by its higher content in the liver and pooled organs. This shows quite clearly that the liver plays a major role in the absorption of fat as well as fatty acids. It is quite possible that many of Frazer's results on the differences in behavior of fatty acids and fats during absorption(4) can be explained on the basis of their different degrees and rates of absorption.

This slow and possibly incomplete absorption of free fatty acids still remains to

8. Lyman, J. F., *J. Biol. Chem.*, 1917, v32, 7.

9. Perretti, G., *Boll. Soc. Ital. Biol. Sper.*, 1935, v10, 873.

be explained. If complete hydrolysis of fats precede absorption it should be expected that fatty acids and glycerides should be absorbed equally well, or that free acid should have an advantage. Since it has been known since the time of Frank's work that ingested fatty acids appear in the lymph as glycerides one is tempted to conclude that it is this synthesis that delays their absorption but that triglycerides need undergo no change and are absorbed as such.

However, there is an alternative interpretation. Thus the important physical state of comparatively large quantities of free fatty acid in the lumen of the intestine may be pronouncedly different from that of fat plus a small amount of fatty acid slowly released by hydrolysis and constantly removed by absorption. The resynthesis of fat from fatty acid is an uncontested fact. The requisite glycerol for this resynthesis is on hand from a recently hydrolyzed fat, but must first be synthesized in or carried to the mucosa for synthesis from free fatty acid. This could conceivably cause a delay in absorption.

Summary. From spectrophotometric examination of the tissue lipids of rats at 10, 16, 24 and 48 hours after being fed free conjugated linoleic acid or its glyceride, it is concluded that glycerides are absorbed more rapidly, and possibly more completely, than free fatty acids but that the pathways of absorption are the same.

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Competitive Elution of Pertussis Hemagglutinin. (18010)

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Keogh, North and Warburton(1) have observed that erythrocytes of various mammalian and avian species are agglutinated by saline suspensions of *Hemophilus pertussis* and related members of this genus. Hemagglutination was also induced by filtrates or cell-free supernatants of liquid-medium cultures of these organisms. Other investigators (2,3) have subsequently demonstrated similar hemagglutination with other bacterial species. Conditions for the production of the hemagglutinating substance in casein-hydrolysate broth, and the relation of the hemagglutinating substance to the antigen or antigens which induce protective antibodies against *H. pertussis* infection in mice, have been described by Fisher(4) and by Keogh and North(5) respectively.

In the course of a study of the behavior and properties of the pertussis hemagglutinin we attempted to elute the hemagglutinin from erythrocytes by various methods. Hemagglutination was obtained with the supernatants of 7- to 9-day-old cultures of virulent strains‡ of *H. pertussis* grown in Cohen and Wheeler's medium(6). The potency of such preparations was determined by adding

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4. Fisher, S., *Australian J. Exp. Biol. and M. Sc.,* 1948, v26, 299.

5. Keogh, E. V. and North E. A., *Australian J. Exp. Biol. and M. Sc.,* 1948, v26, 315.

‡ Strains No. 16945, 18323, 18530 and 18560, kindly furnished by Dr. Pearl L. Kendrick, Michigan Department of Health.

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