age treated with 8 IU PMS. The incidence of mating and the number of implantation sites reported here and those recorded by Strauss are greater than those observed by earlier workers (Cole, PMS(2); Evans and Simpson, FSH or FSH plus HCG (8); Austin, PMS plus HCG(9)).

Summary. A single injection of 20 IU PMS on Day 30 and 1.2 IU HCG intravenously on Day 32 caused superovulation in the rats and, if mated on the evening of Day 32, an average of 22.8 implantation sites on Days 10 to 13 of pregnancy. Thirteen to 26 blastocysts were recovered from the uteri of the rats ovariectomized on Day 3 of pregnancy and given 2 mg of progesterone daily starting on Day 3. Implantation of these blastocysts was induced by a single injection of 1 μg estrone. An average of 17.3 sites was obtained when 20 IU PMS plus 1.2 IU HCG was used, and 7.7 sites when 10 IU PMS was given. The method for inducing superovulation provides the means for obtaining large numbers of blastocysts on Day 4 or 5 post-insemination, and large numbers of delayed blastocysts by a combination of treatments which cause superovulation and delayed implantation.

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Enhancement of Alimentary Hyperglyceridemia by Fructose and Glycerol in Man.* (31411)

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It is well established that administration of glucose either by oral or parenteral route diminishes the alimentary hyperglyceridemia (1,2,3). A similar effect is obtained with insulin(1). There is much evidence to suggest that this phenomenon is attributable to an accelerated rate of elimination of triglycerides from the circulation rather than to a decreased inflow of fat from the gut. Thus, the clearing of intravenously administered fat emulsion is impaired in experimental diabetes and can be normalized with insulin treatment (4,5). The activity of adipose tissue lipoprotein lipase is augmented by glucose(6,7) and diminished in diabetes(8). Also the fasting plasma triglyceride level is rapidly decreased on giving glucose(9,10) or insulin (10,11). On the other hand, it has been shown that glucose promotes the esterification of fatty acids in the intestine(12,13) and could thus be expected to increase the input of triglycerides from the thoracic duct into plasma.

The present study was undertaken because there was good reason to expect that the effects of fructose and glycerol on the alimentary hyperglyceridemia might be different from those of glucose. Both substances provide α-glycerophosphate for esterification of fatty acids in the intestinal mucosa, and, secondly, they stimulate the insulin secretion much less than glucose. Furthermore, it has been recently demonstrated that both fructose and glycerol are able to induce hyperglyceridemia in the rat(14,15).

Material and methods. The experiments

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were performed on patients hospitalized for different minor non-metabolic disorders. They showed no signs of disturbed intestinal function and their fasting plasma triglyceride levels ranged from 72 to 178 mg/100 ml. No special dietary arrangements were made for the study except that the subjects fasted for 12 hours before start of the experiment. During the test no drugs or food other than indicated below was given and the subjects were inmobilized.

Two oral fat "tolerance" tests were performed on each subject with 1 to 3 days' interval. In the first test, 100 ml of cow's cream containing 40 g of fat was given within a few minutes followed by 200 ml of water 1 hour later. Blood samples were withdrawn before and 2, 4 and 6 hours after the fat intake. The second test was otherwise similar but one hour after the cream the subjects received 20 g of either glucose (5 subjects), fructose (8 subjects) or glycerol (9 subjects) orally as a 10% solution. Three subjects received 50 g of fructose. In 2 additional experiments, 50 g of fructose was administered as an intravenous infusion starting one hour after the fat meal and lasting for 3 hours. In the control test these subjects received a similar infusion of physiological saline. In some control tests the subjects were given only the hexoses or glycerol without fat.

Plasma triglyceride was determined in principle according to Carlson(16). Separation of the lipoprotein particles into density classes below and over 1.006 was carried out by layering the plasma under physiological saline and spinning in a Spinco L 50 ultracentrifuge 30 min × 20,000 rpm.

Results. The mean plasma triglyceride increment *vs* time curves for each experimental group are shown in Fig. 1. Oral administration of glucose caused a decrease of plasma triglyceride response in 3 of the 5 cases studied while fructose and, even more, glycerol enhanced the alimentary hyperglyceridemia in each individual subject and at almost every time point studied. No change of plasma triglyceride occurred when the 3 substances were given in the same dosage without fat indicating that the effects observed could not be attributed to alterations

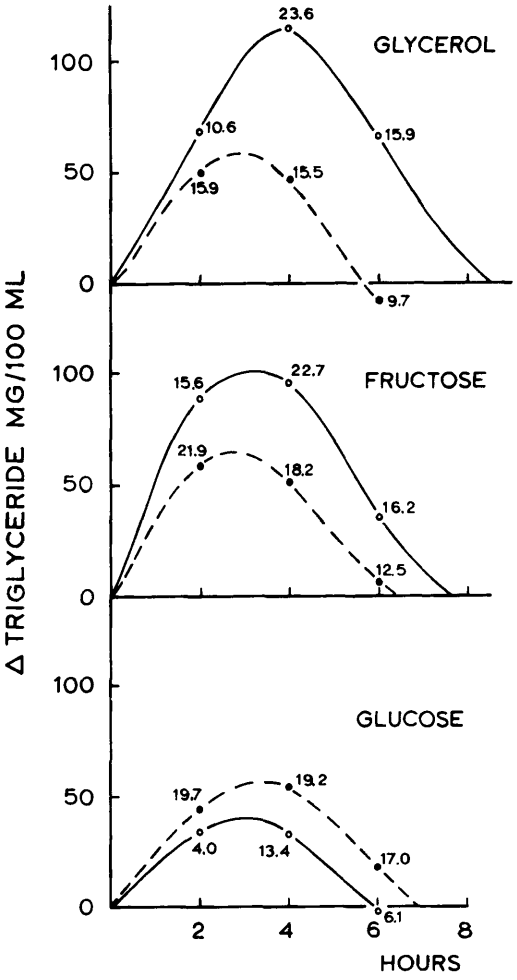

FIG. 1. Mean increase of plasma triglyceride above the fasting level in subjects given 40 g fat alone (●- -●) or in combination with glycerol, fructose or glucose (○——○). The figures give standard error of the mean.

in the basal ("fasting") triglyceride concentration. Increase of the fructose dose from 20 to 50 g did not result in a significantly greater plasma triglyceride response. In the 2 subjects to whom the fructose was given intravenously the hyperglyceridemia was enhanced only insignificantly. The increase of triglyceride in the <1.006 and >1.006 particles followed an essentially similar pattern in the 2 fat loading tests (Table I).

Discussion. The results indicate that glucose, fructose and glycerol alter the magnitude of serum triglyceride response to a constant

TABLE I. Mean Increment of <1.006 and >1.006 Particle Triglyceride Level Above the Fasting Concentration (mg/100 ml) in 3 Subjects Receiving Fat With and Without Fructose and in 3 Subjects Receiving Fat With and Without Glycerol.

	2 hr		4 hr		6 hr	
	<1.006	>1.006	<1.006	>1.006	<1.006	>1.006
Fat	29.3	12.0	27.3	6.1	5.3	—3.7
Fat + fructose	29.3	21.8	56.3	11.2	8.3	— .9
Fat	34.0	11.2	32.0	24.1	6.3	2.0
Fat + glycerol	46.6	18.1	55.0	29.2	20.0	28.1

oral fat load but that the shape of the serum triglyceride *vs* time curve remains essentially unchanged. Such effect may be obtained by altering the triglyceride influx into the blood or the removal efficiency/substrate concentration ratio. What is important to realize in this context is the fact that during the absorption period the amount of triglyceride poured from the thoracic duct into the blood stream and the quantity of triglyceride absorbed from the intestinal lumen are not equal. It has been shown by Karmen *et al*(17) and confirmed by others(18,19) that during the process of fat absorption 20 to 45% of lymph triglyceride fatty acids are of endogenous origin. This means that the fat load directed to plasma during the hours following a fat meal exceeds the intake by an average factor of 1.5. Furthermore, the utilization of endodenous precursors for the intestinal triglyceride synthesis is apparently highly variable, and, therefore, the constancy of exogenous fat dose does not also ensure that blood is exposed to a constant triglyceride load.

The present observations on the diminishing effect of glucose on the alimentary hyperglyceridemia are in accordance with the earlier experience(1,2,3). As mentioned above, glucose may affect the magnitude of plasma triglyceride response in 2 opposite ways. In incubated intestinal preparations glucose added *in vitro*(13) or given to fasted animals *in vivo*(12) stimulates the esterification of fatty acids evidently through providing both α-glycerophosphate and ATP. It is highly probable that this effect results in an increased inflow of endogenous fatty acids into lymph triglycerides. On the other hand, glucose augments the elimination rate of triglyceride from the blood either by changing the metabolic pattern in tissues or through the stimulation of insulin secretion with a concomitant activation of lipoprotein lipase. The available evidence on the low adipose tissue (or postheparin plasma) lipoprotein lipase activity in insulin deficiency states such as fasting(20) and absence of β-cells(8, 21) suggests that insulin might act as a positive effector of lipoprotein lipase increasing its affinity to substrate at below-saturation level. The net effect of glucose clearly shows that the stimulation of the removal mechanism is efficient enough to compensate also for the possible increased influx of endogenous intestine-borne triglyceride.

The finding that fructose and glycerol not only lack the lipemia-reducing capacity of glucose but that they even enhance the alimentary hyperglyceridemia may have two explanations: inhibition of plasma triglyceride removal or stimulation of the intestinal synthesis of triglyceride from endogenous fatty acids. The finding that intravenous was less effective than oral fructose speaks against the former possibility. That fructose does not stimulate chylomicron removal to the same extent as glucose may be ascribed either to a weak stimulation of insulin secretion or to a slow *in vivo* metabolism of fructose in adipose tissue.

A mass of evidence favors the second alternative. Both fructose and glycerol are ample sources of α-glycerophosphate in the intestinal mucosa. In human intestine fructose is predominantly metabolized *via* the fructose-1-phosphate pathway producing triose phosphates(22). Oral fructose-C^{14} is incorporated into the glyceride-glycerol of rat intestinal mucosa to a much greater extent than glucose-C^{14}(23). The presence of glycerokinase in the intestinal mucosa of many animals is well documented and the incorporation of oral

glycerol-C^{14} into lymph triglycerides of man has been demonstrated(24). It is also known that intragastric administration of glycerol increases the *in vivo* esterification of fatty acids by hamster intestinal mucosa(12).

Summary. Single oral doses of fructose and glycerol administered to normoglyceridemic human subjects after a standard fat meal increase the postprandial hyperglyceridemia while glucose given in a similar way decreases the serum triglyceride response. It is tentatively suggested that the effect of fructose and glycerol is due to a stimulation of intestinal synthesis of triglycerides from endogenous fatty acids. The opposite effect of glucose might be attributed to an accelerated elimination of triglycerides from the blood possibly mediated by insulin.

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A Comparison of *n*-Hexadecane and Mineral Oil Emulsions in Induction of Hypersensitivity in Mice.* (31412)

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The adjuvant action of Freund-type water-in-oil(w/o) emulsions for the immunogenicity of antigens is imperfectly understood. It has been attributed to the various performances of emulsion constituents acting singly as well as to their collective effect in determining the characteristics of the entire emulsion(1,2). Mineral oils are superior to animal and vegetable oils in constituting effective Freund emulsions, probably because they are more poorly catabolized than animal and vegetable oils. Shaw *et al*(1) compared the adjuvant activity of various pure constituents of mineral oils in inducing experimental allergic encephalomyelitis in guinea pigs. They found C_{15-20} alkanes to be better than those of either shorter or longer carbon chains. Thus, they showed that crude oils of variable and complex composition can be replaced by such pure chemicals as *n*-hexadecane, a C_{16} alkane.

We have used *n*-hexadecane with excellent results in our experiments on mouse delayed

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