TABLE IV. Leukocytes in CSF of Monkeys Inoculated with Active ECHO Viruses Types 6 and 16.

Cells/mm³ CSF	No. of monkeys								
500-599			1		1				
400-499		2							
300-399		2							
200-299		1		1	2				
100-199		2	2						
21- 99		1	1	1	1	1	1	2	2
0- 20	8					1	3	2	2
	0	2	4	6	7	8	10	15	21
	Day after inoculation								

Maximal WBC count in 2 control monkeys receiving heat-inactivated viruses was 20/mm³.

culture fluid, the highest count observed was 20 cells/mm³.

Microscopic examination of the spinal cords revealed no convincing evidence of neuronal damage. In sections from certain monkeys that received active virus, however, slight round cell infiltration of the meninges was observed in a few areas (Fig. 1).§

Discussion. Mild or moderate muscle weakness has been observed in human beings infected with ECHO types 2(6), 4(4), 6(2), 9(5), and 16(4) virus. It is therefore of interest that in monkeys experimentally inoculated with ECHO 6 and 16 certain muscles were affected. The findings here reported also indicate that, just as in man, these 2 representative ECHO viruses are capable of producing in rhesus and cynomolgus monkeys a mild meningitis characterized by the presence of virus in the spinal fluid and a predominantly mononuclear pleocytosis.

Summary. In monkeys injected intrathecally with ECHO virus 6 and 16, clinical signs of muscle weakness, pleocytosis of cerebrospinal fluid and development of homologous neutralizing antibodies were observed. These reactions were not detected in 2 animals inoculated in comparable manner with suspensions of these viruses which had been heated at 65°C for one half hour.

§ We are indebted to Doctors D. E. Denny-Brown, Joseph M. Foley and John M. Craig for examining sections of CNS tissues.

1. Committee on the Enteroviruses. *Am. J. Pub. Health,* 1957, v47, 1556.

2. Kibrick, S., Melendez, L., Enders, J. F., *Ann. N. Y. Acad. Sci.,* 1957, v67, 311.

3. Karzon, D. T., Barron, A. L., Winkelstein, W. Jr., Cohen, S., *J. Am. Med. Assn.,* 1956, v162, 1293.

4. Hammon, W. McD., Yohn, D. S., Ludwig, E. H., Pavia, R. A., Sather, G. E., McCloskey, L. W., *ibid.,* 1958, v167, 727.

5. Sabin, A. B., *A.M.A. Am. J. Dis. Child.,* 1958, v96, 197.

6. Steigman, A. J., *Ann. N. Y. Acad. Sci.,* 1957, v67, 249.

7. Dalldorf, G., *J. Exp. Med.,* 1957, v106, 69.

8. Habel, K., Loomis, L. N., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 597.

9. Wenner, H. A., Chin, T. D. Y., *Spec. Publ. N. Y. Acad. Sci.,* 1957, v5, 384.

10. Robbins, F. C., Weller, T. H., Enders, J. F., *J. Immunol.,* 1952, v69, 673.

11. Milovanovic, M. V., Enders, J. F., Mitus, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 120.

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Lipemia-Induced Acceleration of Intravascular Clotting.* (25001)

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Reports of changes in coagulability of blood associated with post-prandial lipemia have thus far been based on test tube methods (1,2). Lipemia following ingestion of cream, as well as intravenous infusion of cottonseed oil emulsion were found by one of us to result in significant shortening of whole blood clotting time in siliconized tubes and of plasma clotting time with Russell's Viper Venom(3). The question presented itself as to whether these *in vitro* findings reflected an increased tendency to clot formation *in vivo*. Therefore, a study was undertaken of the effects of

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intravenous administration of a fat emulsion† upon clotting of stagnant venous blood in the anesthetized dog, utilizing the experimental approach previously described by Wessler(4).

Material and methods. All experiments were performed on apparently healthy mongrel dogs weighing 25 to 48 lb, anesthetized with Pentobarbital. Sterile normal saline was infused into an antecubital or crural vein throughout the experiment. This infusion was briefly interrupted (2-3 minutes) only when the fat emulsion (10-40 ml) was injected through the same needle, later in the experimental procedure. Femoral and external jugular veins were widely exposed, and all demonstrable tributaries ligated and cut, thus freeing the exposed vessels from surrounding structures. All manipulations were carried out with greatest possible care so as to avoid injury to the vessel wall. Blood was trapped in these exposed veins by applying seraffine clamps at each end; additional clamps served to subdivide the vein into smaller segments, each containing about 1 ml of blood. At selected intervals, one small vein segment after another was excised; the contents were emptied into 0.1 M sodium citrate solution in Petri dishes and carefully inspected for the presence of a grossly visible clot. The *first* appearance of a fibrin clot, regardless of size, was taken as the end point. The interval from the moment of clamping to removal of the segment which contained the first noticeable clot was designated as *intravascular clotting time* (ICT). Blood samples were usually collected from antecubital vein or artery to coincide with clamping of veins on each side, as well as immediately preceding injection of fat emulsion and toward the end of the experiment, *i.e.*, after completing measurement of ICT on the second (usually the left) side (L in Fig. 1), whereupon the dog was sacrificed. Serum total fatty acids, analyzed in accordance with Bragdon's procedure (5), were utilized as index of the total lipid level. (Serum cholesterol and phospholipids were also measured but showed no significant changes incident to the experiments and are therefore not recorded.)

† Cottonseed Oil, 15.0; Dextrose, 4.4; Purified Soybean Phosphatide, 1.2; Water for Injection, q.s. ad 100 ml. This emulsion was furnished through courtesy of Don Baxter.

TABLE I. Intravascular Clotting Time (ICT) in 10 Fasting Dogs.

Dog No.	Intravascular clotting time (min.) First side	Second side
1	40	L 40
2	L 30	30
3	"	L "
4	L "	"
5	L "	"
6	L "	"
7	L 40	>40
15	"	L 40
16*	"	L >4.0
18*	30	L 1.5

L denotes left side.

* Canine serum was inj. into peripheral vein immediately before measurement of ICT on left side. Vein segments were excised and inspected every 0.5-1 min. In dog 16, receiving 120 ml of serum for 20 min., no fibrin clot was seen in any of 5 available vein segments; in dog 18, receiving 90 ml within 2 min., a large clot was present in second segment inspected.

Results. Fasting values. In 15 fasting dogs (Tables I and II), ICT usually ranged between 30 and 40 minutes. Occasionally, no end point was reached, *i.e.*, no fibrin clot was seen in the last available vein segment (dogs 7, 20, 21, 23). There was remarkable consistency of results for the 2 sides of each dog (Table I).

Comparison of pre- and post-injection ICT values. In each of 5 fasting dogs (Table II), fat emulsion was injected rapidly after ICT had been measured in the jugular and femoral veins of the right side. Two minutes later, clamps were applied to the veins of the left side for estimation of ICT at time of marked lipemia. ICT was now 5 minutes shorter in dogs 8 and 9, which had received 10 ml of emulsion, and at least 10 to 15 minutes

TABLE II. Intravascular Clotting Time before and After Intravenous Injection of Fat Emulsion.

Dog No.	Intravascular clotting time (min.) Before inj.	After inj.*
8	30	25
9	"	"
20	>30	20
21	"	"
23	"	15

* Left jugular and femoral veins were isolated by clamps 2 min. after emulsion had been inj. (10 ml to dogs 8 and 9, 40 ml to the others).

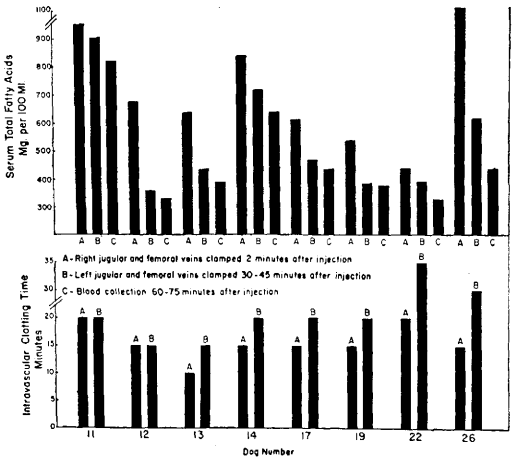

FIG. 1. Relation of intravascular clotting time to hyperlipemia after intrav. inj. of fat emulsion.

shorter in the other 3 dogs treated with 40 ml each.

Relation of ICT values to degree of induced hyperlipemia. In 8 dogs, ICT was determined in the veins of the right side 2 minutes after injection of the emulsion and 30 to 45 minutes later in the veins of the left side. In 6 of the 8 dogs, ICT was distinctly shorter at the peak of induced lipemia than at the time when this lipemia was clearly subsiding, indicating an inverse relationship between ICT and degree of lipemia in the respective dogs (Fig. 1). In all 13 dogs that received the fat emulsion, the immediate post-injection clotting times were below the lower limit of the fasting range, namely, less than 30 minutes (Table II and Fig. 1). This was true, with 2 exceptions (dogs 22 and 26), even of clotting times corresponding to the subsiding phase of lipemia (Fig. 1).

Discussion. The observed acceleration of clot formation in stagnant venous blood in dogs resulting from intravenous infusion of a fat emulsion correlates with the increased *in vitro* coagulability previously found in association with either intravenous or oral administration of fat in humans(3). Wessler(6) has drawn attention to the fact that reduced coagulability, as demonstrated by test tube methods, such as the one-stage (Quick) prothrombin time, does not necessarily mean decreased tendency to thrombosis. He found no delay in intravascular clot formation in dogs with severely reduced prothrombin activity following Bishydroxycoumarin medication. By the same token, shortening of whole blood clotting time or of "Stypven" clotting time cannot be interpreted necessarily as denoting an increased tendency to thrombosis. However, the data just presented *do* support the concept that lipemia results in an *in vivo hypercoagulable* state.

Rapid intravenous infusion of canine serum (not plasma!) into dogs was found by Wessler(7)—and verified by the present authors (Table I)—to cause clot formation in isolated veins in less than 2 minutes, as against 20 to 90 minutes in untreated dogs. His preliminary conclusion that this remarkable clot acceleration was due to active convertin (stable factor) in serum was subsequently disproved by him(6), and the mechanism of serum-induced venous thrombosis is not known. Lipemia-induced acceleration of clot formation, as observed in our experiments, is of much lesser degree but sufficiently distinct and consistent to be accorded potential clinical significance. Thus, when blood flow slows in a narrowed vessel, approaching conditions that exist in an isolated venous segment, namely, stagnation, thrombosis may be facilitated or precipitated by lipemia.

To be sure, this lipogenic clot acceleration may be specific for the fat emulsion used and attributable to certain of its ingredients. Among them, soybean phosphatide appears particularly suspicious since such substances have been shown to have platelet-like and other clot-promoting activity *in vitro*(2). Determination of the possible influence of physiologically induced, that is, alimentary lipemia upon ICT would be of much interest, particularly in view of recent reports describing myocardial and renal infarcts in rats on high-fat diets(8). In spite of the preliminary nature of the data presented, they would seem to suggest added caution in administering intravenous fat emulsions to human subjects, especially when a "temporary thrombotic" (local or systemic) state(9) may be suspected to exist or be impending.

Summary. Intravenous administration of a fat emulsion has been found to accelerate substantially the formation of a fibrin clot in isolated venous segments of anesthetized dogs.

This finding is interpreted as implying that induced lipemia may contribute to, or result in, an *in vivo* hypercoagulable state.

1. O'Brien, J. F., *Am. J. Med. Sc.,* 1957, v234, 373.
2. Poole, J. C. F., *Brit. Med. Bull.,* 1958, v14, 253,
3. Mandel, E. E., Mermall, M. L., Preston, F. W., Silverman, M., *Am. J. Clin. Path.,* 1958, v30, 11.
4. Wessler, S., *J. Clin. Invest.,* 1952, 31, 1011.
5. Colowick, S. P., Kaplan, N. O., *Methods in Enzymology,* Academic Press, 1957, vIII, pp. 318-320.
6. Wessler, S., Ballon, J. D., Katz, J. H., *N. Engl. J. Med.,* 1957, v256, 1223.
7. Wessler, S., *J. Clin. Invest.,* 1955, v34, 647.
8. Hartcroft, W. S., Thomas, W. A., *J.A.M.A.,* 1957, v164, 1899.
9. Wessler, S., Cohen, S., Fleischner, F. G., *N. Engl. J. Med.,* 1956, v254, 413.

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Labeling of Intestinal and Lymph Cholesterol After Administration of Tracer Doses of Cholesterol-4-C^{14}.* (25002)

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In earlier studies(1,2) a tentative mechanism of cholesterol absorption was proposed in which it was postulated that transfer of cholesterol from lumen of intestine to lymph involves complete mixing with a pool of free cholesterol in the intestinal wall. Free cholesterol may be drawn from this pool for synthesis of lymph cholesterol esters and as a structural component of chylomicron. The data in those studies were obtained by feeding 40-44 mg cholesterol-4-C^{14} and determining changes taking place in both chemical and radioactive cholesterol fractions in the lumen, intestinal wall, and lymph. Under such conditions there was a marked increase in total lymph cholesterol in comparison with a fasting animal over 24 hour period, and of total cholesterol, 80-90% was esterified. Administration of 1-3 mg cholesterol-4-C^{14} dissolved in oil does not produce a chemical increase in total lymph cholesterol or change in percent esterified cholesterol over feeding of fat alone (3-5). Comparison of data obtained with administration of 40-44 mg cholesterol-4-C^{14} with that obtained by other workers(3-5) using 1-3 mg suggested that the latter amounts should be considered as *tracer* doses which labeled the endogenous cholesterol in the lumen and later the free cholesterol pool of intestinal wall. Thus, appearance of cholesterol-4-C^{14} in lymph appears to be a measure of reabsorption of endogenous cholesterol when fat alone is fed rather than a measure of exogenous cholesterol absorption. In this context, large doses of cholesterol-4-C^{14} employed in our earlier experiments(1,2) would be considered as *absorptive* doses since they increased pool size and turnover rate of free cholesterol in the mucosa and produced increases in levels of lymph cholesterol fractions. To test above suppositions and supply additional data on free cholesterol pool, the effect of feeding a small (tracer) dose of cholesterol-4-C^{14} on size and labeling of free and ester cholesterol pool of intestinal wall and of cholesterol fractions of lymph have been determined.

Methods and materials. Rats with thoracic lymph fistula were prepared and given saline to drink(1). Twenty-four hours after operation each animal received, by gastric intubation without anesthesia, 3 ml of aqueous emulsion containing 50 mg albumin, 150 mg glucose, and 3.27 mg cholesterol-4-C^{14} (0.5 μc) or one containing these constituents plus 292 mg oleic acid and 279 mg sodium taurocholate. Lymph was collected from 0-6 hours following administration of test meal and animals were sacrificed. The intestine was removed, washed, sectioned, and lipid extracts of intestine and lymph were prepared as de-

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