TABLE V.
Complement fixation Test with Japanese B Encephalitis Virus Antigen and Mouse Hyperimmune Serum. Acetone-ether Extracted Antigens.

Antigen		Dilutions of serum							
Type	Dil.	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Japanese B	1:1	4	4	4	4	3	0	0	0
	1:2	4	4	4	4	4	0	0	0
	1:4	4	4	4	4	4	2	0	0
	1:8	4	4	4	4	4	4	±	0
	1:16	4	4	4	4	4	4	2	0
	1:32	4	4	4	4	4	4	2	0
	1:64	3	3	4	4	4	3	1	0
	1:128	0	±	1	2	1	±	0	0
	1:256	0	0	0	0	0	0	0	0
West. equine	1:1	0	0						

in the highest dilution of antigen which in a box titration gives a 2+ or better reaction; in the example shown, dilution of antigen 1:128 equals one unit.

To conclude, reliable, high-titered antigens for complement-fixation tests with certain neurotropic viruses have been prepared by a simple method of extraction at room temperature of infected brain tissue with acetone and ethyl ether.

16922

Intravenous Infusions of a Combined Fat Emulsion into Human Subjects.*

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In previous papers from this laboratory, an emulsion of fat combined with protein and glucose was reported to have been administered intravenously into dogs with safety and with nutritional benefits.[1,2] The overall advantages of this intravenous emulsion were such that it warranted the present clinical investigation of its effects on human subjects. This however was not done without precedent for Holt had pioneered studies on intravenous fat in a series of pediatric patients whilst others reported sporadic attempts in adult humans.[3,4] The present report is devoted to the method of preparation of the combined fat emulsion and the effects of its intravenous infusion on human subjects.

Preparation of the Combined Fat Emulsion. The successful preparation of this emulsion is dependent upon adequate and thorough homogenization and emulsification which is accomplished in a specially constructed homogenizer adapted to medical usage. Basically the mechanism is that of a high pressure pump which forces the fluid mixture through a minute orifice against the resistance of a strong steel springed valve. The machine consists of three component parts: a reservoir tank with a high speed agitator incorporated

* Aided by grants from the Ben Lewis Fund in Experimental Surgery. Clinical investigation done at Goldwater Memorial Hospital (New York University, Third Surgical Division).

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4 Clark, D. E., and Brunschwig, A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 329.

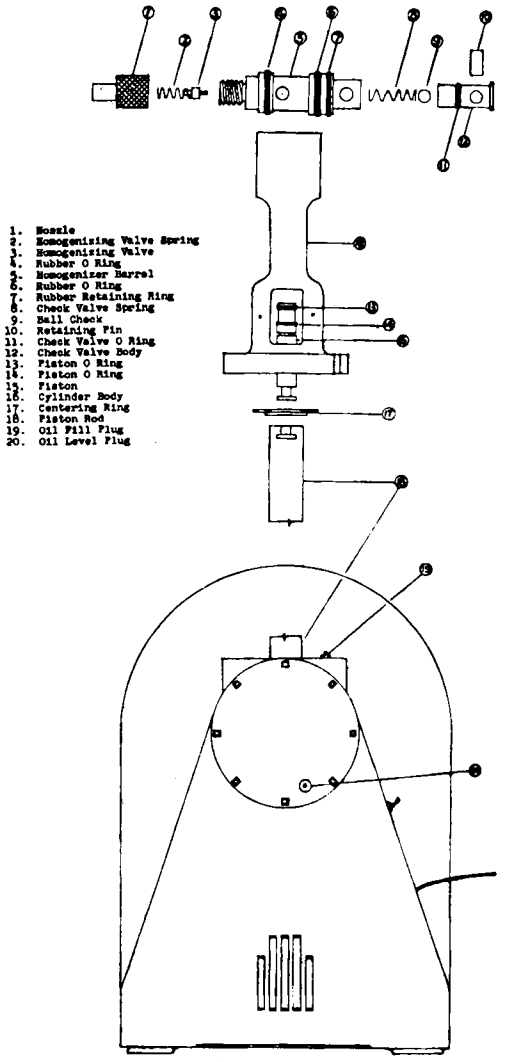

FIG. 1.
Diagrammatic sketch of homogenizer apparatus.

into its cover, a motor and belt mechanism to drive the compressor piston and a homogenizer block fitted with a system of inlet and outlet valves. (See Fig. 1 for diagram of the apparatus.) Within the block a high internal pressure is created which is expended on the homogenization of the mixture. The emulsion is prepared entirely under sterile conditions. The homogenizing block and all the parts in contact with the mixture can be detached as a unit and autoclaved before each batch of homogenate is processed. The ingredients of the mixture already in sterile form are drained from their respective containers into the reservoir tank through a special opening in the cover provided for the purpose. The mixture is converted into a blend or crude emulsion after a 5-minute period of high speed agitation. The blend is then allowed to flow into the homogenizing chamber within which the emulsifying process is completed. The emulsion is continuously recyclized through the block under 2500 pounds pressure for a period of 20 minutes and then collected in sterile liter flasks which are capped and stored ready for intravenous use. Test samples of the homogenate of each batch are examined for particle size and for sterility.

The constituents of the emulsion are solutions of protein hydrolysate (amigen, 10%), 50% glucose, intravenous gelatin (Knox P-20, 6%), and refined coconut oil. All except the latter are available as sterile non-pyrogenic solutions in sealed flasks. The coconut oil is autoclaved before its addition into the tank. The combined fat emulsion used in the present studies is an homogenate of approximately 10% fatty oil, 5% protein hydrolysate, 5% glucose, and 2% gelatin. The caloric value of the emulsion is about 1,300 calories per liter. The pH of the emulsion is about 6.2. The particle size of the emulsion is less than one micron and exhibits active Brownian movement on microscopic examination.

In the present studies intravenous gelatin was used exclusively as the emulsifying agent. After many experiments it was found to be superior to the soy bean phosphatides, lecithin, and a variety of artificial stabilizing agents. Intravenous gelatin *per se* has been proved to be non-toxic and non-allergenic. As an emulsifying agent a relatively small amount, less than 3%, is required for the emulsification of 10% fatty oil. The stability of the 10% combined fat emulsion has been observed for a period of more than two years without "creaming" or "oiling out."

Method of Study. The combined fat emulsion was administered without selectivity to hospital patients who were being treated for a variety of surgical conditions. Many of these patients ran a chronic low grade fever. The studies were started in the morning with

TABLE I.
Immediate Effects from Infusion of One Liter of 10% Combined Fat Emulsion.

Temperature reactions		Constitutional reactions			
Elevation	%	Subjective	%	Objective	%
< 1°	29	Nausea	1	Chills	9
1° to 1.9°	50	Headache	3	Vomiting	2
2° to 2.9°	13	Dizziness	1	Cough	2
> 3°	8	Fatty taste	1	Allergic	1

the patient in the post-absorptive state after a fasting period of 14 hours. Before the infusion was started a sample of blood was taken, the morning urine was obtained, and temperature, pulse, respirations, and blood pressure were noted. The infusion was then given intravenously at a specific rate ranging from 20 to 80 drops per minute varying in different patients. During the course of the infusion and throughout the post-infusion phase until the next morning blood and urine samples were taken and the above mentioned clinical notations were made at regular intervals. The following tests were made routinely on the blood: hemoglobin, red cell count, white cell count with differential, sedimentation time, chylomicron count, and specific gravity. In special cases tests were made for the quantitation of blood lipids with the refractometer and the nephelometer, for blood volume by the Evans blue method and for blood viscosity with the Hess viscosimeter. Clinically, subjective and objective constitutional reactions relative to the intravenous infusion of the emulsion were studied. X-rays of the lungs were taken in series for any lesions due to fatty infiltration, pulmonary irritation or pulmonary emboli.[5] Final check examinations were made 2 weeks after completion of the infusions for hemolytic anemia and for possible liver or kidney damage. Surgical liver biopsies were obtained from several patients who received the emulsion. These specimens were prepared for microscopic analysis by staining with osmic acid, oil-red –O, Sudan IV, and hematoxylin-eosin.

Results. The combined fat emulsion was infused intravenously into 76 human subjects for a total of more than 250 liters. An average of 4 liters of emulsion was administered to each of 35 patients. One patient received a total of 16 liters of the emulsion which was the maximum amount infused into any one patient. Five patients received 2 liters of the emulsion during one infusion period instead of a single liter.

Temperature changes during and immediately after the infusion were computed according to the degree of elevation above the pre-infusion temperature. The results have been classified in Table I on a percentage basis from the protocols of the last 200 infusions. These values were not corrected for temperature changes due to the disease of the patient. Constitutional reactions, subjective and objective, have also been outlined in Table I. The incidence of chills averaged 9% of all infusions, the majority of which came concomitantly with a high temperature reaction. In 2% chills occurred independently, without any temperature elevation. The chill reaction was the severest type of reaction encountered in the present series. Allergenic reactions were usually of the urticarial type due to sensitivity to the amigen component in the emulsion. The latter reaction could be obtained by the infusion of amigen alone and was found to be controllable by antihistaminic drugs given in conjunction with the administration of the emulsion. Cough and vomiting were moderately severe reactions which were usually controlled by reduction of the speed of intravenous administration of the emulsion.

Changes of blood pressure in response to the injection of the emulsion were variable. During the infusion period there was generally a slight elevation of blood pressure followed by a moderate fall in pressure which persisted for 2 hours after completion of the

[5] Jirka, F., and Scuderi, C. S., *Arch. Surg.*, 1936, **33**, 708.

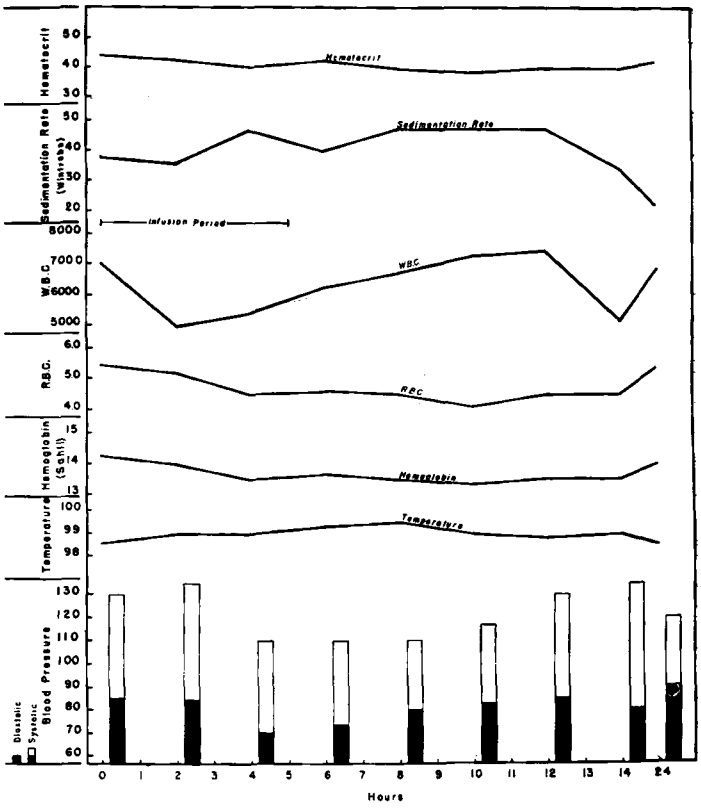

FIG. 2.

Graphs showing the effects of the intravenous infusion of one liter of the 10 per cent combined fat emulsion. The values of the individual curves were averaged from all the protocols.

infusion. The elevation of systolic pressure did not exceed 20 mm Hg and the fall in systolic pressure ranged from 10 to 25 mm Hg. The diastolic pressure tended to fall towards the end of the infusion but also returned to its normal level (Fig. 2).

The values for hemoglobin, hematocrit cell volume and red and white cells tended to fall below their initial values during the infusion phase of the emulsion and returned to their original levels shortly before or after the termination of the infusion. The erythrocyte sedimentation rate showed an increase in rapidity of sedimentation which averaged 35% greater than the pre-infusion sedimentation time, and also returned to its initial level. The differential white count was not indicative of any trend. Leukopenia was found to occur in 2% of infusions concomitant with chill and temperature reactions (Fig. 2 and Table II).

Counts of chylomicrons were made from peripheral venous blood under dark field illumination with a net micrometer in the eyepiece of the microscope. The curve of chylomicron counts beginning from the post-absorptive level through the infusion phase of the emulsion was characteristic. In the post-absorptive state the initial pre-infusion count varied from zero to 10 chylomicrons. After the infusion was started there was a rapid rise in the number of chylomicrons to a peak. At about the mid-point of the infusion the chylomicrons began to decrease in number and return slowly to the initial level. Fig. 3 was drawn from the average values of all the chylomicron counts. The chylomicron curve was found to be modifiable by the speed of infusion, the amount of fat emulsion administered and the oral ingestion of food.

Quantitative microchemical determinations of blood lipids showed a rapid rise of blood fat

TABLE II.
Effects of Intravenous Infusion of One Liter of Combined Fat Emulsion Into a Subject.

Time (min.)	Amt infused (ml)	Temperature Fahrenheit (degrees)	Pulse rate (per min.)	Blood pressure (mm Hg)	Hemoglobin Sahli (g)	Sedimentation time (mm/hr)	R.B.C. count (million)	W.B.C. count	Hematocrit (%)	Plasma refractive index	Chylomicra count ¼ unit	Blood lipids (mg)	Urine acetone
0	—	98.0	80	132/98	14.0	24	4.75	7000	44	1.350	8	800	neg.
60	240	97.8	80										
120	350	98.0	98	140/100	13.0	23	4.75	6800	41	1.349	350	1070	neg.
180	520	98.6	100										
240	625	98.6	96	140/110	13.0	36	4.70	5000	40	1.349	150	905	1+
300	—	98.8	104										
360	1000	98.6	104	140/110	13.2	36	4.75	5600	41	1.351	250	625	1+
420	—	98.2	100										
480	—	99.0	104	130/110	13.4	21	4.90	5800	42	1.350	150	810	neg.
540	—	98.0	104										
600	—	99.0	100	150/110	13.0	36	4.75	6000	41	1.350	100	705	neg.
660	—	99.0	98										
720	—	99.2	100	150/110	13.2	36	4.90	6600	42	1.350	50	770	neg.
24 hr	—	—	—	—	—	28	5.20	6800	43	—	10	685	neg.

and an equally rapid fall to normal limits within 2 hours after completion of the infusion. Nephelometric examinations of plasma samples showed variations in the degree of turbidity comparable to the variations obtained by microchemical analysis and chylomicron counts made on the same samples. Viscosimetric determinations and refractometric analysis showed changes in the blood during the course of the infusion which were consistent with the changes already noted. The specific gravity of the plasma showed a temporary dilution effect due to the infusion followed by a return to normal limits.

Consecutive urine studies were made in 43 infusion tests. In no case was a positive test for blood or urobilinogen obtained which could be attributed to the emulsion. Chemical tests for fat in the urine were negative. Dark field examination of the urine for chylomicrons sometimes showed a moderate elevation in the number of fat particles although the chemical test for fat was negative. A positive reaction was obtained occasionally for acetone varying from 1 + to 2 + in 2 or 3 of the intermediate urine specimens taken during the course of the infusion of the emulsion. When the infusion was completed the positive tests for acetone became negative. A positive test for diacetic acid was found rarely.

Hematologic follow-up studies in patients who received either single or multiple infusions showed no signs of hemolytic anemia. Likewise x-rays of the lungs gave no evidence of fatty emboli or pulmonary irritation. The photographs in Fig. 4 and Fig. 5 were taken to show the microscopic appearance of human liver sections. The first, Fig. 4, was taken a few hours after an infusion and the second, Fig. 5, 36 hours after the last of 9 consecutive infusions of the combined fat emulsion. Liver and kidney function studies showed no pathologic physiology ascribable to the use of the emulsion.

In 5 patients it was necessary to discontinue the infusion of the emulsion before half of the contents of one liter had entered the vein. This was necessary because of the severity of the chill experienced by the patients. All of the 5 patients were in advanced states of malnutrition with severe anemia and with markedly diminished circulating blood volumes. Four of these patients were able to tolerate large volumes of the combined fat emulsion after the anemia and the blood volume were corrected by multiple blood transfusions.

Comments. The combined fat emulsion was administered intravenously to human patients with safety. The immediate and late effects of single and repeated infusions of the

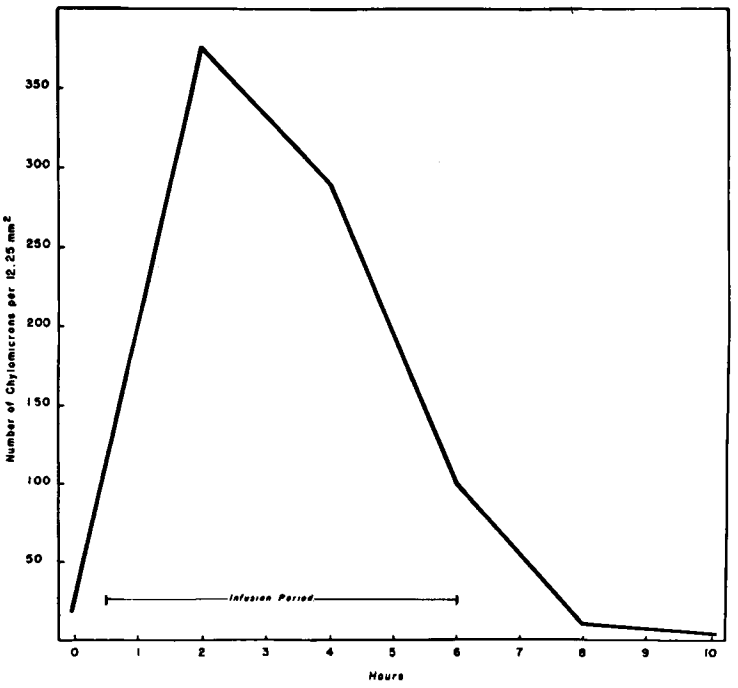

FIG. 3.

Graph of the curve of the chylomicron counts obtained during the infusion and post-infusion periods of the 10 per cent combined fat emulsion. The values on this curve have been averaged from all the chylomicron counts in the protocols.

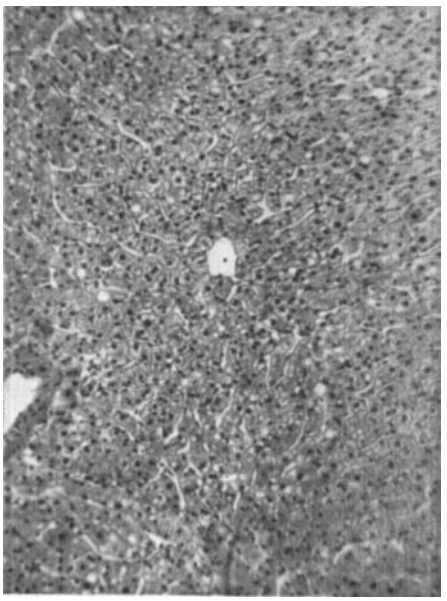

FIG. 4.

Low power magnification of human liver section focused on central vein. Surgical biopsy several hours after patient had received 2 liters of the 10 per cent combined fat emulsion. (H & E stain ×50).

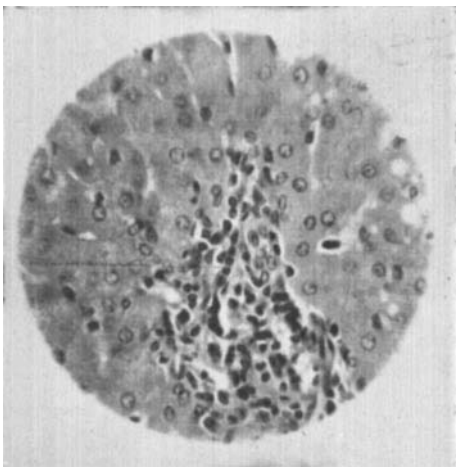

FIG. 5.

High power magnification of human liver section focused on periportal area. (H & E stain ×220). Surgical biopsy 36 hours after patient had received a total of 9 infusions of the combined fat emulsion.

emulsion were studied both from the clinical and the hematologic standpoint. A moderate elevation in temperature as a consequence of

injection of the combined fat emulsion can be accounted for on the basis of an increase in total heat production due to the metabolism of fat plethora. The increased rate of blood sedimentation was believed to be associated with erythrocyte pseudo-agglutination caused by the infused gelatin. The variations in hemoglobin, etc., were related to the dilution effect of the emulsion. The incidence of constitutional reactions compared favorably with reactions encountered after blood transfusions. The sharp rise in the number of chylomicrons followed by the slower fall was indicative of the rate of disappearance of the intravenously injected fat from the blood stream. The appearance of acetone found in an occasional specimen of urine during the infusion phase may be explained on the basis of incomplete oxidation of the fat. In the follow-up we were unable to find any serious toxic effects or late sequelae ascribable to the emulsion.

Summary. The method of preparation of a 10% combined fat emulsion used in the present investigation was described. A combined fat emulsion with a potency of approximately 1300 calories per liter was infused intravenously into 76 human subjects. A variety of laboratory tests and clinical observations provided evidence in favor of its suitability as an intravenous emulsion.

The authors are indebted to the E. F. Drew Co., to the Mead Johnson Co., and to the C. F. Knox Co., for generous supplies of material used for the preparation of the emulsion. The homogenizer was made by the C. W. Logeman Co., New York City.

16923

Influence of Temperature on the Distensibility of the Pubic Ligament.*

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The studies of Hisaw and his collaborators[1,2] have demonstrated that hormonal changes can profoundly influence the distensibility of an apparently physiologically inert tissue such as the pubic membrane within a relatively short time. In the present study we have attempted to determine the relative influence of changes in the physical environment, *i.e.* load and temperature, on the response of the pubic ligament of the guinea pig.

The experiments to be described subsequently, were performed in both intact preparations of non-gravid and in isolated preparations of gravid and non-gravid guinea pigs. In the former series hormonal influences were controlled by ovariectomy and injection of serum from pregnant rabbits. It was soon found that under our experimental conditions such hormonal factors played a lesser part than did slight variations in the environment, as *e.g.* caused by variations in the distance of a source of heat to the preparation.

The method used in studying the effect of temperature on the stretching of the pubic ligament in the intact animal is illustrated in Fig. 1. The animal was kept under deep nembutal narcosis during the entire experiment. Stretch on the pubic ligament was exerted by a weight which pulled a string loop fastened with one end to the pubis, at the other to the ischium. The other half of the pelvis was similarly fastened with a short loop of string to a fixed point. Recording took place with a third loop, fastened to the side on which the weight pulled; this arrange-

* This investigation was carried out with the aid of a grant from the Harlan Shoemaker Fund for Paralytics, Los Angeles, Calif.

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