which contained chiefly 7S antibody might indicate the stage of the epidemic.

These data also demonstrate that 19S *macro*-globulin HI antibodies are the first to appear in sera of young pigs naturally infected with J.E. virus and this, together with the finding that they are replaced by 7S antibodies, indicates that infection stimulates production of the same type of antibody molecules, and in the same order, as those observed in other mammalian species immunized with a variety of soluble or viral antigens (4-8). In addition, the susceptibility of the early antibodies to 2-ME suggests a practical method for handling sera employed in predicting an outbreak of J.E. in man.

Summary. When serum specimens of pigs obtained weekly from a slaughterhouse throughout the summer of 1965 were tested for their HI activity against J.E. virus, an abrupt change was seen from 4% positive in the second week to 46% positive in the third week. By the fourth week, 90% were positive. When the 2-ME sensitivity HI activity of these sera was tested, almost 100% of the specimens obtained through these 3 weeks contained 2-ME-sensitive antibody. However, sensitivity to 2-ME was reduced to 70% in the first week of September, to 44% in the second week, and after the fourth week, none of the tested serum specimens was sensitive. Evidently, 2-ME-sensitive antibody appeared before the 2-ME-resistant antibody and the detection of 2-ME-sensitive antibody against J.E. virus in the early summer gave a reliable basis for prediction of the outbreak among human beings later in that year.

1. Konno, J., Endo, K., Agatsuma, H., Ishida, N., Am. J. Epid., 1966, in press.

2. Clark, D. H., Casals, J., Am. J. Trop. Med. Hyg., 1958, v7, 561.

3. New Diagnostic Procedure for Japanese Encephalitis, 2nd Ed., Japan N.I.H., Tokyo, 1962.

4. Nossal, G. J. V., Szenberg, A., Ada, G. L., Austin, C. M., J. Exp. Med., 1964, v119, 485.

5. Svehag, S-E., Mandel, B., ibid., 1964, v119, 1.

6. Fazekas de St. Groth, S., Webster, R. G., Cellular Biology of Myxovirus Infections, J. & A. Churchill, LTD., London, 1964, 246.

7. Berlin, B. S., Proc. Soc. Exp. Biol. and Med., 1963, v113, 1013.

8. Sell, S., J. Immunol., 1965, v95, 300.

9. Schluederberg, A., Nature, 1965, v205, 1232.

10. Hatano, R., Hinuma, Y., Proc. Soc. Exp. Biol. and Med., 1966, in press.

Received August 24, 1966. P.S.E.B.M., 1967, v124.

Preliminary Observations on Reversal of Hypovolemia with Intravenous Fat Emulsion. (31670)

D. F. FLICK,* JERRY B. SCOTT,† AND ROBERT A. HARDIN‡

Biochemistry Division, US Army Medical Research Laboratory, Fort Knox, Ky. Report 595

A number of solutions have been used to reverse the symptoms of experimental hypovolemia (oligemia). The solutions studied have been either naturally-occurring or synthetic polymers varying in molecular size and composition. Of the naturally-occurring organic polymers the following have been utilized as blood substitutes in hypovolemic studies: dextran(4-10), pectin(11,12), pectin derivative(13), gelatin(1,2,8), gelatin derivatives(8), and levulan, a fructosan(16). Polymeric glucose(3) and polyvinylpyrrolidone(4, 8) are examples of synthetic polymers which have been investigated for plasma-extending properties in the hypovolemic animal. In addition, whole blood(1,2), defibrinated blood (1), serum(1), plasma(7), and saline(1,4,12) have been utilized to reverse effectively the symptoms of experimental hypovolemia.

Miyagawa suggested(14) that preparations of glucose and cod liver oil, and other fats,

* Present address: Nutrition Research Branch, Division of Nutrition, Food and Drug Admin., Washington, D.C.

† Present address: Dept. of Physiology, Univ. of Oklahoma Med. School, Oklahoma City.

‡ Present address: 4503 Melbourne Road, Indianapolis, Ind.

may be of use in blood tranfusions or as parenteral nutriments. The problems associated with parenteral fat administration of various preparations have been published(17, 18).

This report presents observations of the ability of intravenously administered fat emulsion to reverse the symptoms induced by massive hemorrhage and on posthemorrhagic recovery in the dog.

Materials and methods. Male and female mongrel dogs, weighing 10.2-15.3 kg, were used in these studies. After an overnight fast, each dog was anesthetized with sodium pentobarbital and injected with sufficient sodium heparinate to avoid *in vivo* blood clotting for the duration of the experimental procedure of 3½-4 hours. One femoral artery was cannulated with polyethylene tubing for recording the arterial blood pressure utilizing the Sanborn two-channel polygraph. Heart rates were calculated from the tracings which were run at 10 cm/min. The femoral vein of the opposite leg was cannulated for blood removal and for drip-infusion.

Total blood volume was calculated on the basis of 80 ml/kg of body weight(15) in order to control the rate and total volume of blood withdrawn. Control heart rates and arterial pressures were recorded prior to initiation of hemorrhage. In this manner each dog served as its own control. Blood volumes let ranged between 56.0-65.5% of the calculated volumes at bleeding rates of 5.3-13.8 ml/min.

Blood pressure recordings were taken at 5-10-minute intervals within the period of blood withdrawal. A one-hour interval after the end of hemorrhage was adopted as part of the experimental procedure. After the one-hour interval, the intravenous infusion was begun. The rate of infusion was 1.0 ml/min for the first 10 minutes and was then increased to 5.0 ml/min until the equivalent of the volume of blood withdrawn was re-infused. Blood pressures were recorded at 10-minute intervals during the hour-long period of hypovolemia and during the period of infusion.

The animals were divided into 3 groups: a) those which were not infused, b) those infused with a non-fat emulsion (*i.e.*, all the ingredients of the complete fat emulsion but without cottonseed oil), and c) those infused with complete emulsion. Compositions of the emulsions are shown in Table I. The non-fat emulsion contained the components in the same concentrations as those in the fat emulsion. The non-fat infusate was emulsified in a colloid mill and autoclaved before infusion. A comparison of the effects of the 2 emulsions (non-fat *vs.* complete emulsion) was used to evaluate the efficacy of intravenous fat infusion to reverse the symptoms of experimental oligemia. A comparison of no infusion *vs.* infusion was used to evaluate the effect of infusion upon reversal of the oligemic symptoms.

TABLE I. Composition of Non-Fat and Complete Fat Emulsions.

Component	Non-fat emulsion	Complete fat emulsion*
	%	%
Cottonseed oil (W/V)	—	15.0
Glucose (W/V)	4.0	4.0
Phosphatides (W/V)†	1.2	1.2
Inert polymer (W/V)‡	.3	.3
Water (V/V)	94.5	79.5
Calories/ml (calculated)	.21	1.56

* Lipomul IV kindly supplied by Upjohn Co., Kalamazoo, Mich.
† Purified phosphatides marketed as "lecithin."
‡ Polyoxyethylene oxypropylene.

After the period of infusion, each dog was injected (IM) with 300,000 units of procaine penicillin G and on alternate days for the first week. After experimental treatment, dogs were placed in individual cages and observed at daily intervals for 4 weeks and at weekly intervals for 4 months. No observations were made beyond 4 months. During the recovery period, only gross observations were recorded.

Results and discussion. Data on changes in mean arterial pressures and heart rates of the dogs before hemorrhage, during hemorrhage, at the end of the one-hour period of hypovolemia and immediately after completion of the infusion are shown in Table II. The blood pressure depression due to hemorrhage was not equal in all the animals even though the volumes of blood withdrawn were approximately the same. However, there was a marked decrease in the mean arterial pressure as a result of hemorrhage which was suf-

TABLE II. Changes in Mean Arterial Blood Pressure (Femoral) and Heart Rate of Dogs Before Hemorrhage, During Hemorrhage, One Hour After Hemorrhage, and Immediately After Intravenous Infusion of Either Emulsion with No Fat or Complete Fat Emulsion.

	Mean arterial blood pressure (mm Hg)				Heart rate (beats/min)			
Dog No.	Before bleeding	Lowest pressure during bleeding	One hr after bleeding	Immediately after infusion*	Before bleeding	Lowest rate during bleeding	One hr after bleeding	Immediately after infusion*
1	139	61	90	—	159	145	183	—
2	176	48	132	—	206	186	244	—
3	151	44	34	—	165	184	229	—
4	134	48	77	122	152	112	155	162
5	142	37	88	126	214	152	254	204
6	90	40	60	92	160	108	150	165
7	130	80	96	81	220	146	180	191
8	147	88	113	119	202	224	264	244

* Infusion rates: first ten minutes, 1.0 ml/min, then 5.0 ml/min until infusion was approximately equivalent to volume of blood let. For type of infusion, see Table III.

ficient to indicate that hypovolemia had occurred(15). After one hour of oligemia, 7 dogs had an increase in blood pressure. These increases may be related to normal compensatory mechanisms(16) which result in an increase of mean arterial pressure without a blood volume replacement. After intravenous infusion 3 of the 5 dogs responded with a further increase in mean arterial pressure. One dog had a slight decrease (No. 7), and the increase in one dog was only a few mm Hg (No. 8).

The results of the responses in heart rates are also listed in Table II. It is apparent that the heart rates returned to near pre-hemorrhage rates in 4 of the 5 dogs which received an infusion. Dog No. 8 exhibited a marked tachycardia after infusion as did No. 5 at the end of the bleeding period.

Table III lists data on sex, body weight, calculated blood volume, volume of blood withdrawn and bleeding rate. Rate of blood withdrawal was in the range 5.3-13.8 ml/min and total volume of blood let was 56.0-65.5% of the calculated total blood volume(15). These volumes of blood withdrawn were nearly twice the volumes Schechter and Hestrin withdrew from rabbits and mice(16). Dogs 1-3 received no infusion and were bled at volumes commensurate with the animals given infusions. That these animals died within 3½ hours after massive hemorrhage and those infused did not was interpreted to mean that the infused animals survived the period of hypovolemia due to the infusion.

The 2 dogs infused with the non-fat emulsion survived longer than 4 months. Initially, these animals did not respond readily to being called in the post-operative recovery period and whimpered when they were handled. They did not attempt to stand by their own volition within the first 4 or 5 days. After this period they would assume an upright stance only if encouraged. The attempt to stand was noticeably laborious. The food intake of these animals, though not measured, was noticeably less than the dogs infused with the complete emulsion. The latter animals recovered readily after massive hemorrhage and accepted water and a small amount of food immediately after regaining consciousness. Within 24 hours the dogs infused with complete emulsion were able to stand upright. At 2 weeks these dogs showed no outward signs of the previous massive hemorrhage, whereas the dogs infused with the non-fat emulsion were still weak and passive to handling.

Table III also lists the calculated number of calories infused into each dog. The dogs infused with the complete emulsion received several-fold more calories than those infused with the non-fat emulsion. The observations suggest that the high calorie content of the complete emulsion may have contributed to the rapid recovery of these dogs. The probable caloric effect in recovery from massive hemorrhage should be further investigated.

Summary. A total of 8 mongrel dogs was bled 56-65% of calculated blood volume at

TABLE III. Sex, Body Weights, Total Blood Volume, Volumes of Hemorrhage and Infusion, Types of Infusions and Observations of Dogs After Massive Hemorrhage.

Dog No.	Sex	Body wt (B.W.), kg	Total blood vol, ml*	Blood let — Volume, ml	Blood let — % of B.V.	Bleeding rate, ml/min	Type of infusion	Infusion vol, ml	Calories infused — Total	Calories infused — Cals/kg B.W.	Observations
1	♂	11.8	944	610	64.6	9.7	None	—	—	—	Died 3 hr 30 min posthemorrhage
2	♂	10.9	871	525	60.2	13.8	None	—	—	—	Died 3 hr 8 min posthemorrhage
3	♂	15.3	1226	685	56.0	10.9	None	—	—	—	Died 1 hr 6 min posthemorrhage
4	♂	13.4	1072	658	61.4	8.3	Non-fat emulsion	719	151	11.3	Slow recovery, survival >4 mo
5	♀	10.2	818	472	57.8	9.1	Non-fat emulsion	562	118	11.6	*Idem*
6	♀	11.5	920	550	59.8	5.3	Complete emulsion	513	800	69.6	Rapid recovery, survival >4 mo
7	♀	12.7	1016	660	65.0	7.1	Complete emulsion	615	960	75.6	*Idem*
8	♀	10.5	840	550	65.5	11.9	Complete emulsion	564	880	83.8	,,

* Total blood volume (B.V.) was calculated on the basis of 80 ml per kg of body weight(15).

rates of 5-14 ml/min. At the end of 60 minutes in hypovolemia the animals were treated experimentally in the following manner: 1) 3 dogs were given no infusion; 2) 2 dogs were infused with an emulsion containing no fat; and 3) 3 dogs were infused with an emulsion containing fat (15% cottonseed oil). The infusions were administered in a volume equivalent to the volume of blood withdrawn. Dogs which received no intravenous infusion died within 3½ hours after massive hemorrhage. Animals infused with an emulsion containing no fat recovered from the experimental hemorrhage but were not physically active within the first week of the posthemorrhagic period. Dogs infused with complete emulsion recovered rapidly after massive hemorrhage and accepted food and water within a few hours after regaining consciousness. These animals were fairly active during the first week after massive hemorrhage and recovered without event within two weeks. All dogs receiving infusions lived longer than 4 months.

The authors wish to thank Colonel William W. Cox, U. S. Surgeon General's Office, for suggestions which led to the studies of this report. Also, thanks are extended to Dr. Curt Meyer, Upjohn Co., Kalamazoo, Mich., for Lipomul IV used for these studies.

1. Lawson, H., Rehm, W. S., Am. J. Physiol., 1943, v140, 431.

2. Fletcher, A. G., Jr., Hardy, J. D., Riegel, C., Koop, C. E., J. Clin. Invest., 1945, v24, 405.

3. Horvath, S. M., Werner, A. Y., McCannon, D. M., Knapp, D. W., J. Appl. Physiol., 1954, v7, 49.

4. Sjostrom, B., Acta Soc. Med. Upsal., 1953, v59, 17.

5. Wilson, J. S., Estes, E. H., Jr., Doyle, J. T., Bloom, W. L., Warren, J. V., Am. J. Med. Sci., 1952, v223, 364.

6. Waud, R. A., J. Pharmacol. Exp. Therap., 1957, v119, 98.

7. Pirani, C. L., Juster, R., Froeb, H. F., Consolazio, C. F., Ingrahm, R. C., J. Appl. Physiol., 1955, v8, 193.

8. Shaffer, J. R., Artz, C. P., Am. Practitioner, 1954, v5, 545.

9. Semple, R. E., Can. J. Physiol., 1954, v32, 670.

10. Jaenike, J. R., Waterhouse, C., Circulation, 1955, v11, 1.

11. Middleton, S., Wiggers, C. J., Am. J. Appl.

Physiol., 1944, v144, 326.

12. Dworkin, R. M., Proc. Soc. Exp. Biol. and Med., 1944, v56, 20.

13. Handford, S. W., Smythe, C. M., Gilmore, J. P., Leopold, R. S., Kathan, R. H., Arm, H. G., J. Appl. Physiol., 1955, v7, 553.

14. Miyagawa, Y., Japan. J. Exp. Med., 1933, v23, 59.

15. Wiggers, C. J., Physiology of Shock, New York, Commonwealth Fund, 1950.

16. Schecter, I., Hestrin, S., J. Lab. Clin. Med., 1963, v61, 962.

17. Symposium on Intravenous Fat Emulsions, Metabolism, 1957, v6, 591.

18. Di Palma, J. R., Ed., Drill's Pharmacology in Medicine, 3rd ed., McGraw-Hill, New York, 1965.

Received August 24, 1966. P.S.E.B.M., 1967, v124.

Enhanced Vitamin B_{12} Absorption from Rat Intestine by Proteases In the Absence of Intrinsic Factor. (31671)

KUNIO OKUDA

Department of Medicine, Kurume University School of Medicine, Kurume, Japan.

In our search for a readily absorbable form of vitamin B_{12} complex produced during the digestive process of liver, it was found that B_{12} in liver was absorbed from an isolated rat intestine to an extent comparable to that achieved with free B_{12} and homologous intrinsic factor (IF) when Pronase(1), a Streptomyces proteases preparation, was used for digestion. The effect of Pronase which was independent of IF, was demonstrable whether digestion was carried out in test tubes or inside the intestine, as long as it was present in the intestine at the time of absorption. No other pure proteases tested had comparable effects. Absorption of crystalline B_{12} of small doses was similarly enhanced by Pronase from an isolated rat intestine in the absence of IF. Pronase, a mixture of several proteolytic enzymes and one of the most potent preparations available, was used for the purpose of simulating, *in vitro,* the intestinal digestion which is much more efficient than any artificial digestion system using a single crystalline enzyme.

So far, no other compounds have been shown to enhance absorption of small doses of B_{12} under a condition where IF is lacking, and the present observations seem to provide important clues as to the mechanism of intestinal absorption of B_{12} in relation to IF action. This is a preliminary report of our finding.

Methods. Co^{57}-hydroxocobalamin* (Co^{57}-B_{12}) with a specific activity of approximately 8 $\mu c/\mu g$ was injected subcutaneously to adult rats of the Wistar strain, 10 $m\mu g$ daily, for 3 weeks and the liver was removed 4 days after the last injection. The liver was cut into small pieces, dried *in vacuo* over P_2O_5 at 4°C and pulverized. The isotopic dilution of Co^{57}-B_{12} in the material was 50- to 90-fold as determined by the microbiological assay using the Skeggs' medium(2) and *Lactobacillus leichmanii* 4797, and by the radiometric measurement with a gamma scintillation counter. In some experiments, instead of dried liver, fresh liver homogenate was used. It was assumed that most of the radioactivity in such liver preparations represented B_{12} in its natural form(3).

Enzymatic digestion of liver was carried out *in vitro* using crystalline trypsin or a mixture of equal portions of trypsin, α-chymotrypsin and papain in pH 7.6 tris-maleate buffer, Pronase-P† in pH 7.4 phosphate buffer, or pepsin in 1/20 N HCl, in a water bath at 37°C under agitation, as well as *in vivo* using Pronase and trypsin. After digestion *in vitro,* the mixture was centrifuged and the supernatant was applied quantitatively in a rat intestinal loop for absorption measurement.

The effect of IF and digestion on absorption was studied by the intestinal loop meth-

* Kindly supplied by Dr. K. C. Mezey, Merck Laboratories, Rahway, N. J.

† Purchased from Kaken Chemical Co., Ltd., Bunkyo-Ku, Tokoyo. Activity: 45,000 P.U.K./g.