fusions, and sera of ordinary hemophiliacs were negative. Control antigens of purified gamma globulin, human fibrinogen, and albumin gave negative results. These titers were obtained repeatedly, no change being noted throughout the hospital stay, in spite of repeated transfusions.

The anticoagulant was then shown to inhibit directly antihemophilic globulin. Taylor, *et al.* (*6*) have shown the *in vitro* effect of antihemophilic globulin in lowering the clotting time of hemophiliac patients. This could be repeated by us in the case of ordinary hemophiliacs who responded to blood or plasma in the usual fashion. However, in the two patients presented no effect was noted. Also, if the antihemophilic globulin was first incubated with the proper amount of serum from each of these patients, its acceleratory action on the clotting time of ordinary hemophilic blood was lost. If the globulin was incubated with the same amount of normal or ordinary hemophilic serum, the activity of the globulin when added to the blood of an ordinary hemophiliac was unimpaired.

It was evident from these results that the antihemophilic globulin, which alone had such a marked acceleratory effect on ordinary hemophiliac blood, was in some way tied up by the sera of these two patients. In view of the demonstration of definite precipitins in their sera it was felt that the sera probably inhibited or tied up the antihemophilic globulin by means of an antigen-antibody reaction.

On the basis of the evidence presented the following hypothesis was formed to explain the presence of the anticoagulant in these two patients. Each was deficient in, or lacked, the substance known as antihemophilic globulin in his blood—a substance shown to be essential for the coagulation of blood in the normal time. Just where this globulin enters into the coagulation mechanism is unknown, but it would seem that it is necessary for liberation of thromboplastin from platelets in the first stage of clotting. When this globulin is given intravenously to ordinary hemophiliacs, either in the form of fresh blood or plasma or antihemophilic globulin contained in Fraction I of Cohn, it causes a marked acceleration of coagulation (*2*). In these two cases repeated injections of this globulin are thought to have resulted in the formation of antibodies against this globulin. These antibodies, of course, have the ability to inhibit any globulin which is later given. Therefore, these patients became refractory to further transfusion or injection of Fraction I. No beneficial effect resulted from further injections since the active globulin factor which was being given, and which is necessary for normal coagulation, was immediately rendered ineffective by the circulating antibodies. Likewise, the circulating antibodies would exert an anticoagulant effect when the patients' blood was mixed with normal blood *in vitro* by inhibiting the globulin substance present in normal blood.

The explanation proposed for the development of a refractory state to transfusion in these two hemophiliacs, based upon an immunologic response to injections of a globulin fraction deficient or lacking in their blood, may also be the underlying factor in the refractory phase manifested by many hemophiliacs. The appearance of specific antibodies which inhibit antihemophilic globulin, and thus delay the coagulation of normal blood, may depend upon several factors which are not yet clear. Whether or not a complete absence of this globulin from the blood of the hemophiliac receiving transfusions or injections of the globulin is mandatory for the development of these antibodies is not known. Perhaps there are varying degrees of hemophilia accompanied by varying degrees of deficiency of this globulin and varying ability to respond to injections of the globulin. In some of those who have a marked lack, or perhaps a complete absence, of the globulin in their blood the response would be "isoimmunization" with the development of antibodies against the injected globulin. These cases would show a refractory state to further injections of globulin, and their blood would demonstrate anticoagulant activity when added to normal blood.

Further investigation is to be carried on to determine if a mechanism such as that described is more generally applicable to hemophiliacs who become refractory to treatment.

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## A Homogeneous Emulsion of Fat, Protein, and Glucose for Intravenous Administration


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Several investigators (*3*) have reported success in the preparation of various types of fat emulsions, some of which were well tolerated on intravenous injection into animals. Also, experimental evidence has been produced to show that such fat administered intravenously was properly metabolized for energy utilization (*2*) and was not lost in either urine or feces. Intravenous studies of emulsions combining the three primary foodstuffs were attempted only once (*1*), and, had this procedure been routinely practical, it would have constituted another advance in surgical parenteral nutrition.

Egg lecithin, soybean phosphatides, and other chemical agents have been used as stabilizers in the emulsification and homogenization of fat. Such emulsions, although well tolerated in some cases, have not been uniformly dependable for intravenous injection because of varying degrees of toxicity believed to be due to the chemical complexity of the stabilizing agent. Hydrophilic colloids, both acid and alkaline, were used in the stabilization of fat but on biological investigation were discarded because of the frequency of fatal embolism after injection of emulsions prepared in such manner.

In this laboratory two satisfactory types of fat emulsion have been prepared. The first type was an emulsion of coconut oil and serum albumin which, on homogenization, yielded a highly stabile preparation well tolerated intravenously. The


[1] The cooperation of Profs. Mulholland and Co Tui is gratefully acknowledged.

second type, derived from glucose, protein, and fat, was prepared in the manner described below from solutions which are commercially available in sterile containers and from a neutral saturated coconut oil which can easily be sterilized with safety. The following represents the basic proportions of the ingredients which are mixed in a 5-gallon, sterile flask: 1 l. of 6 per cent infusion gelatin (Knox P-20), 1 l. of protein hydrolysate 5 per cent amino acid solution, 200 cc. of 50 per cent glucose solution, and 100 grams of pure, refined, edible coconut oil.

After thorough mechanical agitation, the mixture is put through the Logeman homogenizer, an instrument so constructed as to make possible sterilization of its working parts. After this procedure of emulsification and homogenization, the material is collected in sterile vacuum bottle dispensers without any other processing and stored in the refrigerator. Refrigeration converts the emulsion into a solid gel which can be restored to the liquid state by warming the dispensing flask in hot water. Refrigerated samples have maintained complete stability after two months storage. Test samples of the pH of the emulsion averaged 6.5. The droplets were smaller in size than that of the canine erythrocyte and were comparable to that of the chylomicra. The concentrations of fat protein and glucose were approximately 5 per cent each and averaged 800 cal./l. The gelatin acted as a stabilizer, partially as a source of nutrient energy, and served to maintain colloid osmotic pressure.

This emulsion was administered intravenously to 7 dogs and 12 hospital patients. In the latter group at hourly intervals for a period of 8 hours during and after the infusion, temperatures, blood pressure readings, and blood samples were taken. In both groups there occurred no serious untoward reactions as were indicated by the latter studies. Two of the animals received 150 cc. of this emulsion (approximately 7.5 grams of fat) daily for 35 days. The others were sacrificed at varying times for evidence of fatty degenerative changes in the liver, lung, brain, and other organs. Microscopic examination of osmic acid, Sudan III, and hematoxylin eosin preparations failed to show evidence of lipoid granulomatosis. Three patients were maintained exclusively on this emulsion during the postoperative period with satisfactory results. One malnourished patient received with benefit a total of 5,500 cc. of the emulsion (approximately 200 grams of fat) intravenously for a period of 8 days. The emulsion caused no thrombosclerotic changes in the recipient vein and was nonirritating when infiltrated into tissue. Vitamin B and methyl donators were supplied to ensure utilization of fat. Penicillin could be added to the emulsion for intravenous administration.

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# IN THE LABORATORY

## The Importance of Controlling Cooling Temperatures During Embedding in Paraffin


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Many textbooks of microtechnique (*1*) urge the use of only enough paraffin to cover the specimens and the use of cold water for solidifying paraffin in the process of embedding. Johansen recommends using ice water when embedding during hot weather. Unfortunately, our laboratory has found that this is not always sound advice. The use of cold water for solidifying our particular embedding mass had always resulted in cavitation or numerous low-density areas. Usually the cavities were quite large and were located in the central portion of the block, separated from, or adjacent to, the embedded materials. In an attempt to rectify the situation a series of experiments were carried out. In the light of Johansen's recommendation to use ice water for cooling, it should be stated that the experiments were conducted during the month of August, when temperatures in the room where embedding was done were 90–95° C.

In the first experiment an embedding mass[1] with a boiling point of 54–56° C., heated to 56° C., was poured into paper "boats" 2½ inches long and ¾ inch wide. No specimens were embedded. Some of the "boats" were filled (¾ inch thick), others were poured medium full (½ inch thick), and only a thin layer (¼ inch thick) was poured into others. The paraffin in three such sets was solidified in water at 9° C., 22° C., and 34° C., respectively. In each case the surface of the paraffin was blown upon, and as soon as a supporting crust was formed, the boat was submerged for 12 hours. At the end of the 12-hour period, the boats were dried and the paraffin blocks removed.

Upon cutting the blocks it was found that those medium full and full which had been cooled at 9° C. had large (3 mm. in diameter) continuous or discontinuous cavities throughout the central portion. Both top and bottom surfaces were decidedly concave. The corresponding blocks cooled at 22° C. and 34° C were solid and of desirable texture throughout. The top surface of both sets was concave. The bottom surface of those cooled at 34° C. was either level or slightly convex; that of those

[1] A mixture of 360 grams of Parawax 40 grams of bayberry wax, and 80 grams of stock rubber paraffin (1 pound of Parawax, 10 grams of crude rubber, and ½ gram of asphaltum).