Inspection of the tables shows that the intravenous administration of 15,000 S.T.D. produces a rapidly fatal result in mice. Temperatures as high as 100° C. for short periods of time do not completely inactivate erythrogenic toxin as evidenced by the fatal result following intravenous administration of larger doses of such heated filtrates (Table 2).

Thus, the characteristics described for the agent in broth filtrates of hemolytic streptococci are not distinguishable from those of erythrogenic toxin with respect to heat stability and lethal action in mice. Furthermore, neutralization by erythrogenic anti-toxin completely obliterates the lethal action of broth filtrates of hemolytic streptococci in mice. For these reasons, unless it is shown that the lethal agent described remains in broth cultures after absorption of erythrogenic toxin with antitoxin, it can not be considered as distinct from erythrogenic toxin. Erythrogenic toxin probably is not the only factor involved in the toxic manifestations of hemolytic streptococcus infection in man. This seems quite evident from the observations of Kenny and Colebrook[6] on puerperal sepsis. There is some evidence that certain hemolytic streptococci produce a toxic substance when grown in tissue media.[7] However, due to the diverse biological phenomena presented by the erythrogenic toxin, its presence as a "contaminating factor" must be considered in interpreting the results obtained in animals with any filtrate of the hemolytic streptococcus.

TABLE 2

HEAT STABILITY OF ERYTHROGENIC TOXIN: INTRAVENOUS INJECTION OF TOXIN AND TOXIN-ANTITOXIN IN MICE

Toxin*		Dose (ml)	Result	Toxin neutralized by NY 5 antitoxin†
Streptococcus NY 5 (Type 10)	Unheated	1.0	+	–
	Heated 56° C.– 30 min	1.0	+	–
and	" 80° – 30 "	1.0	+	–
	" 100° – 20 "	1.0	–	–
Streptococcus BFO	" 100° – 20 "	2.0	+	–
(Type 2)	" 100° – 60 "	1.0	–	not done
	" 100° – 60 "	2.0	–	" "
	" 100° –120 "	1.0	–	" "
	" 100° –120 "	2.0	–	" "
Broth controls	Unheated	2.0	–	" "
	Heated 100° C.–120 min	2.0	–	" "

(+) = death within 10 min.
(–) = no reaction.
* Contained approximately 2,000,000 skin test doses per ml. Diluted 1 : 66 to contain approximately 30,000 skin test doses per ml. 1.0 ml. contained 2 M.L.D. (mouse, Table 1).
† Toxins neutralized by equivalent units of antitoxin.

GEORGE E. FOLEY

DEANTIGENATED BEEF BLOOD PLASMA AS A POSSIBLE SUBSTITUTE FOR HUMAN BLOOD PLASMA[1]

SINCE racemization of protein is accompanied by loss of antigenicity, an attempt was made to use this process to deantigenate beef blood plasma with the possibility in mind of using the modified plasma as a therapeutic substitute for human plasma. Beef plasma treated for 15 days at 37° with sodium hydroxide in a concentration of 0.5 normal, after the manner described by Dakin[2] for the racemization of casein, yielded a product which, when neutralized, was highly toxic for guinea pigs on intravenous injection. This toxic action was reminiscent of the action of the anaphylatoxin produced by Vaughan[3] by the treatment of protein with an alcoholic solution of sodium hydroxide. Systematic reduction of the period of incubation of the alkaline plasma progressively reduced the toxicity. It was found that eight hours was the longest period which plasma could be treated without the development of toxic properties manifest on intravenous injection into guinea pigs. The longest period of treatment which gave a product that did not have a primary oxytocic action on the isolated uterus of a guinea pig was one hour. Beef plasma which had been incubated one hour or longer with 0.5 normal sodium hydroxide and then neutralized was no longer antigenic when tested by gross anaphylaxis or by the more sensitive method using uterine strips from guinea pigs sensitized to native beef plasma. Even a five-minute exposure to 0.5 normal alkali destroys most of the antigenicity of beef plasma.

Most of the protein of beef plasma that had been treated for one hour with alkali can be precipitated with acid at pH 4.3 and redissolved in alkali. It can also be precipitated with alcohol, dehydrated with acetone, and the dried powder redissolved in water. In neutral solution the protein can be heated without

[1] From the Department of Pathology and the Otho S. A. Sprague Memorial Institute, University of Chicago.

[2] *Jour. Biol. Chem.*, 13: 357, 1912; 15: 263 and 271, 1913.

[3] "Protein Split Products," Philadelphia, 1913.

[6] M. Kenny and L. Colebrook, *Jour. Path. and Bact.*, 44: 91, 1937.

[7] To be published.

coagulation or apparent alteration of its properties. These facts form the basis of the procedure used in preparing large amounts of the modified plasma for physiologic experiments. Originally ultrafiltration was used to "wash" out the excessive salt formed from the neutralization of sodium hydroxide. This process is slow and bacterial contamination is difficult to avoid. At the present time the routine procedure is as follows. A special citrated beef plasma of low bacterial count is obtained in five-gallon lots (from Armour and Company through the courtesy of Dr. Julius D. Porsche). To each liter of the plasma is added 100 cc of 22 per cent. sodium hydroxide, both of which have been warmed to 37°. The mixture is placed in a 37° incubator for one hour, after which 1.0 normal hydrochloric acid is added slowly with brisk stirring until a reaction of pH 4.3 is reached. The precipitate is separated and washed with a citric acid-disodium phosphate buffer at pH 4.3. The precipitate is then finely suspended in about 700 cc of pyrogen-free distilled water and 30 cc of 22 per cent. sodium hydroxide added slowly as the suspension is vigorously stirred. The protein dissolves very rapidly and as soon thereafter as possible 1.0 normal hydrochloric acid is added until the reaction is pH 7.3–7.4. The volume is then made one liter. If the solution is turbid it becomes clear on warming. It is sterilized by passage through a Berkefeld filter. The final product is a slightly opalescent solution with the appearance and viscosity of serum. An alternative procedure is to neutralize the mixture when it is taken from the incubator and then add 4 volumes of 95 per cent. alcohol. The precipitate is washed several times with 95 per cent. alcohol and finally extracted with acetone until the filtrate is colorless. The protein, when dry, is a fluffy white powder from which solutions of a desired concentration can be prepared by adding saline and heating for about 15 minutes in an Arnold sterilizer. Beef serum can be used, as well as plasma, in the procedure described and gives a satisfactory end product. With serum the solution is lighter in color and can be more easily passed through a Berkefeld filter.

Experiments are under way (in collaboration with Dr. Howard C. Hopps) to find the therapeutic effect of the modified plasma on experimental shock. A normal dog given, while under ether anesthesia, 500 cc of the substance containing 5 per cent. protein showed no change in respiration and a slight rise in blood pressure apparently due to increased blood volume. Allowed to recover, this animal disclosed no untoward effect which might be attributed to the infusion. Dogs brought into shock by massive bleedings have been successively treated by infusion with the modified plasma. Their blood pressure is readily restored and they survive what otherwise would have been fatal shock. An animal used a second time in such an experiment, three weeks after the first experiment, likewise recovered and showed no effect that could be ascribed to a sensitization from the previous infusion.

It is generally believed that the loss of antigenicity which a protein undergoes when treated with strong alkali is due to racemization. The changes produced in beef plasma described above are apparently *not* due to racemization for the following reasons: (1) with complete racemization optical rotation is brought to an irreducible minimum. The optical rotation of modified beef plasma after one hour of treatment with 0.5 normal sodium hydroxide is but slightly altered; (2) with complete racemization the treated protein is no longer digestible by proteolytic enzymes or putrefactive bacteria. Modified beef plasma is readily digested by pepsin and becomes putrefied on standing exposed.

The cause of the loss of antigenicity from short exposure to alkali is not yet fully determined. During neutralization of the alkaline plasma a very strong odor of hydrogen sulfide is given off. This suggests the destruction of cystine and other sulfur-containing amino acids. It is possible that other amino acids, including those that are believed to be necessary for antigenicity of proteins, are also destroyed. If this be true the modified beef plasma may be considered analogous to gelatin which is produced by hydrolysis of collagen. Gelatin is deficient in certain amino acids, a fact which has been related to its lack of antigenicity.

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