The photophobia was so severe and it was so difficult to keep the eyes still that satisfactory colored photographs were obtained only after numerous trials and errors.

A brief case report follows:

It is common knowledge that dogs with severe black-tongue are likely to die suddenly and since we have been able to relieve animals with spontaneous black-tongue,[2] we injected 150 mgs of nicotinic amide as soon as the dog had been carefully examined and illustrations made. Twenty hours later the dog showed remarkable improvement. He was obviously much stronger and ate the food offered. The fiery redness of the oral mucous membranes had faded considerably. Salivation had decreased and the diarrhea had stopped. The appearance of the eyes, however, remained unchanged. Next, 50 mgs riboflavin[5] were injected intravenously. Twenty-four hours later there was less lacrimation and photophobia. The injection had receded and there were fewer dilated and tortuous vessels in the conjunctiva and sclerae. Nystagmus was not present. Seventy-two hours later there was no detectable lacrimation or photophobia, and the redness and general increase in vascularity had subsided. Indeed, only very careful examination revealed "ghost vessels"—vessels which had been engorged but were now only faintly discernible.

SUMMARY

In four dogs, the diarrhea, increased salivation and mucous membrane lesions characteristic of black-tongue were relieved within 24 hours following the administration of 150 mgs nicotinic amide, whereas the lacrimation, photophobia and extreme injection of the eye vessels did not diminish. These severe eye lesions regressed greatly 24 hours after the injection of 50 mgs of riboflavin, and in 72 hours had disappeared.

The simultaneous occurrence of nicotinic acid and riboflavin deficiency in four dogs is evidence that such deficiencies occur as mixed diseases rather than as single entities.

These findings are further evidence of the universality of nutritional deficiencies, since they suggest that deficiency diseases among the pets of families with deficiency diseases are not uncommon. In at least one instance the finding of nutritional deficiencies in a dog eating scraps from the family table led to a better understanding of the ill health of the family, none of whom had diagnostic lesions of deficiency disease at the time, although they all complained of weakness, nervousness, irritability and loss of appetite—the vague and ill-defined symptoms characteristic of deficiency diseases in the early stages.

TOM D. SPIES

SCIENTIFIC APPARATUS AND LABORATORY METHODS

IDENTITY OF A LETHAL AGENT IN BROTH FILTRATES OF HEMOLYTIC STREPTOCOCCI WITH ERYTHROGENIC TOXIN[1]

THE similarity of a recently described lethal agent produced in broth cultures by hemolytic streptococci (Lancefield Group A)[2] to erythrogenic toxin raises the question of their differentiation.

Marked differences in heat stability indicate the existence of at least two kinds of hemolytic streptococcus toxin: the heat stabile erythrogenic and the heat labile hemolytic toxins. The resistance of eyrthrogenic toxin to comparatively high temperatures for considerable periods of time is well established.[3, 4] Since the lethal agent described, which was resistant to temperatures which do not completely inactivate erythrogenic toxin, and was lethal for mice, the effect of intravenous injection of heated and unheated erythrogenic toxin in mice was studied.

The toxins used were unpurified filtrates. Skin test doses per milliliter were determined by toxin-antitoxin flocculation. Table 1 summarizes the results obtained in mice by the intravenous injection of unheated

TABLE 1

DETERMINATION OF M. L. D. OF EROTHROGENIC TOXIN: INTRAVENOUS INJECTION IN MICE

Toxin	Dose (ml)	Skin test doses ×100	Result
Streptococcus NY 5 (Type 10)	1.0	300	+
	0.5	150	+
	0.25	75	−
Streptococcus BFO (Type 2)	1.0	300	+
	0.5	150	+
	0.25	75	−

(+) = death within 10 min.
(−) = no reaction.
Undiluted toxin contained approximately 2,000,000 skin test doses per ml. Toxins diluted 1:66 contain approximately 30,000 skin test doses per ml.
Mice 20–25 g in weight used.

toxins. Table 2 summarizes the results obtained with heated toxins and toxin-antitoxin mixtures. Toxin-antitoxin neutralization with heated and unheated toxins was checked by the intracutaneous rabbit test described by Fraser and Plummer.[5]

[5] A special concentrated product sent to me for experimental trial by Hoffmann-La Roche, Inc.

[1] From the Department of Preventive Medicine, Harvard Medical School and House of the Good Samaritan, Boston, Mass.

[2] T. N. Harris, *Jour. Bact.*, 43: 739, 1942.

[3] H. J. Parish and C. C. Okell, *Jour. Path. and Bact.*, 33: 527, 1930.

[4] G. A. H. Buttle and A. S. R. Lowdon, *Jour. Path. and Bact.*, 41: 107, 1935.

[5] F. H. Fraser and H. Plummer, *Brit. Jour. Exp. Path.*, 11: 291, 1930.

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.