

serum produces little or no acceleration of the disappearance of antigen it seems justifiable to assume that intravascular union plays an unimportant rôle in the mechanism for removal of foreign serum from the circulation, and therefore that the cellular phase is of predominating significance.

It is also evident from the results on the whole group of 28 rabbits studied that individual variation extends over a wide range both in ability to form antibody and in the rate at which foreign serum is removed from the circulation.

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### **The effect of various proteins on streptolysin production.**

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Variations in streptolysin production in horse and rabbit serum media were noted in a previous publication. In horse serum hemolysin was produced in titratable quantities later in the growth of cultures than in rabbit serum broth but the maximum concentration reached was greater. Unless glucose were present the curve of lysin production corresponded closely to that of growth since hemolysin was found in greater concentration during the period in which the bacteria were multiplying most rapidly. On account of these differences which were characteristic of these sera an attempt was made to discover the responsible factors. The albumen and globulin ratio was modified so that the horse serum contained the same proportions of horse-serum albumen and globulin as were found in rabbit serum, and rabbit serum the same percentages as were present in horse serum. Flasks prepared with 20 per cent. of these modified sera in plain infusion with 0.7 per cent. NaCl, were seeded with equal quantities of a 16-hour culture of hemolytic streptococcus in 20 per cent. horse serum broth. Hemolysin titrations were then made at intervals of an hour with a suspension of horse corpuscles in physiological salt.

The percentages of albumen and globulin in normal rabbit

and horse sera were determined by fractioning the diluted serum with ammonium sulfate. The analyses in parts per hundred were as follows:

	Albumen.	Globulin.
Horse.....	2.6	3.5
Rabbit.....	3.41	2.27

Sterile albumen and globulin were obtained respectively from horse and rabbit sera under sterile conditions. The albumen was precipitated with ammonium sulfate. The globulin was prepared by saturating diluted rabbit serum with CO<sub>2</sub> and so did not represent the true globulin fraction of the serum.

The horse serum was diluted until it contained 2.27 grams of globulin per hundred c.c. and sufficient horse-serum albumen added to bring the percentage of albumen to 3.41 grams per cent. Rabbit serum was diluted and rabbit-serum globulin added so that the percentages of albumen and globulin were the same as those of horse serum. Flasks of 20 per cent. media were prepared with the modified sera. When growth and streptolysin estimation were made with growing cultures in these media it was found that the reversed ratio of albumen to globulin had no effect on the type of curve obtained. The curves in the modified sera were similar to those in normal serum media. Hence it appears that albumen and globulin do not enter into any of the peculiarities of growth exhibited by hemolytic streptococcus. This fact was further substantiated by a study of the proteolytic enzyme of these bacteria, because the enzyme acted similar to erepsin and did not digest serum albumen and globulin. Furthermore the addition of large amounts of albumen and globulin did not modify the curves.

Growth and hemolysin were next studied in peptone. Two preparations of peptone were used. These were similar except one was partially hydrolyzed and the other broken until further digestion with active trypsin gave no increase in amino nitrogen. The peptone was boiled, filtered through a 10-pound filter and added to beef infusion. Analyses of the media prepared with these peptones after autoclaving were as follows:

	Total N. Grams per 100.	Amino N. Per Cent. of Total N.
1.....	0.27	14.9
2.....	0.28	41.8

When streptococci which had not been animal-passed were grown in flasks of these media so that comparisons of growth and hemolysin could be made, the growth was more luxuriant, the lag shorter and the hemolysin stronger in the peptone which had been only partially hydrolyzed. This suggests that causes for variations in growth are to be sought in the partially split proteins of the blood serum.

119 (1866)

**The bicarbonate and chloride content of the blood in certain cases of persistent vomiting.**

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These researches were instigated by an interest in the phenomenon of tetany, particularly in that form known as gastric tetany. Very little work has been done in this field on human subjects, none recently; and it seems that no blood analyses have been published. Whatever work was done was not convincing and the old hypotheses such as the dehydration and mechanical theories once offered as a result of clinical studies to explain the condition must be discarded as untenable.

There has been some successful experimental work on dogs, however, in which tetany was produced by obstructing the pylorus and in which various disturbances in the salts of the blood were recorded.

To summarize: McCann<sup>1</sup> (1918) was the one to discover that after pyloric closure there was a rise in the combined carbon dioxide. This was confirmed by MacCallum<sup>2</sup> (1920) and in the surgical laboratories of this college (Hastings<sup>3</sup> and Murray 1921). MacCallum and ourselves also found a markedly diminished chloride content with normal values for calcium. In our laboratory, contrary to expectations, it was found that the H-ion

<sup>1</sup> McCann, W. S. *J. Biol. Chem.*, 1918, xxxv, 553.

<sup>2</sup> MacCallum, W. G., Lintz, J., Vermilye, H. N., Leggett, T. H., and Boas, E. *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 1.

<sup>3</sup> Hastings, A. B., Murray, C. D., and Murray, H. A. *J. Biol. Chem.*, 1921, xlvi, 223.