In 2 persons suffering from coronary artery thrombosis, 3 cc doses of heparin repeated at 3 hourly intervals were given for periods of 5 and 7 days. The coagulation time was kept prolonged throughout this period.

During the course of these experiments some evidence was obtained to indicate that the heparin injected was probably destroyed in the body and not voided in the urine. The bleeding time (Duke method), capillary fragility (Daldorf method) and clot retraction after heparin are normal. Leucocytes and bone marrow pulp left in contact with heparin for periods greater than one hour tend to agglutinate and later disintegrate. There is a tendency for hematomata to form at the site of venipuncture after heparin unless the wound is carefully tamponed.

Summary. Heparin added to whole blood *in vitro* or administered by vein prolonged the coagulation time of whole blood and plasma. The coagulation time was prolonged and maintained by a single large dose of heparin followed by a smaller continuous dose. The mechanism appeared to be independent of the presence and type of disease. Heparin produces agglutination and disintegration of leucocytes and bone marrow pulp *in vitro*.

10302

Serological Studies on Mastitis.

F. R. SMITH AND C. S. MUDGE.

From the Division of Dairy Industry, Davis, California.

One of the great difficulties encountered in studies on the streptococcal type of mastitis is the diagnosis of the chronic form of the disease. Both the chemical and bacteriological methods have their proponents, but as yet, no one test or series of tests can be considered as a final diagnostic measure. For this reason it would seem that any valuable means of diagnosis must bring in methods differing greatly from those already recommended.

Despite the fine work done on certain animal diseases by applying serological methods this particular field seems to have been practically neglected by those interested in mastitis. This appears particularly strange in view of the fact that as early as 1904 Stahli[1] was able to

[1] Stahli, A. T., *Arch. f. wessensch. u. prakt. Tierheilk.*, 1904, **30**, 374.

demonstrate agglutinating antibodies in the serum of a mastitic cow. He also found that these antibodies were absent from the sera of uninfected cattle. The major criticism of this work would seem to be that it was limited to one cow and to a single strain of streptococcus.

Recently Stableforth[2] made an extensive survey on the types and sub-types of *Streptococcus mastitidis (Streptococcus agalactiæ)* in the herds of Great Britain. He found that usually the strains present in a given herd were alike. The author also believed that serological examination may be used as a diagnostic means in mastitis. His method involved isolation of the organism from the suspected cow followed by agglutination-tests with each of his 5 types of antisera. Stableforth states, "The experience gained in the work here reported has shown that serological methods offer a ready and reliable means of recognizing *Str. agalactiæ* and these have, during the past 3 years, gradually replaced biochemical methods in the control of *Str. agalactiæ* infections."

The work of Lancefield[3] has entirely revolutionized the serological study of streptococci, and thus it appeared desirable to apply her methods in the study of mastitis. If the invasion of *Streptococcus mastitidis* into the udder of the cow is followed by the appearance of antibodies in the serum of that cow, and if these antibodies are group-specific then we would have a very valuable and simple means in the diagnosis of the streptococcal type of mastitis. Extracts of any typical Group B organism would then react with positive serum from any cow. If, however, the antibodies produced are type-specific then precipitative and agglutinative procedures will become much more complicated. The testing of type-antisera would probably necessitate the use of a number of strains of *Streptococcus mastitidis*. This number, however, from the work of Stableforth[2] does not appear to be prohibitory.

Two cultures of *Streptococcus mastitidis* were used in this study. One of these was a typical Group B organism isolated from human feces[4] while the other was isolated from an acute case of mastitis in one of the herds under observation. This latter organism was not subjected to the Lancefield test but did correspond to the physiological reactions described by Sherman[5] for *Streptococcus mastitidis*. It would not seem likely that these 2 organisms would belong to the same serological type under Group B.

---

[2] Stableforth, A. W., *J. Path. and Bact.*, 1938, **46**, 21.

[3] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

[4] Smith, F. R., and Sherman, J. M., *J. Infect. Dis.*, 1938, **62**, 186.

[5] Sherman, J. M., *Bact. Rev.*, 1937, **1**, 1.

Sera for these studies were obtained by bleeding from the jugular vein. Precipitin-tests were made according to the method recommended by Lancefield.[5] The agglutinative procedure was essentially that described by Griffith.[6]

Two series of blood samples were taken from Herd I. An interval of 2 months elapsed between the first and second bleedings. This herd was known to contain mastitic cows and it was from one of the cows in this group that the bovine strain of *Streptococcus mastitidis* was isolated. Only one series of blood samples was secured from Herd II. Little was known about the history of this herd except that it had no connection with Herd I.

All Lancefield tests on the Group B streptococcus isolated from human feces were negative. Thus it would seem that group-antibodies are not produced in the sera of cows infected by *Streptococcus mastitidis*.

Serological reactions on the strain isolated from an acute case of mastitis were interesting. These results have been summarized in Table I. There would appear to be no doubt that type-specific immune bodies are present in the sera of certain cows. Since the complete history of the herd was lacking it would be impossible to correlate the presence of these immune bodies with the incidence of infection in the herd. Routine laboratory analysis of Herd I before the serological studies were begun would indicate that the agglutinative method would discriminate against many cows giving apparently normal milk. This is to be expected since the antibody titer of the blood should remain relatively high for some time after symptoms of infection have ceased.

Due to the large number of positive agglutinative reactions with Herd I it would certainly appear that the infection of this herd was

TABLE I.

Serum reactions with *S. mastitidis* isolated from an acute case of mastitis in Herd I		Serum from individual cows in: Herd I 4/6/38	Herd I 6/16/38	Herd II 6/16/38
Agglutination	+	54	36	0
Lancefield	—			
Agglutination	—	0	2	0
Lancefield	+			
Agglutination	+	30	15	0
Lancefield	+			
Agglutination	—	33	64	17
Lancefield	—			

[6] Griffith, F., *J. Hyg.*, 1934, **34**, 542.

due to one specific type of Group B. This conclusion coincides well with the observation of Stableforth.[2]

The total lack of reaction in the case of Herd II is very interesting, and does indicate that there is some correlation between the occurrence of a given type of *Streptococcus mastitidis* in the herd and the presence of antibodies in the sera of that herd.

It is not the purpose of this work to advocate the routine use of serological methods in the diagnosis of mastitis. Considerable work would be necessary before one would be justified in drawing conclusions as to the correlation between mastitis and the presence of agglutinating antibodies. Such data would necessitate a survey such as that conducted by Stableforth in order that we may attain some knowledge of the number of types of *Streptococcus mastitidis* encountered in the United States. Furthermore complete studies should be made on herds in order that we may draw conclusions regarding the correlation between mastitis as shown by our more common tests and the agglutinative response. Until such data have been collected the serological reactions of cow serum can only be of theoretical interest.

Conclusions. Cow sera may contain streptococcal antibodies which appear to be type-specific. There is evidence of a correlation between the presence of *Streptococcus mastitidis* in the herd and the occurrence of agglutinating antibodies in the serum of the individual cows. It appears probable that infection in an individual herd may be due to a specific type under Group B.

10303 P

Sputum Studies in Lobar Pneumonia. Extra-Cellular Encapsulated Pneumococci.

ARTHUR W. FRISCH. (Introduced by W. O. Nelson.)

From Wayne University College of Medicine, Detroit, Michigan.

In a previous report concerning rusty sputum from cases of lobar pneumonia, 3 types of phagocytosis and the effect of serum therapy were described.[1] The present communication deals with the number of extracellular capsulated pneumococci in rusty sputum as an index

[1] Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 473.