shorter in the presence of salicylate than in the controls without salicylate. The density was also less in the former case than in the latter, largely due to the fact that there were fewer individual cocci, although the number of chains or clumps were approximately the same in both cases as shown by the plate count. It is conceivable that this inhibition of a very important biological function of the streptococci is associated with a decrease of their vitality and their virulence.

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## Cephalin Content of Prepared Fibrinogen and Prothrombin Solutions.

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Mills[1] prepared fibrinogen and prothrombin solutions from platelet-free plasma; the fibrinogen remained unclotted by both calcium and cephalin for 24 hours at 40°C.; the prothrombin needed cephalin as well as calcium for its activation. Few other investigators have secured preparations which could fulfil these rigorous tests, and Howell,[2] in particular, has held to the idea that calcium alone is sufficient to activate prothrombin, the more especially since he and Cekada[3] were able to obtain active thrombins that were lipoid-free. In view of Mills' demonstration that (a) the prothrombin prepared by Howell's acetone method contained cephalin which could be extracted with cold benzene, leaving a preparation which needed cephalin as well as calcium for its activation, and that (b) an appreciable amount of cephalin-like lipoid could be extracted by boiling ether from platelet-free goose and dog plasma, we wondered whether Howell's pure thrombins had been tested on fibrinogen solutions free from lipoids. The following experiment emphasizes the need for satisfying this inquiry.

A dog, *not fasted,* was anesthetized with sodium amytal. Blood was collected, via a paraffined cannula in the carotid artery, into

1 Mills, C. A., *Chinese J. Physiol.,* 1927 (several papers).

2 Howell, W. H., *Am. J. Physiol.,* 1910, **26,** 453.

3 Cekada, E. B., *Am. J. Physiol.,* 1926, **78,** 512.

cooled paraffined centrifuge tubes containing sodium citrate (to a final concentration of 0.5%). After immediate low speed centrifugalization to remove red and white cells, the platelets were separated by prolonged high speed centrifugation. The plasma was salted with 15% (solid) NaCl, followed by 18% $(NH_4)_2SO_4$, the respective fibrinogen and prothrombin fractions being purified by washing and reprecipitation, and tested for their coagulation reactions. The final solution of prothrombin was found to be activated by calcium alone, and formed a firm clot with fibrinogen solution in 4-5 minutes. After extraction for a few minutes with cold benzene, calcium was unable to activate the coagulant. The recalcified benzene-extracted prothrombin, however, still clotted unextracted fibrinogen solution, although somewhat more slowly (10 min.). If the benzene extract (from the fibrinogen solution) was layered on top of a mixture of the recalcified 'lipoid-free' reagents, clotting started in about 10 minutes at the benzene surface and slowly spread down the tube through the watery layer. A small quantity of benzene extract, evaporated to dryness, gave an almost invisible residue which, when dissolved in distilled water, readily activated the recalcified extracted prothrombin. Pure cephalin (from brain) acted in the same manner, but lecithin *(de ovo)* was without effect.

We conclude that tests with cephalin-free fibrinogen solutions must be specifically included before evaluating the coagulant properties of highly purified thrombin preparations. Wooldridge[4] was fully aware that the protein fractions obtained by salting contained "lecithin" (the current term for phosphatides). He tried in vain to obtain lipoid-free fibrinogen, and in an approximate analysis determined 3.2% of lecithin (dry weights). Theorell[5] also found 0.623 gm. of ether soluble P per gram of protein in horse plasma fibrinogen obtained by salting with 27% of ammonium sulphate.

[4] Wooldridge, L. C., Collected papers on "The chemistry of the blood," 1893.
[5] Theorell, A. H. T., *Biochem. Z.*, 1926, **175**, 297.