Three additional spots showing a brilliant yellow fluorescence were seen, two more polar and the third slightly less polar than the ultra-violet absorbing spot. All spots were eluted with a 2 : 1 ethyl acetate/methanol solvent. The less polar of the four spots (compound 1) has been tentatively identified as 6β OH cortisol.

Identification of the isolated 6β-hydroxycortisol was based on the properties as listed in Table 1, the results being consistent with those obtained by the reference 6β-hydroxycortisol. The quantity of 6β-hydroxycortisol isolated from liquor amnii was so small that it was impossible to subject the compound to infra-red analysis.

Identification of the other polar steroids isolated from liquor amnii is being actively pursued.

This work was carried out during the tenure of a research grant from the Medical Research Council of Ireland.

M. Lambert
G. W. Pennington

Department of Pharmacology,
Trinity College, Dublin.

[1] Bernstein, S., Dorfman, R. I., and Nadel, E. M., *Arch. Biochem. and Biophys.*, **53**, 307 (1954).
[2] Ulstrom, R. A., Colle, E., Burley, J., and Gunville, R., *J. Clin. End.*, 8 Met., **20**, 1080 (1960).
[3] Katz, F. H., Frautz, A. G., and Jailer, J. W., *Abst. No. 37, Prog. Forty-second Meet. Endocrine Soc., Miami Beach, Florida*, 35 (June 9–11, 1960).
[4] Bush, I. E., *Biochem. J.*, **50**, 370 (1952).
[5] Bush, I. E., *The Chromatography of Steroids*, 184 (Pergamon Press, 1962).
[6] Dixon, P. (personal communication to M. L.).
[7] Zaffaroni *et al.*, *Rec. Prog. Hormone Res.*, **8**, 51 (1953).
[8] Bernstein, S., and Lombard, R. H., *J. Org. Chem.*, **18**, 146 (1953).
[9] Bush, I. E., and Mahesh, V. B., *Biochem. J.*, **71**, 705 (1959).
[10] Bush, I. E., *The Chromatography of Steroids*, 365 (Pergamon Press, 1961).
[11] Bush, I. E., and Mahesh, V., *Biochem. J.*, **71**, 718 (1959).

HÆMATOLOGY

Staphylococcal Isocoagulases

Staphylocoagulase has been assumed to be a single enzyme elaborated by pathogenic *Staphylococci*. Investigators working with purified highly concentrated staphylocoagulase have described their product as either homogeneous[1] or non-homogeneous[2,3] when studied by free boundary electrophoresis, ultracentrifugation, or starch column chromatography.

We have now been able to show that *Staphylococci* in broth culture liberate into the medium a family of staphylocoagulases. A potent coagulase-producing strain of *Staphylococcus aureus* (*S*–58) was grown for 48 h at 37° C in tryptose broth containing 5 mg/l. of thiamine. After centrifugation, staphylocoagulase was precipitated from the supernatant by addition of 3 vol. of 95 per cent ethanol, slowly and at ice-bath temperature. The collected sediment, redissolved in distilled water, clotted oxalated 1 : 10 human plasma in 2 min and contained 50,000 clotting units/mg of protein. This preparation was subjected to electrophoresis on starch gel according to the method of Smithies[4]. Conditions of electrophoresis were: *tris* buffer *p*H 8·6, 4 V/cm, 18 h. The gel was then sliced horizontally and the cut surface placed on sterile fibrinogen agar (3 per cent bovine fibrinogen and 2 per cent rabbit serum[3]).

After 24 h at 37° C, it was found that fibrinogen precipitates at several sites (Fig. 1). The reaction extending from the origin to the first band represents trailing. If a very powerful staphylocoagulase preparation is used we find trailing will obscure the individual bands. Since the reaction at the gel and agar surface is specific, it would seem that five isocoagulases are present. This experiment has been repeated with staphylocoagulase prepared from other strains and, although as many as eight distinct bands have been found at times, we have not found less than five.

Staphylocoagulase thus joins other enzyme systems, such as lactic dehydrogenase, characterized by iso-enzymes. Work is in progress to determine whether there exists differences between various coagulase-positive strains and among the several isocoagulases. Preliminary observations show that the more intensive the 'purification' procedure the fewer the isoenzymes which can be detected. Until it can be shown that the behaviour of each is like that of the others, particularly with regard to co-factor specificity and requirements, caution must be exercised in the design of studies with the staphylocoagulase system. Finally, it is not clear at this time whether the isocoagulases are entirely extracellular or whether one or more represent intracellular enzymes[5].

This work was supported by grant *H*-2478 from the National Institutes of Health.

J. B. Miale
A. R. Winningham
J. W. Kent

Department of Pathology,
University of Miami School of Medicine,
Miami, Florida.

[1] Murray, M., and Gohdes, P., *Biochim. Biophys. Acta*, **40**, 518 (1960).
[2] Tager, M., *Yale J. Biol. and Med.*, **20**, 487 (1948).
[3] Blobel, H., Berman, D. T., and Simon, J., *J. Bact.*, **79**, 807 (1960).
[4] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[5] Duthie, E. S., *J. Gen. Microbiol.*, **10**, 427 (1954).

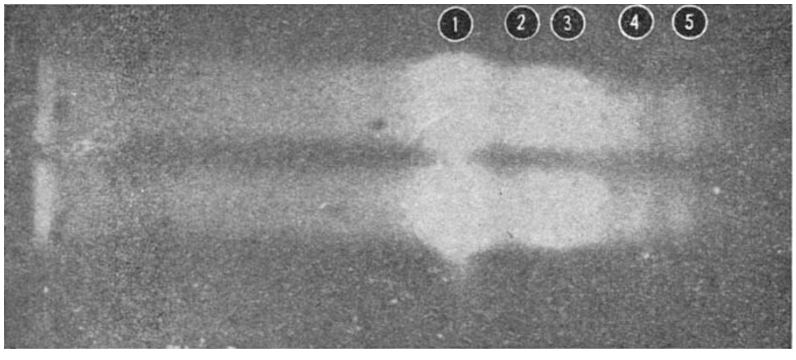

Fig. 1 Staphylococcal isocoagulases. The site of staphylocoagulase isoenzymes separated by starch-gel electrophoresis is marked by precipitation of the fibrinogen in the fibrinogen-agar. They appear as five grey opaque zones

Bivalent Nature of Incomplete Anti-D(Rho)

Erythrocyte antibodies were first demonstrated by their ability to agglutinate erythrocytes suspended in saline; these have been termed complete antibodies. In 1944, Wiener[1] and Race[2] independently showed that certain erythrocyte antibodies were incomplete, possessing the ability to fix to the erythrocyte surface without leading to agglutination. It has been postulated[1], and generally accepted, that the complete saline agglutination antibody is bivalent, and the incomplete coating antibody univalent. Recent investigations in our laboratory using antibodies labelled with iodine-131 have led us to question this hypothesis[3,4]. Our results could best be explained by assuming a bivalent nature of incomplete erythrocyte antibodies.

The assumption of a bivalent nature of incomplete antibodies introduces the problem of explaining the inability of these substances to induce agglutination of saline suspended erythrocytes. A possible explanation would appear to be the unavailability of one or more reactive sites. It may further be postulated that the unavailability of reactive sites is due to the molecular conformation of the incomplete antibody. The experiments described here have been carried out to test this concept. The objective was to change the molecular conformation of