It is intended to use this method in investigating field specimens obtained from suspected cases of leptospirosis in domesticated animals in India.

This test has also been successfully applied to detect antibodies in suspected sera when positive specimens were found to produce a single line of reaction within 2–3 hr. in the slide modification of the gel-diffusion test[7]. Precipitins could be detected by a comparable titre in the plate test. Further investigation is in progress to evaluate the diagnostic sensitivity and reliability of this method, on a comparative basis, the results of which will be reported in detail elsewhere.

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Localization of a Protease in the Cell of *Escherichia coli*

THE cells of *Escherichia coli* contain a protease during their whole growth-cycle. This protease is inactive in living cells and its presence can be demonstrated only after disruption of the cell structure[1]. In connexion with the study of the function of this protease in the metabolism of intracellular proteins its localization in the cell was also investigated.

Spheroplasts were prepared from a 4-hr. culture of *E. coli B* by Lederberg's method[2] using penicillin. 20–30 per cent of the protease activity was transferred to the medium during the preparation of the spheroplasts. Because a part of the spheroplasts was disintegrated and their cellular proteins made soluble at the same time, it can be presumed that a greater part of the enzyme thus liberated is of intracellular origin. The spheroplasts were disrupted by osmotic shock in 0·01 *M tris* at *p*H 8·0 with 5×10^{-3} *M* magnesium chloride. The material was then centrifuged in the cold at 10,000*g* for 10 min. in an MSE centrifuge, the sediment washed with the buffer and again spun down. The combined supernatants were centrifuged for 1 hr. in a cooled Phywe ultracentrifuge at 105,000*g*, the pellet resuspended in the buffer and again spun down. The sediment at 105,000*g* exhibited a characteristic absorption at 257 mμ. The protease activity was determined with casein labelled with iodine-131 substrate[3] and referred to mgm. of protein, on one hand, and expressed as per cent of the whole amount of the enzyme, on the other. A loss of about 20 per cent of the enzymatic activity occurred during these operations.

It was found that the enzyme is present mainly in the cytoplasm and in the fraction which sediments at 10,000*g* and contains the cytoplasmic membrane and

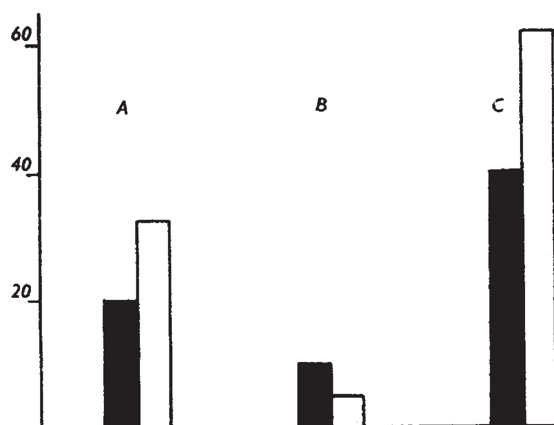

Fig. 1. Localization of a protease in cellular fractions of *E. coli*. *A*, Cytoplasmic membrane fraction; *B*, ribosome fraction; *C*, cytoplasm. Black columns, activity in μgm. of casein hydrolysed by 1 mgm. of protein during 1 hr. at 37° C.; white columns, percentage of total amount of protease

remains of the cell wall. Only a very small part of the whole amount of enzyme was present in the fraction of ribosomes which sedimented at 105,000*g* (Fig. 1). Enzymatic activity in the ribosome fraction probably is not due only to contamination, because the protease activity, related to mgm. of proteins, amounts in this fraction to about one-half of that of cytoplasmic membrane and cannot be completely removed by washing. A certain amount of active protease present in the ribosome fraction in the *E. coli* cell is analogous to the localization of the peptidase[5]. The ribosomes of *E. coli* also contain a ribonuclease[6] which is present in an inactive form and is activated after the disruption of ribosomes by ethylenediamine tetraacetic acid (EDTA) or urea. An attempt was made to verify the possibility that the inactive form of protease was present, and the influence of these substances on the activity of the protease in the fraction which sediments at 105,000*g* was therefore studied. The enzymatic activity was found to be reduced by EDTA at a concentration of 1×10^{-2} *M* by 70–80 per cent and the enzyme was completely inactivated by 4 *M* urea. The decrease in activity caused by EDTA was about the same in the fraction of ribosomes and in the cytoplasm and thus the presence of inactive protease in the ribosomes appears unlikely. The results obtained from fractions isolated from disrupted spheroplasts were checked by using material homogenized in the bacterial press[7]. It was found that in this case the enzyme was also distributed quite evenly between the fractions sedimenting at 10,000*g* and the cytoplasm. Here, too, only a small part of the whole amount of protease was found in the fraction of ribosomes.

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