

show that cobalt is required for nitrogen fixation by the root nodules of these non-legumes.

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- ¹ Ahmed, S., and Evans, H. J., *Biochem. Biophys. Res. Comm.*, **1**, 271 (1959).
² Ahmed, S., and Evans, H. J., *Soil Sci.*, **90**, 205 (1960).
³ Ahmed, S., and Evans, H. J., *Proc. U.S. Nat. Acad. Sci.*, **47**, 24 (1961).
⁴ Reisenauer, H. M., *Nature*, **186**, 375 (1960).
⁵ Hallsworth, E. G., Wilson, S. B., and Greenwood, E. A. N., *Nature*, **178**, 79 (1960).
⁶ Bond, G., in *Nutrition of the Legumes*, edit. by Hallsworth, E. G., 216 (Butterworths, London, 1958).
⁷ Bond, G., and Hewitt, E. J., *Nature*, **190**, 1033 (1961).
⁸ Hewitt, E. J., and Bond, G., *Plant and Soil*, **14**, 159 (1961).
⁹ Hewitt, E. J., *Commonwealth Bureau Hort. Tech. Comm.* 22 (East Malling, 1952).
¹⁰ Broyer, T. C., Carlton, A. B., Johnson, C. M., and Stout, P. R., *Plant Physiol.*, **29**, 526 (1954).
¹¹ Munns, D. N., and Johnson, C. M., *Plant Physiol.*, **35**, 978 (1960).

Isolation of Thermophilic Actinomycetes

To judge from reports in the literature, thermophilic Actinomycetes have proved difficult to isolate. For isolations from mouldy hay we have developed a convenient method which promises to be useful in other contexts¹. The method uses the fact that dry air removes spores of the Streptomycetaceae in preference to bacteria. Instead of suspending the spores in water and plating dilutions in the traditional manner, dry spores are suspended in air and impacted with the Andersen sampler² directly on surface-dried medium. In our work, samples of hay are shaken in a small wind tunnel and the air drawn into an Andersen sampler loaded with Petri dishes which had been poured the day before with half-strength nutrient agar (agar content made up to 2 per cent) containing 0.5 mgm./ml. actidione to suppress growth of mould. A slit-sampler could be used as an alternative to the Andersen sampler, and instead of using a wind tunnel, hay or other material can be shaken

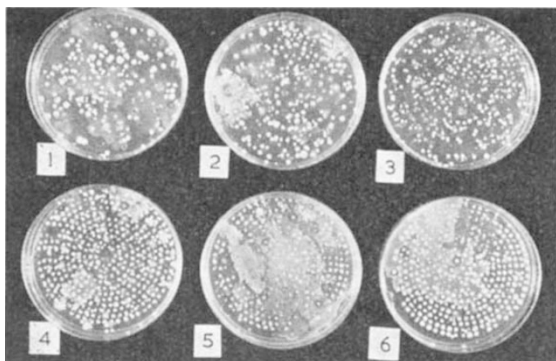


Fig. 1. Petri dishes from stages 1-6 of Andersen sampler exposed to dust from mouldy hay (associated with farmer's lung) for 5 sec. in wind of 4.2 m./sec., incubated at 60° C. for 42 hr. Note antibiotic produced by some colonies of Actinomycetes

in a box (pre-sterilized with propylene oxide if required) and a few minutes allowed for larger particles to settle before sampling the air.

After exposure in the Andersen sampler the plates are incubated at either 40° or 60° C. At 60° white sporing colonies develop overnight (Fig. 1), and at 40° many different forms develop more slowly. At either temperature many slow-growing colonies develop on prolonged incubation. During the past two years many thousands of colonies growing at 60° C. have been obtained in culture by this method from hay associated with cases of farmer's lung.

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¹ Gregory, P. H., and Bunce, M.E., *Rep. Rothamsted Exp. Sta.*, 1960, 127 (1961).

² Andersen, A. A., *J. Bact.*, **76**, 471 (1958).

Collateral Biliary Channels

FOLLOWING ligation of the common bile duct in the rat normal biliary drainage is restored within 7-12 days after operation.

Investigations previously carried out by Trams and Symeonidis¹ described "recanalization" of the common bile duct occurring 14 days after the occlusion. They suggested that it was brought about as follows: (1) Dilation of the duct above the site of ligation producing a distended sac. (2) Prolapse of the walls of the sac around the ligation. (3) Adherence between the walls of the distal part of the bile duct and the prolapsed walls of the sac. (4) Necrosis of the adherent walls restoring the continuity of the common bile duct.

Our results based on a series of animals killed at intervals ranging between 24 hr. and 3 weeks after operation disproved the mechanisms of recanalization suggested by Trams and Symeonidis¹. They show conclusively that normal drainage of bile is brought about by the outgrowth of new channels from pre-existing biliary epithelium. These arise from the bile duct immediately distal to the ligation, encircle the ligature and join up with the distended proximal sac (Fig. 1). This process is initiated immediately after ligation and is complete at 7-12 days after operation.

The findings have been confirmed by (a) radiological and (b) histological observation. The radiological approach was similar to that described previously for the spleen by Braithwaite and Adams². It consisted of injecting a radiopaque medium ('Micropaque', Damancy and Co.) either into the bile duct proximal to the site of ligature (that is, the dilated sac) or alternatively into the bile duct immediately distal to the ligature. This was carried out at varying time-intervals after operation and the passage of medium was observed directly using cineradiography—radiographs were taken at intervals during the course of injection for later examination.

At periods from 7 days after operation the medium passes through the newly formed channels into the distended sac when retrograde injection is performed. When the injection is carried out above the ligation the channels are more difficult to demonstrate.

The outgrowths demonstrated radiologically have been confirmed by histological observation of serial sections of the biliary tract immediately below the