

medium and are now growing at  $4-5 \times 10^6$  per ml. A single attempt at bulk growth with Whitechapel 1 yielded 1.17 gm. from one bottle.

Of special interest is the observation that proteolysed liver B.P.C.<sup>6</sup> can replace the 'Difco' liver infusion called for in the C.P.L.M. medium<sup>4,5</sup>. Attempts to grow the organisms without liver (or a substitute infusion of human red blood cells) were unsuccessful. Proteolysed liver B.P.C. is a completely soluble, inexpensive material and may prove useful as a substitute for liver infusion or as a growth-promoting inclusion in media for the culture of other micro-organisms.

A fuller account of this investigation will be reported elsewhere. I am indebted to Dr. M. G. McEntegart, Liverpool City Bacteriological Laboratory, for his original culture and medium; to Dr. Claude Nicol, Whitechapel Clinic, for clinical specimens; to Messrs. Paines and Byrne, Ltd., Greenford, Middlesex, for generous quantities of 'Pabym' brand proteolysed liver B.P.C.; and to Mr. C. D. Bevis, Lister Institute, for technical assistance.

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### Excess of Molybdenum in Herbage as a Possible Contributory Factor in Equine Osteodystrophia

DEFECTS in nutrition which predispose to osteodystrophic conditions in the horse include a dietary deficiency of phosphorus<sup>1</sup> or disturbance in the vitamin D - calcium - phosphorus relationship. With regard to the influence of trace minerals, Davis<sup>2</sup> found that the presence of small amounts of molybdenum, ranging from 2 to 25 p.p.m. in young growing herbage, was associated with defective bone metabolism in herbivora, consequent on the effects of molybdenum on the utilization of both phosphorus and copper. More recently, Arrington and Davis<sup>3</sup> in limited phosphorus metabolism studies with rabbits reported that molybdenum decreased phosphorus absorption while at the same time increasing the excretion of phosphorus.

On some twelve farms where we have investigated the occurrence of clinical rachitis in one or more of the foals or yearlings, we have found that the molybdenum content of the grazing herbage has been within the range 5-25 p.p.m. The soils on these farms are mainly derived from limestone, being of a similar nature where soil type, manurial treatment and management are concerned to those on which molybdenum-conditioned copper deficiency has previously been identified by us<sup>4</sup>. In addition, readily available molybdenum determinations on these soils

have shown values in the region where excess levels of molybdenum in herbage are likely to occur. While it has been impossible so far to ascertain to what extent molybdenum may be an etiological factor, we suggest that a foal running with its dam on such pastures would receive an excessive intake of molybdenum from the mare's milk and from the herbage. In view of the work referred to above, such a foal would be more susceptible to osteodystrophic conditions as a result of the influence of molybdenum on the metabolism of both phosphorus and copper. Consequently the extent to which molybdenum in moderate excess in pasture may predispose to the development of poor bone in the young horse and afterwards to poor performance in later life is raised.

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### Apparatus for Infiltration of Liquids into Leaves

VACUUM infiltration of liquids into leaves is a technique that has been used for many years. So far as I am aware, the usual procedure is to keep the leaf under liquid while reducing the pressure. Air bubbles then escape from the stomata, and any adhering when the lowest pressure has been attained are dislodged, often with difficulty, by shaking the container; for when the vacuum is afterwards released the adherence of these bubbles would spoil the efficiency of infiltration. The difficulty of bubble removal is greatly increased when a number of leaves are simultaneously infiltrated, so that it is desirable to use as few leaves as possible, or even to treat them singly. To overcome such difficulties it is best to reduce the pressure while the leaves are in air, releasing pressure only after putting the leaves under liquid. A convenient way of doing this is described below.

Two glass or metal containers of suitable strength, each with a side-arm and flange, are stood one upon the other, separated by a wet rubber washer. The leaves are held in chamber *a*, and the infiltrating fluid in *b*; *c* and *d* are clips on rubber pressure-tubing (glass stop-cocks could be used) attached to the side-arms of the chambers. To evacuate the apparatus, *c* is opened, and when the desired degree of evacuation, read on a gauge, is reached, closed again. The whole arrangement is now inverted so that the infiltrating liquid runs on to the leaves. Then *d* is opened so

