of the vole bacillus, or if it should be considered a new species.

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[1] Wells, A. Q., Med. Res. Council Spec. Rep. Ser. No. 259 (1946).
[2] Grasset, E., Murray, J. F., and Davis, D. H. S., *Amer. Rev. Tuberc.*, **53**, 427 (1947).

Microbial Growth Stimulants in Spleen

PREVIOUS studies have revealed that rabbit spleen contains unidentified, water-soluble substances that are growth stimulatory for *Escherichia coli*[1], and also that beef spleen yields heat-resistant, water-soluble extractives stimulatory for species of pathogenic fungi[2]. A blood agar medium incorporating aqueous spleen infusion was formulated for the isolation of pathogenic fungi at 37° C.[2]. The stimulatory effects of spleen extracts on the growth of micro-organisms in chemically defined culture media has been investigated by us.

We have found that bovine spleen contains an active growth-factor which is highly stimulatory for the growth of a variety of micro-organisms when tested in several chemically defined culture media. Some of the species include the yeasts, *Candida albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*; the protozoa, *Ochromonas malhamensis* and *Tetrahymena pyriformis*; and the bacterium, *Lactobacillus casei*. A preliminary purification procedure of the spleen factor was accomplished by following photometrically the turbidimetric growth response of a vitamin-depleted strain of *C. albicans* in a chemically defined liquid culture medium.

One kgm. of fresh, de-encapsulated and de-fatted beef spleen was thoroughly ground and mixed with 2 l. of distilled water. The resulting mixture was infused at 50° C. for 1 hr., occasionally agitated, and then rapidly heated to 80° C. for 5 min. in order to coagulate heat-precipitable proteins. The hot mixture was filtered through a layer each of towelling, gauze and cotton. The filtrate was cooled to 10° C. to congeal any remaining fat, which was removed by filtration through paper. The latter filtrate was autoclaved at 121° C. (15 lb.) for 15 min. in order to produce additional protein coagulation. Autoclaving did not reduce the growth-stimulatory effect of the spleen infusion for *C. albicans*. One litre of aqueous crude spleen extract, which had a *p*H of 6·8, was lyophilized and 23·5 gm. of a tan amorphous powder was obtained. This powder was extracted with one litre of anhydrous methanol at 20° C. for 15 min. The methanol-insoluble residue was microbiologically inactive for *C. albicans* and therefore was discarded. The amber-coloured, methanol filtrate which contained the active principle was mixed in a separatory funnel with an equal volume of pentane (Eastman technical) at 20° C. and shaken gently and intermittently for 10 min. An inactive, white flocculent precipitate which formed in the lower methanol layer was discarded together with the inactive pentane layer. The methanol fraction was reduced in volume in an all-glass distillation apparatus *in vacuo* at 60° C. and then dried over a steam bath until dark brown solids weighing 9·36 gm. were obtained. These were dissolved in double-distilled water and dialysed for 2, 8, 12 and 36 hr. at 5° C. through transparent, seamless, cellulose dialyser tubing (Thomas 4465 A_2) in a stationary water bath of double-distilled water. The four separate dialysates were combined, then concentrated *in vacuo* at 60° C. and lyophilized, yielding 6·66 gm. of light brown, hygroscopic solids. When these solids were assayed in a chemically defined liquid medium with a vitamin-depleted strain of *C. albicans*, they produced characteristic dose response growth-curves which showed an average half-maximal value of 35 μgm./ml.

The active growth-factor is soluble in water, methanol, ethanol and other alcohols, but is insoluble in diethyl ether, chloroform and pentane. In aqueous solution, the factor is unaffected by boiling, freezing or by autoclaving at 121° C., the latter in the presence or absence of dilute hydrochloric acid. Dry solids are stable for at least one year when maintained in a desiccator at 5° C. The spleen factor is also stable in methanol solutions. Studies embracing the further purification and characterization of the stimulatory substance, as well as its relationship to known water-soluble vitamins, are in progress. These will be described in later publications.

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[1] Stapleton, G. E., Sbarra, A. J., and Hollaender, A., *J. Bact.*, **70**, 7 (1955).
[2] Littman, M. L., *Amer. J. Clin. Path.*, **25**, 1148 (1955).

Amino-acid Composition of Sclero-protein of the Sponge *Hippospongia equina*

WE have applied the method of paper partition chromatography to ascertain qualitatively the detailed amino-acid constitution of spongin, using the commercial unbleached honeycomb sponge, gathered in the eastern Mediterranean Sea. Our results differ in important respects from those of Low[1], who used this method to investigate the amino-acid constitution of the barium hydroxide hydrolysate of the sponge, *Spongia officinalis obliqua*, collected in the Gulf of Mexico.

We have examined both acid and alkaline hydrolysates of the sponge, previously washed with water and air dried. The acid hydrolysate was prepared by heating the sponge under reflux for 8 hr. with equal parts hydrochloric acid (6 *N*) and formic acid (85 per cent), while the alkaline hydrolysate was obtained by heating under reflux for 8 hr. with saturated barium hydroxide solution. The solvents and various location agents were all prepared according to Smith[2], while this author's dipping technique was also used. For final confirmation all the spots were extracted and run as one-way chromatograms against known standards.

The amino-acids identified in the acid and alkaline hydrolysates of spongin are given in Table 1. The relative intensity of the amino-acid spots is indicated by representing strong as + + + + and weak as +.

Tryptophan was confirmed with the Ehrlich reagent; tyrosine and histidine with the diazo reagent; iodinated tyrosines with ceric sulphate–