

Sources of Growth Factors Required by Certain "Fastidious" Bacteria. Failure of Ascorbic Acid to Replace Growth-Promoting Principles.\*

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In continuing the study of substances, apparently in the nature of accessory growth factors, which will permit the development of some of the more exacting pathogens in a synthetic medium, it seemed desirable to gain some information concerning the natural sources of such compounds before attempting further chemical purification. A number of different animal and plant tissues were selected as possible sources and tested for the presence of growth promoting principles. The tissues were finely ground, extracted with water and heated to boiling. The coagulum was removed by filtration and the filtrate treated with charcoal to adsorb the growth factors. The charcoal was then extracted in a continuous extractor with hot ethyl alcohol to remove the growth factors, the alcohol evaporated off *in vacuo* and the residue taken up in a measured amount of distilled water. The extracts were sterilized in the autoclave.

The potency of these extracts was tested by adding them in several different dilutions to tubes of synthetic medium and noting the growth which followed inoculation with organisms which ordinarily refused to develop in the synthetic medium. Ten different cultures were used: *Streptococcus hemolyticus* (from scarlet fever), *Streptococcus lactis*, *Staphylococcus aureus*, *Staphylococcus albus*, *Corynebacterium diphtheriae*, *Brucella abortus*, *Pasteurella avicida*, *Salmonella pullorum*, *Eberthella typhosa*, and *Shigella dysenteriae*.

Observations of growth were made at several different intervals over a period of 10 days or occasionally longer. The rapidity of development of the organisms as well as the dilution in which growth occurred served to give a measure of the potency of the extracts. Altogether over 30 preparations were tested in this manner.

The results may be summarized as follows: † Veal infusion +++ ,

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† Comparative potency of the preparations is shown by the plus signs: ++++ very good, +++ good, ++ fair, + poor, and — no effect.

skim milk +++, calf liver +++++, ash from calf liver —, calf thymus +++, calf heart ++, calf spleen +++++, calf kidney +++, calf lung +++, pig embryo ++, chicken liver +++, shad roe +++, human liver with carcinoma +++, human placenta +++, human urine +, oat sprouts ++, wheat germ ++, rice bran +++, tea —, lettuce ++, sprouted soy beans +++, canned tomato juice +++, turnips +++, whole white potatoes +++, ash of potatoes —, potato sprouts +, potato tuber left after removal of sprouts ++, carrots ++, cocoanut milk +++, baker's yeast +++++, several cultures of *Aspergillus* and *Penicillium* + or ++, *Pseudomonas fluorescens* +.

In considering this summary it should be pointed out that the results do not necessarily give a quantitative picture of the relative amounts of growth factor actually in the products. Various considerations such as ease of extraction by the method employed, the possible presence of growth inhibiting substances or substances which might inactivate the growth factor, and the presence of varying amounts of extraneous material might affect the final results. Despite these uncertainties the results appear to have some value in showing the general distribution of growth-promoting principles and also in affording some idea of those products which might be used as sources of material for further attempts at chemical separation and purification.

It appeared of interest, also, to attempt the substitution of purified products for the foregoing extracts of animal and plant tissues. A sample of Merck's ascorbic acid (cevitamic acid) was available. In view of the established relationship of this to vitamin C of animal nutrition it was tested for any effect it might have on the growth of bacteria. The ascorbic acid was added to the synthetic medium used in the previous tests in amounts to give 0.1, 0.01, and 0.001 mg. per cc. of medium. The tubes were then inoculated with the same 10 microorganisms used for the previous tests. However, growth was never observed in any case and ascorbic acid could not be substituted as a growth-stimulating agent for the unknown products extracted from animal and plant tissues.