by the presence, of the added potassium phosphate; but this does not imply that in these cases the cause of rickets was necessarily a deficiency either of potassium or of phosphorus. The quantitative relations of the inorganic ions rather than an absolute deficiency of any one of them, may have been the determining factor. Also it may well be that under certain dietary conditions, rickets may be caused by deficiencies or unbalanced quantitative relationships of organic as well as inorganic food factors. It would appear however to have been demonstrated that rickets may be caused or prevented without change in either the protein or vitamin components of the diet and therefore that neither of these can be regarded as a necessarily predominating factor. It is also of outstanding interest that the rats showing multiple fractures and marked deformity of thorax, which would probably be inincluded under the classification used by some writers as "presenting the gross picture of rickets" but whose bone lesions on microscopic examination were classified as those of osteoporosis, were those which had been subjected to even greater dietary deficiency than those showing typical rickets.

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Growth accessory substances in the nutrition of bacteria.

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In studying the growth characteristics of mucoid bacilli Thjotta observed that *B. influenzæ* will grow in blood-free broth containing the mucoid material from cultures of Friedlander's bacillus and other closely allied organisms. The growth accessory substance or substances which can replace blood and blood derivatives in the cultivation of Pfeiffer's bacillus Thjotta found in both the saline suspensions and watery extracts of the heat killed bacillary material. Furthermore, these bacterial emulsions and extracts can be boiled for ten minutes and filtered through Berkefeld

candles without losing their growth-inducing properties. It was thought not unlikely that the growth-stimulating effect of these bacterial extracts might be due to substances belonging to the class of the so-called vitamines. To test this assumption, similar extracts were prepared from yeast cells which are known to be rich in growth accessory substances. These extracts, even in minute amounts, were found capable of promoting growth. Extraction of the growth accessory substances from another source, namely, green vegetables, was tried; extracts of fresh tomatoes, green peas and string beans were found remarkably active in stimulating growth. These active yeast or vegetable extracts when added to broth greatly accelerate growth of organisms such as *Bacillus influenzæ* and pneumococci, so that within five hours abundant growth is evident. In the case of pneumococcus, a seeding too minute to initiate growth in plain broth alone will amply suffice to induce abundant growth in the same medium if a small amount of extract containing these growth accessory substances is added. In the case of *Bacillus influenzæ*, when seeded from blood media, luxuriant growth occurs in plain broth containing yeast extract, while no growth takes place in the same broth without the addition of extract. The presence of these growth accessory substances in extracts diluted 1 : 1,000 suffices to stimulate growth under these conditions. However, for reasons to be discussed later, continued cultivation fails in broth containing only yeast or vegetable extracts.

While the nature of these growth accessory substances is not known, they are presumably analogous to the so-called vitamines. It has been found that they resist boiling for at least ten minutes, that they are destroyed by autoclaving, that they are extractable from fresh vegetables and from growing bacterial and yeast cells, that they are water soluble, that they pass a Berkefeld filter, and that extracts of these substances contain but little nitrogen—about 0.116 per cent.

In the application of this principle to bacterial nutrition, particular attention has been given thus far to the nutritional requirements of *Bacillus influenzæ*, since this organism belongs to a peculiar group of bacteria which heretofore have been considered obligate hemophiles.

Although *Bacillus influenzæ* will grow luxuriantly when transplanted from blood medium to plain broth containing yeast extract, cultivation cannot be continued more than one or two transfers in yeast broth alone. This suggested that possibly some other substance may be carried over from the original blood culture in an amount sufficient to supplement the yeast broth and that growth fails in succeeding cultures because this substance is either exhausted by growth or lost by dilution on subsequent transfers. For purposes of discussion this substance may be referred to as the X factor and the vitamine-like substance in the extracts as the V factor. Neither of these two factors by itself can sustain growth of *Bacillus influenzæ*. Evidently both are essential to growth and both occur in blood which is always used in the cultivation of *Bacillus influenzæ*. As previously pointed out, the V factor it destroyed by autoclaving. If, therefore, blood medium is autoclaved, it should no longer be able to support growth of *Bacillus influenzæ*. This is actually the case. If, however, the X factor has not been destroyed by heating then this same medium should be reactivated by the addition of fresh yeast extract. This also is the case. The growth accessory substance (the V factor) which is destroyed by autoclaving blood, can be supplied from other sources, such as yeast; and this substance is capable of reactivating a medium in which, as a result of heating, the X factor alone remains. Search is being made for the X substance in material other than body tissue. That crystalline hemoglobin itself, however, does not contain both of these essential substances, is shown by the fact that pure crystalline hemoglobin when added to broth fails to support growth of *Bacillus influenzæ* unless yeast or its equivalent in V substance is also present. This fact indicates that crystalline hemoglobin contains some of the X factor. Further studies are planned to determine the importance of this principle in the cultivation of other species of bacteria. From analogy with animal nutrition, it seems not unreasonable to suppose that nutritional deficiency in the cultivation of bacteria may be overcome by the addition to culture media of the appropriate growth accessory substances.