If the animals are fed on the posterior lobe of hypophysis growth is not only not stimulated, but even greatly retarded as may be seen from two live animals, a control and a posterior lobe fed animal, both descendants of the same female and of the same age.

Feeding of anterior lobe causes (1) a very marked acceleration of growth and (2) a continuation of growth beyond the specific size of the species resulting thus in hypophyseal gigantism. Feeding of posterior lobe has neither of these two effects, but even retards growth.

Since in these experiments the hypophysis was fed without the addition of normal food and in large doses, one may think that the results were caused not by the action of a specific substance contained in the hypophysis but merely by the greater food value of the gland. Part of the acceleration of growth may have been actually due to merely quantitative differences in the food substances; but it should be pointed out that it is impossible to renew growth by feeding even large quantities of normal food after growth has come practically to a standstill. As regards the continuation of growth beyond the normal size of the species, it is obvious that the alteration of this specific character of growth cannot be due to an increased amount of food and it seems, therefore, that at a stage where growth ceases or is greatly diminished under normal conditions, cell proliferation can be actually enforced by the specific growth-promoting substances contained in the anterior lobe of the hypophysis.

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Observations on bacterial metabolism.

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In the course of an investigation of the cultural requirements of certain of the pathogenic bacteria, a substance which occurs in meat infusion, and also in some of the proteins has been found to be essential for the growth of the streptococcus, and for certain

strains of the pneumococcus. While it has thus far not been possible to isolate the compound in pure form, perhaps enough has been learned of its occurrence and properties to warrant a short note.

If an infusion of beef, or better, of beef heart muscle be prepared by boiling a pound of the chopped muscle in a liter of water, straining and filtering, and if 0.1 per cent. glucose and a nitrogen free inorganic salt mixture be added, it is found that the broth thus prepared is quite suitable for growth of the hemolytic streptococci. A P_H of 7.2–7.6 is most favorable, and no peptone or other nitrogenous material need be added. If, however, the meat infusion be mixed with 2 per cent. of wood charcoal, of the commercial brand called "Norit," and boiled for fifteen minutes and filtered, the streptococcus will no longer grow on the filtrate, after adding glucose, and salts, and adjusting the reaction. Evidently a substance has been quantitatively removed from the infusion by the charcoal which is required by the streptococcus for growth. The addition of 1 per cent. commercial peptone to such a charcoal treated infusion now renders it again suitable for growth, although the peptone itself, plus glucose and salts, will not give growth with the streptococcus.

Since the material which is removed by the charcoal is apparently present in commercial peptone, it seemed most probable that an amino-acid or polypeptide was in question. The addition of a sulphuric acid hydrolysate of casein to the charcoal treated infusion was next tried, and found to be quite as effective as the peptone. The hydrolysate is prepared by 18 hrs. boiling with 33 per cent. H_2SO_4, and the acid then removed with baryta. To rule out as far as possible the presence of non-protein impurities in the casein, a purified specimen was prepared by reprecipitating three times with acetic acid from Na_2CO_3 solution, and finally washing thoroughly with alcohol and ether. An acid hydrolysate of this casein proved equally active when added to the inactive infusion. Similarly, active preparations could be obtained by the use of a sulphuric hydrolysate of edestin and meat residue, and very weakly active preparations from egg white, but the hydrolysates of wheat gluten, gelatine, wool and silk were quite inactive. Published analyses of these proteins did not show any amino

acid common to the ones furnishing active hydrolysates and lacking in the others. Acid hydrolysates of yeast and of salmon sperm were also inactive, showing that none of the constituents of nucleic acid were concerned.

A separation of the amino acids from a casein hydrolysate was therefore undertaken, first into groups by the butyl alcohol extraction method of Dakin. By this method, the monoamino acids are extracted almost quantitatively, and crystallize out of the alcohol as a yellow, granular material, easily filtered out and dried. The proline remains dissolved by the alcohol, while the hexone bases and the dibasic acids remain dissolved in the aqueous phase, unextracted by the alcohol. The active material passed over almost quantitatively with the monoamino acids.

Various methods of separating this mixture of monoamino acids have been tried. The only reagent so far obtained which precipitates the active material from the mixture of monoamino acids is mercuric sulphate in sulphuric acid solution. This reagent precipitates the following known amino acids: tryptophane, tyrosine, cystine and histidine. Pure preparations of all four of these amino acids have been tested with charcoal treated infusion and found negative.

For further separation of the compounds precipitated with mercuric sulphate, considerable quantities of casein had to be used, and the preliminary extraction of the monoamino fraction by Dakin's method was to be avoided if possible. It was found that mercuric sulphate precipitated the active material from the original hydrolysate, and further, that it was not even necessary to remove the sulphuric acid with baryta, but that one could neutralize the excess acid with crude sodium hydroxide. After filtering off the melanin thus precipitated, the active material could be thrown down by the addition of mercuric sulphate dissolved in 5 per cent. sulphuric acid. Up to this point the separation has been made repeatedly. After removing the Hg from the precipitate by H_2S, the activity of the preparation varies, probably with the amount of $HgSO_4$ used, and the concentration of H_2SO_4 in the mixture. The optimum conditions for precipitation have not yet been exactly determined.

Further purification of the precipitate, freed from mercury,

has so far been unsatisfactory. Fractional crystallization leaves the active substance in a syrupy filtrate. Precipitation with $AgSO_4 + Ba(OH)_2$, of histidine and a syrupy material giving the histidine diazo test, leaves most of the active material in the filtrate. The addition of an excess of baryta causes some, but not all, to be thrown down together with impurities as the silver compound. Phosphotungstic acid apparently destroys the activity of the compound, although this requires further verification. It has not been possible to obtain active material either from the phosphotungstic precipitate or filtrate. The phosphotungstic acid has been removed both by baryta and by extracting with amyl alcohol and ether.

In attempting other methods, also, the activity has gradually diminished and been entirely lost, and it may prove impossible to obtain the material in pure form by methods at present available. It is hoped, however, that further work with larger quantities of material will result in the separation of this compound, which may prove to be of more general interest than simply from the standpoint of bacterial nutrition.

To sum up: the experiments here reported indicate that casein and certain other proteins contain a hitherto undescribed component, which also occurs in an infusion of beef and beef heart. It is essential to the growth of the hemolytic streptococcus and probably the pneumococcus, and is absorbed from the beef infusion by charcoal, and precipitated from the casein in an impure form by mercuric sulphate. The chemical nature of the substance has not yet been determined.

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The cause of the parallelism between the gram reaction and the gentian violet reaction.

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In previous studies, published at intervals since 1912, it has been shown that a striking parallelism exists between the Gram