different strains of typhoid so that the results cannot be attributed to the idiosyncrasies of one strain. The strains have been plated out repeatedly to see if irregular colonies carrying the lytic principle could be obtained from the normal culture, but up to the present time no lytic principle has been isolated without the interaction of tissue enzymes with the typhoid bacilli.

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Growth-determining substances in bacteriological culture media.

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Some months ago a report was made of a series of experiments based on the observation that, while a peptone-free meat infusion broth would produce abundant growth of hemolytic streptococci, short boiling with charcoal removed this property entirely. The addition of commercial peptone or of a sulphuric acid hydrolysate of certain proteins, such as casein or meat, reactivated the charcoal-treated infusion and heavy cultures of streptococci could be obtained on the mixture, while neither one alone gave the slightest trace of growth. It was shown that the activating material in the protein hydrolysate was precipitated by mercuric sulphate, and that it had not been possible to identify it with any of the amino acids known to be precipitated by this reagent either alone or in combination. It is the purpose of the present communication to describe the further purification of this activating material.

Much of the work has been done using a commercial preparation called "aminoids" in place of an acid hydrolysate of casein. This consists of an enzyme digest of milk proteins continued until the product is biuret free. It has been used simply as an economy of time since in handling large quantities the acid hydrolysis is somewhat cumbersome. Every step in the separation, however, has been checked on an acid hydrolysate and it is believed that there is no essential difference in the factors involved.

In attempting to separate the active material from the mercuric sulphate precipitate, fractional precipitation with the same

reagent was tried. This led to the discovery that there were two factors in the precipitate, both of which were necessary to reactivate the charcoal-treated infusion. One of these must be carried down by adsorption in the original mercuric sulphate precipitate, or else its solubility is influenced by the presence of other substances, for it is not reprecipitated, to any extent, from the mixture by the addition of mercuric sulphate. If the filtrate from the first precipitation is tested for this factor, it is found to be present in moderate concentration, although apparently less than in the precipitate.

There is some difficulty in making this separation quantitative with mercuric sulphate, and a more convenient reagent was found in silver sulphate and baryta. The original mercury precipitate is freed from mercury by H_2S, and after boiling out the H_2S and cooling, silver sulphate solution is added in slight excess, and barium hydroxide solution to moderately alkaline reaction to litmus. The precipitate, which is freed from silver by H_2S, contains histidine and considerable brown sticky material, in addition to an active fraction which may be briefly referred to as "X." If necessary, the second factor, or "Y," most of which remains in the silver filtrate, may be removed more completely by reprecipitating with silver sulphate and baryta.

In several experiments it was found possible to precipitate the histidine and the pigment with phosphotungstic acid, leaving the active "X" in the filtrate. Evaporation to dryness after removing the phosphotungstic acid, yielded a semi-crystalline material, but also destroyed the activity of this fraction. These experiments were made with a single solution of phosphotungstic acid. With all the subsequent preparations of the reagent complete loss of activity has resulted during the precipitation of the crude "X" fraction, and neither the precipitate nor the filtrate nor both together have given growth. It is possible that oxidation may explain this change in properties, and further work must be done in attempting the isolation of this factor.

The silver sulphate filtrate, or "Y" fraction, does not give a precipitate with phosphotungstic acid. Mercuric sulphate throws down a rather abundant precipitate, composed probably of tryptophane and tyrosine. The filtrate from this precipitate

contains the "Y" factor. This can be concentrated on the water bath to a small bulk, filtered from tyrosine after standing overnight on ice, and concentrated further with the addition of alcohol, to beginning crystallization. On standing, crystals separate consisting of microscopic spheres apparently made up of finely interwoven needles. These crystals are exceedingly soluble in water, and quite soluble even in 70 to 80 per cent. alcohol, and there is considerable loss on recrystallization. In one preparation the yield after one recrystallization from strong alcohol was 0.012 g. from 200 g. aminoids. Growth was given with 0.000,01 g. of these crystals in 25 c.c. of media. Further recrystallization apparently either eliminates the active factor from the main bulk of crystals, or else alters the chemical nature of the substance, since growth becomes very slow and scanty. The mother liquors, still containing considerable quantities of crystalline material, together with some amorphous brown substance, likewise show diminished activity, so that there is probably in the case of the "Y" factor, as with the "X," a certain amount of lability as the preparations become purer. I do not wish to state definitely at this time the belief that the crystals just described are in fact the active "Y" material, and further work with larger quantities must be done. There is, indeed, no assurance that the crystals are pure because of the difficulty in recrystallizing caused by the high solubility.

After two recrystallizations, the crystals when dried at 100° are light and powdery. They give a moderately strong reaction with Folin's phenol reagent, but no color with the nitro-prusside test. Nitrogen is present to the amount of 10.6 per cent. by the micro-kjeldahl method, and qualitative tests for sulphur are positive after fusion with sodium, but the lead-acetate test on boiling with NaOH is negative. Phosphorus and halogens are not found. Sufficient material for complete quantitative analysis has not yet been prepared.

In the first report on this work, it was stated that the hydrolysates of certain proteins, such as gelatine, were not capable of reactivating the infusion. At that time, it was not recognized that two substances were involved, and in the light of that fact, a further investigation should be made as to whether those pro-

teins are deficient in both the "X" and the "Y" factors, or whether one may occur without the other. A few preliminary tests have indicated that gelatine and egg proteins contain the "Y" and are deficient only in "X," but the results were not clean cut, and it is possible that other factors came in. Lack of time has prevented the extending of these observations.

Without more definite knowledge of the chemical nature of these two substances, speculation as to the manner in which they induce growth of the streptococci does not seem warranted. There is no means at present of knowing whether they act as "building-stones" in supplying some necessary grouping in the synthesis of the bacterial protein, or whether they simply initiate or accelerate some essential vital process. Perhaps, in the light of much recent work dealing with the effect of vitamines on bacterial and yeast growth, it is not unwarranted to believe that still other phases of animal metabolism may be cleared up in part through work on the metabolism of lower forms of life. In the case of the study of the streptococcus, there are at least three factors, still unidentified, which determine growth; namely, some substance in the charcoal-treated infusion, the "X" fraction, and the "Y" fraction. It is by no means felt that all or any of these factors if isolated, will prove to be new physiological compounds, but if such should be the case, one must believe, in order to explain their occurrence in meat, milk, etc., that they also play a part in animal metabolism.

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The supposed relation between alkalosis and tetany and similar conditions.

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Examination of the work of Wilson, Stearns and Thurlow[1] shows that their conception of an "alkalosis" as one of the consequences of parathyroidectomy rests essentially upon the sup-

[1] Wilson, D. W., Stearns, T., and Thurlow, M. de G., *Journal of Biological Chemistry*, 1915, xxiii, 89.