The amount of copper added to the extract in the first precipitation was 0.01816 gm. per liter. The first supernatant fluid had a copper content of 0.0146 gm. per liter. Thus the precipitate had in it 0.00356 gm., representing one liter of extract. Fifty cubic centimeters of the dissolved third precipitate showed no detectable copper when the organic matter was destroyed by nitric acid, the material evaporated to dryness, taken up in concentrated hydrochloric and again evaporated to dryness, taken up in very dilute hydrochloric acid and examined by the ferrocyanide method.

The above tests would seem to preclude agglutinin being a protein, or even a protein hydrolysis product. The small quantity of nitrogen may be in agglutinin, or may be due to a small amount of nucleic acid present as an impurity from the bacteria.

Since it is known that the method of extraction, namely weak alkali, will extract nucleic acid from bacteria, and since the nucleic acids are precipitable by copper, the nitrogen present may well be in the form of nitrogenous base as an impurity derived from the bacteria. If so, possibly the agglutinin is represented by carbohydrate. On the other hand, the carbohydrate itself may also be derived from the bacterial nucleic acid. In this case the chemical factors representing the agglutinin may be so minute in amount as to escape detection entirely. Analyses now in progress may throw some light on this question.

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The isolation of a crystalline substance (M. P. 223°C) having the properties of "bios."

(Preliminary report).

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A crystalline substance melting sharply at 223° C. has been isolated by the use of differential adsorbents from autolyzed yeast which in very small amounts (0.005 mg. per cc. of Ful-

mer's medium F.) produces during a 24 hour incubation at 31° C. a volume of yeast growth fifteen to twenty times that in the controls.

Microphotographs made with the petrographic microscope were exhibited to demonstrate the homogeneity of the crystalline product together with evidence obtained by recrystallizing the product first from water and then twice from alcohol.

The recrystallized product retains its constant melting point and shows no loss of activity.

Curves based on yeast tests made with varying concentrations of autolyzed yeast and of the crystals were shown to demonstrate that the form of curve is identical in the two instances, both showing an optimum concentration and lesser stimulation for concentrations on either side of the optimum concentration.

The yield of the product was about 0.03 per cent of the dry weight of the autolyzed brewer's yeast used. The carbon and hydrogen percentages have been determined and are: Carbon, 43.29 per cent; hydrogen, 8.31 per cent. The nitrogen content has not yet been accurately determined but will apparently run in the neighborhood of 25 per cent. The substance gives no ninhydrin or biuret reaction. It is freely soluble in cold water, in acid and alkaline solutions and in dilute ethyl alcohol. In cold 95 per cent alcohol it is sparingly soluble but passes readily into solution when the alcohol is heated. It is only slightly soluble in 100 per cent acetone but very slight dilution of the acetone with water brings about its solution.

The essential feature of the method of isolation consists in a preliminary purification of autolyzed brewer's yeast with fullers earth which removes other substances than bios, and then the use of ferric hydroxide in colloidal sol which under specific conditions devised by Dr. Kerr removes the bios and can later be dissolved and the bios freed from cantaminating ions. Details of the process are still undergoing further refinement and will appear in a later publication.

The expense of this investigation was met in part by a grant to the University by the Fleischman Company, and we are also indebted to the Jacob Ruppert Company for the brewer's yeast used in the studies.