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A new sulphur-containing amino acid isolated from casein.

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In a report on a study of the cultural requirements of streptococci made last year before this society, the writer stated that a compound containing sulphur had been isolated from casein, which was apparently not related to cystine, and which seemed to be required for the growth of the test organisms. Although subsequent work has shown that this sulphur compound, when pure, is apparently not concerned in the growth of streptococci, it seemed desirable to make a study of the substance, both because of the uncertainty of the nature of non-cystine protein sulphur, and also in order to be able to effect a separation of this compound from the bacterial growth inducing factor in the amino acid fraction under investigation. While there are still many points to be cleared up in connection with the substance, perhaps enough information has been obtained to warrant a preliminary report.

There have been a number of difficulties met with in the work. The yield is very small, and probably not by any means quantitative, and further, no insoluble compounds suitable for separation have so far been found, so that purification has consisted largely in methods for the removal of impurities.

In order to obtain sufficient material for analyses, thirty pounds of commercial Argentine casein were used. Briefly, the method consists in hydrolysis with sulphuric acid, neutralization with sodium carbonate, and precipitation with mercuric sulphate solution. From the washed precipitate, freed from electrolytes, a considerable quantity of other material is removed by a second precipitation with mercuric sulphate, the sulphur compound this time remaining in the filtrate. Further purification is effected by precipitation of the filtrate, after removing electrolytes, by silver sulphate and barium hydroxide, and the compound itself is obtained from the silver filtrate, freed, of course, from Ag and Ba, by fractional crystallization, finally from dilute acetone. The yield from thirty pounds was about 10 grams.

The compound crystallizes from water, dilute alcohol and dilute acetone, in shining white microscopic plates or rosettes of rather indefinite crystal form. As a criterion of purity, amino nitrogen determinations were carried out after successive crystallizations until constant values were obtained. At this time the proportion of N to S was 2:1, and it was assumed that the preparation was pure. Analyses of this product indicated the formula $C_{11}H_{23}SN_2O_4$. Unfortunately, when most of the amino acid had been used up, it was found that the remaining material contained as an impurity a substance forming a hydrochloride relatively insoluble in concentrated HCl. Since the entire preparation had not been crystallized at the same time, and only the first portions, used for quantitative analyses, had been checked up by amino nitrogen and sulphur determinations, it is possible that the impurity was not present in the material which was analyzed. From the few tenths of a gram remaining, the insoluble hydrochloride was removed as completely as possible by dissolving in boiling HCl and allowing to crystallize on ice for several days. The filtrate, containing the sulphur compound, was freed from HCl by evaporation and by Ag_2SO_4, and the sparingly soluble copper salt prepared by boiling the solution with $Cu(OH)_2$ and filtering. The salt separates as microscopic, pale blue platelets. About 0.25 gram of this salt was obtained, and a single combustion and amino nitrogen determination gave the formula $C_{11}H_{21}SN_2O_4Cu$, corresponding exactly with that obtained for the amino acid in the earlier analyses. The combustion was done by the Dennstedt method, permitting the simultaneous determination of carbon, hydrogen, sulphur and copper.

The nitrogens are both in the form of amino groups, and since the solution of the compound in water is practically neutral, it is probable that two COOH groups are present. A single formol titration gave results which were slightly low, but in fair conformity with this supposition. The sulphur is not in the lead-blackening form, and is not readily split off as sulphate by boiling with acids or alkalis. The number of hydrogen atoms present suggests a straight chain compound, but no direct evidence of the structure has been obtained.

In order to rule out the possibility of the material used for analyses having been impure, it is planned to prepare another lot

of about the same quantity, after which complete analytical data and a detailed account of the preparation will be published elsewhere.

When the composition and properties of the amino acid are definitely established, it will be necessary to show that it is really a primary component of protein. It is possible that the sulphur has been introduced into the molecule either from the sulphuric acid used in hydrolysis, or from the H_2S used throughout the preparation. The possibility of introducing sulphur from sulphuric acid is rather remote, since the amino acid has not the properties of a sulphonic acid. However, it can be excluded only by the use of enzyme digestion or alkali hydrolysis in the primary breaking down of the protein, and this has not yet been attempted. In the preparation as outlined above, it is apparently not possible to avoid the use of H_2S, and this factor can be ruled out only by the elaboration of a method based on quite different principles, or by careful quantitative determinations of total sulphur throughout the various fractions, which is not likely to prove very satisfactory. It is not apparent, however, just how sulphur from H_2S could be introduced into any of the known amino acids to give a compound of the above formula.

Should it be possible to exclude these sources of extraneous sulphur, this compound will probably account for a part at least of the non lead-blackening sulphur known to be present in certain proteins. The amount present may well be considerably in excess of the present yield, since the method is obviously not quantitative.

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The inheritance of susceptibility to implants of splenic tissue in mice.

1. Japanese waltzing mice, albinos, and their F_1 generation hybrids.

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The use of the terms "auto," "homio," and "hetero" transplantation has been general and of great value in the long series