a powerful growth-stimulating hormone for *Str. hæmolyticus*. He was able to demonstrate a marked reduction in the 'lag phase' during the growth of this bacterium in beef infusion broth to which had been added 0·1 per cent beet juice. Since beets are a rich natural source of glutamine, the connexion is suggestive.

In an attempt to grow the freshly isolated strain of *Str. hæmolyticus* in a medium of completely defined chemical composition, including glutamine, the peptone in McIlwain's medium was replaced with an amino-acid mixture (Fildes[4]) frequently used in this laboratory for nutritional studies. No growth was obtained under these conditions, nor was growth observed when the peptone was replaced with acid-hydrolysed casein. In view of these findings, it seems likely that there is present in peptone another factor (or factors) which must be available before freshly isolated strains of *Str. hæmolyticus* can be cultivated in a medium of known chemical composition.

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Nature of a Group in Papain Essential to its Activity*

MUCH of the literature on the activation and inactivation of papain[1,2,3,4] supports the hypothesis that there is an essential SH group in the active enzyme.

The active crystalline enzyme[5], however, does not give a positive nitroprusside test or a satisfactory titration with porphyrindin[6]. Crystalline papain, like certain other proteins, was observed to give a positive nitroprusside test in the absence of cyanide only after denaturation. If, however, the native protein was first inactivated with iodoacetate, then separated from the excess of iodoacetate by precipitation with salt and finally denatured as before, no nitroprusside test without cyanide was obtained. In agreement with this observation, hydriodic acid was detected in the mother liquor from the salt precipitation. Titration of the denatured protein in urea solution by iodine gave results which indicate that only one sulphur group (of about ten in the papain molecule) is involved and that it disappears when the enzyme is inactivated by iodoacetate or cystine. This is supported by the observation that inhibition of papain activity is produced by one molecular equivalent of iodoacetate. Papain is still 'native' after reaction with cystine; that is, the solubility is apparently unaltered and the protein can still be crystallized.

It may be concluded that the SH group that appears on denaturation is blocked from reaction with some but not with all reagents while the protein is in the native state. Dr. M. L. Anson informed us by private communication that he has observed that SH groups in certain proteins react with iodoacetamide, but are not attacked by oxidizing agents.

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Results of titrating denatured papain SH groups with iodine are shown in the accompanying table:

Enzyme preparation	Treatment of protein	Equivalents of protein × 10^7		Equivalents of sulphydryl × 10^7	SH per equivalent of active protein
		Total	Active		
A	None	37	30	28	0·93
A	Iodoacetate	37	2	5	—
A	None	19	15	17	1·13
A	Iodoacetate	19	1	1	—
B	None	32	23	27	1·17
B	Cystine	32	10	11	1·1
B	Cystine	31	7·3	11	1·5
B	Iodoacetate	31	1	2	—

The equivalents of protein are calculated using a molecular weight of 30,000[7]. The enzyme preparation treated with iodoacetate contained 21 milk clotting[7] units of papain per mgm. of protein nitrogen when activated with cyanide, and the one treated with both cystine and iodoacetate contained 27 units.

After the native protein had been freed of the 5–10 fold excess of iodoacetate or the excess of solid cystine, it was denatured by heating at 70–75° C. for 10 minutes. The protein was centrifuged out, washed and finally dissolved in strong urea solution. This solution was treated essentially by the method described by Lavine[8] for the estimation of cystine except that the temperature was 0–5° C. The sodium thiosulphate solution was 0·01 *N*. The blanks agreed to within 0·02 ml.

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Glutamic Acid as a Hydrogen Carrier in Animal Tissues

IT has previously been shown that α-ketonic acids liberate carbon dioxide when added to animal tissues under anaerobic conditions[1]. The evolution of carbon dioxide was found to be due to a 'dismutation'.

(1) $2\ R.CO.COOH + H_2O \rightarrow R.CH(OH).COOH + R.COOH + CO_2$.

We have now found that the anaerobic carbon dioxide formation from α-ketoglutaric acid in kidney and in heart muscle is greatly increased by the addition of ammonium chloride. The analysis of this effect showed that α-ketoglutaric acid 'dismutes' with α-iminoglutaric acid (which arises on addition of ammonium salt to α-ketoglutaric acid)[2] as follows:

(2) $COOH \cdot (CH_2)_2 \cdot CO \cdot COOH$ (α-ketoglutaric acid) + $COOH \cdot (CH_2)_2 \cdot CH : (NH) \cdot COOH + H_2O$ (α-iminoglutaric acid) = $COOH \cdot (CH_2)_2 \cdot CH(NH_2) \cdot COOH$ (glutamic acid) + $COOH \cdot (CH_2)_2 \cdot COOH + CO_2$ (succinic acid).