not appear to be consistent with the hypothesis outlined above.

We have described techniques for measuring the rate of incorporation of various radioactive amino-acids such as glycine and alanine into the protein of tissue slices, and have outlined reasons for believing that this is closely related to biological protein synthesis[6]. Using these techniques, we have incubated α-aminobutyric acid-1-^{14}C with rat liver slices. When the protein is isolated and the amino-acids separated by chromatography[7], we find that the labelled aminobutyric acid has been incorporated into the protein to only a very slight extent, if at all. The ratio of alanine incorporation to aminobutyric acid incorporation is at least one hundred and fifty to one, and may be much higher when improved chromatographic techniques are used. We find, however, that these two amino-acids are oxidized to the α-keto-acids and to carbon-14 dioxide at a similar rate by the liver slices, and that there is, at the end of the experiment, about the same intracellular level of aminobutyric acid as of alanine. Clearly, we have shown that aminobutyric acid is as actively carried into the cell as alanine, and as effectively utilized in some reactions, but that those enzymes which are responsible for protein synthesis in the living cell are sufficiently specific to reject aminobutyric acid, the next higher homologue of alanine and the next lower homologue of valine.

Such specificity is striking and is in agreement with the concept, which has gained so much support from the recent work of Sanger and Tuppy[8] on the structure of insulin, that a protein is a specific compound with a perfectly definite amino-acid sequence. One may ask whether every protein of the cell has such a definite structure.

Are the proteolytic enzymes of the cell sufficiently specific to reject α-aminobutyric acid 150 to 1 with respect to alanine? It is inherent in the theory of catalysis that the catalyst should not affect the equilibrium of a reaction; hence that it must accelerate the forward and reverse reactions equally. The catalytic activity of a given enzyme in synthesizing aminobutyrylglycine can be roughly estimated by measuring the rate of hydrolysis of the dipeptide. To be precise, the rate of hydrolysis corresponds to the rate of synthesis from two free amino-acids, the rate of ethanolysis corresponds to synthesis from the ethyl ester and one free amino-acid, etc.

In all cases that have been reported, aminobutyric acid-peptides are split at rates comparable with the corresponding alanyl-peptides. However, the enzymes used were extracellular. Smith and Polglase[9] found that α-aminobutyric acid amide was split by leucine amino-peptidase from intestinal mucosa at a rate between alanyl- and leucyl-amide. We have measured the rates of hydrolysis of alanyl-, aminobutyryl-, and leucyl-glycine by the proteolytic enzymes of freshly homogenized rat liver, that is, precisely the tissue which rejects aminobutyric acid for protein synthesis. The accompanying results show that aminobutyrylglycine is split by these intracellular enzymes at about the same rate as alanylglycine under these conditions.

While this may be taken as evidence against the participation of proteolytic enzymes in normal protein synthesis, it is not conclusive. A wide range of aminobutyric acid peptides should be tested. It is also possible that the great degree of specificity of protein synthesis is found in the enzymes acting on the larger peptides. We feel, however, that unless an enzyme system does discriminate against aminobutyric acid or an aminobutyric acid peptide, the system being studied should not be considered as part of the mechanism of biological synthesis of protein.

HYDROLYSIS OF PEPTIDES

Substrate	Concentration	% Hydrolysis
DL-Alanylglycine	0·16 *M*	41
	0·08 *M*	85
DL-α-Aminobutyrylglycine	0·17 *M*	37
	0·09 *M*	63
DL-Leucylglycine	0·12 *M*	10
	0·06 *M*	16

1 gm. of fresh rat liver homogenized in 10 c.c. 0·1 *M* phosphate buffer, *p*H 8·2; suspension centrifuged, supernatant diluted 1 : 40 with same buffer, and used as source of enzyme. Incubation mixture: 1 c.c. of enzyme solution, 1 c.c. of peptide solution, 0·01 c.c. of toluene. Temp. 37°.

Incubation time, 6 hr. Hydrolysis determined by ninhydrin method (ref. 10). Per cent calculated for L-isomer only; hydrolysis of D-isomer considered to be negligible.

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A Disulphide Interchange Reaction

IN experiments designed to determine the position of the —S—S— bonds in insulin, cystine peptides were separated from a partial acid hydrolysate, and their structures determined after oxidation to the corresponding cysteic acid peptides. It was found that many more cystine peptides were present than could be accounted for by any one unique structure for the insulin molecule, and it seemed likely that a transfer reaction of the type:

$$R^1\text{—S—S—}R^2 + R^3\text{—S—S—}R^4 \rightleftharpoons R^1\text{—S—S—}R^3 + R^2\text{—S—S—}R^4$$

had taken place during the hydrolytic treatment, leading to a random rearrangement of the —S—S— bonds. That such a reaction can take place was

illustrated by the following model experiment, in which *mono*-2 : 4-dinitrophenyl-cystine is formed from a mixture of cystine and *bis*-2 : 4-dinitrophenyl-cystine.

1 mgm. *bis*-2 : 4-dinitrophenyl-cystine and 10 mgm. cystine were incubated with 2 ml. of 12 *N* hydrochloric acid. After 24 hr. at 37°, 5 ml. water and 10 ml. ether were added and the mixture shaken. Nearly all the yellow colour was found in the aqueous layer, indicating the presence of *mono*-2 : 4-dinitrophenyl-cystine. In a control experiment without cystine, all the colour which was due to *bis*-compound was in the ether layer. The reaction-rate is rapid in strongly acid or in neutral solution and is minimal in dilute acid (about 0·1 *N* hydrochloric acid). This probably explains the fact that insulin is inactivated more readily by heating in neutral than in dilute acid solution[1], if the rearrangement of the —S—S— bridges is assumed to cause inactivation.

The mechanism of the reaction is not clear at present and is under investigation. It may involve the intermediate formation of an —SH compound by reduction or hydrolysis, in which case the actual rearrangement would be brought about by the reaction :

$$-R^1\text{—SH} + R^2\text{—S—S—}R^3 \rightleftharpoons R^2\text{—SH} + R^1\text{—S—S—}R^3.$$

In this case the R^2—SH formed would be available to react with a further disulphide, so that only a catalytic amount of an —SH compound could bring about considerable rearrangement[2].

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Structural Features of Antitumorigenic Corticoids

The antifibromatogenic potency of deoxycorticosterone—prevention of œstrogen-induced abdominal fibroids—diminishes through the substitution $O{=}C_{11}$ (Kendall's compound *A*) and especially through $OH{-}{-}C_{17}$ (Reichstein's compound *S*)[1]. The antifibromatogenic potency of cortisone, which differs from deoxycorticosterone by both these substitutions, is also strikingly diminished ; there were fibroids even with as much as 1,000 μgm. of cortisone acetate per day. But, on the other hand, there apparently was some antifibromatogenic activity of compound *F*, or 17-hydroxy-corticosterone ($OH{-}{-}C_{17}$ and $OH\text{—}C_{11}$); we had thus to raise the question of a 'protective' action of $OH\text{—}C_{11}$ against $OH{-}{-}C_{17}$. However, in view of the variations of the fibrous tumoral effect met with in similar experiments, and especially in view of the small number of experiments performed with compound *F*, the conclusion as to the supposed 'protective' action of $OH\text{—}C_{11}$ against $OH{-}{-}C_{17}$ remained doubtful[1]. In the meantime, we have been able to settle this question in what seems to be a definite manner, thanks to the kindness of Messrs. Merck and Co., who put at our disposal the necessary quantities of compound *F* acetate.

Ten animals (castrated female guinea pigs) were used in the experiment, which lasted 91–92 days. An average of 81 μgm. of œstradiol per day was absorbed from subcutaneously implanted tablets ; 268–447 μgm. (average 350) of compound *F* acetate was absorbed per day from two tablets (a new implantation six weeks after the first one). There were fibroids in nine out of ten animals ; average fibrotumoral effect, 5·2 ; average weight of uterus, 4·6 gm. The quantities of compound *F* were indeed smaller than in some animals of the former series, in which there apparently was prevention ; but there is the fact that fibroids were present in the new series, even with quantities as large as 350–447 μgm. of compound *F* acetate per day. It is thus evident that $OH\text{—}C_{11}$ does not afford 'protection' against $OH{-}{-}C_{17}$, by which antifibromatogenic potency is so greatly diminished (compound *S*).

Although we must drop our original concept of 'protection' by $OH\text{—}C_{11}$ against $OH{-}{-}C_{17}$ with reference to antifibromatogenic corticoids, it is very remarkable that the same concept seems to be valid with reference to the inhibition of the growth of the chick embryo, as evidenced by certain findings of Stock[2]. The effective growth-inhibiting dose of deoxycorticosterone is 250 μgm. ; that of compound *S* ($OH{-}{-}C_{17}$) is as high as 5,000 μgm. ; but the effective dose of compound *F* ($OH{-}{-}C_{17}$ and $OH\text{—}C_{11}$) is only 20 μgm.

Attention may also be directed to a comparative discussion of the structural features of antifibromatogenic and antilymphomatogenic corticoids[3].

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Multi-spots in Paper Chromatograms

During investigations on the synthesis of nucleotides in this laboratory, extensive use has been made of the paper chromatography of phosphorus-containing compounds. In the early experiments it was observed that in a descending pyridine – ethyl acetate – water system, disodium hydrogen phosphate gave two clear spots of approximately equal intensity with R_F 0·17 and 0·55 on spraying with molybdate reagent[1]. Identical spots were obtained with sodium or potassium mono- or di-hydrogen phosphates, whereas ammonium phosphate and free orthophosphoric acid gave only the faster-running spot. Trisodium phosphate showed largely the slower spot with some trailing. (These R_F values are quoted to give an indication of the positions of the spots. The temperature was not strictly controlled.) Other experiments showed the effect was not due to complexes with pyridine as the same double-spot phenomenon occurred in a butanol – water system. By buffering the phosphates with acid and alkali, it was found that the number of spots depended on the *p*H of the solution in which the phosphate was applied to the paper. Potassium dihydrogen phos-