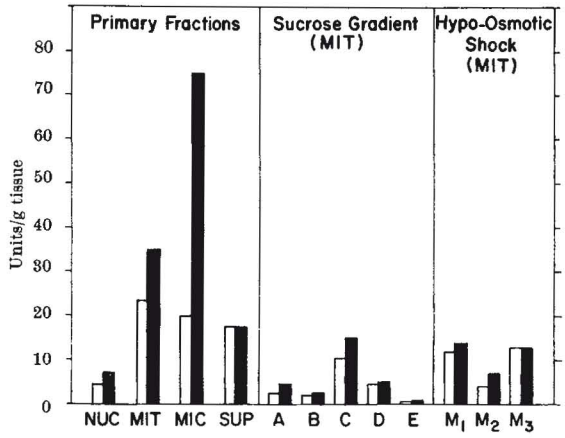

Fig. 2. Effect of 'Triton X-100' on unmasking of latent phosphodiesterase activity in various subcellular fractions. Fractions were prepared as usual and each fraction was divided into two, one serving as control; the other was diluted with 0·32 molar sucrose to a protein concentration of 5 mg/ml. and then treated with 0·2 per cent 'Triton X-100' (final concentration). White block, control; solid block, 'Triton'. Separate experiments established that this concentration of 'Triton' exposed all latent activity (Cheung, to be published). Both sets of samples were kept at 0° for 30 min before enzymatic assay. The activity of each fraction is expressed as units/g tissue. The total activity in the control exhibited 77 units/g tissue while that treated with 0·2 per cent 'Triton' 122 units/g tissue. Results are average of two experiments using a pool of ten brains in each experiment. Recovery of phosphodiesterase from the various fractions in these experiments was 80–85 per cent.

activities. With the exceptions of SUP and M_3, all fractions display an increase in activity. MIC was most pronounced, giving a 4-fold increase. The increase in all other fractions could be caused by the small contamination by microsomes which would be expected. In the presence of 'Triton', MIC accounts for more than half the activity in the whole brain. The fact that there was no increase of activity in the soluble enzyme in SUP and M_3 would suggest that 'Triton' did not activate the enzyme as such, but possibly exposed the enzyme which would otherwise have been inaccessible during the assay. In order to see whether phosphodiesterase was associated with lysosome-type particles, we submitted the microsomal fraction to vigorous mechanical disruption in a 'VirTis 45' homogenizer for times up to 9 min. Samples were taken at various intervals and no increase was observed in any of them. Repeated freezing and thawing of the microsomal fraction also caused no appreciable increase. More than 90 per cent of the activity was still attached to the sediment ($100{,}000g \times 45$ min) after such physical disruption. Parallel treatment of the microsomes with 'Triton' gave the usual burst of activity. It seems that the latent enzyme might be embedded in the lipoprotein matrix of microsomes in such a manner that it could only be exposed by the detergent. In this respect, the behaviour of phosphodiesterase is different from acid phosphatase, a typical lysosomal enzyme, the latent activity of which could be released by all the treatments described above[12]. It is also different from cholinesterase in that the latter is a microsomal enzyme but shows no latent activity[13].

Both MIC and M_1 are composed of membrane fragments, accounting for more or less the same amount of protein. Whereas there is virtually no effect of 'Triton' on the activity associated with M_1, the increase of activity in MIC amounts to 400 per cent, suggesting that phosphodiesterase might be preferentially distributed in certain membrane structures.

Drummond and Perrott-Yee[14] reported that a cyclic 3′,5′-nucleotide phosphodiesterase from rabbit brain was localized entirely in the $100{,}000g$ supernatant. Nair[15] noted that a similar enzyme prepared from frozen dog heart was present exclusively in the supernatant. These observations are at variance with our data and those of Butcher and Sutherland[4], who found that phosphodiesterase from beef heart is mostly particulate. The reason for such a discrepancy is not apparent to us.

Our results indicate that phosphodiesterase as fractionated in isotonic sucrose solution does not exhibit its full potential activity. The majority of the activity in the microsomes is latent and is unmasked by the action of 'Triton', but not by mechanical disruption or repeated freezing and thawing. In the presence of 'Triton', microsomes account for more than half the total activity. The remaining activity distributes about equally among synaptoplasm and a $100{,}000g$ supernatant.

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Aminoacyltransferase Stimulation of Protein Synthesis by Pig Adrenal Polysomes

ADMINISTRATION of adrenocorticotrophin *in vivo* increases the incorporation of ^{14}C-glycine into protein by the supernatant of adrenal homogenates centrifuged at $15{,}000g$[1]. A factor of the soluble cell fraction ($105{,}000g$ supernatant) has been shown to be rate limiting for *in vitro* protein synthesis and its activity is doubled by ACTH administration in rats. This factor was reported to be proteinaceous; its elution pattern from DEAE-cellulose columns[2] was identical with liver aminoacyltransferase[3]. The increased activity of the microsomal fraction by treatment with ACTH was reported to be delayed compared with the soluble cell fraction[4]. Thus it was of interest to find whether polysomes, which are polymeres of ribosomes and messenger-RNA[5,6], or the protein from the soluble adrenal cell fraction were rate limiting for protein synthesis.

When polysomes were incubated with constant amounts of soluble cell fraction, the incorporation of ^{14}C-glycine increased only slightly and by no means in proportion to the amount of polysomes added. Thus incubation of polysomes with 0·4 ml. of $105{,}000g$ supernatant (80 mg-equivalents) showed the following incorporations. Polysomes: 0·1 ml. (240 c.p.m.); 0·2 ml. (269 c.p.m.); 0·4 ml. (337 c.p.m.); polysomes omitted (179 c.p.m.) (for incubation conditions see Table 1). We conclude that the amount of polysomes and therefore the amount of messenger RNA present in the incubation mixture did not limit the rate of amino-acid incorporation.

The protein factor prepared from pig soluble adrenal cell fraction (Fig. 1) and likely to be aminoacyltransferase[2] did, however, stimulate the incorporation of ^{14}C-glycine by constant amounts of polysomes in approximate proportion to the amount added. Samples VI and VII (Fig. 1) con-

Table 1. INCORPORATION OF ^{14}C-GLYCINE BY ADRENAL POLYSOMES AS STIMULATED BY AMINOACYLTRANSFERASE

Sample (Fig. 1)	0·2 ml.	0·4 ml.
V	270	208
VI	515	1,288
VII	754	1,835
VIII	210	311
IX	255	–

Polysomes were prepared from fresh pig adrenals[6,7] and 0·1 ml. = 160 mg-equivalents (containing 0·78 mg protein[8] and approximately 1·8 mg RNA[5]) were incubated with 0·3 ml. 105,000*g* supernatant of fresh pig adrenals (60 mg-equivalent). ^{14}C-glycine, ATP, GTP, PEP and P-kinase were added as in Fig. 1. In this system 0·2 or 0·4 ml. of samples V–IX (Fig. 1) were assayed for stimulation of ^{14}C-glycine incorporation in a total volume of 1·05 ml. (60 min, 37° C). The complete system with no addition showed an incorporation of 209 c.p.m.; 105,000*g* supernatant omitted and 5 c.p.m. when polysomes were omitted. Addition of 0·2 ml. of samples produced the following incorporations: VI, 176 c.p.m.; VII, 342 c.p.m.; VIII, 108 c.p.m.; IX, 206 c.p.m.

tained aminoacyltransferase and stimulated amino-acid incorporation when they were added to incubation mixtures (Table 1).

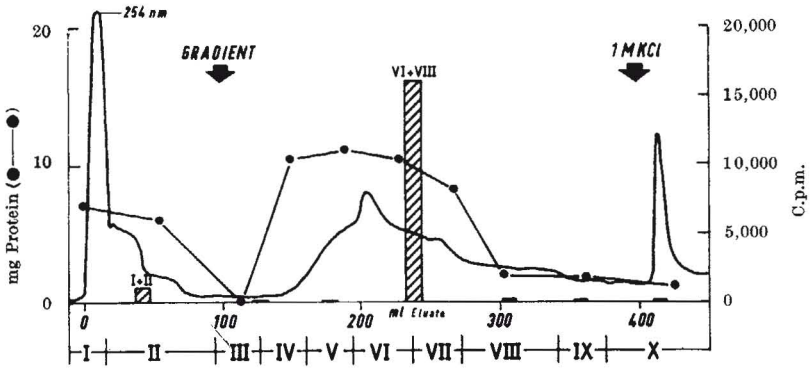

Fig. 1. Preparation of aminoacyltransferase from pig adrenals. 13·7 g fresh pig adrenals were homogenized in 68·5 ml. medium *A* (0·25 molar sucrose, 0·025 molar potassium chloride, 0·005 molar magnesium chloride, 0·05 molar *tris* hydrochloric acid buffer, *p*H 7·5) as described[2] and the 105,000*g* supernatant was passed through dextran gel ('Sephadex *G*-25'). It was then concentrated by ultrafiltration and subjected to anion exchange chromatography on a DEAE-cellulose column using a linear gradient made from I: 0·03 molar potassium chloride, 0·02 molar *tris* hydrochloric acid buffer, *p*H 7·5, and II: 0·5 molar potassium chloride, 0·02 molar *tris* hydrochloric acid buffer, *p*H 7·5 (ref. 2). The eluate was recombined into samples I–X as indicated, concentrated by ultrafiltration, dialysed against medium *A* and portions assayed for stimulation of protein synthesis in a cell-free system. This contained 1 μc. ^{14}C-glycine (0·014 μmoles), 10 μmoles phosphoenol-pyruvate, 1 μmole ATP, 0·25 μmoles GTP, 60 μg pyruvate kinase and 80 mg-equivalent of 15,000*g* supernatant of pig adrenal homogenate in a total volume of 1·05 ml. (37° C, 60 min). The assay of ^{14}C-glycine incorporated into protein was described earlier[3]. The data represent the total stimulation of incorporation in each sample (c.p.m.)

Adrenocorticotrophin administered to rats leads to increased protein synthesis in a cell-free system because of elevated activity or amount of aminoacyltransferase[2]. Only after continued treatment with ACTH is the activity of polysomes stimulated[4], but aminoacyltransferase remains the rate limiting factor for *in vitro* protein synthesis.

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IMMUNOLOGY

Inter Heavy–Light Chain Disulphide Bridge in Immune Globulins

THE immune globulins are a heterogeneous family of proteins made up of light and heavy* chains joined by disulphide bridges. Immunological techniques[1,2] and chemical investigation[3] indicate that most or all of the normal population of light chains is a mixture of two types of polypeptide—κ and λ. The majority of heavy chains can be classified as γ, μ or α. In humans, the γ chains have been further subdivided, making use of common antigenic determinants, into four major sub-classes: γ2a (or Ne), γ2b (or We), γ2c (or Vi), and γ2d (or Ge)[4,5].

Combination of either κ or λ chains with γ, μ or α chains gives rise to immune globulins IgG (gamma-globulins), IgM (macroglobulins), or IgA. It has been postulated[3] that a single disulphide bridge linking κ or λ chains to heavy chains occurs through the light chain C-terminal or next to C-terminal cysteine residue, and this has been based mainly on the arrangements of disulphide bridges in light chain monomers and dimers produced in the urine of myeloma patients (Bence-Jones proteins). A direct demonstration that this is the case has now been obtained by isolating disulphide-bridged peptides containing the C-terminal peptide of the light chain and a half-cystine peptide from the heavy chain of different types of human pathological immune globulins. In addition, a radioactive technique for the isolation of the corresponding carboxymethyl-cysteine peptides has been developed. This communication presents a comparison between peptides derived from different classes and sub-classes. Pooled normal IgG is shown to contain in good yields the peptides found in the two chief sub-classes of γ-chains.

Disulphide-bridged peptides were isolated by the performic acid oxidation diagonal technique[6,7] after peptic (1/20 enzyme–substrate ratio in 5 per cent formic acid, 14 h at 37° C) and then tryptic digestion (1/50 enzyme–substrate ratio in *p*H 7·0, 0·1 molar ammonium acetate buffer, 6 h at 37° C). Peptides were purified by paper electrophoresis at *p*H 6·5, and *p*H 3·5 before and after oxidation; with the macroglobulin Ale, a preliminary fractionation of peptides on a 'Sephadex *G*-25' column in 2 per cent acetic acid was necessary. Peptides with the sequences shown in Table 1 were isolated from three different pathological immune globulins.

Table 1. CYSTINE PEPTIDES CONTAINING THE DISULPHIDE BOND BETWEEN LIGHT AND HEAVY CHAINS OF SOME PATHOLOGICAL IMMUNE GLOBULINS

Protein	Heavy chain type	Light chain type	
Ale	μ		Pro.Leu.Val.Ser.Cys.Glx.Asx.Ser(Asp,Ser$_2$,Thr,Pro)
			|
		κ	Gly.Glu.Cys
Fie	γ		Ser.Cys.Asp.Lys
			|
		κ	Gly.Glu.Cys
Vin	γ		Pro.Leu.Ala(Cys,Ser,Pro)
			|
		λ	Pro.Thr.Glu.Cys.Ser

The occurrence of the sequences derived from Fie and Vin in pooled normal IgG and in other myeloma proteins

* The nomenclature used for immune globulin molecules, chains and fragments is that recommended in ref. 24.