to be time-dependent, to be inhibited by chloramphenicol and to be energy-dependent. Another control experiment established that RNA from repressed cells was totally inactive for this synthesis. The activity of RNA from the induced cells was largely associated with the 23S fraction of rRNA when RNA was isolated from sucrose density gradients before use in the assay.

The *N*-terminal amino acid of tryptophanase is methionine and the *C*-terminal amino acid is valine (Morino & Snell, 1967). Incubations with either [³⁵S]methionine or [³H]valine were employed to establish whether these amino acids are terminal in the product synthesized *in vitro*. It was established that methionine is the *N*-terminal amino acid in tryptophanase synthesized in the RNA-S-30 fraction system, but not in tryptophanase synthesized in the polyribosome system; conversely it was found that the *C*-terminal valine was not present in the tryptophanase synthesized in the RNA-S-30 fraction system but was present in that synthesized in the polyribosome system.

Radioactive tryptic-peptide 'maps' obtained from protein synthesized with either system (with labelling with either [¹⁴C]leucine or [³⁵S]methionine) were superimposable with the 'map' obtained from purified tryptophanase except that for the protein obtained from the RNA-S-30 fraction incubation ten of the 32 tryptic peptides were very weak. We conclude that essentially complete tryptophanase subunits are synthesized in both systems, but that the polyribosome system does not initiate protein synthesis and that the RNA-S-30 fraction system produces molecules with incomplete *C*-termini. Nevertheless the chromatographic analysis forms a satisfactory assay for tryptophanase mRNA.

The properties of the protein synthesized in the RNA-S-30 fraction system were examined in greater detail to establish whether the incomplete *C*-termini affected the tertiary or the quaternary structure. By using material purified in the absence of tryptophanase carrier we showed that the newly synthesized protein did bind the cofactor (pyridoxal phosphate), but then when analysed by sucrose-density-gradient centrifugation it sedimented largely as subunits.

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Lincomycin as a Chloroplast Probe

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Chloroplasts contain a protein-synthesizing machinery involving 70S ribosomes, but it is not clear which proteins are made on these ribosomes (Kirk, 1970). Studies with chloramphenicol have given conflicting results, probably because of the lack of specificity of action of this compound in some plant tissues (Ellis, 1969). One of the similarities between isolated chloroplast and bacterial ribosomes is that protein synthesis is inhibited by lincomycin; protein synthesis by cytoplasmic ribosomes on the other hand is not inhibited (Ellis, 1970). Lincomycin is a highly effective inhibitor of chloroplast development in greening pea apices (Ellis & Hartley, 1971) and can be used to determine the sites of synthesis of chloroplast proteins in this tissue.

When etiolated pea apices are detached and illuminated with white light for 48h, several enzymes increase in specific activity as follows: ribose phosphate isomerase, threefold; phosphoribulokinase, 15-fold; ribulose diphosphate carboxylase, sixfold; phosphoglycerate kinase, twofold; NADP-specific triose phosphate dehydrogenase, sixfold; RNA polymerase, threefold. The specific activities of NAD-specific triose phosphate dehydrogenase and succinate dehydrogenase are unaffected by illumination of the apices. The rate of incorporation of [¹⁴C]leucine into protein by plastid pellets increases eightfold on the basis of fresh weight and threefold on the basis of weight of protein; it has previously been shown by polyacrylamide-gel electrophoresis that chloroplast ribosomal RNA components increase in amount on illumination of the apices (Ellis & Hartley, 1971).

When the apices are illuminated in the presence of 5 μ M-lincomycin all the enzymes except ribulose diphosphate carboxylase show similar increases in specific activity as the controls, even though chlorophyll synthesis is depressed by 75%. The increase in activity of the carboxylase is inhibited by 50% by 1 μ M-lincomycin. The increase in the leucine-incorporating ability of plastid pellets is totally prevented by 5 μ M-lincomycin.

These results suggest that chloroplast ribosomes synthesize some component of ribulose diphosphate carboxylase and at least some of the chloroplast ribosomal proteins. The other enzymes appear to be synthesized in the cytoplasm, even though some of them are located exclusively in the chloroplasts and are involved in photosynthesis. These results support the conclusion derived from genetic studies that chloroplasts are not autonomous (Kirk & Tilney-Bassett, 1967).

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neutral aqueous buffers. In the absence of rabbit plasmin the fragmentation can be achieved by the addition of human plasmin (Kabi Pharmaceuticals Ltd., Stockholm, Sweden) after acid treatment of the IgG.

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A New Enzymic Fragment (Facb) of Rabbit Immunoglobulin G

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IgG* prepared from rabbit serum by repeated ammonium sulphate precipitation and chromatography on DEAE-cellulose contains traces of a proteolytic enzyme that is probably plasmin. The immunoglobulin is not digested by the enzyme even during prolonged incubation in neutral solutions, but it can be made susceptible to the enzyme by preliminary incubation at low pH.

A solution of rabbit IgG (20mg/ml in phosphate buffer, pH 7.0) was adjusted to pH 2.5 with 1.0M-hydrochloric acid. The solution was incubated at 30°C for 60min and then adjusted to pH 7.0 with 1.0M-sodium hydroxide. On further incubation at 30°C for 30–120min the immunoglobulin was quantitatively hydrolysed, yielding two fragments that were detected by starch-gel electrophoresis. These fragments differed in size and could be separated by gel filtration on Sephadex G-150 in 6M-urea–0.1M-sodium acetate buffer, pH 4.7. Characterization of the fragments showed that the point of cleavage was between lysine-326 and alanine-327 in the heavy chains, 108 residues from the C-terminus. The smaller fragment therefore resembles the Pep III fragment described by Utsumi & Karush (1965). The remainder of the immunoglobulin molecule makes up the larger fragment, which will be designated Facb (fragment antigen- and complement-binding).

The Facb fragment was prepared from IgG from rabbits hyperimmunized with ovalbumin. The fragment formed an immune precipitate with ovalbumin and the quantitative precipitin curve was almost indistinguishable from that of the original IgG. The ovalbumin-fragment complex was found to have complement-fixing activity that was approx. 60% of that of the corresponding ovalbumin-antiovalbumin complex.

Rabbit IgG can be freed of plasmin activity by repeated passage through Sephadex G-200 in

Antigenicity of Sheep Cortical-Bone Matrix Proteins

By I. R. DICKSON. (*Institute of Orthopaedics, Stanmore, Middx., U.K.*)

The antigenicity of sheep cortical-bone proteins was studied as a preliminary to evaluation of the feasibility of using monospecific antibodies, labelled with radioactive isotopes or fluorescent reagents, to study bone protein metabolism and also to estimate new bone formation in grafts and implants. Sheep cortical-bone powder was extracted with EDTA, pH 8.0, by the method of Herring (1964). Chromatography of the concentrated extract (fraction I) on CM-cellulose columns (10mm-potassium acetate buffer, pH 5.0) yielded a fraction (fraction II) that was retained on the column and one that was not adsorbed. The latter was ultra-centrifuged (80000g for 48h at 4°C) in a caesium chloride solution ($\rho = 1.5$); fraction III was recovered as a brown gel from the supernatant solution, and the remaining fractions were pooled (fraction IV). Fraction II consisted mainly of soluble collagen and a glycoprotein with the mobility of serum albumin on cellulose acetate electrophoresis (0.12M-sodium barbiturate buffer, pH 8.6). Fraction III had an electrophoretic mobility of 0.9 relative to serum albumin and contained 0.3% hydroxyproline, 0.7% uronic acid and 2.8% sialic acid. Fraction IV contained 8.7% uronic acid, 7.3% sialic acid and 0.4% hydroxyproline; it stained with Alcian Blue but not protein stains and it had an electrophoretic mobility of 2.0 relative to serum albumin.

Antisera, raised in rabbits to fraction I, sheep serum, sheep γ -globulin and a 3M-magnesium chloride extract of powdered sheep articular cartilage, were used to study the bone fractions by immunoelectrophoresis, double diffusion and 'tanned' erythrocyte haemagglutination techniques. Serum albumin was present in fractions I, II and III; fractions I and II also contained immunoglobulin G. Precipitin lines were obtained when antisera to fraction I were double-diffused against fraction I and its fractions: this was due to anti-(serum protein) activity. When this activity was adsorbed out with sheep serum no precipitin lines were

* Abbreviation: IgG, immunoglobulin G.