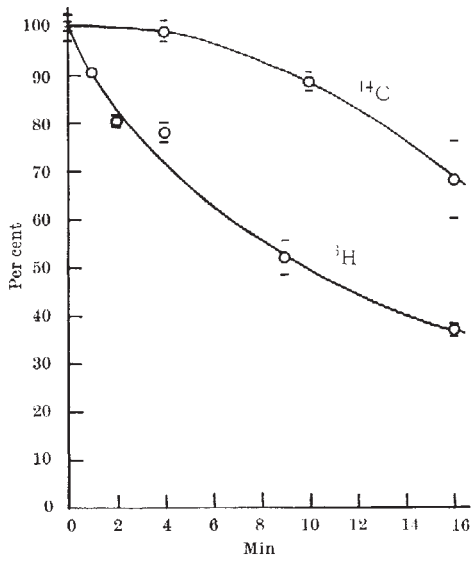

Fig. 1. Percentage of specific activity remaining in labelled nuclei after digestion with 160 μg/ml. DNase I, as a function of time.

acid. DNA was estimated by spectrophotometry after extraction by a modified Schmidt-Tannhauser procedure[3]. Radioactivity was estimated in a 'Tracerlab' *LSC* 30 liquid scintillation counter. Similar experiments were also performed on nuclei which had been labelled with tritiated thymidine by the addition of the isotope in eight fractions of 0·01 μc./ml. spread over the 70 h period of growth. A fractionated treatment was used because there is evidence that cell suspensions degrade thymidine[4]. The specific activities of the DNA were expressed as a function of the control (untreated nuclei), and plotted against time of enzyme action (Fig. 1).

It is apparent that the enzyme preferentially removes labelled DNA, particularly in the case of DNA labelled with tritium. A high concentration of enzyme was used here to emphasize the effect, but a noticeable decrease in specific activity was also obtained after the first few minutes with concentrations of 30 μg/ml. Some preliminary results along similar lines have been obtained using kidney nuclei from rats treated with folate[3] which were flash-labelled with tritiated thymidine 1 h before they were killed. The fall in specific activity after treatment with DNase was much less than with the cultured cells exposed to label for 70 h, but still appreciable, and this effect is being further investigated.

The explanation of this effect is unknown at present, but two possibilities may be considered. The nuclear DNA may be so damaged by the radiation from the isotope label that the active regions are more intrinsically labile to enzyme attack. Alternatively, the damaged DNA is undergoing repair and the action of the DNase I is to hydrolyse already excised material. The greater effect of the tritium label as compared with the carbon-14 label is probably due to the greater energy absorption from the tritium β particle, although in murine lymphoma cell cultures the number of bases labelled is about ten times higher in the case of carbon-14. It has recently been reported elsewhere that loss of tritium activity from acid-precipitable material after labelling with tritiated thymidine can occur over the course of some hours[5]. This was attributed to the localized absorption of energy from tritium disintegration, because it did not occur after labelling with carbon-14.

The use of carbon-14 or tritium precursors can thus produce cells in which the chromosomal material is changed such that a proportion of the DNA is more labile to enzyme attack. Whereas this effect may have implications in the use of labelling techniques, it is not yet certain what the biological consequences of such lability might be.

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Formation of Blue Oxidation Product from Psilocybin

Gilmour and O'Brien[1] have reported that a blue colour develops when psilocybin (4-phosphoryl-*N*,*N*-dimethyltryptamine) is incubated with a preparation of rat brain mitochondria. The colour is apparently not dependent on oxygen for its formation, is associated only with the particulate matter and cannot be brought into solution by treatment with various organic solvents, detergents, acid or alkali. The reaction is inhibited (80 per cent) by EDTA.

Experiments carried out in this laboratory and elsewhere provide a reasonable explanation for this phenomenon. Psilocin, the dephosphorylated derivative of psilocybin, forms a deep blue colour on incubation with ceruloplasmin—the copper oxidase of mammalian serum or with a copper oxidase obtained from the gill plates of *Mytilus edulis*. The reaction is accompanied by the uptake of oxygen[2,3]. The colour has a spectral maximum at about 620–625 mμ and a smaller peak at 400 mμ. Although 5, 6 and 7-hydroxyindole derivatives are also oxidized in the presence of both enzymes, only psilocin yields the blue colour. The blue material is a fairly stable free radical (Blumberg, W. E., and Peisach, J., personal communication), while the free radicals formed from other ceruloplasmin substrates are rather short lived[4]. The reaction catalysed by ceruloplasmin is unaffected by EDTA. This is in contrast to the reactions with other substrates such as *p*-phenylenediamine, which are inhibited by about 50 per cent by EDTA (refs. 5 and 6).

During the formation of blue material in the presence of the *Mytilus* enzyme the colour becomes associated principally with particulate matter and resists extraction with various non-polar solvents. Much of the colour can, however, be brought into solution with molar hydrochloric acid. If psilocin is oxidized in a system with very little protein the blue oxidation product appears to remain soluble.

The oxidative formation of blue colour can also be elicited without enzyme in the presence of ferric ion, which acts as an electron acceptor. As would be expected, EDTA and other chelating agents block this non-enzymatic reaction. When treated with alkali, the oxidation product of psilocin turns pale green, and can readily be extracted into butanol, where the deep blue colour reappears. The blue product can be re-extracted into 0·1 molar hydrochloric acid. The hydrochloric acid solution at this point contains no detectable iron, and the blue colour cannot therefore be due to a metal complex.

With psilocybin neither formation of blue colour nor uptake of oxygen occurs during incubation with ceruloplasmin or with ferric ions. Thus a free, non-esterified phenolic hydroxyl group is required for reactivity. These reactions could be related to those described by Gilmour and O'Brien in two possible ways. First, their psilocybin may have been contaminated with a small amount of psilocin. No evidence for purity was presented. The colour is so intense that even a slight contamination would produce visible colour in their system. Second, because prolonged incubation was required for the colour to

form (16–20 h at 5° C) it seems likely that during this period of time a phosphatase could remove the phosphate and thus expose the free phenolic hydroxyl to oxidative attack either by a copper enzyme or by a metal ion such as the ferric ion. Horita and Weber[7,8] described the dephosphorylation of psilocybin by homogenates of various mammalian tissues, as well as by purified alkaline phosphatase. They found that the psilocin produced in this reaction is transformed into a deep blue colour. The latter reaction may be catalysed by cytochrome oxidase which readily oxidizes psilocin to the blue product at physiological *p*H (7·4), although the optimum *p*H for this reaction is 9 (ref. 9). Because Gilmour and O'Brien found EDTA inhibited the formation of blue colour, the reaction probably involves some iron which is either free or loosely bound so as to be accessible to chelating agents. This would also explain why the reaction could take place without oxygen.

I therefore propose that the formation of blue colour from psilocybin in the presence of the brain fractions described by Gilmour and O'Brien be attributed to the following reaction.

$$\text{psilocybin} \xrightarrow{\text{phosphatase}} \text{psilocin} \xrightarrow[\text{or copper oxidase or } Fe^{3+}]{\text{cytochrome oxidase}} \text{blue product}$$

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Primary Structure Heterogeneity in Ribosomal Proteins from *Escherichia coli*

FUNCTIONAL heterogeneity of ribosomal proteins from *Escherichia coli* is extensive and *in vitro* polypeptide synthesis requires at least six different protein fractions[1]. The structural heterogeneity of ribosomal proteins is even more impressive; on gel electrophoresis of 70*S* ribosomal protein from *E. coli* about thirty protein bands separate and opinion favours the presence of at least as many distinct proteins[2–10]. Little is known, however, about the extent to which the primary structure of these proteins is related. In this communication a comparison between the amino-acid composition and tryptic fingerprints of eight simplified protein fractions, isolated from 70*S* ribosomal proteins of *E. coli*, will be made. In addition the purification and some properties of three single ribosomal proteins, one acidic and two basics, will be presented. Fig. 1 gives the results of applying 1 g 70*S* ribosomal protein (prepared using glacial acetic acid[2,10]) to a column of CM cellulose. Four different peaks were reproducibly resolved. The electrophoresis patterns on polyacrylamide gels (15 per cent) of selected fractions are shown in Fig. 2. As Table 1 shows, the amino-acid compositions of these fractions, determined on a Technicon autoanalyser, are quite distinct. Each fraction contains at least one amino-acid, the percentage of which is substantially greater or less than the percentage of that amino-acid in any other fraction. The relative difference in the contents of basic over acidic amino-acid residues increases regularly from a value of −7 mole per cent to 12 mole per cent. Tryptic fingerprints confirm the presence of extensive differences in primary structure between each of these fractions; with the exception of fractions 1*a* and 1*b*, each fraction shows a different tryptic fingerprint with strong spots uniquely missing or present on comparison with any of the other fingerprints (Fig. 3).

The chief component in peak 1 (compare Fig. 1), called ribosomal *A*-protein, was purified on DEAE cellulose and hydroxyl apatite until this protein moved as a single band in polyacrylamide gel electrophoresis. From the

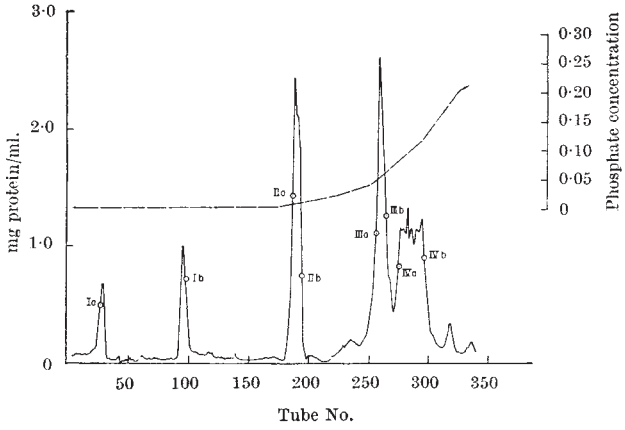

Fig. 1. Chromatography of *E. coli* ribosomal protein on CM cellulose. The column (43 cm × 3·2 cm) at 14° C was loaded in two portions: 400 mg of ribosomal protein, dissolved in 20 ml. of starting buffer, 0·005 molar ammonium phosphate, 8·0 molar urea, *p*H 8·0, was applied to the column and the acidic protein portion was eluted with the same buffer. At tube 60, the second portion, 550 mg of ribosomal protein was applied in a similar manner. The gradient, 600 ml. each of 0·005 molar, 0·10 molar and 0·30 molar ammonium phosphate buffer, all 8·0 molar urea, *p*H 8·0, was started at tube 140. Protein concentrations were determined by the Lowry method[11]. Circles refer to the tubes (8 ml.) selected for further characterization. Recovery from the column was 88 per cent of the input. Pooled fractions, after dialysis and freeze drying, were recovered in 94 per cent yield. Peak I, 8·7 per cent, Peak II, 14·8 per cent, Peak III, 23·7 per cent, Peak IV, 26·2 per cent, Peak V, 4·1 per cent, Peak VI, 1·0 per cent.

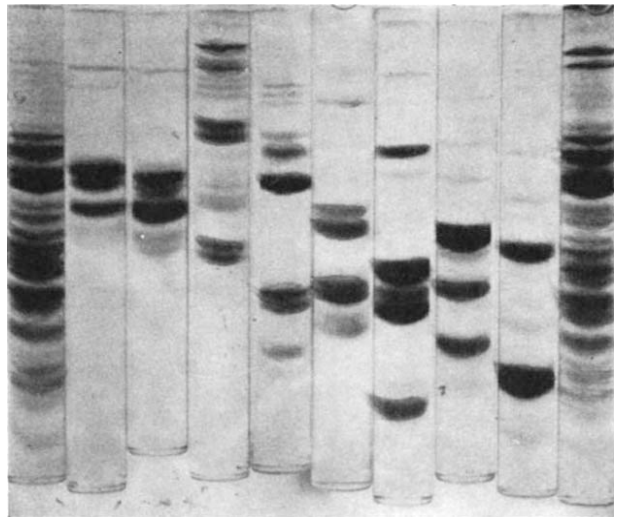

Fig. 2. Electrophoresis on polyacrylamide gels of the CM cellulose fractions indicated by the circles in Fig. 1. The cathode is at the bottom and the origin is indicated by a faint line near the top. Solvent was 8·0 molar urea, 0·3 molar formic acid and 0·06 molar potassium formate, *p*H 3·5 (ref. 9). Gels of unfractionated ribosomal proteins appear on either end as a control. There is similarity between the band patterns of fractions I*a* and I*b*.