

different from the phospholipid substance described by Green and Wilson⁴.

When the properties of the growth-promoting substance from yolks were reported, it seemed to us worth while to examine the possible identity of this factor with the material derived from tissues of tumour-bearing animals. Application of the procedures used in the isolation of tumour-enhancing factor from animal tissues to the unsaponifiable portion of yolks gave a considerable quantity of a fluorescent substance the chromatographic behaviour and the absorption spectra of which were identical with those given by material from rat tissues. These facts strongly support our concept that the growth-promoting factor in egg-yolk and the tumour-enhancing factor are identical.

It is hoped to determine the chemical nature of this substance shortly, because considerably larger quantities of this material are available in egg-yolk than in rat tissues.

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¹ Szepsenwol, J., *Proc. Soc. Exp. Biol. Med.*, **96**, 332 (1957).

² Denton, C. A., Lillie, R. J., and Sizemore, J. R., *Fed. Proc.*, **13**, 455 (1954).

³ Menge, H., Lillie, R. J., and Denton, C. A., *Fed. Proc.*, **16**, 392 (1957).

⁴ Green, H. N., and Wilson, R., *Nature*, **178**, 851 (1956).

Efficient Trace-labelling of Proteins with Iodine

In the methods of iodination currently used only the cationic portion of the iodine molecule becomes bound to the ring structure of tyrosine, so that the theoretical efficiency of labelling is 50 per cent. In practice, efficiencies are always lower than this and may be only a few per cent when the ratio of iodine to protein used is less than one atom per molecule. Values greater than 50 per cent can be obtained by adding oxidizing agents to liberate iodine from iodide, but most if not all of these appear to affect adversely the properties of the labelled protein.

Iodine-131 monochloride was therefore prepared and used in dilute aqueous solutions for labelling proteins. Efficiencies were twice those obtained with labelled elementary iodine in similar circumstances. Essentially the same result was achieved by merely adding carrier-free radioactive iodine to inactive carrier iodine monochloride, provided the radioiodine used was free of reducing agent. (Iodine-131 condensed into *N*/50 sodium hydroxide can be obtained for this purpose from the Radiochemical Centre, Amersham, subject to an extra handling charge. Very little appears to be known about the ionic equilibria obtaining in this solution.) The radioiodine may be added to the carrier iodine monochloride, in dilute hydrochloric acid solution, before or after conversion of the iodine monochloride to hypoiodite. This step, which is indicated by the loss of the characteristic yellow colour of iodine monochloride, appears to be a prerequisite for substitution of iodine in the benzene ring of tyrosine. The conversion is best carried out by injecting a glycine buffer of *pH* 8.5 into the iodine-131 monochloride solution just prior to rapid mixing with the protein. The *pH* of the protein solution should not be higher than 9.5 since hypoiodite is unstable above this *pH*. Using this procedure 60–80 per cent of the radioactivity used was bound to proteins provided the molar ratio of iodine

monochloride to protein was greater than 2. This applied to plasma albumins and globulins of various species, including some antibodies and pathological human globulins.

Lower efficiencies were obtained using lower molar ratios because of preferential utilization of the iodine monochloride for non-specific oxidations and especially of sulphhydryl. The hydroxyl group of tyrosine is not ionized at *pH* 4.5 so that iodination does not occur, while oxidations proceed normally at this *pH*. Protein solutions which had been in contact with excess iodine at *pH* 4.5 and were afterwards passed through an ion exchange column to remove iodine and iodide then reacted with iodine-131 monochloride at *pH* 8–9 giving 90 per cent incorporation of the label at all molar ratios greater than 2, and 50–60 per cent incorporation at molar ratios as low as 0.1. Alternatively the approximate amount of iodine monochloride required for non-specific oxidation in each case was determined in a preliminary tracer experiment at *pH* 8–9 and this amount of inactive iodine monochloride added to a fresh portion of protein. The iodide formed was removed by ion exchange and the oxidized protein then iodinated with iodine-131 monochloride at full efficiency.

These increased efficiencies make labelling with iodine-131 a more economical procedure and one that is less hazardous to the operator since iodine monochloride has a much lower volatility than iodine. In addition, they offer the practical possibility of labelling only a small proportion of all protein molecules present. The biological behaviour of protein molecules labelled in severely competitive conditions in this way is now being studied.

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Separation and Determination of Millimicrogram Amounts of Cobalt and Microgram Amounts of Copper in Biological Material

THE chemical determination of metals chemically bound in biological material at concentrations less than 10^{-6} gm./100 gm. raises considerable difficulties. This is due to the fact that not all sensitive methods of determining metals in homogeneous material can be adapted to the treatment of samples containing organic compounds in the presence of an excess of other metals.

When undertaking the investigation of biological material, it is necessary first to separate the mineral components from the substrate by ashing the organic substances present therein, and then to select sufficiently sensitive methods for the determination of each of the various elements after they have been separated from one another. To exemplify the procedure followed, the determination of the cobalt and copper contained in whole blood has been chosen.

Available data point to a concentration of cobalt¹ of the order of 10^{-8} gm. and of copper² of 10^{-6} gm. in 100 gm. of whole blood. Since the amount of blood in clinical tests usually does not exceed 10 gm., the cobalt and copper contents in a sample would be of the order of 10^{-9} gm. and 10^{-7} gm. respectively.

We have separated both elements by paper chromatography from other metals (in the first place