

Table 2. Comparison of second-order rate constants for the reaction of glycylglycine and DL-leucylglycine with hydrazine, methylhydrazine and aqueous hydrochloric acid

The rate constants for the reactions with hydrazine (see Table 7, Bradbury, 1958a) and methylhydrazine were obtained at 60°. Harris, Cole & Pon (1956) determined the hydrolytic rate constants in HCl at 99°.

| Peptide | Uncatalysed rate constants $10^5 k''$ (l.mole ⁻¹ min. ⁻¹) in | | Catalysed rate constants $10^5 k_c''$ (l.mole ⁻¹ min. ⁻¹) in | | Hydrolytic rate constants $10^3 k''$ (l.mole ⁻¹ min. ⁻¹) |
|------------------|--|-----------------|--|-----------------|--|
| | Hydrazine | Methylhydrazine | Hydrazine | Methylhydrazine | |
| Glycylglycine | 41 | 2.12 | 3040 | 28.7 | 3.5 |
| DL-Leucylglycine | 1.81 | 0.112 | 145 | 2.20 | 0.7 |

reasonable to suppose that the ratio $k''(\text{Gly. Gly})/k''(\text{Leu. Gly})$ gives an approximate estimate of the relative selectivities of the reagents towards the splitting of peptide bonds. A simple calculation from the results in Table 2 gives a value of the ratio of 5 for aqueous hydrochloric acid and average values of 16 for methylhydrazine and 22 for hydrazine. Therefore the selectivity of the reagents towards peptide bonds is in the order aqueous acid < methylhydrazine < hydrazine.

The lower reactivity of methylhydrazine as compared with hydrazine towards peptide bonds precludes its use as a reagent for the determination of the C-terminal amino acids of proteins, as shown by the failure of the two experiments with insulin. However, it may be useful as a solvent for proteins, since it appears to combine the good solvent properties of hydrazine (as shown by the solubility tests with four proteins) with decreased reactivity towards peptide bonds. For physical measurements on proteins it may be possible to use methylhydrazine in place of hydrazine in order to avoid the degradation which accompanies the use of the latter (Rees & Singer, 1956; Bradbury, 1958a).

SUMMARY

1. The kinetics of the uncatalysed and acid-catalysed reaction of glycylglycine and DL-leucyl-

glycine with methylhydrazine were examined by techniques recorded in a previous paper (Bradbury, 1958a).

2. Contrary to expectations based on an intuitive approach to steric effects it has been found that methylhydrazine is no more selective in its fission of peptide bonds than is hydrazine.

3. The slow fission of peptide bonds by methylhydrazine, as compared with hydrazine, precludes its use for the determination of the C-terminal amino acids of proteins, but is advantageous in its application as a protein solvent.

It is a pleasure to thank Mr H. Groll for assistance with the experimental work.

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Fractionation of Proteins of the Microsomes of Rat Liver by Means of a Non-Ionic Detergent

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(Received 27 February 1958)

Labelled amino acids have been shown to be rapidly incorporated into the proteins of the microsome fraction and particularly into those which are closely associated with ribonucleic acid (Allfrey, Daly & Mirsky, 1953; Hultin, 1955; Littlefield, Keller, Gross & Zamecnik, 1955; Simkin & Work, 1957). Little is known of the nature of the micro-

somal proteins into which this incorporation occurs. Littlefield *et al.* (1955) found that during short periods after the administration of a radioactive amino acid to a rat, proteins of high specific radioactivity remained insoluble after treatment of the microsomes with sodium deoxycholate. Hultin (1955) and Simkin & Work (1957) obtained micro-

somal protein fractions of different specific radioactivities by extractions with inorganic salts at various pH values. Recently it was shown (Cohn & Butler, 1957*a, b*) that treatment of the microsome fraction with a non-ionic detergent released about one-half of the proteins into solution without solubilizing any of the ribonucleic acid. In preliminary experiments it was found that 2 min. after the administration of [¹⁴C]phenylalanine the proteins remaining insoluble had a higher specific radioactivity than the solubilized proteins. However, 6 min. after the injection the solubilized proteins were more highly labelled than those which remained insoluble.

It was thought that further fractionation would throw some light on the problem whether the easily solubilized proteins are derived directly from proteins which remain associated with ribonucleic acid. The investigation gave evidence that under the conditions used a number of protein fractions of different specific radioactivities become labelled practically simultaneously.

MATERIALS AND METHODS

Animals. Male Wistar albino rats (200–350 g.) bred at this Institute were kept on a mixed diet. They were fasted for 18–20 hr. before death.

Radioactive amino acids. DL-[3-¹⁴C]Phenylalanine (2 mc/m-mole) was prepared by Dr V. C. E. Burnop. For confirmatory experiments generally-labelled L-[¹⁴C]phenylalanine (L-[G-¹⁴C]phenylalanine) (11.7 mc/m-mole) was obtained from the Radiochemical Centre, Amersham, Bucks.

Administration of radioactive amino acids. The amino acid, dissolved in 0.85% NaCl soln., was injected either intraperitoneally or into the right femoral vein. In preliminary experiments administration into the tail vein was attempted. Low and inconsistent results suggested that administration by this route was often only partly successful. Before an intravenous injection the rat was lightly anaesthetized with ether.

Removal of liver. The rat was perfused with 0.25M-sucrose under anaesthesia induced by pentobarbitone sodium. The liver was then quickly removed, plunged into ice-cold sucrose (0.25M) and chopped with scissors.

In experiments designed to examine the incorporation of the radioactive amino acid the rat was killed by breaking its neck, and the liver cut out and chopped after having been plunged into ice-cold 0.25M-sucrose. These operations took 30–45 sec. to complete.

Preparation of microsome fraction

Tissue suspensions. To each gram of liver 3 ml. of 0.25M-sucrose was added and the whole placed in the ice-cold glass tube of a Potter-Elvehjem-type homogenizer into which fitted a polythene pestle. The difference between the internal diameter of the tube and the diameter of the pestle was 0.30 mm. Twenty up-and-down strokes were carried out while the pestle was driven at 1700 rev./min. Tissue suspensions and all subcellular fractions were maintained at 0–5° throughout.

Centrifugal fractions. The tissue suspension (11.5 ml. into each tube of the no. 40 head, Spinco centrifuge, model L) was centrifuged at 18 000 g (average *g*) for 10 min. A volume (6.7 ml.) of the supernatant liquid was withdrawn from each tube. All these samples from one liver were pooled, and 6.5 ml. was pipetted into fresh centrifuge tubes and 0.25M-sucrose (4.5 ml.) added. The microsome fraction was obtained by centrifuging at 105 000 g for 45 min. All but approx. 0.1 ml. of this, the final, supernatant liquid was withdrawn, and the inside of the tube was wiped with filter paper.

Solubilization of microsome fraction

Use of Lubrol W. Lubrol W (Imperial Chemical Industries Ltd.), a condensate of cetyl alcohol and polyoxyethylene, was used at a concentration of 0.25, 0.40 or 0.50% in a 0.25M-sucrose solution. The microsome pellet was dispersed in this solution with the aid of a loosely fitting polythene pestle. Sufficient Lubrol W solution was added to make up the volume to 11 ml. The liquid became clear within about 2 min. Centrifuging at 105 000 g for 2 hr. produced a pellet, described below as the Lubrol pellet, which had the same appearance as the microsome pellet. The supernatant liquid, referred to as the Lubrol supernatant liquid, was withdrawn except for about 0.1 ml. next to the Lubrol pellet.

Use of sodium deoxycholate. The microsome pellet or the Lubrol pellet was solubilized by means of sodium deoxycholate (British Drug Houses Ltd.) according to the technique of Littlefield *et al.* (1955). The liquid (11 ml.) was then centrifuged at 105 000 g for 2 hr. The pellet was usually pale brown in colour.

Use of sodium perfluoro-octanoate. The Lubrol pellet was suspended in 0.25M-sucrose. The addition of 0.1M-sodium perfluoro-octanoate (Minnesota Mining and Manufacturing Co., St Paul 6, Minn., U.S.A.) dissolved in 0.25M-sucrose, pH 7.2, produced a rapid clarification of the liquid. It was then centrifuged at 105 000 g for 2 hr. The pellet so produced was either light brown or pink in colour.

Fractionation of Lubrol supernatant liquid

After the Lubrol supernatant liquid had been withdrawn MgSO₄·7H₂O was added to saturation. Next morning the turbid liquid was centrifuged at 10 000 g for 10 min. to give a pad of solid on top of a clear pale-yellow liquid. This liquid was carefully drawn into a syringe fitted with a long needle so as not to disturb the solid, which contained fraction *M_s* (Fig. 1). The clear liquid was then dialysed (Visking tubing 32/32 in.) against water until free from MgSO₄. During dialysis a fine sediment (fraction *W_s*) appeared which was subsequently separated by centrifuging. The clear supernatant liquid (*W_L*) was treated with an equal volume of a saturated (NH₄)₂SO₄ solution. The light flocculent precipitate (fraction *AS₅₀*) was collected by centrifuging (22 000 g for 45 min.). The supernatant liquid was then saturated with (NH₄)₂SO₄. The following day the light flocculent precipitate (fraction *AS₁₀₀*) was collected by centrifuging (44 000 g for 60 min.). The sediments containing fractions *AS₅₀* and *AS₁₀₀* were each dialysed (Visking tubing 32/32 in.) against water until free from sulphate. During dialysis fractions *AS₅₀* and *AS₁₀₀* redissolved. After dialysis the liquids were freeze-dried.

*Analysis of tissue suspensions and
subcellular fractions*

Nitrogen. A micro-Kjeldahl method was used to determine total nitrogen.

Phosphorus. After the destruction of organic matter with 60% (w/w) HClO_4 (A.R.) the phosphorus was determined colorimetrically by the method of Fiske & Subbarow (1925), HClO_4 being used instead of H_2SO_4 .

Protein. Total protein in each sample was precipitated with 0.4N- HClO_4 and treated as described by Littlefield *et al.* (1955). The Lubrol supernatant liquid was treated with an equal volume of 20% (w/v) trichloroacetic acid (A.R.). If the Lubrol supernatant liquid was frozen solid before the addition of 10% (w/v) trichloroacetic acid, the proteins appeared to be more readily precipitated. Sometimes the Lubrol supernatant liquid was freeze-dried and excess of detergent removed by extraction with acetone. The protein of fraction M_s was also precipitated with trichloroacetic acid. When much Lubrol W was present trichloroacetic acid appeared to precipitate the whole protein fraction more readily than HClO_4 .

Lipid phosphorus. Phosphorus extracted by ethanol-ether- CHCl_3 (2:2:1, v/v) (Littlefield *et al.* 1955) was taken to be the total lipid phosphorus.

Ribonucleic acid. The extinction at 260 $m\mu$ of acidified alkaline digests (Littlefield *et al.* 1955) served for the estimation of ribonucleic acid. Analyses for ribonucleic acid phosphate showed that this procedure could satisfactorily apply to all further fractions obtained from the microsome fraction.

Treatment of radioactive protein fractions

Precipitation of proteins. DL- or L-Phenylalanine (3 mM) was included in both HClO_4 and N-NaOH solutions to facilitate the washing of adsorbed radioactive material off the precipitate. In one experiment a suspension of rat liver was prepared in the presence of DL-[3- ^{14}C]phenylalanine (0.5 $\mu\text{C/g}$. of fresh liver). The proteins of the microsome fraction and of the final supernatant liquid were precipitated and isolated as described above. The radioactivity recovered with the protein fraction was negligible

irrespective of whether or not the microsome pellet was suspended in sucrose before the addition of HClO_4 or whether DL-phenylalanine was present in the HClO_4 and N-NaOH solutions. However, protein fractions were isolated in the presence of DL- or L-phenylalanine as a routine.

Isolation of protein fractions soluble in magnesium sulphate. Fraction W_s was left in the centrifuge tube and freeze-dried. Samples of fraction W_L , AS_{50} and AS_{100} were also obtained by freeze-drying the respective dialysate.

Radioactive assays. The final protein residue was assayed for radioactivity on polythene planchets (1 or 2 cm^2) in an end-window counter connected to a scaling unit. When the amount of sample was insufficient to give infinite thickness the proportion of the maximum radioactivity assayed was ascertained from a saturation curve. Since samples of the fractions of the Lubrol supernatant liquid contained considerable amounts of non-protein matter, mostly Lubrol, their specific radioactivities were all calculated to the same nitrogen content (14.3%).

RESULTS

Fractionation of microsomal proteins

Solubilization of microsomal proteins with Lubrol W. When the microsome fraction was resuspended in a solution of Lubrol W of a concentration above 0.25% the pellet obtained on centrifuging contained almost all the ribonucleic acid, about one-half of the proteins and between 20 and 40% of the lipid phosphorus of the microsome pellet (Cohn & Butler, 1957a).

Treatment of Lubrol pellet with sodium perfluorooctanoate. When sodium perfluorooctanoate was added to a Lubrol pellet resuspended in 0.25M-sucrose soln. the pellet obtained after recentrifuging contained 80–90% of the ribonucleic acid, 20–30% of the protein and 25% of the lipid phosphorus of the microsome fraction, if the final concentration of sodium perfluorooctanoate was

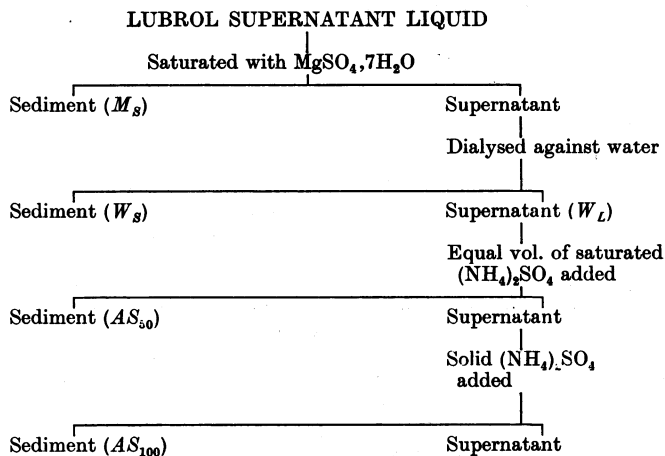


Fig. 1. Fractionation of Lubrol supernatant liquid.

Table 1. *Effect of concentration of Lubrol W on the specific radioactivities of proteins of the pellet and supernatant liquid*

DL-[3-¹⁴C]Phenylalanine (2 mc/m-mole) was administered intraperitoneally (0.20 mc/kg.) and the liver microsomes were subsequently isolated and partly solubilized with Lubrol.

| Expt. no. | Period after injection (min.) | Concn. of Lubrol (%) | Specific radioactivity of proteins (μ C/g.) | |
|-----------|-------------------------------|----------------------|--|---------------------------|
| | | | Lubrol pellet | Lubrol supernatant liquid |
| 1 | 6 | 0.25 | 0.78 | 1.33 |
| | | 1.0 | 0.70 | 1.16 |
| 2 | 31.5 | 0.25 | 2.47 | 5.40 |
| | | 1.0 | 2.45 | 5.12 |
| | | 1.0 | 2.40 | 5.85 |
| 3 | 55 | 1.0 | 2.40 | 5.24 |
| | | 2.0 | 2.41 | 5.36 |

0.32% (7.5 mm). These constituents of the perfluoro-octanoate pellet were thus present in the ratios ribonucleic acid:protein:phospholipid (1:2-3:1.5). The mean phosphorus content of the phospholipids was taken to be 4% (Swanson & Artom, 1950). The amounts of protein and phospholipid in the pellet increased when the concentration of sodium perfluoro-octanoate was lowered. At concentrations above 0.32% increasing amounts of ribonucleic acid as well as protein and phospholipid remained in the supernatant liquid.

Lubrol supernatant liquid. On saturating the Lubrol supernatant liquid with magnesium sulphate the precipitate that formed (M_s) contained about 20-25% of the proteins, most of the lipid phosphorus and about half the Lubrol W present in the liquid. The proteins soluble in water constitute 40-50% of the Lubrol supernatant liquid. The fraction precipitated by half-saturated ammonium sulphate (AS_{50}) forms approximately two-thirds of the water-soluble proteins, the remainder being fraction AS_{100} .

Distribution of specific radioactivities among cell fractions after the administration of [¹⁴C]phenylalanine. Table 1 gives some representative results to demonstrate the effect of three different concentrations of Lubrol W on microsomal proteins labelled by previous administration of [¹⁴C]phenylalanine. These results show that within the range of concentrations 0.25-2.0% (w/v) the specific radioactivities of the protein of the pellet and the supernatant liquid remain constant. For very short periods of incorporation the proteins of the Lubrol pellet appeared to have a higher specific radioactivity than those of the supernatant liquid (Table 2). If the rat was allowed to live for at least 6 min. after the injection the specific radioactivity of the proteins of the Lubrol supernatant liquid

rose to values more than twice as high as those of the pellet.

Table 2 shows the effect of time on the incorporation of [¹⁴C]phenylalanine into the proteins of those microsomal fractions insoluble in Lubrol W. The specific radioactivities of the total proteins of the microsome pellet, the Lubrol supernatant liquid and the final supernatant liquid remaining after removal of the microsomes are given for comparison. It can be seen that the specific radioactivities of proteins of both the microsome fraction and the final supernatant liquid rise during the period examined in the manner previously described by Keller, Zamecnik & Loftfield (1954). For short periods of incorporation the specific radioactivity of the protein remaining with the pellet after treatment of the Lubrol pellet with sodium deoxycholate is greatly increased, showing that proteins of low specific radioactivity are removed. The pellet obtained in this way had the highest specific radioactivity of any microsomal protein fraction examined (Expt. 2). The action of sodium perfluoro-octanoate (Expt. 1) was similar, but the proteins of the pellet had a somewhat lower specific radioactivity than with sodium deoxycholate. The sodium perfluoro-octanoate pellet would thus appear to contain some proteins of the Lubrol pellet of relatively low specific radioactivity which are solubilized by sodium deoxycholate. Two minutes after the administration of [¹⁴C]phenylalanine the ratio of the specific radioactivity of the protein of the sodium perfluoro-octanoate pellet to that of its supernatant liquid was 4:1. When the period of incorporation was 6 min. (Expt. 4) the proteins of the sodium perfluoro-octanoate pellet were about 2.5 times as radioactive as those of its supernatant liquid. When the rat was killed 10.5 min. after the injection further solubilization of the Lubrol pellet with sodium deoxycholate (Expt. 5) produced two fractions of equal specific radioactivities. The figures for Expt. 7 show that 30.5 min. after the injection of [¹⁴C]phenylalanine the specific radioactivity of the proteins of the pellet after treatment with sodium deoxycholate is well below that of the supernatant liquid. The specific radioactivity of the proteins of the sodium perfluoro-octanoate pellet, however, can be seen to be slightly above that of the proteins of the supernatant liquid, both 10.5 and 30.5 min. after the administration of [¹⁴C]phenylalanine. It is obvious that the ratio of the specific radioactivity of the proteins in the pellet and supernatant fractions produced by sodium perfluoro-octanoate falls with time after injection from its maximum to a value close to unity. In contrast, treatment with sodium deoxycholate causes, 30 min. after injection, a drop to 0.61 in the ratio of the specific radioactivity of the proteins in the pellet and supernatant fraction.

Table 2. Specific radioactivities of microsomal fractions not solubilized by Lubrol W
DL-[3-¹⁴C]Phenylalanine (2 mc/m-mole) was injected into the right femoral vein (dose was 0.20 mc/kg., except for rat no. 4 which was 0.12 mc/kg.).

| Rat no. | Body wt. (g.) | Period (min.) | Final supernatant liquid† | Specific radioactivities of proteins (μc/g.) | | | | | | | | | | Rat no. in Table 2 | | |
|---------|---------------|---------------|---------------------------|--|--------|---------------------|--------|----------------------------|--------|------------------------------|--------|----------------------------|--------|--------------------|------|---|
| | | | | Lubrol | | Sodium deoxycholate | | Sodium perfluoro-octanoate | | Lubrol pellet resuspended in | | Sodium perfluoro-octanoate | | | | |
| | | | | Microsomes | Pellet | Supernatant liquid | Pellet | Supernatant liquid | Pellet | Supernatant liquid | Pellet | Supernatant liquid | Pellet | Supernatant liquid | | |
| 1 | 320 | 2 | 0.14 | 0.65 | 0.77 | — | — | — | 1.16 | — | — | — | — | — | 0.29 | 1 |
| 2 | 350* | 2 | 0.11 | 0.71 | 0.90 | 0.46 | — | 0.39 | — | — | — | — | — | — | 0.28 | — |
| 3 | 255 | 2 | 0.19 | 1.03 | — | 1.00 | — | — | 1.71 | — | — | — | — | — | 0.35 | — |
| 4 | 270 | 6 | 0.25 | 1.03 | 0.56 | — | — | — | 0.92 | — | — | — | — | — | — | — |
| 5 | 280 | 10.5 | 0.68 | 2.22 | 1.34 | 2.91 | — | 1.38 | — | — | — | — | — | — | — | — |
| 6 | 320 | 10.5 | 0.47 | 1.85 | — | — | — | — | 1.08 | — | — | — | — | — | 0.92 | — |
| 7 | 320† | 30.5 | 0.76 | 1.94 | — | — | — | 1.45 | — | — | — | — | — | — | 0.88 | — |

* Injection into tail vein.
† L-[G-¹⁴C]Phenylalanine (11.7 mc/m-mole) was injected into the right femoral vein (0.037 mc/kg.).
‡ Final supernatant liquid remaining after removal of microsomes.

Table 3. Microsomal proteins solubilized and not solubilized by Lubrol W; comparison of radioactivity of fractions
DL-[3-¹⁴C]Phenylalanine (2 mc/m-mole) or L-[G-¹⁴C]phenylalanine (11.7 mc/m-mole) was injected into the right femoral vein.

| Expt. | Radioactivity of injected phenylalanine (μc/kg.) | Period (min.) | Specific radio-activity of microsomes (μc/g.) | Relative specific radioactivity of proteins of microsomal fractions (total proteins of microsomes = 100) | | | | | | | | | | Rat no. in Table 2 | | | | | |
|-------|--|---------------|---|--|--------|---------------------|--------|----------------------------|--------|------------------------------|--------|----------------------------|--------|--------------------|----------------|----------------|------------------|-------------------|---|
| | | | | Lubrol pellet | | Sodium deoxycholate | | Sodium perfluoro-octanoate | | Lubrol pellet resuspended in | | Sodium perfluoro-octanoate | | | | | | | |
| | | | | Lubrol pellet | Pellet | Supernatant liquid | Pellet | Supernatant liquid | Pellet | Supernatant liquid | Pellet | Supernatant liquid | Pellet | Supernatant liquid | W _s | W _L | AS ₅₀ | AS ₁₀₀ | |
| A | 200 (DL) | 2 | 0.65 | 118 | — | — | — | — | — | — | — | — | — | — | 37 | 148 | 65 | 202 | 1 |
| B | 33 (L) | 2 | 0.63 | 83 | — | — | — | — | — | — | — | — | — | — | 82 | 148 | 83 | —* | — |
| C | 120 (DL) | 6 | 1.03 | 54 | — | — | — | — | — | — | — | — | — | — | 106 | 204 | 135 | 344 | 4 |
| D | 45 (DL) | 6 | 0.62 | — | — | — | — | — | — | — | — | — | — | — | 123 | 202 | 140 | 229 | — |
| E | 10 (L) | 6 | 0.11 | 86 | — | — | — | — | — | — | — | — | — | — | 133 | 114 | 100 | 354 | — |
| F | 100 (L) | 6 | 2.84 | 58 | — | — | — | — | — | — | — | — | — | — | 116 | 161 | 165 | 252 | — |
| G | 200 (DL) | 10.5 | 1.85 | — | — | — | — | — | — | — | — | — | — | — | 62 | 207 | — | — | 6 |
| H | 37 (L) | 30.5 | 1.94 | — | 45 | 75 | — | — | — | — | — | — | — | — | 105 | — | 241 | 402 | 7 |

* Sample was insufficient for radioactive assay.

The results of the salt fractionation (Fig. 1) of labelled proteins of the Lubrol supernatant liquid are shown in Table 3. The values for the radioactivities of all the fractions are expressed as specific radioactivities relative to those of the total microsome proteins (= 100), so that the results of experiments with different doses of [¹⁴C]phenylalanine may be compared. The specific radioactivity of fraction W_s is appreciably less than that of fraction W_L . Fraction AS_{50} has a lower specific radioactivity than fraction AS_{100} . This final fraction AS_{100} , which has the highest specific radioactivity of any fraction in the Lubrol supernatant liquid, can be seen to be slightly more radioactive than the proteins of the sodium perfluoro-octanoate pellet even 2 min. after the administration of [¹⁴C]phenylalanine (Expt. A). On the whole the same pattern of distribution of radioactivity is shown at all the times examined. The behaviour of fraction M_s is somewhat variable and no regularity has been noticed. When the specific radioactivities of these protein fractions of the Lubrol supernatant liquid are compared with those of the Lubrol pellet the figures show that, with the exception of the experiments at 2 min. and also Expt. E, which is anomalous, the Lubrol pellet and its fractions have a lower radioactivity than the fractions of the Lubrol supernatant liquid. The results in Table 3 show that whereas the relative specific radioactivities of the Lubrol and the sodium perfluoro-octanoate pellets decreased with the length of the period of incorporation, the relative specific radioactivities of the fractions of the Lubrol supernatant liquid give evidence of a rise taking place between 2 and 6 min. after injection.

DISCUSSION

The present investigation has given further evidence of the existence of different microsomal protein fractions distinguishable by the extent of incorporation of a labelled amino acid. The first step in the process of fractionation, the treatment with a non-ionic detergent to produce a soluble protein fraction almost free from ribonucleic acid, may be compared with the solubilization of microsomal proteins which Littlefield *et al.* (1955) achieved by means of sodium deoxycholate. In the present investigation Lubrol W solubilized about one-half by weight of microsomal protein as compared with five-sixths which was solubilized by sodium deoxycholate. However, the protein solubilized by Lubrol W was more highly labelled than the larger quantity of protein solubilized by sodium deoxycholate, showing the presence of a fraction of low radioactivity in the sodium deoxycholate solution.

There is so far no evidence to suggest if and how such fractions as AS_{100} or its constituent proteins are derived from possible precursors in ribonucleoproteins. It was noteworthy, however, that the specific radioactivities of fractions M_s , W_s , AS_{50} and AS_{100} all rose at approximately the same rate as the specific radioactivity of total microsomal proteins. This simultaneous labelling of proteins should be contrasted with the results of Simkin & Work (1957), who found that the pattern of the specific radioactivities of all their protein fractions changed with time over a period of 3 hr. after injection of labelled amino acids. Although no information is available at present on the morphological side of this problem, the presence of ribonucleic acid in all the fractions examined by Simkin & Work (1957) would suggest that sodium chloride at different pH values breaks up microsomal protein and nucleoprotein complexes in a manner different from that of solubilizing agents. Since guinea pigs were used by Simkin & Work and rats in the present work a difference in behaviour of microsomes between these species cannot be ruled out.

The chemical nature of the different fractions of the present work has not been investigated further, but the solubility in half-saturated ammonium sulphate solution of the final fraction (AS_{100}) suggests that it may be an albumin, and it would be interesting to discover if it is related to serum albumin. In this connexion it may be significant that serum albumin having a high specific radioactivity has recently been detected by an immunological technique in liver microsomes (Campbell, Greengard & Kernot, 1958).

SUMMARY

1. The microsome fraction from rat liver was treated with the non-ionic detergent Lubrol W, which solubilized approximately one-half of the protein, 60–80% of the lipid phosphorus and a negligible amount of ribonucleic acid.

2. When rats were killed 2 min. after the injection of [¹⁴C]phenylalanine a higher specific radioactivity was found in the protein of the Lubrol pellet than of the supernatant liquid. Six minutes or longer after injection the specific radioactivity of the protein of the Lubrol supernatant liquid was more than twice that of the protein of the pellet.

3. Treatment of the Lubrol pellet with either sodium perfluoro-octanoate or sodium deoxycholate released proteins which, 2 min. after injection, possessed a low specific radioactivity. The pellets so obtained had a higher specific radioactivity than almost all other microsomal protein fractions examined.

4. The proteins of the Lubrol supernatant liquid were separated into four fractions, differences in solubility in strong solutions of magnesium sulphate, ammonium sulphate and water being utilized. The specific radioactivities of these fractions showed the same pattern of distribution, whatever the time interval after the injection of [^{14}C]phenylalanine. The final fraction containing these proteins least readily precipitated became labelled very quickly; 2 min. after injection it had a specific radioactivity as high as that of the proteins of the sodium perfluoro-octanoate pellet.

5. The present results indicate that a number of microsomal proteins which can easily be solubilized become labelled simultaneously.

The authors thank Mrs J. Tapley for technical assistance and Imperial Chemical Industries Ltd. for providing a sample of Lubrol W.

This investigation has been supported by grants to the Royal Cancer Hospital and the Chester Beatty Research Institute from the British Empire Cancer Campaign, the

Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

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Preparation of some Deoxyribonucleic acid-Protein Complexes from Rat-Liver Homogenates

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(Received 24 February 1958)

Preparation of deoxyribonucleic acid from rat-liver homogenates by the action of various salts and phenol (Kirby, 1957) showed that deoxyribonucleic acid free from protein could be isolated when the salt was *p*-aminosalicylate but that about 1% of residual protein remained attached to the deoxyribonucleic acid when benzoate was used. These results suggested that some deoxyribonucleic acid may be bound to the protein through metal linkages, and it was thought that further information could be obtained by isolation and analysis of deoxyribonucleic acid produced by the action of different salts.

For this investigation fluoride and azide were chosen as typical inorganic salts with considerable power of complexing with metals. The action of diethyldithiocarbamate was compared with that of diethylacetate and the action of 2-nitroso-1-naphthol-4-sulphonate with 1-naphthol-4-sulphonate, since the first and third of this group have considerable chelating abilities. *cyclo*Hexanediamine-*NNN'*-tetra-acetate was examined because this agent was effective in releasing deoxyribonucleic acid from rat liver whereas a very

similar chelating agent, ethylenediamine-*NNN'*-tetra-acetate, was not (Kirby, 1957). Finally the effect of benzoate at different concentrations was investigated to determine the effect of ionic concentration on the amount of residual protein attached to the deoxyribonucleic acid. It had been demonstrated previously that deoxyribonucleic acid extracted in the presence of 0.15M-salts contained more protein than when 0.3M-salts were used.

MATERIALS AND METHODS

Sodium salts were used in all cases and adjusted to pH 6.5-7.0 with NaOH or acetic acid with narrow-range indicator papers or a pH meter.

Isolation procedure. This was essentially as described previously. The animals were killed and their livers excised and dropped on to solid CO_2 . The livers (usually 50-75 g.) were broken up in the appropriate salt solution (400-600 ml.) in a Waring Blender for about 45 sec. The mixture was stirred and an equal volume of 90% phenol (w/w) was added immediately and stirring continued for 40 min. The conditions of centrifuging depended upon the viscosity of the aqueous layer, which was much more viscous when