Amino Acid Incorporation into the Protein of Mitochondrial Preparations from Cerebral Cortex and Spinal Cord

By H. S. BACHELARD*

Department of Biochemistry, Monash University, P.O. Box 92, Clayton, Victoria, Australia

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1. Washed guinea-pig cerebral-cortex mitochondria incorporate [^{14}C]leucine into their protein at a rate comparable with the rates reported for liver or heart mitochondria only if the mitochondria are separated from myelin and nerve endings by density-gradient centrifugation. 2. The non-mitochondrial components (myelin and nerve endings) of brain mitochondrial preparations incorporated [^{14}C]leucine at a negligible rate. 3. The mitochondria do not require an exogenous supply of energy or a full supply of amino acids to support the process. 4. The incorporation rate was linear up to 2 hr. aerobic incubation at 30° and was inhibited by chloramphenicol, only slightly by actinomycin D and not by penicillin or pretreatment with ribonuclease. The observed incorporation is considered to be unlikely to be due to contaminating cytoplasmic ribosomes or bacteria. 5. The process was also studied in mitochondrial preparations from rabbit cerebral cortex and spinal cord.

In recent years evidence has been presented in support of the claim by Simpson & McLean (1955) that mitochondria are capable of independent protein synthesis. McLean, Cohn, Brandt & Simpson (1958) demonstrated this capability in muscle and liver mitochondria *in vivo* and *in vitro*. Some studies have indicated that the mitochondrial systems might be as active as microsomal preparations in muscle (Simpson & McLean, 1955), mammary tissue (Fraser & Gutfreund, 1958) and yeast cells (Cooper, Harris & Neal, 1962). However, in brain tissue *in vivo*, the microsomal fraction was found to be the most highly labelled by radioactive amino acids (Waelsch, 1957; Furst, Lajtha & Waelsch, 1958; Clouet & Richter, 1959).

Studies on the incorporation of amino acids into the protein of isolated mitochondria from mammalian tissues have been restricted mainly to liver (Roodyn, Reis & Work, 1961; Braun, Marsh & Drabkin, 1963; Kroon, 1964) or muscle (Kalf, 1963, 1964; Kroon, 1964; Truman, 1964), and very little attention seems to have been paid to mitochondria from brain and nerve, which pose special problems. Mitochondrial preparations from brain, as usually obtained by differential centrifugation of sucrose homogenates, contain relatively few mitochondria and are heavily contaminated with non-mitochondrial material, in particular myelin and nerve endings (Whittaker, 1959; De Robertis, Pellegrino de Iraldi, Rodriguez de Lores Arnaiz & Salganicof, 1962). Klee & Sokoloff (1964) studied the incorpora-

tion of [^{14}C]leucine into such mitochondrial preparations in the presence of the microsomal and soluble fractions and found that the mitochondria from immature rat brain were considerably more active than those from the mature brain.

The view (Roodyn *et al.* 1961) that the possibility of independent protein synthesis in mammalian mitochondria should not be disregarded is of interest in considerations of general protein turnover in nervous tissue, particularly in peripheral nerve. Evidence has been presented in support of a local axonal mechanism for protein synthesis in peripheral nerve (Clouet & Waelsch, 1961; Koenig, 1965a), yet ribosomes are apparently absent (Palay & Palade, 1955; van Breeman, Anderson & Roger, 1958) and the RNA concentration, though it may be of ribosomal type, has been reported to be only 0.1–0.2% of the concentration in the nerve-cell bodies (Koenig, 1965b). On the other hand, mitochondria are known to occur in the axoplasm of peripheral nerve (Hess & Lansing, 1953; Webster, 1962).

EXPERIMENTAL

Preparation of mitochondria

Initial dispersions. Guinea pigs (400–500 g.) were stunned by a sharp blow on the back of the neck and killed by exsanguination; adult rabbits were killed by the injection of air into the ear vein. After removal of the whole brain the cerebral hemispheres were separated from the white matter, weighed on an August Sauter (Ebingen, Germany) torsion balance and placed immediately in 9 vol. of ice-cold 0.32 M-sucrose–2 mM-EDTA–20 mM-tris-HCl buffer, pH 7.4,

* Present address: Department of Biochemistry, Institute of Psychiatry, Maudsley Hospital, London, S.E. 5.

in a Teflon-pestle homogenizer (A. H. Thomas Co., Philadelphia, Pa., U.S.A., type B; internal diam. 20 mm., clearance 0.13–0.18 mm.). Grinding involved 12 passages of the pestle, rotating at 2000 rev./min., during 30 sec., with intermediate periods of cooling in ice–water.

When preparations from spinal cord were required, the whole spine was removed immediately after the death of the rabbits and was chopped into 1–2 in. lengths. The spinal cord was extruded with a steel knitting needle and treated exactly as described for the cerebral cortex.

Crude mitochondrial fraction. Centrifugal forces are expressed below as average g values calculated to the centres of the tubes. The whole dispersion was centrifuged at 900 g for 10 min. in the SS 34 head of a Servall RC2 centrifuge. The pellet was washed once by resuspension in the original volume of tri-buffered sucrose and recentrifugation. The pellet was discarded. The combined supernatant and washings were centrifuged at 11 000 g for 15 min. The supernatant was discarded and the pellet was washed five times in the original volume of buffered sucrose by resuspension and centrifugation. The pellet was finally suspended in the buffered sucrose (3 ml./g. of original tissue).

Mitochondrial subfractions. The crude mitochondrial fraction was subfractionated in a discontinuous gradient of sucrose with the following steps: 1.4 M, 1.2 M, 1.0 M and 0.8 M. This gradient was left for 8–10 hr. at 3° before use. The crude mitochondrial fraction, dispersed in buffered sucrose as 33% of the original tissue weight, was layered carefully on top of the density gradient and then centrifuged for 2 hr. at 50 000 g in the no. SW 25 head of a Spinco model L ultracentrifuge, by the procedure described by De Robertis *et al.* (1962). The four subfractions (*A*, *B*, *C* and *D*) that appeared in layers in the gradient were withdrawn with fine pipettes and were diluted to a sucrose content of 0.32 M. The pellet (*E*) was suspended in 0.32 M-sucrose. Each subfraction was centrifuged at 20 000 g for 15 min. in the no. 40 head of the Spinco model L ultracentrifuge and the pellets were resuspended in the original buffered sucrose (3 ml./g. of original tissue).

Incorporation studies. The mitochondrial fractions were used for the incorporation studies on the day of preparation. The basal incubation medium contained: KCl (75 mM), KH_2PO_4 (45 mM), MgSO_4 (7.5 mM), EDTA (1 mM) and tris (75 mM). All constituents except the MgSO_4 were mixed, adjusted to pH 7.4 with 2 N-HCl and autoclaved. The MgSO_4 solution was autoclaved separately and the two solutions were mixed under sterile conditions immediately before use. No further adjustment of pH was found to be necessary. In some experiments a sterile synthetic amino acid mixture was included, containing alanine (6.6%), arginine (2.0%), aspartic acid (4.9%), asparagine (1.6%), cystine (8.8%), glutamic acid (10.9%), glutamine (8.4%), glycine (5.5%), histidine (2.9%), isoleucine (1.5%), lysine (5.8%), methionine (11.2%), phenylalanine (1.7%), proline (2.1%), serine (5.9%), threonine (4.4%), tryptophan (3.8%), tyrosine (7.5%) and valine (4.4%) (Kroon, 1963).

The mitochondrial preparations (0.4 ml.) were incubated with 1.0 ml. of the basal medium, plus any additions as indicated in the text, in 1 in. tubes at 30° with vigorous shaking. The reactions were started, 5 min. later, by the addition of L-[U- ^{14}C]leucine (0.5 μC in 20 $\mu\text{l.}$) and stopped after the required time-interval by the addition of 6.6% (w/v) trichloroacetic acid in 33% (v/v) ethanol (5 ml.).

Ribonuclease pretreatment. A solution of ribonuclease was heated at 80° for 5 min., cooled, added when relevant to the mixture of mitochondria and medium to give a final concentration of 20 $\mu\text{g.}/\text{ml.}$ and incubated at 37° for 30 min. immediately before equilibration at 30° and the addition of L-[^{14}C]leucine.

Bacterial contamination. For measurements of bacterial contamination, samples of the final reaction mixture after 2 hr. incubation were diluted with sterile 0.9% NaCl and plated on agar plates of a malt extract–glucose–salts medium (Slonimski, 1953) adjusted to pH 7 with 2 N-KOH. Colonies were counted after 18 hr. incubation at 37°.

Reagents and analyses

Protein purification and estimation. The reaction mixtures containing the precipitated mitochondrial protein were quantitatively transferred to tapered centrifuge tubes and centrifuged in an MSE Minor bench centrifuge. The pellet was washed by resuspension in 6% (w/v) trichloroacetic acid and centrifugation. It was then suspended in 2 ml. of N-NaOH (which contained non-labelled L-leucine, 1 mg./ml.) and held at 37° for 30 min. After cooling and the addition of N-HCl (2 ml.) and 12% (w/v) trichloroacetic acid (4 ml.), the mixture was heated at 85–90° for 15 min., cooled to 0° and centrifuged. The pellet was washed by resuspension in 6% (w/v) trichloroacetic acid and centrifuged. After dehydration by two washes with acetone, the protein was defatted by incubation in ethanol–ether (2:1, v/v) at 60° for 15 min. and the mixture was cooled and centrifuged. The purified protein was washed with ether and dispersed finely and evenly over the tapered part of the centrifuge tube by leaving a little of the ether in the tube, dispersing the precipitate evenly through the ether and removing the ether at 40–45° with constant swirling. The protein was finally dissolved in formamide (0.6 ml.) and samples (10–50 $\mu\text{l.}$) were estimated according to the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard. Similar concentrations of formamide were included in the reagent blank and albumin standards as it diminished colour formation.

Radioactivity measurements. The radioactivity of the formamide solutions of the purified protein was counted in a Packard Tri-Carb model 314 EX liquid scintillation counter. The protein solution (0.4 ml.) was mixed with 10 ml. of the scintillator described by Bruno & Christian (1961). The formamide had no quenching effect; the absolute efficiency was 63–65%. In all experiments a zero-time assay was carried out by adding the [^{14}C]leucine and the 6.6% (w/v) trichloroacetic acid in 33% (v/v) ethanol to an unincubated mixture of the medium and the relevant mitochondrial preparation. The protein was purified by an identical procedure and the resultant specific radioactivity value was subtracted from the values for the incubated mitochondria in the same experimental run.

Succinate dehydrogenase. Estimations for succinate-dehydrogenase activity were carried out manometrically according to the method of Aldridge & Johnson (1959) with the modifications described by Schwartz, Bachelard & Mellwain (1962).

Electron microscopy. Samples of the mitochondrial preparations were centrifuged at 20 000 g for 15 min. and resuspended in 0.32 M-sucrose (the combination of fixative and the EDTA in the original sucrose was found to have

deleterious effects on the mitochondria during subsequent fixation). The sucrose suspensions (0.2–0.5 ml.) were added to centrifuge tubes containing 5 ml. of modified Dalton's fixative (Richardson, 1962) at 0°. After 90 min., further fixative was added to fill the tubes, which were centrifuged at 80 000*g* for 20 min. in the no. 40 head of the Spinco model L ultracentrifuge. Small pieces of the pellets were removed and treated as small tissue blocks. After a brief wash in water the pieces were dehydrated serially in aqueous ethanol of decreasing water content and finally in absolute ethanol. The pieces were embedded in methacrylate and sectioned on an LKB Ultratome. The sections (600–800 Å) were stained on grids with 0.3% (w/v) lead acetate in 0.1*N*-NaOH for 30 sec. and observed with a Siemens Elmiskop I electron microscope.

Reagents. All reagents, unless otherwise specified, were of A.R. purity. High-specific-activity L-[U-¹⁴C]leucine (41.8 or 155 mc/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks., and was diluted with non-labelled L-leucine to a specific activity of 10 μ C/ μ mole before use.

Ribonuclease, ADP, ATP and α -oxoglutaric acid were purchased from the Sigma Chemical Co., St Louis, Mo., U.S.A.

Lead acetate for electron microscopy was obtained from K and K Laboratories, Plainview, N.Y., U.S.A.

Fig. 1. Course of amino acid incorporation into crude mitochondrial suspensions prepared from sucrose homogenates by differential centrifugation. Mitochondria were incubated with 0.5 μ C of L-[¹⁴C]leucine (10 μ C/ μ mole) as described in the Experimental section. O, Untreated mitochondria; ●, after pretreatment with ribonuclease.

Pencillin G was supplied by Drug Houses of Australia (Laboratories) Pty. Ltd., Sydney, N.S.W., Australia, and chloramphenicol (Chloromycetin) by Parke, Davis and Co., Sydney, Australia. Actinomycin D was generously given by Professor A. W. Linnane, Department of Biochemistry, Monash University.

RESULTS

Incorporation of [¹⁴C]leucine into the protein of mitochondrial preparations from guinea-pig cerebral cortex. The crude mitochondrial fraction from the cerebral cortex of young guinea pigs incorporated [¹⁴C]leucine at a relatively low rate (Fig. 1) compared with the results obtained for the mitochondria from other mammalian tissues (Table 1). The rate (15 μ moles of leucine/mg. of protein/hr.) was linear over a period of 2 hr. and was not affected by preincubation with ribonuclease at 37° for 30 min.

The lack of inhibition of the incorporation rate by pretreatment with ribonuclease and the linearity of the rate over 2 hr. render it unlikely that the observed rate was due to contamination by cytoplasmic ribosomes.

The relatively low incorporation rate into the crude mitochondrial protein is understandable in view of the well-established heterogeneity (Whittaker, 1959; De Robertis *et al.* 1962) of such mitochondrial preparations. The small proportion of mitochondria in the crude mitochondrial fraction used in the present study is evident from the electron micrograph (Plate 1). The fact that the amino acid is incorporated into the mitochondrial protein is demonstrated in Fig. 2, where the incorporation rates into the various mitochondrial subfractions are presented. The highest incorporation rate was observed in subfraction *E*—the subfraction enriched in mitochondria; very little incorporation occurred in other subfractions (less than in the crude preparations). Electron microscopy (Plate 2) of subfraction *E* indicated that, apart from very few intact nerve endings, the preparation consisted almost entirely of mitochondria that were well-preserved, considering the lengthy purification

Table 1. Comparison of [¹⁴C]leucine incorporation into the protein of mitochondria from different mammalian tissues

Tissue	Incubation temp.	Incorporation rate (μ moles/mg. of protein/hr.)	Reference
Ox heart	30°	50*	Kroon (1963, 1964)
Ox liver	30	75*	Kroon (1964)
Rat heart	37	50	Kalf (1963)
Guinea-pig cerebral cortex (crude)	30	15	Present work
Guinea-pig cerebral cortex (subfraction <i>E</i>)	30	60–75	Present work

* My calculation from reported data.

Fig. 2. Course of amino acid incorporation into subfractions of the crude mitochondrial preparation after separation by sucrose-density-gradient centrifugation. □, Subfraction A (myelin fragments); ■, subfraction B (myelin fragments, vesicles); ○, subfraction C (nerve endings, vesicles); ∇, subfraction D (nerve endings); ●, subfraction E (mitochondria; Plate 2).

procedure involved. The rate ($70 \mu\mu\text{moles/mg. of protein/hr.}$) was very similar to the rates reported in the literature for mitochondria from other mammalian tissues (Table 1).

Certain mitochondrial preparations have been found to require exogenous ATP or ATP-generating systems (McLean *et al.* 1958; Roodyn *et al.* 1961; Kalf, 1963), whereas others have not (Kroon, 1963). Enhancement of incorporation to various degrees by a synthetic amino acid mixture has also been reported (Roodyn *et al.* 1961; Kalf, 1963; Kroon, 1963), but Truman (1964) found this to be unnecessary. The requirement of the purified mitochondria from guinea-pig cerebral cortex for exogenous substrates or energy sources was tested (Table 2). ATP, ADP or a mixture of ADP and α -oxoglutarate had no effect on the incorporation of L-[^{14}C]leucine. It seems reasonable to conclude that the endogenous substrates in the mitochondria were capable of maintaining the energy required for the amino acid incorporation, at least over the time-period (1 hr.) tested. The addition of a synthetic amino acid mixture also had no effect on the incorporation rate.

Table 3 shows the effects of antibiotics. Large doses of penicillin (330 units/ml.) had no detectable inhibitory effect. Since penicillin inhibits the growth of many bacteria by interfering with the synthesis of the bacterial cell wall (Gale, 1963)

Table 2. Effect of additions to the basal incubation medium on incorporation of amino acids

Purified mitochondria (subfraction E) from guinea-pig cerebral cortex were incubated in the basal medium with $0.5 \mu\text{C}$ of L-[^{14}C]leucine ($10 \mu\text{C}/\mu\text{mole}$) (see the Experimental section) for 1 hr. at 30° .

Addition	Radioactivity in protein	
	(counts/min./mg. of protein)	(% of control)
None	735	(100)
ADP (2 mm)	704	96
ATP (0.1 mm)	750	102
None	665	(100)
ADP (0.6 mm) + α -oxoglutarate (15 mm)	683	104
None	920	(100)
Amino acid mixture ($50 \mu\text{g./ml.}$)	952	104

Table 3. Effect of antibiotics on incorporation of amino acids

Purified mitochondria (subfraction E) from guinea-pig cerebral cortex were incubated in the basal medium with $0.5 \mu\text{C}$ of L-[^{14}C]leucine ($10 \mu\text{C}/\mu\text{mole}$) (see the Experimental section) for 1 hr. at 30° .

Antibiotic	Radioactivity in protein	
	(counts/min./mg. of protein)	(% of control)
None	920	(100)
Penicillin (330 units/ml.)	908	99
None	625	(100)
Actinomycin D ($40 \mu\text{g./ml.}$)	525	85
Chloramphenicol ($10 \mu\text{g./ml.}$)	284	45

rather than by inhibiting general protein synthesis, this result may be taken as part evidence that the observed incorporation rates were not due to bacterial contamination.

Actinomycin D, which is considered to inhibit protein synthesis by inhibiting the formation of messenger RNA (Goldberg & Reich, 1964), caused only slight inhibition (15%) at dose levels ($40 \mu\text{g./ml.}$) higher than those normally used. The concentration of actinomycin D used would be expected to suppress bacterial growth completely. Chloramphenicol, which is believed to inhibit protein synthesis by blocking the attachment of messenger RNA to the ribosome (Weisberger & Wolfe, 1964), actively inhibited the amino acid incorporation into the mitochondrial protein.

Amino acid incorporation into the protein of mitochondrial preparations from rabbit cerebral cortex and spinal cord. Crude mitochondrial suspen-

Electron micrograph of the crude mitochondria, prepared by differential centrifugation of sucrose homogenates, showing mitochondria, nerve endings and myelin fragments. Magnification $\times 42\,750$.

Electron micrograph of subfraction *E*, prepared by sucrose-density-gradient centrifugation of the crude mitochondria, consisting almost exclusively of mitochondria. Magnification $\times 28000$.

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Table 4. Incorporation of L-[^{14}C]leucine into the protein of mitochondrial preparations from rabbit cerebral cortex and spinal cord

Mitochondria were prepared and incubated with L-[^{14}C]leucine (0.5 μC) at 30° as described in the Experimental section.

Tissue	Mitochondria	Incorporation (counts/min./mg. of protein)		Succinate- dehydrogenase activity ($\mu\text{moles of CO}_2/\text{mg}.$ of protein/hr.)
		At 30 min.	At 60 min.	
Cerebral cortex	Crude	—	85	11.0
	Subfraction E	138	261	19.9
Spinal cord	Crude	—	49	4.1
	Subfraction E	114	239	16.6

sions and subfractions were prepared from the cerebral cortex and spinal cord of adult rabbits and incubated in the basal medium with 0.5 μC of L-[^{14}C]leucine as described in the Experimental section.

The rates of incorporation into the crude and subfraction E preparations are shown in Table 4. The purified mitochondria (subfraction E) from adult cerebral cortex and spinal cord incorporated the leucine into protein at similar rates, which were lower than those observed for purified mitochondria from guinea pigs (Fig. 2). The low rate of incorporation by the crude mitochondria from rabbit spinal cord and the relatively low succinate dehydrogenase activity (Table 4) suggest that the preparation from spinal cord is even more heavily contaminated with non-mitochondrial material and emphasizes the advantage to be gained by working with the relatively uncontaminated preparations obtained by sucrose-density-gradient centrifugation.

DISCUSSION

As pointed out by Roodyn *et al.* (1961), precise calculations of the weight of amino acid incorporated into mitochondrial protein cannot be made in ignorance of the size of the intramitochondrial amino acid pool. However, the approximations that can be made serve as a basis for comparison of the incorporation rates observed in different studies where the same labelled amino acid and similar conditions have been used. The rate of incorporation of [^{14}C]leucine into freshly prepared guinea-pig brain mitochondrial protein was found to be comparable with the rates reported for mitochondria from other mammalian tissues only if the brain mitochondria were separated from non-mitochondrial material (myelin and nerve endings) that contaminated the usual preparations made by differential centrifugation of sucrose homogenates. The non-mitochondrial contaminants had consider-

ably less activity than the purified mitochondria, and, further, the subfractions (C and D) that contain nerve endings (De Robertis *et al.* 1962) showed very little activity (Fig. 2). The results also suggest that the myelin in these preparations was not an important site of protein synthesis, since those subfractions (A and B) that contain myelin (De Robertis *et al.* 1962) showed virtually no activity under the conditions studied.

The rate of amino acid incorporation into subfraction E, the purified mitochondria (60–75 $\mu\text{moles of leucine/mg. of protein/hr.}$), was not far below the rates (150 $\mu\text{moles/mg. of protein/hr.}$) reported for [^{14}C]leucine (Zomzely, Roberts & Rapaport, 1964) and [^{14}C]lysine (Suzuki, Korey & Terry, 1964) incorporation into brain ribosomes. The ineffectiveness of ribonuclease pretreatment and the linearity of the rate up to 2 hr. (Figs. 1 and 2) render it unlikely that the rates observed in the present study were due to contamination by cytoplasmic ribosomes.

The possibility of bacterial contamination in such studies has been seriously considered by many workers to be negligible (Roodyn *et al.* 1961; Kalf, 1963; Kroon, 1963).

In the present study, attempts were made to limit bacterial contamination by using sterile media wherever possible. The level of bacterial contamination detected (400–1000 colonies/ml. after an incubation period of 2 hr. at 30°) was well below the levels considered insignificant by Roodyn *et al.* (1961), who found that the rate of amino acid incorporation into liver mitochondria did not vary with the number of bacteria present. The lack of inhibition by large doses of penicillin and the slight inhibition by relatively high concentrations of actinomycin D provide further evidence against the observed rates being due to contaminating micro-organisms. The view that the mitochondrial activity is not an artifact is supported further by the differences observed between mitochondria from normal and regenerating liver (Braun *et al.* 1963) or

between mitochondria from immature and mature rat brain (Klee & Sokoloff, 1964), since in both cases the bacterial contamination might be expected to be relatively consistent.

The purified mitochondrial preparation from guinea-pig cerebral cortex showed no requirement for exogenous sources of energy, in agreement with the reports by Kroon (1963) for ox-heart mitochondria, but different from the requirements shown by other heart and liver mitochondrial preparations (McLean *et al.* 1958; Roodyn *et al.* 1961; Kalf, 1963).

Evidence has been presented for the occurrence of DNA (Kalf, 1964), RNA, aminoacyl-RNA-synthetase activity (Wintersberger, 1965) and DNA-dependent RNA-polymerase activity (Luck & Reich, 1964) in mitochondria; Elaev (1964) has reported the isolation of ribosomes from rat-liver mitochondria. It is possible therefore (Luck & Reich, 1964) that independent mitochondrial protein synthesis might be directed by a mechanism similar to, but separate from, that occurring in the ribosome. In that case, the lack of real inhibition of the brain mitochondrial activity by actinomycin D, which is believed to act on DNA-dependent RNA nucleotidyltransferase (Goldberg & Reich, 1964), suggests that a relatively stable messenger RNA is involved in the brain mitochondrial system, assuming adequate permeation of the antibiotic into the organelle.

Chloramphenicol, which has been reported to be inactive on microsomal protein synthesis (Mager, 1960), actively inhibited the brain mitochondrial system, as was found to be the case with mitochondria from other sources (Mager, 1960; Kroon, 1963; Linnane, Biggs, Huang & Clark-Walker, 1966). This antibiotic is considered to act by preventing the attachment between messenger RNA and ribosomes (Weisberger & Wolfe, 1964) and it has been suggested, from chloramphenicol-induced changes observed in mitochondrial ultrastructure, that in higher organisms it is the mitochondrion that is the actual site of action of the drug (Linnane *et al.* 1966).

The significance of mitochondrial protein synthesis in the context of general cellular metabolism is difficult to assess since it is not clear which mitochondrial proteins are involved. Studies on disrupted mitochondria (Roodyn *et al.* 1961; Kroon, 1963; Truman, 1964) have indicated that it is structural rather than soluble proteins that are labelled. However, other reports have presented evidence that soluble mitochondrial proteins might be involved (Kalf & Simpson, 1959; Kalf & Grece, 1964).

Though purified nerve-tissue mitochondrial preparations are comparable in amino acid-incorporating ability with the most active mitochondria from other sources, the significance of this system in

considerations of protein synthesis in peripheral nerve is dependent on the type of protein labelled.

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