Thus another instance confirming the fact that enzymic steroid hydroxylations occurs by way of a simple displacement of the hydrogen of the position oxygenated has now been documented. Previous instances are those at carbons 11α and 11β from these laboratories[1], and C-11α[4] and C-7α[5] from other laboratories.

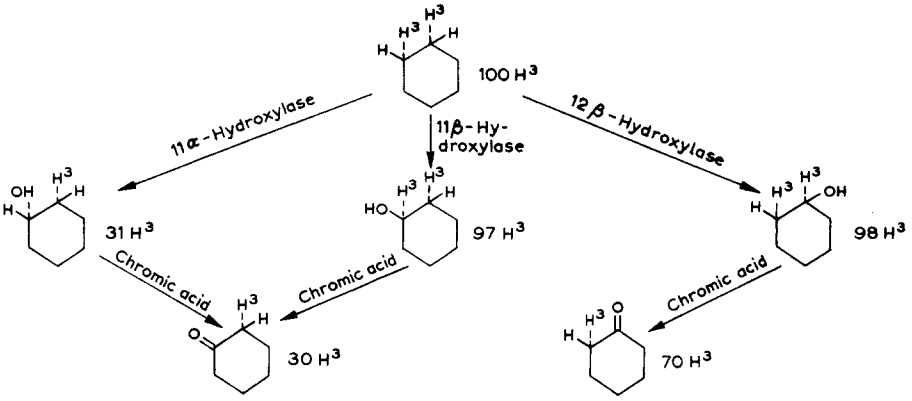

Fig. 1.

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The role of cell sap in the incorporation of ^{14}C-labelled leucine into proteins of isolated rat-liver mitochondria

The rate of incorporation of intravenously injected amino acid into the microsomal nucleoproteins of rat liver greatly exceeds that into other subcellular fractions and it has been suggested that most or all of the proteins of the living cell are synthesised in this particulate fraction[1]. This hypothesis has received support from the fact that

when microsomes are incubated in the absence of other particulate fractions, an active incorporation of amino acids into the microsomal protein takes place[2]. However, an energy-dependent incorporation of amino acids into proteins, in the absence of appreciable amounts of microsomes, has been observed when mitochondrial preparations are incubated either aerobically[3] or anaerobically[4].

The object of the present study was to compare some of the requirements for amino acid incorporation into the proteins of isolated mitochondria with those for microsomal incorporation.

ZAMECNIK AND KELLER[5] found that cell sap is required for the energy-dependent incorporation of amino acids into liver microsomal protein. In the presence of guanosinetriphosphate (GTP) and a suitable energy source, the cell sap can be replaced by a fraction which precipitates at pH 5 ("pH 5 fraction")[6] and contains both protein and ribonucleic acid. In the present study rat-liver mitochondria were incubated anaerobically with ^{14}C-labelled leucine in the presence of phosphoenolpyruvate (PEP) and adenosine triphosphate (ATP), with and without fractions of the cell sap, and the specific radioactivity of the mitochondrial protein was determined. For the purpose of comparison similar experiments were carried out simultaneously with microsomes. The results are shown in Table I.

TABLE I

THE EFFECT OF CELL-SAP FRACTIONS ON THE AMINO ACID UPTAKE BY MITOCHONDRIAL AND MICROSOMAL PROTEINS

Rat-liver mitochondria[8] or microsomes[8] (8–9 mg protein) were incubated with 0.5 μC [^{14}C]leucine, in a medium containing 0.01 *M* $MgCl_2$, 0.02 *M* potassium phosphate (pH 7.8), 0.03 *M* $KHCO_3$, 0.025 *M* KCl, 0.35 *M* sucrose and PEP-Kinase, at 37°, under 95% N_2/5% CO_2, in a total vol. of 1 ml. Whole cell sap, when added, represented that obtained from 0.1 g liver. When "pH 5 fraction" was used, 0.25 μmole GTP was also included in the incubation mixture. After 50 min incubation the particulate fractions were isolated from the medium by centrifugation. The extracted and washed[9] proteins were plated on 0.28 cm^2 discs and their radioactivity determined at infinite thickness in a windowless flow counter. The figures in parentheses are corrected for the presence of microsomes as described in the text.

Cell sap components present:	*Radioactivity of protein (counts/min)*			
	Mitochondria		*Microsomes*	
	Control	*2 µmoles ATP 10 µmoles PEP*	*Control*	*2 µmoles ATP 10 µmoles PEP*
None	30	76 (82)	28	42
Boiled cell sap	27	80 (87)	28	40
Whole cell sap	36	216 (199)	43	637
"pH 5 fraction"	24	132 (101)	20	604

It can be seen that the extent of leucine incorporation into mitochondrial protein in the absence of cell sap is similar to that in the presence of boiled cell sap, as is the case with microsomes. Under these conditions the two preparations differ, however, in that the mitochondrial exhibits a higher energy-dependent incorporation than the microsomal, both in absolute value and, even more so, relatively to the extent of incorporation in the presence of whole cell sap. It was observed in the course of these studies that the quantity of "pH 5 fraction" bringing about maximal incorporation was the same for mitochondria and microsomes and in the experiments

given in the Table this optimal amount, containing 1.8 mg protein and 0.04 mg ribonucleic acid (RNA), was used. The difference between the two preparations with respect to the "pH 5 fraction" is that the mitochondrial incorporation is considerably lower with this fraction than with whole cell sap, whereas the microsomal incorporation is as high.

The mitochondrial preparations used in the present studies contained 2 mg RNA/100 mg protein. It is possible that this is a result of a contamination by microsomes and on this basis the maximum extent to which the amino acid incorporation by such mitochondria may be due to the microsomes present has been calculated. Since the RNA content of microsomes is 12.8 mg/100 mg protein[7], about one sixth of the "mitochondrial protein" might originate from microsomes. In the absence of cell sap or in the presence of boiled cell sap, the incorporation into microsomal protein is lower than into mitochondrial protein, so that any contamination by microsomes could only have reduced the mitochondrial incorporation. It has been found previously[4] that mitochondria inhibited by about 50 % the microsomal incorporation catalysed by cell sap, and, therefore, the specific radioactivity of the contaminating microsomes would be only one half of that shown in Table I. Thus, the maximum contribution made by the contaminating microsomes would reduce the figure obtained for the energy-dependent incorporation into mitochondria with whole cell sap by 8 % and with "pH 5 fraction" by 24 %.

From similar experiments using lysine instead of leucine as the labelled amino acid, essentially the same picture emerged.

Two points can be emphasised from the results of these experiments. First, a significant energy-dependent amino acid incorporation into the proteins of isolated mitochondria takes place in the absence of cell sap, the extent of which is not much more than doubled by the presence of the whole cell sap. Secondly, only a small part, if any, of the amino acid incorporation into the protein of isolated mitochondria appears to proceed under these conditions by a pathway involving the "pH 5 fraction". This fraction does not seem to include all the factors responsible for the stimulating effect of whole cell sap upon mitochondrial incorporation.

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