again poorly eluted from silica gel and insufficient material was obtained for further purification. Table II gives the specific activities at each stage, and the results confirm those already obtained from the experiment *in vivo* that the ratio of the labelling in ubiquinone and ubichromenol lies somewhere between 2 and 4 to unity. If ubichromenol were an artefact the specific activities of the two compounds must be, within experimental error, identical. In a recent publication, JOSHI *et al.*[14] have suggested that ubichromenol may be a precursor of ubiquinone in the rat. Our results do not support this hypothesis: rather they suggest that either ubichromenol arises from ubiquinone or that the two substances have a common precursor.

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[1] D. L. LAIDMAN, R. A. MORTON, J. Y. F. PATERSON AND J. F. PENNOCK, *Biochem. J.*, 74 (1960) 541.
[2] A. T. DIPLOCK, E. E. EDWIN, J. GREEN, J. BUNYAN AND S. MARCINKIEWICZ, *Nature*, 186 (1960) 554.
[3] D. E. M. LAWSON, E. I. MERCER, J. GLOVER AND R. A. MORTON, *Biochem. J.*, 74 (1960) 38P.
[4] E. E. EDWIN, A. T. DIPLOCK, J. BUNYAN AND J. GREEN, *Biochem. J.*, 79 (1960) 91.
[5] J. LINKS, *Biochim. Biophys. Acta*, 38 (1960) 193.
[6] H. H. DRAPER AND A. S. CSALLANY, *Biochem. Biophys. Res. Commun.*, 2 (1960) 307.
[7] J. STEVENSON, P. J. HAYWARD, F. W. HEMMING AND R. A. MORTON, *Nature*, 196 (1962) 1291.
[8] D. MCHALE, J. GREEN AND A. T. DIPLOCK, *Nature*, 196 (1962) 1293.
[9] A. T. DIPLOCK, J. GREEN, E. E. EDWIN AND J. BUNYAN, *Biochem. J.*, 76 (1960) 563.
[10] A. T. DIPLOCK, E. E. EDWIN, J. BUNYAN AND J. GREEN, *Brit. J. Nutr.*, 15 (1961) 425.
[11] C. MARTIUS, *Quinones in Electron Transport*, Ciba Symposium, Churchill Ltd., London, 1961, p. 312.
[12] O. WISS, U. GLOOR AND F. WEBER, *Quinones in Electron Transport*, Ciba Symposium, Churchill Ltd., London, 1961, p. 264.
[13] O. WISS, U. GLOOR AND F. WEBER, *Am. J. Clin. Nutr.*, 9 (1961) 27.
[14] V. C. JOSHI, J. JAYARAMAN AND T. RAMASARMA, *Biochem. J.*, 88 (1963) 25.

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On the incorporation *in vitro* of [^{14}C]leucine into rat pancreas

On incubation *in vitro* of a number of pooled rat pancreases we found a poorly reproducible incorporation of [^{14}C]leucine into the nuclei and microsomes, isolated from the tissue after incubation. We presumed that this was caused by functional differences between the pancreases used.

Therefore we tried to bring the pancreases that were to be pooled to the same functional state by using rats that had been brought into the same condition. In one typical experiment the animals had fasted for 48 h. In a second set of experiments, the rats that had fasted for 48 h were given a pilocarpine injection. By these measures a depletion of the pancreas and a subsequent synchronization and enhancement of the synthetic processes were envisaged.

White Wistar rats, 3–4 months of age, were used throughout these experiments. A dose of 3 mg pilocarpine·HCl in 0.5 ml saline[1] was injected subcutaneously, 15 min before sacrifice.

The pancreas was removed from the animal immediately after decapitation, cleaned, and cut into pieces of about 2 mm × 2 mm × 2 mm with a sharp razor blade. Ten pancreases were worked up at a time and the pieces were washed three times with ice-cold Krebs–improved Ringers-III medium. The tissue was divided into four portions and these portions were put into four Cr-Ni gauze containers, which fitted into a glass vessel containing the incubation medium. This glass container was shaken in a water bath at 37°. The incubation mixture consisted of about 3 g of pancreas pieces in 16 ml medium. After 10 min preincubation 4 ml of a [^{14}C]leucine solution in saline (10 μC, specific activity 7.2 mC/mmole) were added.

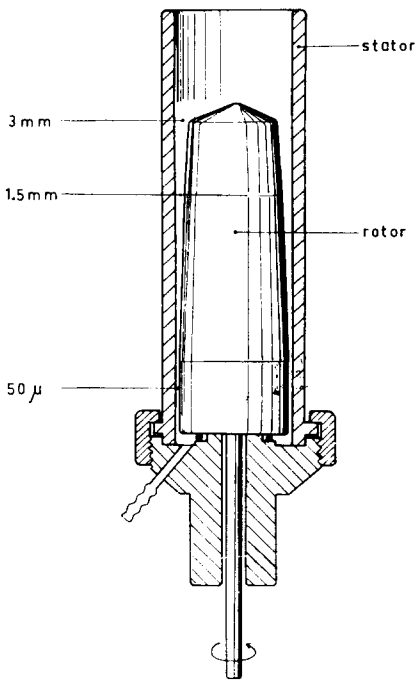

Fig. 1. Homogenizer.

At 5-min intervals after the addition of the leucine, the Cr-Ni gauzes were lifted from the glass container, the pancreas was washed three times with an excess of ice-cold medium, brought into 70 % glycerol, and the nuclei and the microsomes were immediately isolated from the tissue.

In order to be able to perform the homogenization procedure at 5-min intervals, we had to modify the technique previously described[2]. To this end we have constructed a new apparatus, especially suitable for the homogenization of pancreas in 70 % glycerol. This homogenizer consists (Fig. 1) of a partly conical, partly cylindrical rotor within a cylindrical stator. The clearance between the cylindrical parts of the two should be about 50 μ. This apparatus can be used, dismantled, cleaned, and reassembled within 5 min.

The isolation of the nuclei from the homogenate takes place in the same manner as in our previous publication.

TABLE I

EFFECT OF PRETREATMENT OF THE LIVING RAT ON THE INCORPORATION *in vitro* OF LEUCINE INTO MICROSOMES AND NUCLEI

The activity is expressed in counts/min in an infinite layer (diameter 6 mm) of dry microsomal and nuclear powder. The means of three experiments for each pretreatment are given with the extreme values within parentheses.

Pretreatment	*Fraction*	*5 min*	*10 min*	*15 min*	*20 min*
None	microsomes	145 (94–207)	168 (103–221)	264 (170–349)	267 (175–336)
	nuclei	14 (11–22)	34 (26–38)	48 (36–58)	49 (39–55)
Fasted (48 h)	microsomes	109 (78–144)	160 (117–191)	209 (120–308)	228 (146–299)
	nuclei	10 (6–14)	20 (17–24)	29 (21–42)	33 (20–47)
Fasted (48 h) + pilocarpine injection	microsomes	63 (60–66)	118 (117–118)	152 (130–174)	189 (143–234)
	nuclei	7 (6–7)	15 (14–15)	21 (20–22)	27 (26–28)

The microsomes were isolated from the first supernatant in the procedure for the preparation of nuclei. This supernatant was diluted with an equal volume of 10% glycerol, resulting in a 40% glycerol suspension, from which the mitochondria were removed by centrifugation in a Spinco L centrifuge at an average centrifugal force of $30000 \times g$ for 7 min. The microsomes were collected from the supernatant by centrifugation at $105000 \times g$ for 90 min.

The isolated nuclei and microsomes were washed twice with 50% ethanol and dried at 50°. The samples were powdered in a mortar and brought on to an Al counting plate with a diameter of 6 mm. Care was taken that the quantity of material, in which the radioactivity had to be measured, amounted to at least 12 mg/cm², so that the conditions for measurement at infinite thickness were met to a good approximation[3].

The results are represented in Table I. Fasting of the animals did not markedly improve the reproducibility of the results. The same applied to the pretreatment consisting of fasting plus pilocarpine administration except for the nuclei in which the spread in the results was definitively lower than in untreated animals.

It is clear that the rates of incorporation *in vitro* into microsomes and into nuclei were influenced in a negative sense by fasting of the living rat. It is surprising that pilocarpine injection after fasting caused a further decrement of the incorporation rates, pilocarpine being a well-known stimulating agent for the pancreas in the living animal.

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[1] A. K. LAIRD AND A. D. BARTON, *Biochim. Biophys. Acta*, 27 (1958) 12.
[2] C. POORT, *Biochim. Biophys. Acta*, 23 (1957) 34.
[3] R. W. HENDLER, *Science*, 130 (1959) 772.

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