by 82%. In the isolated perfused rat heart, α -bromopalmitate (25.8mg./100ml.) depressed the oxidation of [1^{14}C]palmitate by 53%.

The effects of α -bromopalmitate (25.8mg./100ml.) on the metabolism of [$U^{14}\text{C}$]glucose were studied in hearts from fed, 48hr.-starved and alloxan-diabetic rats perfused in a recirculation apparatus (Morgan, Henderson, Regen & Park, 1961), modified to permit collection of metabolic $^{14}\text{CO}_2$. Perfusion media were as described by Garland, Newsholme & Randle (1964). Results are expressed as μmoles of glucose equivalents/g. wet wt./hr. (means of five hearts $\pm$ S.E.M.).

Glucose uptake by fed-rat hearts was unaffected by α -bromopalmitate in the presence or absence of added insulin (0.1 units/ml.). In starved-rat hearts glucose uptake was increased by α -bromopalmitate if insulin was present from 31.1 ± 9.4 to 54.9 ± 6.7 ($P < 0.01$). In hearts from alloxan-diabetic rats the increase was from 7.8 ± 3.9 to 19.5 ± 6.7 ($P < 0.01$) in the absence of added insulin and from 8.9 ± 2.2 to 39.4 ± 5.6 ($P < 0.01$) with insulin.

α -Bromopalmitate stimulated the oxidation of [$U^{14}\text{C}$]glucose to $^{14}\text{CO}_2$ in all hearts and the effect was increased by added insulin. The greatest increase was observed in hearts from alloxan-diabetic rats, with a fivefold stimulation from 3.5 ± 1.4 to 17.1 ± 4.2 ($P < 0.01$).

Incorporation of [$U^{14}\text{C}$]glucose into glycogen was depressed by α -bromopalmitate; the maximum effect was in hearts from starved rats with added insulin, where incorporation fell from 4.1 ± 0.3 to 1.8 ± 0.8 ($P < 0.01$).

Gluconeogenesis by pigeon liver homogenates from [2^{14}C]lactate was stimulated by 1mM-DL-carnitine (48%) and by carnitine + 0.5mM-palmitate (104%), but neither protected against the potent inhibition by 0.5mM- α -bromopalmitate (90%) (cf. Delisle & Fritz, 1967; Corredor, Brendel & Bressler, 1967). Similar results were obtained with [$U^{14}\text{C}$]alanine or [$U^{14}\text{C}$]aspartate as substrates for gluconeogenesis.

In intact rats, α -bromopalmitate, at 50mg./kg. either intravenously or intraperitoneally, significantly lowered blood glucose, depressed [1^{14}C]palmitate oxidation by 68% and stimulated [$U^{14}\text{C}$]glucose oxidation by 52%.

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Studies on the Isoniazid-Copper Interaction

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Although Sorkin, Roth & Erlenmayer (1952) have reported that an isoniazid-copper blue complex has an enhanced tuberculostatic activity *in vitro* compared with that of isoniazid, this conclusion has not been supported by Pothmann & Stüttgen (1955).

Evidence is now presented suggesting that the different reports on the enhancement of isoniazid activity by copper could be attributed to the fact that the blue complex reported to have an absorption maximum at 760m μ (Sorkin *et al.* 1952; Fallab & Erlenmayer, 1953) may correspond to an incomplete isoniazid-copper interaction. It has now been found that such a complex shows a progressive hyperchromicity during a 30hr. period accompanied by an absorption shift to shorter wavelengths on a much broader region (680–720m μ). A comparable transition, in which the blue complex turns immediately into the brown complex obtained upon standing, is observed after autoclaving even for 5min. Examination of the 1:1 and 2:1 isoniazid-copper complexes by electron spin resonance detected shifts in the corresponding spectroscopic splitting factors indicating that the 2:1 complex has a more covalent nature than the 1:1, this effect being more consistent with comparable displacements observed in the absorption maximum of the corresponding optical spectra. The amplitude of the cupric spin resonance signal in the complexes studied, as compared with that given by CuSO_4 , shows that under aerobic conditions the reduction of cupric ions on interaction with isoniazid does not exceed 20%. Assays of the biological activity of the 1:1 complex against a streptomycin-resistant mutant of *Mycobacterium smegmatis* A.T.C.C. 607 shows that compared to isoniazid or copper alone, the complex is far more bactericidal. The present results demonstrating the gradual formation of the isoniazid-copper complex are consistent with the report by Winder (1960), who observed a marked increase in the inhibitory effect of isoniazid on the catalase of *Mycobacterium tuberculosis* BCG upon preincubating isoniazid and copper for 3hr., compared with the same experiment without preincubation. It is concluded then that isoniazid reacts progressively with copper, forming a complex which has a considerably enhanced mycobactericidal activity compared with that of isoniazid or copper alone.

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Assay of a Microsomal Marker Enzyme: Rat Liver Arylsulphatase C

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At least three arylsulphatase enzymes (aryl-sulphate sulphohydrolases, EC 3.1.6.1) distinguishable by their activity towards various substrates, their tissue distribution and intracellular localization have been shown to co-exist in certain mammals (Dodgson, Spencer & Thomas, 1955). In rat liver, two of the enzymes, arylsulphatases A and B, are localized mainly in the lysosome fraction of the cell (Viala & Gianetto, 1955), whereas the third one, arylsulphatase C, is in the microsome fraction, where it is present in a highly insoluble form (Dodgson, Spencer & Thomas, 1954; Dodgson, Rose & Spencer, 1957). The combined activity of arylsulphatases A and B measured under arbitrary conditions is frequently used in cell-fractionation studies as a marker for the lysosome fraction and various studies on arylsulphatase C (e.g. Spencer, Dodgson, Rose & Thomas, 1955; Dodgson *et al.* 1957) have indicated that this enzyme would be useful for the identification of the microsome fraction. This latter possibility has now been investigated fully following the establishment of experimental conditions for the specific assay of the enzyme.

Potassium *p*-nitrophenyl sulphate has frequently been used as a chromogenic substrate for the assay of arylsulphatase C (cf. Dodgson *et al.* 1955), but is unsuitable for use in fractionation studies on liver since the liberated *p*-nitrophenol is not quantitatively recoverable from whole homogenates of this tissue. Furthermore, the activity of the rat liver enzyme towards this substrate is affected by chloride, which is sometimes used as a component of the fractionation and assay media. However, potassium *p*-acetylphenyl sulphate, the substrate chosen originally by Dodgson, Spencer & Thomas (1953) for measuring arylsulphatase activity in rat tissues, shows none of these limitations in that the liberated phenol is completely recoverable from whole homogenates and enzyme activity towards the substrate is not affected by chloride. It has now been shown that this substrate can be used for the assay of arylsulphatase C, provided that the reaction is performed in the presence of a concentration of phosphate ions that is sufficient to inhibit arylsulphatases A and B completely.

The spectrophotometric assay is performed according to the directions of Dodgson & Spencer (1953) with some minor modifications and using a final concentration of potassium *p*-acetylphenyl sulphate of 40 mM in 0.1 M-sodium phosphate buffer, pH 8.0 (optimum conditions).

Under these conditions it has been confirmed that arylsulphatase C sediments in the microsome fraction. Subfractionation of the microsome pellet into rough and smooth endoplasmic reticulum by the method of Dallner, Siekevitz & Palade (1966) has demonstrated that the enzyme is associated with the membrane component of the system (cf. glucose 6-phosphatase).

The intracellular distribution pattern of arylsulphatase C, combined with the relative simplicity of the assay procedure, suggests that the enzyme is a useful marker for the membrane component of the endoplasmic reticulum of the rat liver cell. Whether it can be used with other tissues and species remains to be tested.

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The Formation of Rough Vesicles by the Interaction of Reticulosomes, Phospholipids and Ribosomes

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Reticulosomes as isolated from rat liver are non-membranous granules consisting of protein and phospholipid (protein/phospholipid ratio 8:1), and in the presence of micellar phospholipid they form a lipoprotein complex, which, when viewed with the electron microscope, resembles a membranous reticulum (Pollak, Ward & Shorey, 1966).

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