et al., 1963), which may explain its slower effect on fatty acid and ketone body concentrations. Galactose is only slowly converted into glucose by the perfused liver (Ross, Hems & Krebs, 1967), and in this investigation galactose re-feeding produced no change in blood glucose. The eventual fall of ketone body and fatty acid concentrations may be explained by galactose inhibition of fatty acid release from adipose tissue (see Buckle, Rubinstein, McGarry & Beck, 1961).

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Pathways of Lipid Synthesis in the Sheep Intestine

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In monogastric animals dietary triglycerides are hydrolysed in the lumen of the small intestine to monoglycerides and free fatty acids, which are then absorbed into the mucosal cell. Re-synthesis of triglyceride occurs by two pathways, the monoglyceride pathway accounting for 75%, and the α -glycerophosphate pathway for 25%, of resynthesized triglyceride (Mattson & Volpenhein, 1964). In the ruminant animal, because of lipolysis by rumen micro-organisms, most of the dietary fat reaches the small intestine in the form of free fatty acids (Garton, 1960) and only negligible amounts of monoglyceride are detected in the intestinal contents of the sheep (Leat & Harrison, 1967). It was therefore of interest to determine if the monoglyceride pathway was functional in the ruminant intestine.

Segments (200 mg. wet wt.) of the small intestine of lambs and sheep were incubated *in vitro* basically according to Kern & Borgstrom (1966) and the synthesis of lipids from various combinations of the following substrates was determined: [9,10-³H]-palmitic acid, [1-¹⁴C]oleic acid, [1-¹⁴C]glycerol and [1-¹⁴C]glycerol-labelled [³H]monopalmitin. Similar studies were made *in vivo* in anaesthetized sheep by using isolated intestinal loops. Lipid extracts of the intestinal tissue were subjected to thin-layer

chromatography to determine the nature and extent of lipid synthesis.

It was found that the ¹⁴C-labelled glycerol from both the 1- and the 2-[³H]monopalmitate was incorporated into triglycerides by the intestine of the sheep with little quantitative difference between the foetus, lamb and adult. The triglyceride synthesized via the monoglyceride pathway at times exceeded 50% of the total glyceride synthesis, whereas the incorporation of free glycerol on an equimolar basis was on average less than 10% of that of monoglyceride glycerol. The 2-monopalmitate was incorporated into triglycerides to a greater extent than was the 1-monopalmitate, partly because there was less hydrolysis of the 2-isomer. In homogenates of sheep intestine, however, monoglycerides have been reported to be ineffective as precursors of triglyceride (Bickerstaffe & Annison, 1968).

The incorporation *in vivo* of 1- and 2-monopalmitate into triglyceride in the intestinal mucosa in intestinal loops was similar to that observed *in vitro* with intestinal segments, with little difference found between sheep and rats.

It may be concluded that, although under normal conditions the contents of the small intestine of the sheep contain negligible amounts of monoglyceride, the intestinal wall still maintains the ability to absorb monoglycerides intact and incorporate them directly into triglycerides.

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Some Effects of α -Bromopalmitate, an Inhibitor of Fatty Acid Oxidation, on Carbohydrate Metabolism in the Rat

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There is evidence that the depression of carbohydrate utilization by muscle and the stimulation of gluconeogenesis by the liver observed in conditions of high lipid mobilization are caused by products of fatty acid oxidation (Randle, Newsholme & Garland, 1964; Williamson, 1967). The effects of α -bromopalmitate, an inhibitor of fatty acid oxidation, on some aspects of carbohydrate metabolism have therefore been studied.

α -Bromopalmitate (0.25 mM) inhibited the oxidation of [1-¹⁴C]palmitate by rat heart homogenates

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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