Hearse, D. J., Powell, G. M., Olavesen, A. H. & Dodgson, K. S. (1967). *Biochem. J.* **105**, 33P.
Hearse, D. J., Powell, G. M., Olavesen, A. H. & Dodgson, K. S. (1968). *Biochem. J.* **107**, 24P.

Identification of the Amino Acid Residues that Bind Azo-dye in the Soluble Azo-Dye Carcinogen-Binding Proteins of the Rat Liver

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Metabolites of azo-dye carcinogens bind covalently to specific soluble proteins in the rat liver (Miller & Miller, 1947; Sorof, Young, McCue & Fetterman, 1963). A basic azo-dye-binding protein has been isolated in apparently pure form and a small-molecular azo-dye-binding protein fraction has been substantially purified (Ketterer, Ross-Mansell & Whitehead, 1967). Considerable interest attaches to the identity of the amino acid(s) involved in carcinogen binding. A sulphur amino acid has been implicated by Andersen (1964) and evidence has been published that this is methionine (Scribner, Miller & Miller, 1965; Higashinakagawa, Matsumoto & Terayama, 1966).

Previous workers have used whole homogenate as starting material. The present authors have studied azo-dye-bound protein fragments obtained from purified azo-dye-binding proteins into which label has become incorporated from either [^{35}S]-methionine or [^{35}S]cysteine.

Fifty mg. of 4-dimethylamino-3′-methylazobenzene in 2ml. of corn oil and 60μc of labelled amino acid were injected into rats 40hr. and 16hr. respectively before they were killed. Basic and small-molecular azo-dye-binding proteins were isolated and digested with Pronase. Azo-dye-bound peptides were extracted and degraded further by alkaline hydrolysis. These fragments were purified by 'fingerprinting', eluted with formic acid, estimated spectrophotometrically and their specific radioactivities determined. The specific radioactivity of methionine and cysteine obtained from the same protein fractions was also determined.

After the administration of [^{35}S]methionine the specific radioactivity of azo-dye-bound protein fragments from basic azo-dye-binding protein, small-molecular azo-dye-binding protein, cysteine and methionine were all of the same order. After the administration of [^{35}S]cysteine, however, there was radioactivity in the azo-dye-bound fragments from the basic protein and cysteine only.

Thus, although the azo-dye binds to methionine in the small-molecular azo-dye-binding protein fraction, in the case of the basic azo-dye-binding protein, which is the major soluble dye-binding protein, it binds to cysteine.

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Andersen, R. A. (1964). Ph.D. Thesis: University of Wisconsin.
Higashinakagawa, T., Matsumoto, M. & Terayama, H. (1966). *Biochem. biophys. Res. Commun.* **24**, 811.
Ketterer, B., Ross-Mansell, P. & Whitehead, J. K. (1967). *Biochem. J.* **103**, 316.
Miller, E. C. & Miller, J. A. (1947). *Cancer Res.* **7**, 468.
Scribner, J. D., Miller, J. A. & Miller, E. C. (1965). *Biochem. biophys. Res. Commun.* **20**, 560.
Sorof, S., Young, E. M. & Fetterman, P. L. (1963). *Cancer Res.* **23**, 864.

Blood Concentrations of Ketone Bodies and Non-Esterified Fatty Acids in Starved and Re-Fed Rats

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Fritz (1961) suggested that the rate of ketone-body formation by the liver was dependent on the plasma concentration of non-esterified fatty acids; experimental support for this idea was obtained by Ontko & Zilversmit (1966). However, Foster (1967) has recently proposed that ketone-body formation may be independent of the fatty acid concentration. This problem has been investigated by force-feeding 48hr.-starved rats (males weighing 250g.) with solutions of glucose, fructose, galactose or casein (0·5–2g./rat) under light ether anaesthesia; at various times (10–240min.) after re-feeding, blood was collected by aortic cannulation under ether anaesthesia. Blood fatty acids and ketone bodies were estimated by titration and by enzymic analysis respectively.

Glucose, fructose or casein feeding resulted in a rapid decrease in both fatty acids and ketone bodies; in a systematic time-course study the decrease in blood ketone bodies was always accompanied by a decrease in the plasma concentration of fatty acids. Re-feeding with galactose also caused a decrease in ketone bodies and fatty acids, but this occurred only after several hours.

These results support the hypothesis that the rate of ketone body production by the liver *in vivo* is dependent on the plasma concentration of fatty acids; it does not, however, preclude the possibility that the liver can regulate the rate of ketone body production independently of the fatty acid concentration.

The rapid fall in the fatty acid concentration after re-feeding with fructose and casein may be explained by the observations that these compounds can stimulate insulin release (Berger, Joondeph & Rachmeler, 1964; Floyd, Fajans, Conn, Knopf & Rull, 1966; Grodsky *et al.*, 1963). However, galactose does not cause insulin release (Grodsky

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).

Berger, S., Joondeph, H. C. & Rachmeler, B. (1964). *J. Lab. clin. Med.* **64**, 841.
Buckle, R. M., Rubinstein, D., McGarry, E. E. & Beck, J. C. (1961). *Endocrinology*, **69**, 1009.
Floyd, J. C., jun., Fajans, S. S., Conn, J. W., Knopf, F. R. & Rull, J. (1966). *J. clin. Invest.* **45**, 1479.
Foster, D. W. (1967). *J. clin. Invest.* **46**, 1283.
Fritz, I. B. (1961). *Physiol Rev.* **41**, 52.
Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B. & Smith, D. F. (1963). *Amer. J. Physiol.* **205**, 638.
Ontko, J. A. & Zilversmit, D. B. (1966). *Proc. Soc. exp. Biol., N.Y.*, **121**, 319.
Ross, B. D., Hems, R. & Krebs, H. A. (1967). *Biochem. J.* **102**, 942.

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. Resynthesis 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 (200mg. 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-^{3}H]-palmitic acid, [1-^{14}C]oleic acid, [1-^{14}C]glycerol and [^{14}C]glycerol-labelled [^{3}H]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 ^{14}C-labelled glycerol from both the 1- and the 2-[^{3}H]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.

Bickerstaffe, R. & Annison, E. F. (1968). *Biochem. J.* **107**, 27P.
Garton, G. A. (1960). *Nutr. Abstr. Rev.* **30**, 1.
Kern, F., jun. & Borgstrom, B. (1965). *Biochim. biophys. Acta*, **98**, 520.
Leat, W. M. F. & Harrison, F. A. (1967). *Biochem. J.* **105**, 13P.
Mattson, F. H. & Volpenhein, R. A. (1964). *J. biol. Chem.* **239**, 2772.

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 (0·25mM) inhibited the oxidation of [1-^{14}C]palmitate by rat heart homogenates