White adipose tissue lipogenesis showed a similar response to that of brown fat after glucose and insulin treatment in the three conditions, though the rates were severalfold lower in white adipose tissue.

The differences in the basal lipogenesis in brown fat in the virgin, lactating and weaned states may be related to the plasma insulin concentration. Plasma insulin decreases during lactation (Robinson et al., 1978), but increases on weaning (Agius et al., 1979), and brown-adipose-tissue lipogenesis shows a similar pattern. The failure of glucose to elicit the same increase in lipogenesis during lactation as insulin suggests that the insulin response to a glucose load may be decreased during lactation and weaning or that brown fat has a decreased sensitivity to insulin.

The fate of the newly synthesized fatty acids in brown adipose tissue after a glucose load is not known. However, if the newly synthesized lipids are catabolized within the tissue, the decreased rate of lipogenesis during lactation may be important in the direction of substrates to the mammary gland by preventing the ultimate oxidation of lipogenic precursors in brown fat.

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A second site of vasopressin action on [1-14C]oleate metabolism in isolated rat hepatocytes:
Increased formation of 14CO2

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Vasopressin increases conversion of oleate into esterified products in hepatocytes from fed rats and decreases ketogenesis (Williamson et al., 1980). The present communication indicates that, in addition to this cytoplasmic action of vasopressin, the hormone may act on intramitochondrial sites, namely increased 14CO2 production from [1-14C]oleate. The increased 14CO2 production is dependent on the presence of Ca2+ ions in the incubation medium.

Hepatocytes were prepared from fed female rats as described by Williamson et al. (1980). The incubation procedure and measurements of 14CO2 production from labelled substrates were as described by Whitelaw & Williamson (1977). Rates were calculated from plots of the values at 20, 40 and 60 min.

2-Oxoglutarate and citrate were determined in neutralized extracts by the methods of Bergmeyer & Bernt (1974) and Dagley (1974) respectively, by using an Aminco DWII double-beam spectrophotometer.

In the presence of extracellular Ca2+ (2.4 mM), addition of vasopressin to hepatocytes from fed rats increased oxidation of [1-14C]oleate to 14CO2 by 54% (Table 1). Vasopressin also increased 14CO2 production from [U-14C]proline (Table 1).

Increased 14CO2 production from [U-14C]proline was observed in both the presence and the absence of unlabelled oleate (1 mM), but the stimulation was greater in the former case (47% versus 21%; see Table 1). The increased 14CO2 production from either [1-14C]oleate or [U-14C]proline was markedly decreased when Ca2+ was omitted from the incubation medium (Table 1).

Vasopressin causes a transient stimulation of the O2 uptake of perfused livers from fed rats (Hems et al., 1978). The data presented here suggest that this may be a consequence of increased oxidation of tricarboxylic acid-cycle intermediates to CO2 in the presence of vasopressin. Thus the oxidation to CO2 of both oleate (which is metabolized to acetyl-CoA) and proline (which is metabolized via glutamate to 2-oxoglutarate) was increased by vasopressin. Increased oxidation of oleate to CO2 may also contribute to the observed inhibition by vasopressin of ketone-body production from oleate (Williamson et al., 1980).

The mechanism by which vasopressin increases 14CO2 production from [1-14C]oleate and [U-14C]proline is not known. Such increases might result from increased tricarboxylic acid-cycle turnover. If tricarboxylic acid-cycle turnover is inhibited by β-oxidation of long-chain fatty-acyl-CoA to acetyl-CoA (possibly by competition for electron acceptors), then the effects of vasopressin on 14CO2 production might be explained by relief of this inhibition. This might arise as a consequence of stimulation of respiratory chain activity. Such a mechanism might explain why the effects of vasopressin on [U-14C]proline oxidation are less marked in the absence of
oleate. Alternatively, increased \(^{14}\text{CO}_2\) production might result from activation of enzymes of the tricarboxylic acid cycle, possibly NAD-isocitrate dehydrogenase and/or 2-oxoglutarate dehydrogenase. It has been reported that both NAD-isocitrate dehydrogenase (Denton et al., 1978) and 2-oxoglutarate dehydrogenase (McCormack & Denton, 1979) are activated by Ca\(^{2+}\) ions. As the increase in \(^{14}\text{CO}_2\) production from oleate or proline in the absence of extracellular Ca\(^{2+}\) is considerably smaller (Table I), it is possible that vasopressin might activate one or both of these enzymes by an increase in mitochondrial Ca\(^{2+}\) concentration secondary to an increase in the cytoplasmic Ca\(^{2+}\) concentration. In this regard, vasopressin causes a decrease in the concentration of 2-oxoglutarate in hepatocytes from fed rats incubated with 1 mM-oleate. For control incubations, the 2-oxoglutarate concentration was \(0.53 \pm 0.06 \mu\text{mol/g fresh wt. of cells}\); in the presence of vasopressin the 2-oxoglutarate concentration was \(0.27 \pm 0.02 \mu\text{mol/g fresh wt. of cells}\) (results expressed as means \(\pm \text{S.E.M. for nine observations, } P < 0.005\)). This decrease is not observed when Ca\(^{2+}\) is absent from the incubation medium (control (7) 0.48 \pm 0.03 \mu\text{mol/g fresh wt. of cells}; plus vasopressin (7) 0.53 \pm 0.06 \mu\text{mol/g fresh wt. of cells}). Citrate concentrations are elevated by vasopressin in the presence of Ca\(^{2+}\) (control (3) 0.51 \pm 0.01 \mu\text{mol/g fresh wt. of cells}; plus vasopressin (3) 0.77 \pm 0.07 \mu\text{mol/g fresh wt. of cells, } P < 0.05\). These concentration changes are consistent with Ca\(^{2+}\)-dependent activation of 2-oxoglutarate dehydrogenase activity by vasopressin. Such activation might occur directly (see McCormack & Denton, 1979) or indirectly, possibly by stimulation by Ca\(^{2+}\) ions of respiratory-chain activity.

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The mode of action of insulin at the molecular level is a matter of controversy (Goldfine, 1977, 1978; Kahn, 1979). There is accumulating evidence for the entry of insulin into cells, where much of it is degraded. However, there also appear to be insulin receptors on the membranes of various intracellular organelles, and it is possible that interaction with intracellular receptors is a necessary prerequisite for some aspects of insulin action. The degradation of \(^{125}\text{I}-\text{labelled insulin}\) when incubated with whole cells or subcellular fractions has been measured by the appearance of low-molecular-weight breakdown products, which are soluble in trichloroacetic acid (Ansorge et al., 1971; Izzo et al., 1972; Izzo, 1975; Terris & Steiner, 1975). Very few