have shown that small amounts of estrogens stimulate growth of mammary tumors in ovariectomized rats, while large quantities block their growth.

*Summary.* Stilbestrol diphosphate is rapidly hydrolyzed by the phosphatases present in homogenates of rat prostate, liver, kidney and spleen, and to a lesser extent by brain tissue. A convenient chemical procedure is described for assay of free stilbestrol in plasma. Following administration of stilbestrol diphosphate to rabbits, appreciable amounts of free stilbestrol appear in the blood.

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## Control of Plasma Non-Esterified Fatty Acids in Pregnancy and the Puerperium.* (26327)

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As term approaches the concentration of non-esterified fatty acids (NEFA) in peripheral blood plasma is significantly increased in normal human pregnancy. Prompt return toward normal non-pregnant values occurs within 48-72 hours after delivery. The NEFA fraction thus participates in the hyperlipidemia of pregnancy, although the physiological basis for the gestational increase in blood lipids remains unknown. We have suggested, however, that the lipid change may reflect altered carbohydrate utilization as another aspect of the diabetogenic influence of pregnancy(1).

The studies of Dole(2-3) and of Gordon (4-5) have related the plasma concentration of NEFA to carbohydrate utilization. Increase in plasma non-esterified fatty acids is observed when carbohydrate is either unavailable or cannot be utilized because of metabolic error. In either case, as Gordon has suggested, "caloric homeostasis" tends to be preserved due to the extremely rapid rate of NEFA utilization even at low plasma levels (5). Although in general NEFA release from fat depots to plasma reflects the prevailing level of carbohydrate utilization, the following observations relative to experimental modification of plasma NEFA in pregnancy and the puerperium suggest that control of fatty acid concentration in blood may be quite complex.

This complexity was first suspected when it was observed that degree of change in plasma NEFA after insulin treatment did not

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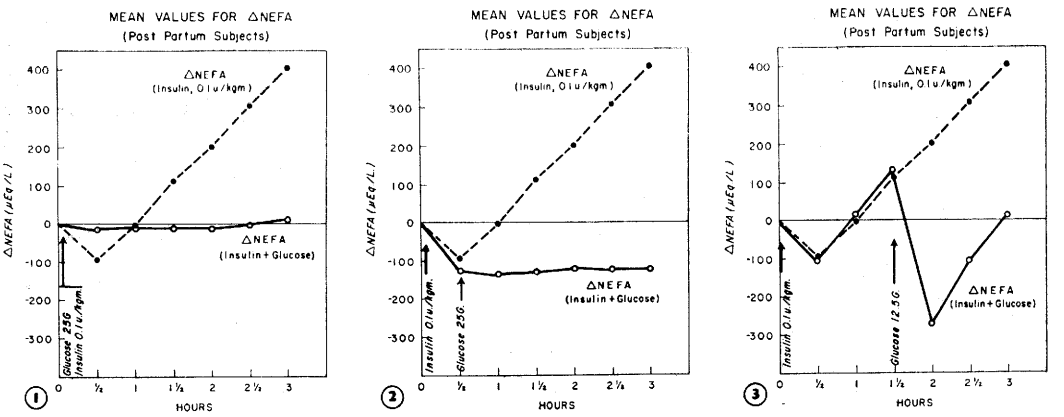


FIG. 1-3.

parallel development of insulin resistance in late pregnancy. Following a standard dosage of 0.1 U/kg regular insulin free of hyperglycemic-glycogenolytic factor, the decrease in plasma NEFA was essentially the same in non-pregnant and pregnant subjects despite significant attenuation of hypoglycemia and hypophosphatemia in the latter group(6). In the early puerperium (4th or 5th post partum day) even with recovery of insulin sensitivity, NEFA decrements were significantly less than observed in late pregnancy, degree of change in NEFA concentration departing from that of glucose.

*Method.* In the present investigation normal puerperal women were studied on the 4th or 5th post partum day after uncomplicated vaginal delivery. One group of 20 subjects were given by vein 0.1 unit of glucagon free regular insulin/kg body weight. A second group of 20 received with the insulin 25 g 50% glucose in water. A third group of 20 first were treated with insulin and then given 25 g glucose one half hour later. Finally a group of 20 puerperal subjects received 12.5 g glucose 1½ hours after the standard insulin dose. A limited number of other observations were made on pregnant and non-pregnant subjects with various combinations of insulin and glucose treatment. These data will be referred to but not constitute the basis of this report. Blood samples were obtained before insulin treatment and at half hourly intervals thereafter for 3 hours. After separation of formed elements by centrifugation NEFA was determined in duplicate on plasma aliquots by the method of Dole(2).

*Results.* A characteristic metabolic response in the early puerperium was the complete suppression of NEFA change when 25 g glucose was given I.V. with the insulin, although this amount of glucose was without effect on the level of fatty acids when given alone(7). This is shown in Fig. 1 where the NEFA mean values for 2 groups of 20 normal postpartum subjects are plotted. One group received insulin and responded by the typical biphasic change in NEFA concentration during the 3 hour period of observation. The other group received 25 g glucose with the insulin which resulted in a stabilizing effect on NEFA and gave almost quantitative inhibition of the biphasic response.

In Fig. 2 is shown the result of 25 g glucose given ½ hour after insulin administration. Attention is directed to the effect of this amount of glucose which suppressed recovery and "rebound" above fasting NEFA values. This effect was observed not only in the early puerperium but for all normal pregnant and non-pregnant subjects studied. If the glucose is given 1½ hours after the insulin plasma NEFA concentration becomes fixed or stabilized at the level attained at that time.

When a fraction of the 25 g glucose is given (*i.e.* 12.5 g) with the insulin the decrease in NEFA concentration is exaggerated. This was demonstrated for all categories of subjects studied whether pregnant, puerperal or non-pregnant. In contrast to the 25 g glucose load the 12.5 g glucose dosage did

not stabilize NEFA when given at ½ or 1½ hours after insulin. On the contrary, further abrupt decrements in NEFA were observed (Fig. 3).

*Comment.* It appears that in certain circumstances the amount of available carbohydrate can profoundly influence the effect of insulin on concentration of non-esterified fatty acids in peripheral blood plasma. Whether these effects are related to release or to utilization of NEFA is uncertain but the changes in NEFA concentration may depend upon the relative amount of carbohydrate presented to the tissues at various levels of available insulin from endogenous or exogenous sources. Recent studies suggest that glucose is metabolized preferentially by the peripheral tissues(8.9) and it is possible that the balance between fatty acid and glucose metabolism in fasted subjects receiving insulin is determined by the amount of carbohydrate available. Small amounts of glucose (*i.e.* 12.5 g) in presence of exogenous insulin or insulin of pancreatic origin resulting from glucose administration itself are insufficient to suppress NEFA utilization. This effect together with direct suppression of NEFA release from adipose tissue by insulin as shown by radioisotope studies(10) results in a net decrease in NEFA concentration. With a larger glucose load (25 g) perhaps a new balance is established with relatively less NEFA utilized because of the greater amount of carbohydrate available. In this situation NEFA concentration tends to "stabilize" at the point in our experiments when the glucose is given. The relative effects of insulin upon fatty acid synthesis and release may also bear on our data as well as the "recycling" of NEFA as described by Laurell(11) and by Fredrickson and Gordon(12).

Whatever factors are involved, the preliminary observations reported here suggest that plasma level of NEFA is subject to a regulatory mechanism that is dependent not only upon ability to metabolize carbohydrate and availability of insulin but upon quantity of carbohydrate present. It can be inferred also that plasma concentration of non-esterified fatty acids is not solely determined by the rate of release of these acids from fat depots.

*Summary.* The amount and timing of glucose administered to fasting post partum women receiving intravenous insulin determines the amount and direction of change in plasma NEFA. Twenty-five grams of glucose inhibits the characteristic response of NEFA to insulin while half this dosage either enhances the insulin effect or causes secondary decrements in non-esterified fatty acid. These results suggest that the regulation of plasma NEFA is a complex function that may involve fatty acid synthesis, utilization and recycling as well as controlled release from fat depots.

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