to diabetic and non-diabetic patients results in a marked increase in the di-enoic acid content of the cholesterol esters, in association with a lowering in the mono-enoic acid content. When ethyl esters of oleic acid are substituted for the ethyl linoleate the opposite pattern is observed. A high carbohydrate, fat-free diet produces much the same pattern of cholesterol ester composition as that seen during oleate ingestion.

Grateful acknowledgement is made to Procter and Gamble Research Labs. for supplies of purified oleate and linoleate.

1. Kinsell, L. W., Friskey, R., Splitter, S., Michaels, G. D., *The Lancet*, 1958, v1, 334.
2. Michaels, G. D., Fukayama, G., Chin, H. P., and Wheeler, P., PROC. SOC. EXP. BIOL. AND MED.
3. Michaels, G. D., *Am. J. Clin. Nutr.*, in press. (1958)
4. Bragdon, J. H., *J. Biol. Chem.*, 1951, v190, 513.

Received June 16, 1958. P.S.E.B.M., 1958, v98.

Ketosis in the Rat Fetus.* (24199)

ROBERT O. SCOW, SIDNEY S. CHERNICK AND BETTE-BARRON SMITH
Laboratory of Nutrition and Endocrinology, Nat. Inst. of Arthritis and Metabolic Diseases, P.H.S., U. S. Depart. of Health, Bethesda, Md.

Although placental transfer of many substances has been demonstrated(1-3), such studies of ketone bodies have not been made. Since ketosis may readily develop in the pregnant animal when deprived of insulin(4,5) or when food intake is limited(5-7), it seemed of interest to determine if the ketone body level in fetal blood was related to that in maternal blood.

Methods. Sprague-Dawley rats pregnant 17 to 20 days and fed Purina Chow were used. Five of these rats were "totally" pancreatectomized(8) after 17-hour fast and tested 23 hours later. They were not fed postoperatively. Controls consisted of pregnant normal rats fasted 17 and 40 hours. Maternal blood samples for glucose determinations were taken from the tail. Immediately thereafter, mother rats were anesthetized with ether. Fluid was drawn from amniotic cavity with a hypodermic needle and syringe. Fetal blood samples were obtained by removing the fetus from the uterus, cutting open the chest and heart, and collecting the blood on a spot plate with heparin. Generally a single fetus was used for each determination. After obtaining fetal blood samples, blood for determination of maternal levels of ketone bodies and fat was drawn from the aorta with a syringe and needle. Glucose was measured by the method of Haslewood and Strookman(9), ketone bodies by the method of Bessman(10) and total lipids by the method of Bragdon(11).

Results. There was a marked elevation of glucose, ketone bodies, and fat in blood of the pancreatectomized rats (Fig. 1). Blood ketone bodies of the fasted normal pregnant rats were also increased, whereas their glucose levels were less than those in the fed rats. There was no significant difference in blood ketone body levels between pancreatectomized and 40-hour fasted normal pregnant rats. Preliminary observations indicate that the marked ketosis observed in the fasting pregnant rat does not occur before 14th day of gestation. Blood fats of some of the fasted normal pregnant rats were also increased.

In agreement with observations of others (1,2), maternal and fetal blood glucose levels were essentially the same. In the diabetics maternal glucose levels (260-420 mg/100 ml) averaged 60 mg/100 ml higher than fetal levels. These data reflect the known rapid transfer of glucose across the placenta(2). Glucose levels in the amniotic fluid of both normals and diabetics were 20 to 30 mg/100 ml lower than those in the fetal blood.

Maternal blood ketone body levels ranged from 0.6 to 114 mg/100 ml (Fig. 1). Over

* The excellent technical assistance of Barbara Guarco and Theresa R. Clary is gratefully acknowledged.

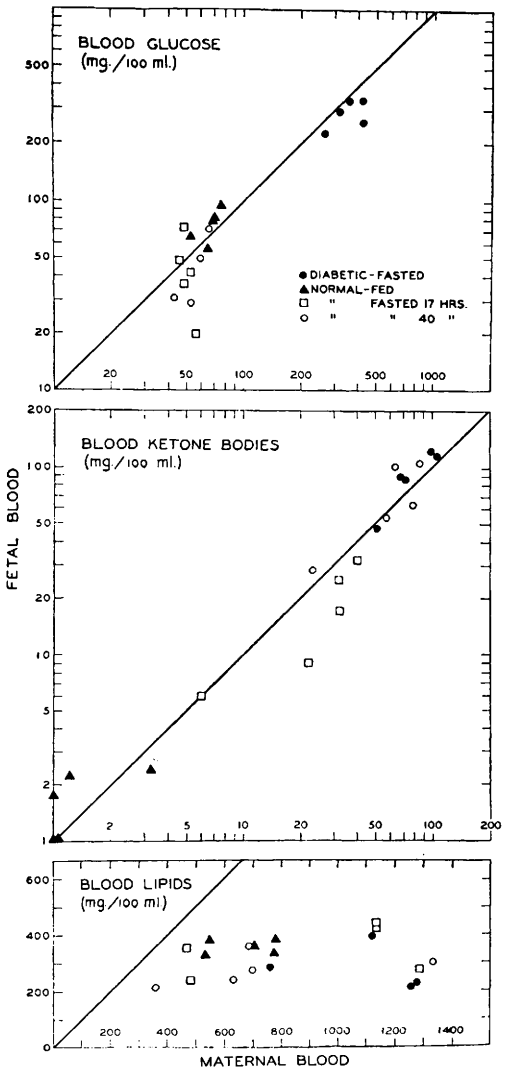

FIG. 1. Comparison of levels of glucose, ketone bodies (as acetone) and total lipids (as palmitic acid) in fetal blood with those in maternal blood. The diagonal line at 45° angle represents the theoretical distribution of observations if there were no differences between fetal and maternal blood.

this wide range, blood ketone body concentration of the fetus equalled that of its mother. The very close agreement of ketone body levels in fetal and maternal blood strongly suggests that ketone bodies are rapidly transferred across the placenta. The ketone body concentration in amniotic fluid also paralleled that in maternal blood.

It has been demonstrated that fats cross the placenta very slowly(1,3). In agreement with these observations, fetal blood lipids were found to be independent of maternal levels (Fig. 1). There were no detectable amounts of fat in the amniotic fluid.

Discussion. The cause of increased severity of fasting ketosis during the last third of pregnancy is not known(5-7). Since blood glucose is not elevated and glucose administration readily corrects the ketotic state(7), the ketosis cannot be attributed to insulin deficiency as in the diabetic. MacKay and Barnes have shown that the adrenal gland is necessary for ketosis in fasting pregnant rats (6). Others have observed that plasma level of 17-hydroxycorticosteroids and urinary excretion of glycogenic corticoids are markedly increased during the last third of pregnancy (12-14). It is well-known that cortisone is antiketogenic by virtue of its gluconeogenic action(15,16). However, in severe insulin deficiency, in which carbohydrate utilization is impaired, cortisone is strongly ketogenic(16). If the rate of gluconeogenesis in the fasting pregnant animal is inadequate, a severe carbohydrate deficiency may develop in maternal tissues due to high priority of the fetus for nutrients(17). In such a case it is very likely that the elevated level of corticoids in the mother would result in severe ketosis. The effects of ketosis in the fetus are not known.

Summary. Blood levels of ketone bodies, glucose, and fats in pregnant rats were altered by fasting and by pancreatectomy. The concentration of ketones and glucose in fetal blood changed with maternal level, whereas the lipids were independent of maternal level. Ketone bodies appear to cross the placenta as rapidly as glucose.

1. Needham, J., *Chemical Embryology,* Cambridge Univ. Press, 1931, v3, 1485.

2. Davies, J., *Am. J. Physiol.,* 1955, v181, 532; 1957, v188, 21.

3. Goldwater, W. H., Stetten, D., Jr., *J. Biol. Chem.,* 1947, v169, 723.

4. Campbell, J., Best, C. H., *Metabolism,* 1956, v5, 95.

5. Gray, C. H., *Lancet,* 1938, v235, 665.

6. MacKay, E. M., Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1936, v34, 682.

7. Fraser, A. H. H., Godden, W., Snook, L. C., Thomson, W., *J. Physiol.,* 1939, v97, 120; Parry, H. B., *Toxaemias of Pregnancy,* Ciba Foundation Symposium, Blakiston, Philadelphia, 1950, p85; Phil-

lipson, A. T., *ibid.*, p94.
8. Scow, R. O., *Endocrinol.*, 1957, v60, 359.
9. Haslewood, G. A. D., Strookman, T. A., *Biochem. J.*, 1939, v33, 920.
10. Bessman, S. P., Anderson, M., *Fed. Proc.*, 1957, v16, 154.
11. Bragdon, J. H., *J. Biol. Chem.*, 1951, v190, 513.
12. Paterson, J. Y. F., *J. Comp. Path.*, 1957, v67, 165.
13. Venning, E. H., *Endocrinol.*, 1946, v39, 203.
14. Lloyd, C. W., Hughes, E. C., Lobotsky, J., Rienzo, J., Avery, G. M., *J. Clin. Invest.*, 1952, v31, 1056.
15. Scott, J. L., Jr., Engel, F. L., *Endocrinol.*, 1953, v53, 410.
16. Scow, R. O., Chernick, S. S., Guarco, B. A., *Fed. Proc.*, 1958, v17, 144.
17. Hammond, J., *Proc. Nutrition Soc.*, 1944, v2, 8.

Received June 18, 1958. P.S.E.B.M., 1958, v98.

Fat Deposition in Rat Tibia Due to Lysine Deficiency.* (24200)

EDWARD L. ROHDENBURG
(Introduced by T. F. Zucker)
Stine Laboratory, E. I. du Pont de Nemours and Co., Newark, Del

Recent publications(1-5) have shown that amino acid composition of the diet may have a decided effect on bone structure. Bavetta *et al.*(1,2) described pathological changes in bones and teeth of rats fed diets deficient in lysine or tryptophan. Specific effects should also be evident in the chemical composition of bone. Harris *et al.*(6) previously observed an abnormal, fatty bone marrow in histological sections from rats fed lysine-deficient gliadin. Quantitative changes in fat content of the tibiae of rats fed diets containing various amounts of lysine are described in this report.

Methods. Rats reared on stock diet(7) were weaned at 21 days with average weight of about 50 g. They were caged individually and fed the experimental diets, using litter mate distribution with 3 males and 3 females for each diet. At termination of the 5-week feeding period the animals in each group were weighed, killed, and their tibiae analyzed. For this purpose, the tibiae were dissected from tissues of right hind legs. After cleaning, the raw weight for the pooled group of 6 tibiae was obtained. All analyses were calculated on the basis of this weight. For moisture determination the bones were dried at 100°C. For fat determination the bones were then boiled in 95% alcohol under reflux for 24 hours and extracted with ether in a Soxhlet for same period of time. The bones were dried again and fat content calculated by difference. Finally the dried fat-free bones were ashed at 800°C. Having established amount of water, fat and ash present, the remaining constituents of raw bone have been called "organic residue" by Chick *et al.* (8). The basal diet used consisted of 90% commercial bread, dried at room temperature, supplemented with vitamins, minerals and fat. The bread contained 4% non-fat milk solids. This diet is low in lysine and was previously used by Rosenberg and Rohdenburg(7) to investigate improvement in growth and protein efficiency attainable by supplementation with lysine. An additional group of rats was fed the stock diet to study normal bone composition.

The results of adding increasing amounts of L-lysine monohydrochloride† to the bread diet are summarized in Table I. Fat content of tibiae of rats fed the unsupplemented bread diet was greater than that observed when stock diet was fed. Addition of lysine to the bread diet improved both growth and bone composition. With increasing lysine supplementation there was a decrease in fat content

* Our attention was originally called to this problem by Dr. Theodore F. Zucker of Columbia University, to whom we wish to express our appreciation.

† Du Pont L-lysine monohydrochloride, containing 95% L-lysine monohydrochloride and 5% D-lysine monohydrochloride.