found in all of the rabbits nursed by N_1, but none were found in any of the rabbits nursed by D_1.

Due to a number of circumstances an exact repetition of the above experiment was not possible. Different, but related, bucks had to be used in both strains. While both original does were used, this time their babies were exchanged with the offspring of 2 other does (Fig. 2B and C). No lesions were found in rabbits nursed by the resistant, but unrelated does, D_1 and D_2. Lesions were present in the animals nursed by the non-resistant does, N_1 and her daughter N_{a_1}.

Summary and conclusion. Newborn rabbits were exchanged between does previously shown to be members of families that were either "resistant" or "non-resistant" with respect to incidence of spontaneous, non-lipid, medial lesions. Lesions were found at 8 weeks of age only in the rabbits nursed by does of the non-resistant family. These data suggest that a factor is present in rabbit milk which either enhances the formation of the non-lipid, medial aortic lesion found in weanling rabbits of non-resistant strains or which, in resistant rabbits, protects against an ubiquitous lesion-inducing agent.

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Changing Activity of Erythrocyte Glucose-6-Phosphate Dehydrogenase and Tolerances to Glucose and Tolbutamide in Growing Sheep.* (31913)

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A metabolic syndrome—low erythrocyte glucose-6-phosphate dehydrogenase (G6PD) activity coupled with diabetic type tolerances to glucose and tolbutamide—first reported in man(1) has now been found to develop in growing sheep. Previous workers have demonstrated that blood glucose concentration and glucose tolerance decrease progressively in young sheep(2-4), and that erythrocyte G6PD activity is low in adult sheep(5,6). We now report that erythrocyte G6PD activity and tolbutamide tolerance of young lambs resemble those of normal man, and gradually change during 100 days of extrauterine life to values considered pathologic in man. This enlarges the group of metabolic conditions in ruminants which may serve as models for medical problems in man(7).

Methods. Standard clinico-chemical methods were used for the estimation of G6PD activity(8), biuret protein(9), glucose concentration(10), intravenous glucose tolerance (2-4) and tolbutamide tolerance(11). Packed cell volumes were read after centrifugation at 1500 *g* for 25 minutes. Erythrocyte glucose concentration was calculated from whole blood and plasma concentrations without correction for trapped plasma in the packed cell volume. Glucokinase activity (V of hexokinase using glucose as substrate) was assayed by NADPH generation(12) in 50% saturated ammonium sulfate precipitates of the hemolysates prepared for G6PD assay(8). The same activity was found with 1 or 100 mM glucose in the cuvette. We studied 6 adult sheep (crossbred females) and 4 growing lambs. They were fed poor quality grass hay *ad libitum* and 0.5 kg of grain concentrate daily per adult. The lambs had free access to the feed or to suckle.

Results. Erythrocyte G6PD activity was 95-190 U/100 ml red cells in the lambs when

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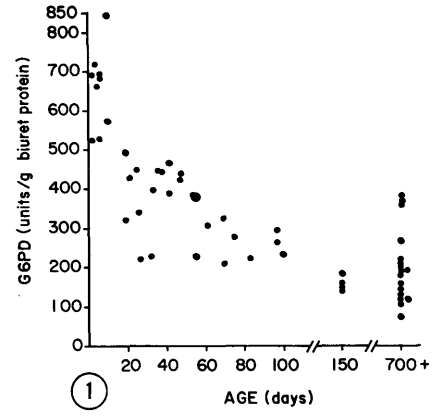

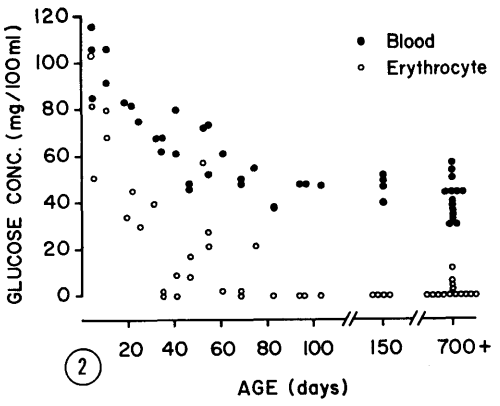

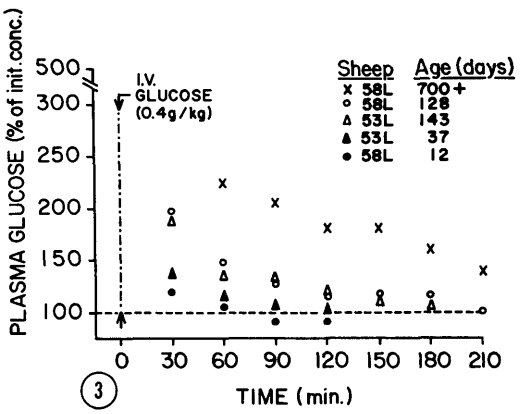

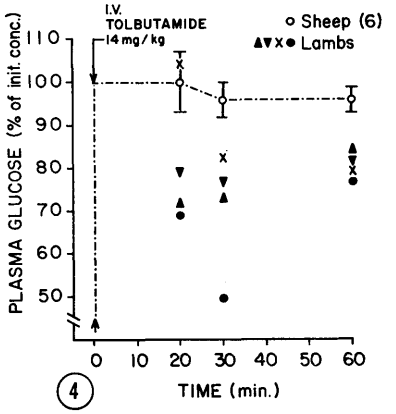

FIG. 1. Erythrocyte glucose-6-phosphate dehydrogenase (G6PD) activity in 4 lambs growing from 1 to 150 days of age and in 6 adult sheep, 700+ days. Samples were taken from the lambs twice a week at first then once a week, from the adults 3 times each.

FIG. 2. Glucose concentration in whole blood and erythrocytes of 4 growing lambs (1-150 days) and 6 adult sheep (700+ days). The blood samples were used also for G6PD assays, Fig. 1.

FIG. 3. Intravenous glucose tolerance test in lambs 12-143 days old and an adult sheep. The test in the young lambs resembles that of normal man, while that of adult sheep would suggest diabetes if found in man.

FIG. 4. Intravenous tolbutamide test in 4 lambs 21-45 days old and the mean ± S.D. of tests on 6 adult sheep. Two hypoglycemic responses (● and ▲) would be normal in man, the 2 other tests on lambs (▼ and ×) would suggest borderline diabetes, and the tests in the adult sheep would indicate severe diabetes if found in man.

1-11 days of age and 26-93 U/100 ml in the adult sheep. If biuret protein is used as the reference base, the average G6PD activity is 659 U/g in the young lambs and 191 U/g in the adult sheep, a 3.5 fold difference. The fall in G6PD activity ran parallel with blood glucose concentration as the lambs grew older (Fig. 1 and 2). The tolerances to glucose and tolbutamide in lambs 20-30 days of age resembled those of normal man, but came to resemble diabetic man in older lambs (Fig. 3 and 4). Erythrocyte GK activity did not change with age (Fig. 5).

Discussion. Compared to normal adult values for the species, erythrocyte G6PD activity is 240% higher in young lambs (Fig. 1) but only 25% higher in newborn humans (13,14). The 25% decline in man has been ascribed to a higher proportion of young cells at birth or to maturation of erythrocyte metabolism occurring gradually after birth(13); G6PD activity is high in very young erythrocytes and it decreases with aging *in vivo*(15). The 240% decline in sheep may also be associated with erythrocyte maturity and/or it may be an adaptation of the enzyme to a

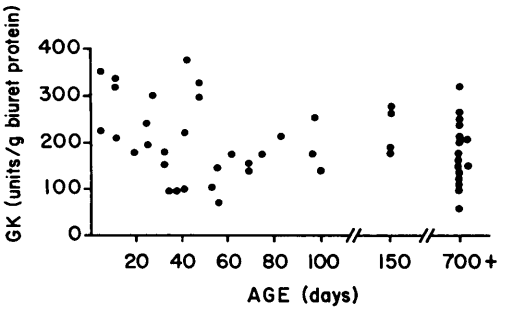

FIG. 5. Erythrocyte glucokinase (GK) activity in 4 lambs growing from 1 to 150 days of age and 6 adult sheep, 700+ days. The same blood samples were used for G6PD assays, Fig. 1.

diminishing availability of glucose (compare Fig. 1 and 2). Pertinent to the first possibility is the change in average red cell life span in growing sheep, 15-36 days in young lambs and 76-133 days in adult sheep(16). Pertinent to the second is the well known adaptive behavior of hepatic G6PD (*e.g.*, 17). Hepatic G6PD activity falls with advancing age in lambs(18), and it is low in erythrocyte G6PD deficient humans(19). The lack of change in GK activity (Fig. 5) is not surprising, since the enzyme appears to have a high affinity for glucose; only glucokinase with a low affinity for glucose has been found adaptive (20).

Several metabolic changes in growing sheep can be explained by the hypothesis that tissues which are usually sensitive to insulin (*e.g.*, liver, muscle and adiposa) become progressively insensitive. This hypothesis is suggested by the changes in the tolerance tests (Fig. 3 and 4) and supported by progressively diminished and delayed responses of blood glucose to exogenous insulin and of blood pyruvate to intravenous glucose(21-23). This apparent loss of sensitivity to insulin develops at the same time as the loss of erythrocyte G6PD activity in growing sheep.

In human G6PD deficiency, there has been a conflict in responses of different subjects to specific types of glucose tolerance tests. The first report associated a diabetic type of tolerance to oral glucose with G6PD deficiency(1). A subsequent report failed to confirm this in 3 different groups of G6PD deficient subjects(24). However, the tolerance to oral glucose following the administration of cortisone was significantly different from normal(24), resembling the response found in the prediabetic state(25). Glucose tolerance tests in general involve responses of the pancreas to the glucose load and of target tissues to the insulin secreted. Cortisone appears to decrease the response of some tissues (*e.g.*, muscle) to insulin(26), thus providing a more severe test of the insulinogenic response to glucose(25). It seems to us that the diminished tolerances of some G6PD deficient subjects to oral glucose alone and of others to cortisone-glucose are less likely to represent the presence in different subjects of two qualitatively different abnormalities than to represent a difference in degree of the same metabolic abnormality. Analogy with ontogenetic changes in sheep suggests that this may be a relative insensitivity to insulin.

Summary. Erythrocyte glucokinase and glucose-6-phosphate dehydrogenase activities, glucose concentration, and tolerances to glucose and tolbutamide were measured in 6 adult sheep and 4 lambs growing from 1 to 150 days. The young lambs had G6PD activity and tolerances like normal man. The adult sheep had low G6PD activities and tolerances like diabetic man. Erythrocyte G6PD and glucose diminished gradually in growing lambs to approximate adult values by 60-100 days. The changes in the tolerances tests can be explained by a progressive development of insulin resistance, previously demonstrated in growing lambs.

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Effect of Hypophysectomy or *p*-Hydroxypropiophenone on Hepatic Precancerous Changes in Rats Given Thioacetamide or 3'Methyl-4-Dimethylaminoazobenzene. (31914)

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Prior to the onset of demonstrable malignant neoplasia in rats fed 3'methyl-4-dimethylaminoazobenzene (3'Me-DAB) hepatic adenylic acid (5'AMP) deaminase (E. C. 3.5.4.6.) activity of the liver was increased (1) and the deaminase activity of primary liver tumors which subsequently developed was higher than normal liver(2). Hypophysectomy, which inhibits liver tumor induction in rats fed 3'Me-DAB(3), inhibited increases in 5'AMP deaminase activity of precancerous liver(1).

Single intraabdominal injections of 3'Me-DAB also caused increases in hepatic deaminase activity and these increases were accompanied by decreased incorporation of labeled orotic acid into hepatic nuclear ribonucleic acid (RNA)(4). When hypophysectomized animals were given single injections of 3'Me-DAB, enzyme activity was not appreciably increased and incorporation of labeled orotic acid was not decreased(5). Thus, both tumor induction(3) and metabolic lesions in precancerous liver(1,2,4,5) caused by 3'Me-DAB were prevented or delayed by altering the endocrine status of the rat.

Thioacetamide is hepatocarcinogenic in rats (6-8). Like 3'Me-DAB, it too caused increases in hepatic 5'AMP deaminase activity (9) and changes in hepatic nuclear RNA metabolism(8-11). The purpose of the present work was to ascertain whether these effects of thioacetamide upon hepatic tissue were influenced by altering the endocrine status of the rat.

Materials and methods. Rats used in this study were Holtzman females weighing 150-180 g or hypophysectomized females weighing 110-140 g (purchased from Hormone Assay Laboratories, Chicago). They were fed Rockland mouse and rat diet or a semi-synthetic diet(12). Hepatocarcinogens were either fed or injected intraabdominally. When fed, 3'Me-DAB was added to the semi-synthetic diet(12) at 0.06%; thioacetamide was added at 0.066%. When injected, 3'Me-DAB was dissolved in corn oil and injected once at 250 mg/kg body weight; thioacetamide was dissolved in 0.9% NaCl and was injected daily at 50 mg/kg body weight. Orotic acid-