

PROGESTERONE EFFECTS ON GALACTOSE METABOLISM IN PREPUBERTAL PATIENTS WITH CONGENITAL GALACTOSEMIA AND IN RATS MAINTAINED ON HIGH GALACTOSE DIETS *

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In a recent publication (1) it was reported that *in vitro* addition of certain steroid hormones causes a 200 to 300 per cent stimulation of galactose-1- C^{14} oxidation to $C^{14}O_2$ by rabbit liver slices. Progesterone, androsterone and testosterone produced maximal stimulatory effects. Further data have been obtained (2) which demonstrate *a*) that of many tissues tested, only liver and intestine respond to the steroids; *b*) the same steroid specific effect can be obtained in a $100,000 \times G$ supernatant fraction of rabbit liver homogenates; and *c*) the *in vitro* steroid stimulation of galactose oxidation is mediated by an indirect stimulation of the uridinediphosphogalactose-4-epimerase (UDPGal-4-epimerase) reaction.

During the course of these investigations some *in vivo* effects of progesterone on galactose oxidation were investigated. The results of progesterone administration to three prepubertal galactosemic subjects and to young rats maintained on high galactose diets serve as the basis for this report.

MATERIALS AND METHODS

Case Histories¹

Case 1: L.W.J., 11.5 year old white male. The patient was the product of a normal full term pregnancy and delivery. There was no family history of metabolic disorder. At the age of two weeks the patient began to have frequent episodes of vomiting and diarrhea in spite of frequent changes in formula. Because of continued gastrointestinal irritability and failure to gain weight,

at age 2.5 months he was admitted to Sinai Hospital in Baltimore where after careful study (3) the diagnosis of congenital galactosemia was made. The patient was placed on a galactose-free diet and when seen again three months later, had gained weight and showed regression of cataracts and improvement of liver function tests. During the succeeding years the patient did quite well except for difficulty with vision and poor performance in school. On admission to the Clinical Center the patient exhibited bilateral nuclear cataracts, slight hepatomegaly and mental retardation with a mental age of seven years and an I.Q. of 59. The hemogram and blood serology were normal. Blood urea nitrogen was 9 mg. per 100 ml., fasting blood glucose 84 mg. per 100 ml., total cholesterol 182 mg. per 100 ml., cephalin flocculation was negative and total serum proteins and albumin to globulin ratio were normal. Urinalysis on several occasions revealed no reducing substance or proteinuria. The hemolysate assay for galactose-1-phosphate uridylyltransferase (P-Gal transuridylylase) of Anderson, Maxwell and associates (4, 5) substantiated the diagnosis of congenital galactosemia.

Case 2: E.L.W., 11.5 year old white male. This patient was also the product of a normal pregnancy and delivery. There was no family history of metabolic disorder. The mother is known to have discoid lupus erythematosus. The patient manifested no abnormalities in feeding or development during the neonatal period. An injury at the age of three months prompted hospital admission and he was diagnosed as having malnutrition and hepatomegaly. He was empirically placed on a diet of Mulsoy® with elimination of milk and milk products. Addition of milk to this regimen produced vomiting and diarrhea. He was hospitalized again at the age of two years because of pneumonia. At this time he was again noted to have hepatomegaly and showed evidence of mental retardation. He apparently did fairly well until age 10 years when he began to have difficulty with his vision. About 1.5 months prior to his admission to the Clinical Center, a physical examination was arranged at the Johns Hopkins Hospital. Bilateral cataracts were noted and following admission and thorough work-up, including galactose tolerance and the hemolysate assay for P-Gal transuridylylase, the diagnosis of congenital galactosemia was made and the patient was placed on a rigid milk-free diet for the first time in his medical his-

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tory. The patient was then transferred to the Clinical Center for further study.

Case 3: J.J.O., 7 year old white male. This patient was the product of a normal pregnancy and delivery. Two siblings also have been diagnosed as having congenital galactosemia. There is no family history of other metabolic disorder. Following development of neonatal vomiting, diarrhea, jaundice and failure to gain weight, the patient was hospitalized and the diagnosis of galactosemia was made on the basis of abnormal galactose tolerance. Subsequent confirmation of the diagnosis has been obtained by the hemolysate assay for P-Gal transuridylase. Because of rigid adherence to a completely galactose-free dietary regimen, this patient has done very well, manifesting little clinical evidence of galactosemia.

During the course of these studies all three subjects were maintained on galactose-free diets.

Procedures

Galactosemic studies. Two $\mu\text{c.}$ of galactose-1- C^{14} was administered intravenously following an overnight fast, and samples of expired air were collected in Douglas bags at intervals during the six hour period immediately following administration of the isotope. These samples were analyzed for total CO_2 content by the method of Fredrickson and Ono (6). The volume of expired air

was measured using a wet-test gas meter after removal of CO_2 by bubbling through CO_2 -free NaOH . Carbonate was collected by precipitation with 20 per cent BaCl_2 . The resulting BaCO_3 was washed with water until the supernatant solution was no longer alkaline, and dried *in vacuo* at 110°C. overnight. CO_2 was liberated from a weighed amount of BaCO_3 , diffused into hyamine base and specific activity determined by assaying for C^{14}O_2 content using a Tricarb² Liquid Scintillation Spectrometer (7). The cumulative expiratory excretion of C^{14}O_2 was then calculated according to Berlin, Tolbert and Lawrence (8).

Following a control study, as outlined above, Subject 1 received daily 10 mg. intramuscular injections and Subjects 2 and 3 received daily 20 mg. intramuscular injections of an aqueous suspension of progesterone (Syn-gesterone®, Pfizer) for a period of six days. On the seventh day (24 hours following the last injection of the hormone) an identical C^{14}O_2 excretion study was performed. Subject 1 received galactose-1- C^{14} of specific activity 1.76 $\mu\text{c.}$ per mg. and Subjects 2 and 3 received galactose-1- C^{14} of specific activity 4.72 $\mu\text{c.}$ per mg. In all cases galactose of equal specific activity was used in the control and postprogesterone studies. Galactose-1- C^{14} was purchased from the National Bureau of Standards

² Packard Instrument Company, LaGrange, Ill.

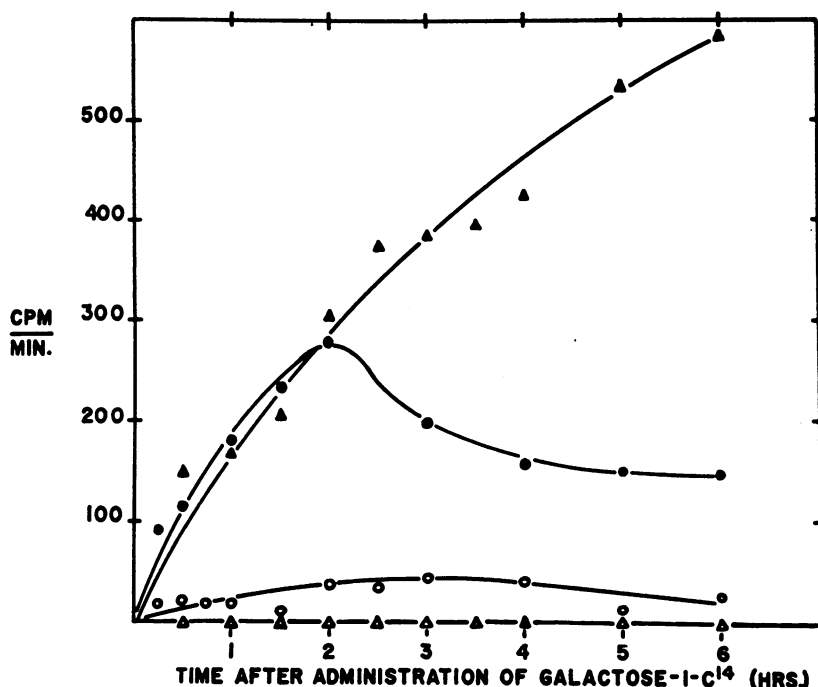


FIG. 1. EXPIRATORY EXCRETION OF C^{14}O_2 BY GALACTOSEMIC PATIENTS AFTER PROGESTERONE ADMINISTRATION

Subject 1, L.W.J., (○) before progesterone administration; (●) after progesterone administration.

Subject 2, E.L.W., (△) before progesterone administration; (▲) after progesterone administration.

and was sterilized as a saline solution by filtration prior to use.

Cataract studies. Young Sprague-Dawley male rats weighing 70 to 80 Gm. for galactose and 30 Gm. for xylose experiments were randomly divided into groups of 10 and maintained *ad libitum* on a 30 or 40 per cent galactose diet or a 30 per cent xylose diet prepared by adding the carbohydrate weight for weight to finely ground Purina® Rat Chow. An aqueous suspension of progesterone (Syngesterone®, Pfizer) was administered daily to the treated groups by intramuscular injection of 2.5 mg. per animal. Animals were observed daily for cataract development. Cataracts were counted only when nuclear opacification was complete and clearly visible to the unaided eye. Cumulative incidence of cataract formation was followed rather than animal incidence making a total of 20 possible cataracts in each group of 10 rats. D-galactose and D-xylose were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio or Pfanstiehl Chemical Company, Waukegan, Illinois.

RESULTS

Effect of progesterone administration on the expiratory excretion of $C^{14}O_2$ by galactosemic subjects following administration of galactose-1- C^{14}

The expiratory excretion of $C^{14}O_2$ following intravenous injection of galactose-1- C^{14} before and after progesterone administration is shown in Figure 1. During the control period in both cases, no significant amount of $C^{14}O_2$ could be detected in the expired air. Following six days of progesterone administration a significant amount of the administered isotope appeared in the expired air. The calculated cumulative excretion during the six hour collection period following treatment is 7 per cent of the injected C^{14} for Subject 1 and 12 per cent for Subject 2. The third patient studied in this manner is not shown on this graph because the study was not carried out for the complete six hour collection period. However, the results obtained during a two hour control period and a four hour post-treatment period are similar to the results obtained from Subject 2. Since the results are based on samples containing relatively small amounts of radioactivity, samples of red cell hemolysates were analyzed for galactose-1-phosphate³ (gal-1-P) to see if the increase in excretion observed after progesterone administration

³ We are indebted to Dr. H. N. Kirkman of the National Institute of Arthritis and Metabolic Diseases who kindly carried out these determinations.

was due to falling levels of gal-1-P following hospitalization and institution of a strict galactose-free diet. Within the limits of the method (9) the erythrocytic gal-1-P levels did not change between the control and post-treatment isotope studies. The difference in the shape of the two post-treatment excretion curves cannot be explained at this time but may be related to the fact that different absolute amounts of galactose were administered to the two patients and the dosage of progesterone was different in the two experiments. Furthermore, since the post-treatment excretion curve of Subject 2 is incomplete, the ultimate excretion of $C^{14}O_2$ should be much greater than the 12 per cent observed during the first six hours.

It would be of interest to know what the nongalactosemic prepubertal excretion curve is following administration of galactose-1- C^{14} . However we have not been able to justify performance of this study because of the ethical implications attendant to the administration of radioactive isotopes to children when such studies are not directly related to diagnosis or possible therapy.

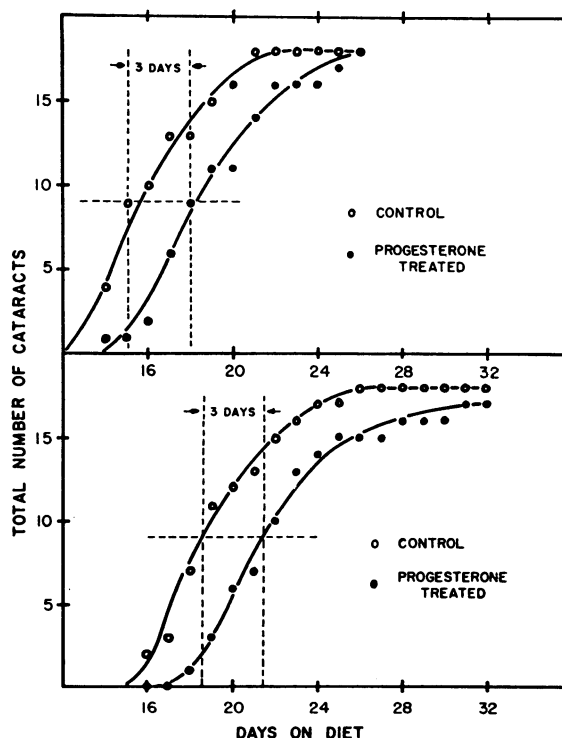


FIG. 2. EFFECT OF PROGESTERONE ADMINISTRATION ON CUMULATIVE INCIDENCE OF CATARACTS IN RATS MAINTAINED ON 40 PER CENT GALACTOSE DIETS

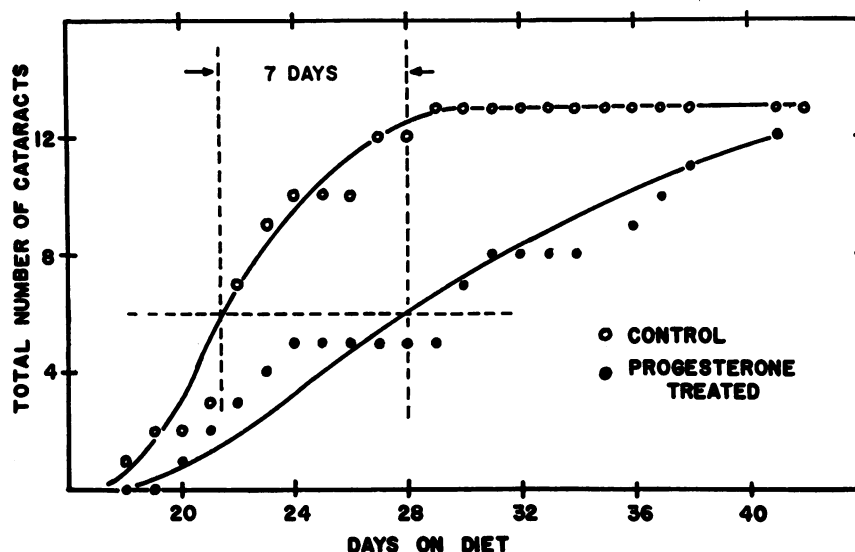


FIG. 3. EFFECT OF PROGESTERONE ADMINISTRATION ON CUMULATIVE INCIDENCE OF CATARACTS IN RATS MAINTAINED ON 30 PER CENT GALACTOSE DIETS

Effect of progesterone administration on cumulative incidence of cataract formation in rats maintained on high galactose and xylose diets

The results of two replicate experiments using a 40 per cent galactose diet are shown in Figure 2. It can be seen that the total number of cataracts was the same in both the control and treated groups. However, there is a three day delay in the time taken for half the total number of cataracts to develop in the progesterone-treated group. In an attempt to accentuate this difference, an experiment was conducted using a 30 per cent galactose diet. These results are shown in Figure 3. Using this lower galactose content diet, the total number of cataracts was less and again it can be seen that the same number of cataracts ultimately developed in both groups. However, the time for development of half the total number of cataracts is now delayed seven days by treatment with progesterone.

Another parameter of the progesterone effect on cataract formation is shown in Figure 4. Daily incidence of cataract development is plotted against day of the cataract development period. Thus, day 1 of this period becomes the day of observance of the first cataract in all experiments independent of the dietary regimen. Not only is there a significant delay in the peak incidence but there is also a decrease in the daily incidence of cataract development, $t = 2.50$, $p < 0.02$. This is more ap-

parent in the group on the 30 per cent galactose diet. No differences were observed between control and treated groups with regard to weight gain, dietary consumption or general appearance. That

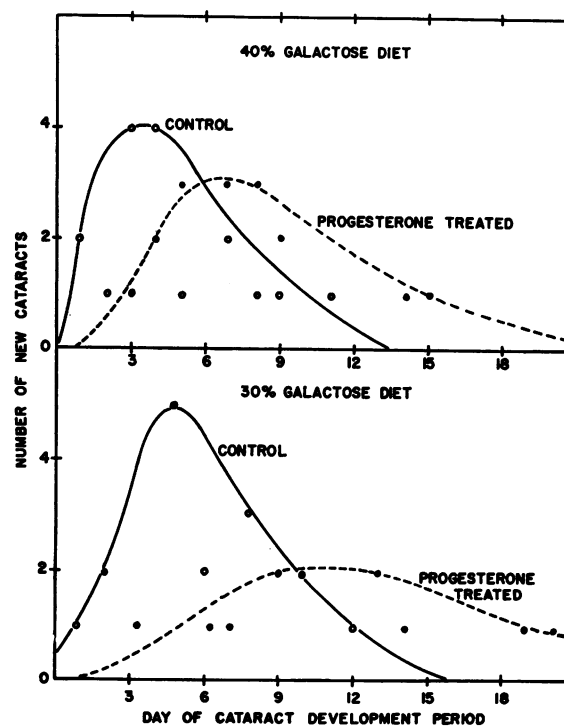


FIG. 4. EFFECT OF PROGESTERONE ADMINISTRATION ON DAILY INCIDENCE OF CATARACTS IN RATS MAINTAINED ON 30 AND 40 PER CENT GALACTOSE DIETS

TABLE I
Effect of progesterone on daily incidence of cataracts in rats maintained on a 30 per cent xylose diet

Days on diet	No. cataracts	
	Control	Progesterone
1-6	0	0
7	5	6
8	8	8
9	8	8
10	10	11
11	10	11
12	10	11

this is a specific effect on galactose metabolism is suggested by the fact that progesterone administration had no effect on cataract development in rats maintained on a 30 per cent xylose diet (Table I).

DISCUSSION

Congenital galactosemia is a disease characterized by the development of cataracts, mental retardation and liver damage (10). These manifestations are thought to be the result of excess intracellular accumulation of gal-1-P (11, 12). The biochemical lesion in this disease is a genetically determined deficiency of the enzyme P-Gal transuridylylase as has been demonstrated by Isselbacher and associates (13) and Kalckar (14). Figure 5 depicts the known reactions (15) in the conversion

of galactose to CO_2 via the 6-phosphogluconic acid pathway. Galactose is first phosphorylated by galactokinase. Gal-1-P then reacts with uridine diphosphoglucose (UDPG) in the presence of P-Gal transuridylylase to yield uridine diphosphogalactose (UDPGal) plus glucose-1-phosphate (G-1-P). The UDPGal is then epimerized by the UDPGal-4-epimerase to UDPG which then can react with another molecule of gal-1-P or be pyrophosphorylytically cleaved to yield G-1-P which then enters the glycolytic pathway. In the galactosemic individual, the transferase enzyme is lacking or deficient and consequently gal-1-P accumulates within the cell. However, small amounts of galactose can be metabolized (16) either via the small amount of P-Gal transuridylylase present or via an alternate enzyme recently described by Isselbacher (17). In this latter instance the P-Gal transuridylylase deficiency can be circumvented by an additional pyrophosphorylytic reaction. Gal-1-P under these circumstances is converted to UDPGal by the enzyme UDPGal pyrophosphorylase (17) (Step A, Figure 5). UDPGal is then epimerized by the UDPGal-4-epimerase to UDPG which in turn can be converted to G-1-P by UDPG pyrophosphorylase (Step B, Figure 5). The extent to which such an alternate pathway operates in the normal or in the galactosemic individual is not known.

The mechanism by which progesterone exerts its stimulatory effect on the oxidation of galactose-1- C^{14} to C^{14}O_2 in the galactosemic individual is unknown. These studies were conducted before it was known that the UDPGal-4-epimerase reaction, an enzymatic step beyond the metabolic block in galactosemia, is the site of the *in vitro* progesterone stimulation of galactose metabolism (2). The *in vivo* observations may be the result of a completely different type of mechanism such as induction of the deficient or an alternate enzyme. It is therefore apparent that the explanation of this effect must await further experimental evidence.

At the present time there is no good experimental animal in which to study the complete picture of galactosemia other than the human subject. However, one of the parameters of the disease is the development of cataracts and it is a well established observation that high galactose and xylose diets will produce cataracts in young rats

◀ GALACTOSE METABOLIC PATHWAY ▶

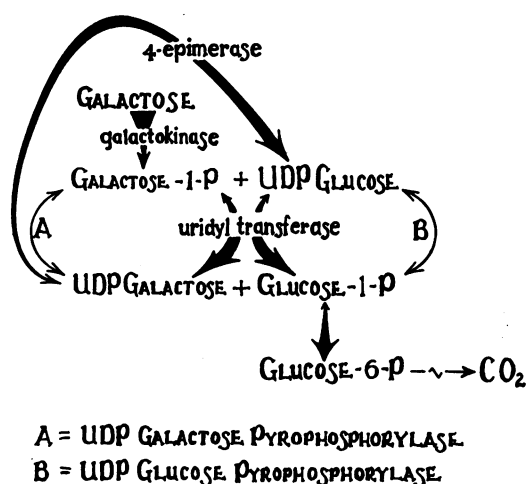


FIG. 5. REACTIONS INVOLVED IN THE CONVERSION OF GALACTOSE TO CO_2 VIA THE PHOSPHOGLUCONIC ACID OXIDATIVE PATHWAY

(18-20). This is not meant to imply a relationship between the mechanism of production of cataracts in the two different situations. However, the observation that progesterone administration delays the onset and decreases the *daily* incidence of cataract development in young rats maintained on high galactose diets does indicate that progesterone in some way acts to ameliorate the toxicity of galactose in high concentration. This is supported by the observation that progesterone exerted no effect on the development of cataracts by rats maintained on high xylose diets. Here again a relationship cannot be drawn between the *in vitro* effect of progesterone on the rate of the UDPGal-4-epimerase reaction and the *in vivo* effect on cataract formation. Several attempts were made to study the effect of progesterone on the rate of galactose-1-C¹⁴ conversion to C¹⁴O₂ by lens but the rate of oxidation was so low that no reliable data could be obtained.

It is of interest to the interpretation of these results that Schwarz and Golberg (21) have reported increased accumulation of gal-1-P in the optic lens capsule of rats fed high galactose diets. These levels of gal-1-P were similar to those reported to occur in erythrocytes of patients with congenital galactosemia (11).

At the present time it is not possible to ascribe any therapeutic benefit for the galactosemic patient to the administration of progesterone based on the results of these tracer or dietary studies. Such a conclusion must await further careful evaluation. Even under optimal conditions progesterone administration could not be expected to replace the present management of galactosemia. However, progesterone might favorably act to minimize the progression of cataract formation and mental deficiency, especially during the periods of the disease when patients are acutely ill as a result of ingestion of high milk diets, or during exacerbations when cataract development and mental retardation seem to be progressive.

SUMMARY

Administration of progesterone to three prepubertal patients with congenital galactosemia resulted in a significant increase in ability to oxidize a tracer dose of galactose-1-C¹⁴ to C¹⁴O₂. Furthermore, administration of progesterone to young

male rats maintained on high galactose diets caused a delay in the onset and a decrease in the *daily* incidence of cataract formation. These observations are discussed in light of *in vitro* effects of progesterone and in terms of possible therapeutic implications in congenital galactosemia.

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