Variation of the oral galactose tolerance test with age

Fifty-one oral galactose tolerance tests were performed on 44 subjects whose ages ranged from 2 days to 37 years. Compared with older children and adults, newborn infants less than 8 days of age showed lower fasting blood glucose levels and a greater rise of blood glucose during the first half hour of the test; galactose was much less frequently demonstrated in the blood by paper chromatography. It is suggested that these findings may indicate that in the newborn infant galactose is more readily converted to glucose than in older subjects, and that this may compensate for the hypoglycemia of infancy.

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HARTMANN and his co-authors[1, 2] stated that in infants who have been fed milk as much as 40 per cent of the total blood sugar may be galactose. They also found that after large doses of galactose (1.75 Gm. per kilogram body weight) by mouth the rise in blood sugar might be entirely due to galactose, the glucose level sometimes falling and then rising again as the galactose fell. To distinguish between glucose and galactose in the blood these workers used the true sugar method of Somogyi before and after yeast fermentation.

With a glucose oxidase and a copper reduction method Dormandy Leak, and Grant[3] reached similar conclusions. They found that in full-term and premature infants who had been fed breast milk the difference between the total blood sugar and the blood glucose was sometimes as much as 60 to 80 mg. per 100 ml. and they assumed that the difference was due to the presence of galactose in the blood.

We have described findings different from the above workers.[4] In 11 infants who had received lactose-containing feedings, we could not demonstrate galactose in the blood in amounts greater than 10 to 20 mg. per 100 ml. by paper chromatography despite differences between total blood sugar and blood glucose levels amounting to 20 to 30 mg. per 100 ml. in several infants. These observations led us to investigate the oral ga-

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lactose tolerance test in newborn infants and to compare the results with those obtained in older children and adults.

CLINICAL MATERIALS AND METHODS

Tests were performed on 44 subjects who were divided into groups depending on their age:

	No. of cases
Group I	
Newborn (aged 2 to 7 days)	
a. Mature (birth weight 2,632 to 3,850 grams)	10
b. Premature (birth weight 1,330 to 2,449 grams)	10
Group II	
Older infants (aged 8 days to 2 months)	
a. Mature	7
b. Premature	6
Group III	
Children (aged 6 months to 8 years)	6
Group IV	
Adults (aged 16 to 37 years)	5

Each group contained approximately an equal proportion of males and females.

The newborn infants were all apparently normal and were born in the Women's Pavilion of the Winnipeg General Hospital. The older children were inpatients at the Winnipeg Children's Hospital who were convalescing from acute illnesses or were suffering from minor cutaneous lesions or orthopedic abnormalities. None had had elevated temperatures for several days preceding the test, none were taking drugs which might have an effect on their metabolism, and none were suffering from any metabolic disorder. They were all observed to have average appetites and were eating a standard hospital diet. The adults were all normal and in good health.

The tests on the infants were usually performed at the normal feeding times, i.e., 4 hours after the previous feeding or, in the case of smaller premature babies, 3 hours after feeding. All the older children and adults were fasted for at least 7 hours before the tests.

After a capillary blood sample had been obtained, galactose in water was given by mouth in a dose of 1.75 Gm. per kilogram body weight (maximum dose 50 Gm.). Many of the newborn infants were fed by gavage. No subject took longer than 7 minutes to drink the sugar solution and none vomited. Capillary blood samples were taken ½, 1, and 2 hours after the galactose had been administered.

Capillary blood was collected into dry heparinized capillary tubes and stored on ice until the conclusion of the test when, after centrifuging, all samples of plasma were analyzed for total sugar and glucose and by paper chromatography.

Total sugar was estimated by the method of King[5] which uses a sodium tungstate and copper sulfate precipitant; the filtrate is treated with a copper reagent and the color produced with phosphomolybdic acid. The standard deviation of 21 duplicate samples by this method was found by us to be ±2.04 mg. per 100 ml. Glucose was estimated by the glucose-oxidase method of Huggett and Nixon[6] as modified by us.[4] This modification is found to yield linear results, when concentration is plotted against optical density over the range of glucose concentrations studied (standard deviation of 21 duplicate samples ±2.07 mg. per 100 ml.).

After the chemical analyses had been performed, the remaining plasma was used for paper chromatography, the method having been described previously.[4] This volume varied depending upon the ease of blood collection and the cell volume of the blood. Fifty microliters of plasma filtrate was the minimum used for chromatography and with this volume galactose could just be detected in a concentration of 0.01 per cent. Two, three, or four times this volume was available in many instances, thus greatly increasing the sensitivity of galactose detection. When galactose was detected it was not thought justifiable to estimate, even approximately, its concentration. If no galactose was found, it was recorded as being absent in concentrations greater than 10 or 5 or 2.5 mg. per 100 ml.

Table I. Mean total blood sugar (T) and blood glucose (G) levels (in mg./100 ml.) with standard error (S.E.) and range of 51 galactose tolerance tests on 44 subjects divided into groups by age

Group	No. tests		Fasting		½ hour		1 hour		2 hours	
			Mean ± S. E.	Range	Mean ± S. E.	Range	Mean ± S. E.	Range	Mean ± S. E.	Range
Ia	10	T	59.5 ± 5.2	29 to 79	109.0 ± 8.3	76 to 163	103.1 ± 15.0	81 to 198	88.1 ± 4.8	70 to 125
		G	56.6 ± 5.1	30 to 80*	100.7 ± 5.8	75 to 134	95.2 ± 12.1	72 to 156	82.8 ± 3.9	69 to 114
Ib	10	T	42.5 ± 5.2	18 to 74	101.2 ± 9.8	52 to 149	116.4 ± 8.1	68 to 156	82.4 ± 4.4	63 to 100
		G	39.7 ± 5.3	8 to 65	83.8 ± 8.1	38 to 112	100.0 ± 6.4	62 to 123	71.0 ± 3.1	60 to 86
IIa	10	T†	74.2 ± 2.5	62 to 87	109.4 ± 5.6	85 to 132	120.3 ± 4.2	108 to 144	89.6 ± 5.0	68 to 127
		G	70.5 ± 2.4	62 to 83	95.0 ± 4.7	74 to 109	97.8 ± 4.8	78 to 132	84.1 ± 4.4	65 to 118
IIb	10	T†	63.0 ± 3.0	51 to 82	112.5 ± 8.5	89 to 178	119.0 ± 4.7	101 to 152	74.8 ± 5.3	49 to 104
		G	59.8 ± 3.3	49 to 78	85.4 ± 4.1	74 to 108	92.3 ± 4.5	75 to 113	70.9 ± 5.4	47 to 99
III	6	T	77.0 ± 5.7	62 to 101	112.8 ± 12.2	70 to 151	122.8 ± 11.1	80 to 154	114.5 ± 12.6	76 to 163
		G	75.8 ± 3.8	63 to 85*	85.3 ± 4.2	72 to 102*	77.3 ± 2.9	71 to 81	78.5 ± 2.6	71 to 80
IV	5	T	86.4 ± 3.0	78 to 97	117.4 ± 8.9	103 to 148	128.6 ± 13.2	99 to 172	88.4 ± 2.8	79 to 95
		G	86.2 ± 2.6	78 to 93	96.4 ± 4.0	87 to 108	84.2 ± 4.1	71 to 96	83.6 ± 3.9	75 to 97*

*Occasional instances in which the glucose oxidase method yielded results slightly higher than the copper reduction method are explicable in terms of the standard deviations of the methods.

†Three subjects in Group II and four in Group II b had previously been tested when their ages allotted them to Group I a and I b, respectively.

RESULTS

A total of 51 tests were performed on the 44 subjects since 7 infants (3 mature and 4 premature ones) were each tested on 2 occasions, initially during the first 7 days of life and again when about 3 weeks old. Thus the results of the first tests on these subjects appear in Group I and those of the second tests in Group II.

Blood sugar levels. The mean values with standard error and range for the total blood sugar and blood glucose levels are shown in Table I. There were no statistically significant differences between the mean fasting, one half, one and two hour values obtained in the children and adults. For further analyses, therefore, Groups III and IV have been combined.

There were statistically significant differences in the mean fasting blood glucose levels of Group Ia and b. ($0.01 > P > 0.001$) and between both a and b subgroups of Group I and Group II and Groups III and IV. The difference in the mean fasting blood glucose level between Group II a and b was not significant.

The mean rise in blood glucose from the fasting to the half hour level of Group I a was 44.1 mg. per 100 ml., of Group I b 44.1 mg. per 100 ml., of Group II a 24.5 mg. per 100 ml., of Group II b 25.6 mg. per 100 ml., and Groups III and IV 9.8 mg. per 100 ml. There were no statistically significant differences between a and b subgroups of Groups I and II, however, there were significant differences of mean rise between Group I, Group II, and Groups III and IV. When the fasting blood glucose levels were compared with the rise of glucose during the first half hour after galactose administration in all groups, a statistically significant correlation was shown. For instance, of 17 subjects having a fasting glucose level of less than 60 mg. per 100 ml., 11 showed a rise of 30 mg. per 100 ml. or more, whereas of 34 subjects having a fasting glucose level of 60 mg. per

100 ml. of greater, only 10 showed a rise of 30 mg. per 100 ml. or more ($0.05 > P > 0.02$).

The mean values for total sugar and glucose in Group I a and Groups III and IV are represented in Fig. 1 which emphasizes the difference between the two curves; namely, the difference in the fasting sugar levels and the much greater rise of the blood glucose during the first half hour with the subsequent slow fall for the next one and a half hours in the newborn group. In contrast the glucose levels of Groups III and IV had risen to only a slight degree after half an hour and during the second half hour fell below the fasting level. The total blood sugar levels in Group I a paralleled the glucose levels, whereas in Groups III and IV the total sugar rose to very much greater extent.

Chromatography. Galactose was not found in any fasting blood sample. Table II shows the results of the chromatographic examination of the blood samples of one half, one, and two hours. It may be seen that galactose was only identified in the blood of 11 of the 20 newborn babies tested (Group I a and b) and in only 19 of the 60 blood samples chromatographed. In Group II, the older infants, galactose was identified in every test (38 of 60 blood samples), and in Groups III and IV in 10 of 11 tests (26 of 32 samples). The subject in the latter groups who showed no galactose in any blood sample was a 2-year-old girl with a mild dermatitis but who was otherwise healthy. In all instances when no galactose was recorded this indicated a concentration of less than 10 mg. per 100 ml., and in many, when a larger volume of plasma was available for chromatography, less than 2.5 or 5 mg. per 100 ml. The very infrequent finding of galactose in the blood of Group I subjects as compared with those in Group II and Groups III and IV is of course highly statistically significant. The number of one half and one hour blood samples containing galactose was not statistically significantly different in Group II and Groups III and IV, although fewer two hour samples of blood contained galactose in Group II, i.e., 2 of the 20 two hour samples in Group II as compared with 7 of 11 in Groups III and IV. This difference is statistically significant ($P < 0.01$).

Repeated tests on the same baby. As mentioned 7 babies (3 mature and 4 premature) were each tested on 2 occasions, first when 7 days of age or less and again when between 2 and 3 weeks of age. The results obtained from these babies are included in Groups I and II depending on the age of the baby when the tests were performed. Figs. 2, *A* and *B*, represent the mean total blood sugar and blood glucose levels of the three mature and four premature babies, respectively. The curves show differences that have previously been enumerated in comparing Groups I and II, namely, the lower fasting blood glucose levels and the infrequent demonstration of galactose in the newborn group.

DISCUSSION

The results of the oral galactose tolerance tests in the newborn infants (Group I) showed three statistically significant differences from those obtained in the older infants (Group II) and the children and adults (Group III and IV). These differences were: (1) a lower fasting blood glucose level, (2) a greater rise of blood glucose during the first half hour after the administration of the galactose, (3) the infrequent appearance of galactose in the blood. The tests in Group II also showed significant differences from Group I and Groups III

Table II. Results of paper chromagraphy of one half, one and two hour blood samples of 51 oral galactose tolerance tests

Group	*No. tests*	*No. tests showing galactose*	*No. blood samples chromatographed*	*No. samples showing galactose*
Ia	10	5	30	7
b	10	6	30	12
IIa	10	10	30	19
b	10	10	30	19
III and IV	11	10	32	26

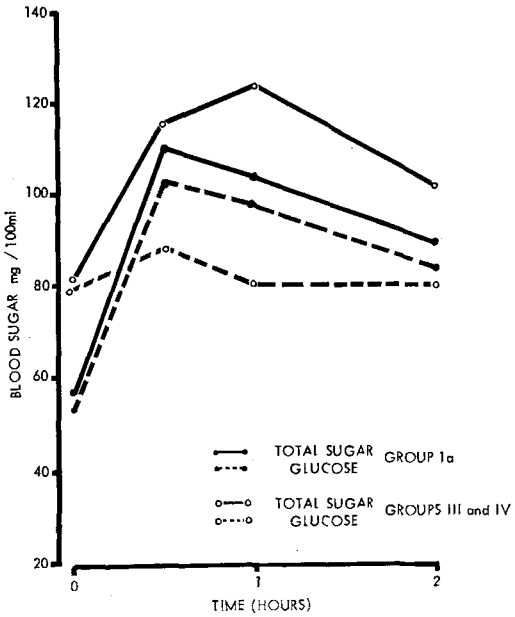

Fig. 1. Mean blood glucose and total blood sugar levels of Group I a and Groups III and IV during oral galactose tolerance tests.

and IV with respect to *1* and *2*. In addition fewer two hour blood samples in Group II contained galactose as compared with Groups III and IV. Except for the lower fasting blood glucose levels of the newborn premature infants (Group I b) no statistically significant differences were found between the results of the premature and full-term infants.

Galactose after absorption from the gut is transported to the liver where it enters the glucose metabolic pathway at the glucose-1-phosphate level. Only small amounts are metabolized in other tissues.[7] If galactose reaches the liver in an amount greater than the capacity of that organ to metabolize it, the excess escapes into the general circulation. After conversion to glucose-1-phosphate, the sugar may be stored as glycogen, converted to glucose-6-phosphate by mutase activity, and be oxidized or released as free glucose, presumably depending upon the immediate needs of the individual. The greater rise of blood glucose within half an hour of feeding galactose, which was found in the Group I infants as compared with Group II and Group III and IV, suggests a greater release of glucose into the circulation and this might have been expected considering the lower initial blood glucose levels of Group I and to a lesser extent of Group II. There was indeed a statistically significant correlation between the fasting blood glucose level and the rise of glucose within the first half hour. In the subjects of Groups III and IV with higher fasting blood glucose levels the blood glucose was probably prevented from rising to any degree by insulin which would be released by any significant elevation of the blood glucose and also by the rapidly rising blood galactose level, the latter sugar being known to initiate release of the hormone as well as the former.[8] Insulin was probably released to a very much lesser degree in Group I, first because the blood glucose had to rise considerably to reach the theoretical threshold for insulin release and second because very little galactose appeared in the blood.

The possibility was considered that the greater rise of blood glucose in the infants was in part due to the stress of the manipulations and needle pricks during the performance of the tests. In 22 children of different ages we performed fasting blood glucose estimations half an hour apart. Although newborn infants showed a mean rise of blood glucose of 6 mg. per 100 ml. over the half hour period, whereas older children showed no mean change, the variation was considerable, and no constant pattern could be observed. Certainly this factor could not have been a significant factor in contributing to the different blood sugar curves at different ages.

The less frequent detection of galactose in the blood of the Group I infants as compared with Group II and Groups III and IV was a striking finding. In Group I it was only demonstrated in 19 out of 60 blood samples chromatographed (Table II). In 7 newborn babies who were tested during the first week, galactose was only found in 1 half hour sample and in 1 of the one hour samples. When the same babies were retested at 3 weeks of age, galactose was found in all half hour samples and in 6 of the seven one hour

samples (Fig. 2). Thus it seems that after an oral dose of galactose, the sugar does not appear in the peripheral blood to any degree during the first week of life, but after that age there is a fairly rapid change and at 3 weeks it may be found in high concentration.

The infrequent finding of galactose in the blood of newborn babies might be due to two main factors: (1) slower absorption of the sugar or (2) greater conversion to glucose in the liver. The latter seems more likely in view of the considerable rise in blood glucose that occurred within half an hour of administration of the sugar. A greater conversion of administered galactose into glucose during the first week of life could only be proved by intravenous galactose tolerance tests. Mulligan and Schwartz[9] administered galactose intravenously to infants 3 to 5 hours of age and reported diminished elimination of the sugar from the blood as compared with that of older individuals. Vink[10] on the other hand, performed intravenous galactose tolerance tests in subjects of different ages and found a greater disappearance rate of galactose in youth; apparently he did not study newborn infants, however. Clearly more information about the intravenous galactose tolerance test in infancy is required and we plan to study this. Some of the factors which might cause infants to metabolize galactose at a different rate from older subjects were discussed by the above authors. We considered the possibility that the difference might be hormonal in origin. It has been found that certain steroids increase the oxidation of galactose with progesterone, androsterone, and testosterone having an especially marked effect.[11, 12] Since the newborn baby has relatively larger amounts of circulating progesterone for the first week of life, probably derived from its mother,[13] it seemed possible that this hormone might be responsible for the differences in the galactose tolerance test we had found at this age. We performed, therefore, galactose tolerance tests on 6 infants of 3 weeks of age, following which progesterone was administered over the next

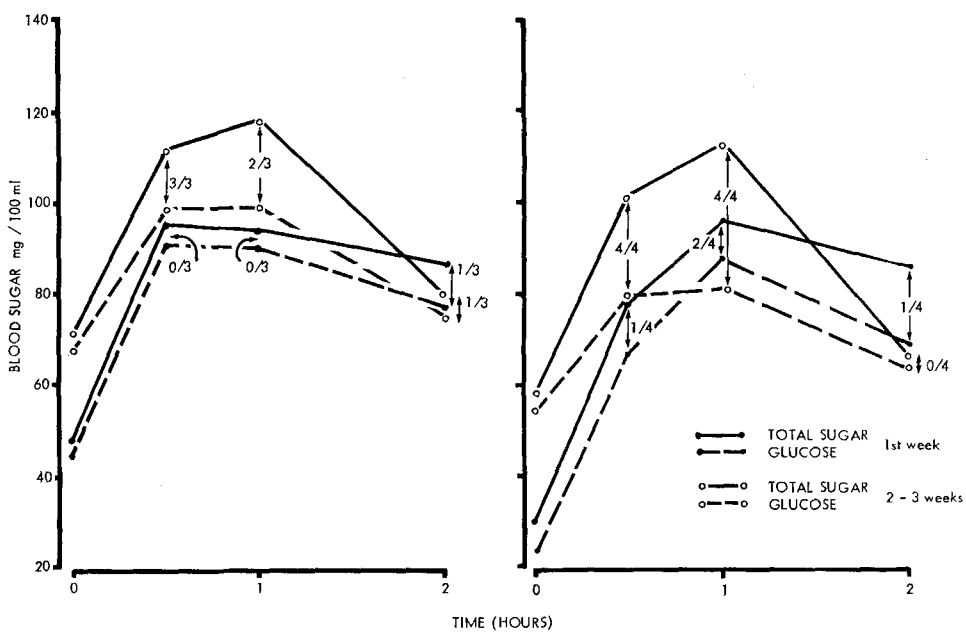

Fig. 2. Mean blood glucose and total blood sugar levels during oral galactose tolerance tests on *(A)* 3 mature and *(B)* 4 premature infants within the first 7 days of life and again when 2 to 3 weeks old. The figures on the graphs represent the proportions of blood samples chromatographed in which galactose was detected.

24 hours. The galactose tolerance tests were then repeated with the thought that a change to the newborn pattern might be shown. In fact no difference in galactose tolerance could be demonstrated after administration of the hormone.

SUMMARY

Fifty-one oral galactose tolerance tests were performed on 44 subjects ranging in age from newborn infants to adults.

It was found that newborn infants of 7 days of age or less had lower fasting blood glucose levels and greater rises of blood glucose within the first half hour after administration of galactose than did a group of children and adults (aged 6 months to 37 years). Fasting blood glucose levels and the rise of blood glucose during the first half hour in a group of infants aged 8 days to 2 months were intermediate between the newborn group and the older children and adults. These differences are statistically significant.

Galactose was much less frequently found in the blood of infants aged 7 days or less after administration of the sugar as compared with that of the older subjects. It is suggested that this may indicate that the newborn infant has the ability to metabolize galactose more completely than older individuals. This ability is rapidly lost after the first 7 days of life.

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