SOME EFFECTS OF LACTOSE AND SUCROSE ON THE GROWTH INTELLIGENCE, BRAIN COMPOSITION, AND HEMOGLOBIN OF YOUNG RATS

by

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INTRODUCTION

It has long been known that the central nervous system contains galactose in the form of cerebrosides. These cerebrosides, which yield sphingosin, galactose, and a fatty acid on hydrolysis, make up a large proportion of the lipid material of the myelin sheath. Since galactose is one of the two monosaccharoses formed on hydrolysis of lactose, the disaccharose found in milk, nature has provided a liberal supply of galactose for the young mammal at the time when the myelin sheath is developing rapidly.

It is interesting to speculate as to the possible relationship of this large content of galactose in the ration of the young when they are building the myelin sheath to the composition of the sheath. If the body could not synthesize galactose, and if the ration were galactose free, it would not be possible to build these galactosides which play such an important part in the central nervous system.

Large quantities of galactose, and of no other sugar, are found in the nyelin sheath. It was for the purpose of seeing what effect a ration low in galactose would have on the development of the young that this investigation was carried out. The purpose of this study was to compare rats receiving no lactose or galactose with animals receiving

20 percent of lactose. This was never achieved, for Sørensen and Haugaard (1933) found that casein itself contains 0.30 percent galactose. The rations containing no lactose, may therefore have contained 0.06 percent galactose.

When the effect of galactose on the development of the nervous system is being studied by comparison of high-and-low-galactose rations, all other factors should be kept constant. The energy available per gram of feed should be the same in both rations, and there should be no disturbance in metabolism in either group. These ideal conditions may have not been attained in these experiments, for the addition of lactose to a ration sometimes introduces several radical changes in the nutritional status of the rats, as has been indicated by the observations of various workers in this field.

To obtain an accurate measure of the intelligence of rats by the maze method, the incentive, or hunger of animals on the test rations must be the same. This will be so if the two rations differ only slightly or if the compound being tested has no effect on hunger. If the addition of lactose to the ration causes differences in hemoglobin, rate of growth, absorption of calculi, etc., the incentives of the two groups are similar only if this sugar is added in very small amounts. The high percentage of lactose, 20 percent, was used before some effects now described in the literature had been studied, and having begun with this amount, it was of doubtful advantage later to reduce it.

REVIEW OF LITERATURE

Feeding Sugars

Feyder (1935) found that rats fed sucrose gain weight more rapidly than those given the same amount of glucose. Sucrose gives fructose and glucose upon hydrolysis so the difference between the two rations may be the fructose in the sucrose ration. Lactose hydrolyzes to form galactose and glucose, and animals receiving this disaccharose do not get the fattening sugar fructose. This may help to explain the more rapid growth rate of the sucrose rats in our own experiment.

Koehler and Allen (1934) fed rats on a sub-maintenance ration, then made up the deficiency in calories by adding various carbohydrates. The rate of gain of the lactose fed animals was only 61 percent that of the sucrose fed rats. It was evident that lactose fed rats did not gain in weight as rapidly as sucrose fed animals.

Cori (1928) found that the rate of absorption of sugar did not depend on concentration, but was a constant and different for each sugar. In the order of their rate of absorption, he lists the sugars thus: galactose, glucose, fructose, mannose, xylose, and arabinose.

Cori also observed that glucose when fed to rats in as large amounts as fifteen grams per kilogram, did not lead to a sugar excretion in the urine. On the other hand, close to 50 percent of the total amount of galactose absorbed was eliminated in the urine. This may explain why lactose fed rats did not gain in weight as rapidly as the sucrose animals. The galactose, produced by hydrolysis of the lactose, may not have been available for energy.

Ariyama and Takahasi (1929) studied the relative ease of assimilation of various sugars and found that fructose and sucrose were more quickly absorbed than galactose or lactose. The sugar assimilated per 100 grams body weight was as follows: galactose, 0.10 grams; lactose, 0.16 grams; fructose, 0.50 grams; and sucrose, 0.76 grams. This work may explain why a lactose fed animal did not make as rapid weight as a sucrose fed animal.

Hartwell (1926) made a study of growth and reproduction on synthetic rations. The ration which she used consisted of the following: caseinogen, potato starch, salt mixture, marmite, and "a little cod liver oil" with the addition of various fats. She reports: "Maximum growth was not obtained on any of the synthetic diets used, and reproduction was poor... The general condition of the rats fed on these synthetic diets was good except for a characteristic loss of fur." Hartwell also tells us that reproduction did not occur in rats fed on 20 percent bacon fat or lard.

Evans and Burr (1926) report some difficulty with a ration high in lactose. They write: "The use of sucrose has been decided upon after a comparison of this sugar with chemically pure glucose and chemically pure lactose. The former offers no advantage, and lactose proved entirely unsuitable since the animals soon declined and died, apparently because of intestinal disturbances." The ration used by Evans and Burr consisted of: casein, 50 parts; sucrose, 150 parts; salts, 8 parts; cod liver oil, 2-3 drops daily; and yeast, 700-1000 milligrams daily.

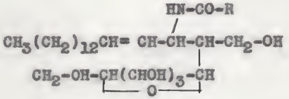
It is quite evident either that lactose in this ration was not utilized by the rat or that the sugar upset the digestive system, causing failure to grow normally. For this reason, the amount of lactose added to a basal ration should be very small.

Randoin and Lecoq (1927) studied the effect of sugars on the vitamin B requirement of pigeons and rats and found that the more sugar the ration contained, the greater was the requirement of vitamin B. They also found that more vitamin B was required in a lactose or galactose ration than with glucose.

Chemistry of the Brain

Previous to a study of effects of various rations on brain composition, a brief review of the chemistry of the brain may be helpful. Brain lipids consist for the most part of cerebrosides, phosphatides, and cholesterol. These constituents are fat-like materials soluble in such solvents as alcohol, ether, pentane, or petroleum ether. Together with water these lipids form the major part of the brain.

Three cerebrosides--kerasin, phrenosin (or cerebron), and nervon-- have been isolated. These cerebrosides are thought to be identical in general structure, and to differ only in the fatty radicals which they contain. On complete hydrolysis each yields sphingosine, galactose, and a fatty acid. They contain no glycerol or phosphorus.

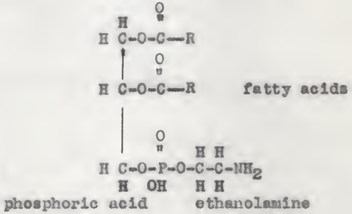


fatty acid
sphingosine
galactose

Cerebroside

The cerebrosides are divided into two classes: saturated cerebrosides whose fatty acids are saturated, and unsaturated cerebrosides whose fatty acids contain one or more double bonds. According to Harrow and Sherwin (1935), the structure of the fatty acids which occur in cerebrosides has not yet been established with certainty.

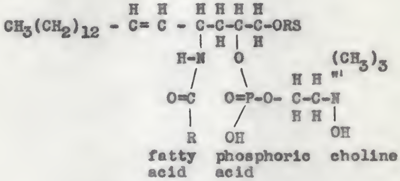
Phosphatides are compounds which contain glycerol, two fatty acids, phosphoric acid, and a nitrogen base. One of the principal classes of phosphatides is made up of the cephalins.



Cephalin

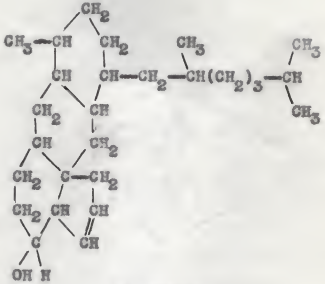
Upon hydrolysis a cephalin yields glycerol, two fatty acids (whose alkyl groups are represented in the formula by "R"), phosphoric acid, and ethylamine. There are as many cephalins as there are possible "R" groups. The lecithins make up another class of phosphatides, the various lecithins differing only in the nature of the "R" groups. A lecithin upon hydrolysis yields glycerol, two fatty acids, phosphoric acid, and choline (trimethyl, hydroxy ethyl ammonium hydroxide).

Sphingomyelins are phosphatides which on hydrolysis give a fatty acid, choline, phosphoric acid, and the base sphingosine. No glycerol is present.



Sphingomyelin

Cholesterol is considered a lipid because of its physical properties. It is insoluble in water, dilute acids, or alkali, and is soluble in alcohol, ether, petroleum ether, and chloroform. It is very stable toward hydrolytic agents such as acids and alkali. It is easily attacked by oxidizing agents.



Cholesterol

Methods of Brain Analysis

The usual method of brain analysis consists of drying the tissue, extracting it with alcohol, ether, pentane or petroleum ether, isolating the constituents of the extracts, and finally measuring the amount of material found in the isolated product. Three of the methods of analysis which have been used will be described briefly.

1. A common method of inorganic analysis is to precipitate the ion to be determined and then to dry and weigh the precipitate. This method has been used in brain analysis with but little success because precipitation of the lipids with metallic ions such as platinum and lead often breaks up the lipid molecule to form compounds which

do not precipitate with the metallic ion.

2. If the empirical formula of a compound, such as sphingomyelin, is known, if the compound contains phosphorus or nitrogen, and if it has been isolated from other compounds containing phosphorus or nitrogen, then the amount may be calculated from the nitrogen or phosphorus which the sample contains. After isolating the saturated and unsaturated phosphatides, the saturated and unsaturated cerebrosides, and cholesterol, by Backlin's method, one might determine the formula of the saturated phosphatides and find the amount of phosphatide from the phosphorus content of the sample. Such a method is not convenient because of the difficulty of determining the formula, because cholesterol has neither phosphorus nor nitrogen, and because there is a possibility of obtaining contaminating material containing phosphorus or nitrogen.

3. A third method used in the separation of the lipids depends upon their solubility. The solubility of any one lipid is affected by the presence of other compounds of lipid nature, and is therefore not constant. Despite this disadvantage, Backlin (1930) has been able to work out a method of brain analysis based on the solubility of the sodium salts and fatty acids in water, of cholesterol in petroleum ether, and of cerebrosides in alcohol.

The modification of his method used in this study is described by Cribbett (1934).

EXPERIMENTAL METHODS

Method of Raising Rats for the Experiment

Animals for the experiment were raised by rats fed on a stock colony ration composed of the following: Ground wheat, 45 parts; ground yellow corn, 25 parts; milk powder, 14 parts; meat scrap, 9 parts; alfalfa leaf meal, 4 parts; cod liver oil, 1 part; dry yeast, 1 part; and salt, 1 part.

Stock colony females were bred in groups and were given the above ration. The young rats were weaned at 14 to 28 days of age, were marked by ear, and placed five in a cage. The cages were 2 x 1½ x 1½ feet in dimensions, and were made of wire. The temperature of the room was maintained at approximately 20 to 26 degrees Centigrade. Weighings were made when the rats were placed on the artificial ration, and for the hemoglobin studies at intervals of one week thereafter. Rats born within three or four days were evenly distributed between rations being compared.

Method of Determining Intelligence of Rats

Alm of Kansas State College, measured the intelligence of the rats by the maze method (1936). The intelligence of the animals was determined when they were about 70 to 120 days of age. In order to provoke sufficient hunger to cause rapid running in the maze, only five grams of feed were given to each animal daily, for several days previous to the test. This feed was given at the same hour each day, at the time of day at which the test was later to be made. After several days of the training ration, the animals were allowed to run the maze daily until they learned to find the food without hesitation. Their intelligence was measured by the time required to reach the food chamber and the number of errors made, an error consisting of entrance into an alley not leading to the food.

Method of Brain Analysis

The rats were killed, the brain removed and macerated by passing from one glass syringe to another. The glass syringes contained a copper mesh to remove connective tissue and assist in more uniform mixing. About 30 milligrams of the sample was spread immediately on weighed strips of filter paper previously extracted with pentane and the sample was weighed. Practically no moisture was lost in the maceration process and the weighing was completed before moisture loss was appreciable. The samples were dried by placing in an ordinary vacuum desiccator for one hour and were then extracted for 40 minutes with cold pentane, then for one hour with boiling alcohol. Unsaturated cerebrosides, unsaturated phosphatides, and some of the saturated cerebrosides and phosphatides dissolve in the cold pentane. The rest of the saturated cerebrosides and phosphatides, and cholesterol dissolve in the boiling alcohol. The method, described fully by Cribbett (1934) depends upon the solubility of the sodium salts and fatty acids in water, of cholesterol in petroleum ether, and of cerebrosides in alcohol.

The saturated cerebrosides, unsaturated cerebrosides, saturated phosphatides, unsaturated phosphatides, and cholesterol, were separated and the extract of each rinsed into small test tubes from which the solvent was evaporated. The tube and contents were weighed on a micro balance to 0.000002 gram. A rack built at the rear of the balance held six tubes which could be lifted off singly and placed in a cradle attached to the left balance pan. Weighings were made without opening the balance and the zero point and sensitivity were determined for each group of test tubes.

Method of Hemoglobin Determination

Hemoglobin was determined weekly when it was observed that there was a hemoglobin difference in the two groups of animals. The Dare hemoglobinometer was used because this instrument can detect differences of 10 percent, and differences smaller than that would not be significant.

EXPERIMENTAL PROCEDURE

Experiment I. Raising Rats on Different Sugars

The object was to raise rats on rations high and low in galactose. The rats were fed in metal cages, six to ten rats in a cage. Iron dishes were used for the feed and water. One group of animals was given ration I with sucrose while another group, litter mates of the first group, was given ration I with lactose or galactose. The animals did not have the appearance of normal health. Both lactose and sucrose animals were rough and emaciated and often had blood at the tip of the nose. The sucrose rats declined in weight and died in many cases. The data of table 1 show that the sucrose rats did not grow as well as the lactose animals.

To be certain that 2 percent yeast was sufficient as a source of the B vitamins, an experiment was tried in

Table 1. Composition of Ration I.

	Parts
Casein (commercial	20
Butter oil (melted over night)	20
Sugar (lactose, sucrose, etc.)	20
Starch (cornstarch)	33
Osborne-Mendel Salt Mixture	4
Yeast, dry	2
Cod liver oil	1
Total	100

which 4 percent yeast was compared to 2 percent yeast in the sucrose ration. The yeast was added at the expense of the cornstarch, which was 31 percent in the higher yeast ration and 33 percent in the lower yeast ration. The data of table 1 show that the animals on the 2 percent yeast gained as much as the animals on 4 percent yeast.

Conclusion of experiment I: The sucrose ration was unsatisfactory, for rats could not be raised to maturity.

Experiment II. Study of Butter Oil Preparation

The object of this experiment was to find a ration which would enable both groups of rats to make normal weight gains. The first variation in the ration was the method of making the butter oil. In the preliminary test, butter was melted in the 110 degree oven and kept in a melted condition over night by placing on top of the oven. It was suspected that the butter of former experiments, kept in the melted condition for 12 hours, had become rancid. Table 2 shows the beneficial effect of feeding a sucrose ration containing butter oil which was mixed with the feed within two hours after melting the butter. The butter was melted in a metal can submerged in boiling water. As the butter melted, the liquid was poured into a large glass container warmed by hot water to maintain the oil in the melted condition. When the curd had settled to the bottom leaving a clear oil, the oil was siphoned off and was mixed with the feed which was stored in a refrigerator at a few degrees above freezing. In both sucrose and

Table 2. Growth of Rats in Experiment I on Ration I with Various Sugars.

Sugar fed	No. of animals	Aver. daily gain in grams	Duration of test in days	Age in days when placed on ration
Sucrose	3	1.45	17	34
Lactose	3	2.36	17	34
Sucrose	13	1.30	20	19
Lactose	11	2.10	20	19
Galactose	7	1.55	18	20-24
Lactose	5	2.70	18	20-24
Lactose with 2% yeast	5	2.09	12	20
Lactose with 4% yeast	5	1.91	12	20

lactose groups, the animals showed excellent growth when the method of preparing the butter oil was changed.

Table 3. Composition of Ration II.

	Parts
Casein (commercial)	20
Butter oil (mixed with feed within two hours after melting butter)	20
Sugar (lactose or sucrose)	20
Cornstarch	33
Osborne-Mendel salt mixture	4
Yeast, dry	2
Cod liver oil	1
Total	100

Table 4 shows that the animals did not lack the unsaturated fatty acids, for linseed oil did not cause greater gains in weight. Liver was beneficial to a slight degree.

Table 4. Growth of Rats on Ration II with and without Liver or Linseed Oil.

Ration	Weight when placed on the sucrose diet II at 60 days of age after feeding 39 days on ration I	Final weight after 25 days on sucrose ration II	Daily gain in grams	Number of animals
Sucrose II plus 2% linseed oil	45 grams	104 grams	2.36	1
Sucrose II plus 5% liver	44.5	126	3.28	2
Sucrose II	47.5	121	2.94	1

Conclusion of experiment II: It is possible to raise rats successfully on the sucrose ration II. The animals did not lack the unsaturated fatty acids, and liver did not improve the ration materially.

Experiment III. The Effect of Liver and the Unsaturated Fatty Acids.

It was desirable to have more accurate knowledge regarding the effect of adding liver to the sucrose ration. The yeast was at the same time increased to 10 percent to provide a more ample supply of the B vitamins. The liver was ground, mashed through an ordinary window screen to remove fibrous material and was weighed and mixed with the ration. The feed was kept in the refrigerator and enough was made up to last a week.

Table 5. Composition of Ration III.

	Parts
Casein (commercial)	20
Sugar (sucrose or lactose)	20
Butter oil (mixed with ration within several hours after melting butter	20
Cornstarch	23
Yeast, dry	10
Osborne-Mendel salt mixture	4
NaCl	1
Cod liver oil	1
Bone meal	1
Total	100

Table 6 shows that liver did increase the rate of gain but that the animals made quite satisfactory gains without it. To be sure that the animals did not lack the unsaturated fatty acids, linseed oil was added to the diet of one group. The linseed oil did not increase the rate of gain. The animals were placed on the experiment at 29 days of age and were on the test 12 days. They were divided equally as to sex as far as possible.

Table 6. Growth of Rats on Ration III.

Ration	Average gain per day in grams	Number of animals
Sucrose	3.45	4
Sucrose plus 5% liver	3.89	4
Sucrose plus 2% linseed oil	3.58	4
Sucrose plus 5% liver and 2% linseed oil	3.87	3

Summary of experiment III: Rats made satisfactory gains on ration III with or without liver or linseed oil.

Experiment IV. First Study of the Ration in Relation to Intelligence

The object of the experiment was to raise one group of rats on the sucrose ration and one group on the lactose ration and to compare the intelligence and brain composition of the two groups. The rats were placed on the artificial ration at 21 days of age and were given this ration for 32 days. They were then placed on a stock feed for 14 days and were tested in the maze. Upon completion of the intelligence test, the brain was analyzed for saturated and unsaturated cerebrosides, saturated and unsaturated phosphatides, and cholesterol. The animals were distributed evenly as to sex as far as possible.

Table 7. Composition of Ration IV.

	Parts
Casein	20
Sugar	20
Butter oil	20
Yeast, dry	10
Cornstarch	24
Osborne-Mendel salt mixture	4
NaCl	1
Cod liver oil	1
Total	100

Table 8. Growth of Rats on Ration IV.

Ration	Gain per day in grams	Number of animals
Sucrose	3.29	7
Lactose	3.33	8

Table 9. Chemical Analysis of Rat Brains (males) in Experiment IV.

	Lactose	Sucrose
Average body weight	233.5 gm.	203.5 gm.
Average brain weight	1.80	1.77
Percent cholesterol	1.39 ± 0.02	1.47 ± 0.17
Percent unsaturated cerebrosides	0.22 ± 0.09	0.21 ± 0.08
Percent unsaturated phosphatides	1.66 ± 0.005	1.69 ± 0.03
Number of animals	2	2

Table 10. Learning Scores of Male Rats in Experiment IV.

	Lactose	Sucrose	Std. Dev.	Critical ratio
Errors, average number				
Trials 1-80	235±93	674±50	59	7.4
Trials 7-80	168±76	569±17	40	10.0
Time, average seconds				
Trials 1-80	482±61	834±285	204	1.7
Trials 7-80	288±59	641±118	88	4.0
Number of animals	4	2		

Summary of experiment IV: (1) Animals grew as well on the lactose ration as on the sucrose. (2) Ration did not affect brain composition appreciably. (3) The number of animals used was too few for general conclusions, but the rats tested showed the lactose animals to be superior in intelligence.

Experiment V. Feeding and Giving Learning Tests to Rats on Ration IV and Comparison of Intelligence and Brain Composition

The object of the experiment was to feed groups of rats on the sucrose and lactose rations IV during the period of test, as well as the growth period, and to compare the intelligence and brain composition of the two groups. Litter mates were placed on ration IV at 16 days of age. Table 11 shows the average weight of each group at various stages in their growth.

The average daily gain of the sucrose rats was 3.77 grams, and of the lactose animals, 3.46 grams.

Table 11. Weights of Rats in Experiment V on Ration IV.

No. of animals	Age in days	16	22	31	39	46	71
				Grams			
8	Sucrose	28.8	37.8	77.6	115.7	122.3	236.4
8	Lactose	28.7	39.2	71.0	102.1	117.4	219.0

Bogart suggested that we measure the hemoglobin of the two groups of rats. Table 12 shows the results.

Table 12. Hemoglobin Readings of Rats in Experiment V.

Age in days	46	52	62	71
Sucrose (8)	97	110	120	120
Lactose (8)	112	115	120	117

Table 13. Chemical Analysis of Rat Brains (males) in Experiment V.

	Lactose	Sucrose
Average body weight	193 gm.	193 gm.
Average brain weight	1.80	1.69
Percent cholesterol	1.83±0.14	1.80±0.20
Percent unsaturated cerebrosides	0.31±0.03	0.31±0.11
Percent unsaturated phosphatides	1.40±0.06	1.75±0.47
Number of animals	3	5
Age in days at analysis	105-107	105-107

Table 14. Learning Scores of Male Rats in Experiment V.

	Lactose	Sucrose	Std. Dev.	Critical ratio
Errors, average number:				
Trials 1-70	268+17	421+14	12.7	11.2
Trials 7-70	189±18	351±13	12.2	13.3
Time, average seconds				
Trials 1-70	471+84	474+90	66.0	0.0
Trials 7-70	213±17	288±57	30.0	2.5
Number of animals	3	4		

The rats were tested in the maze on the same artificial ration which they had been receiving in the experiment.

The learning scores show that the lactose animals ran the maze with fewer errors and in less time than the sucrose animals. The lactose rats averaged 277 total errors while the sucrose animals made 421 errors, or 1.5 times as many errors as the lactose group. The critical ratio for all the trials is 11.2, which shows that the difference in number of errors is significant. A critical ratio of more than four shows a significant difference.

Considering all the trials, there is no appreciable difference in the time. If we discard the first six trials on the grounds that the rats were not yet accustomed to the maze, we find that the sucrose animals required more time to run the maze: 288 seconds for the sucrose and 213 seconds for the lactose. The first six trials should be disregarded, for the rats would investigate each corner of the runways, all of which required much time. After the first few times in the apparatus, the rats ran rapidly from the point of starting to the proper turns in the pathways.

The chemical analysis of the brain showed no appreciable difference. The results of all the experiments showed that young lactose animals consistently have higher hemoglobin than sucrose fed animals.

Conclusions of experiment V: This test indicated that the intelligence of the lactose animals was greater than that of the sucrose group. This is particularly significant when we consider that the animals were kept on the artificial rations while given the learning tests. There was no appreciable difference in brain composition at 105 days of age. The hemoglobin in the lactose group during the age of rapid growth was significantly higher than in the sucrose group.

Experiment VI. Study of a Ration Containing Lard

The object of the experiment was to raise rats on sucrose and lactose rations V and to compare intelligence, brain composition, and hemoglobin of the rats.

It is known that the most rapid changes in brain composition occur at about 15 to 20 days of age and also at about 35 to 40 days of age. Placing rats on a galactose free ration at 14 days of age should avoid the normal deposition of galactosides occurring at 19 days, if galactose is not synthesized by the animal. For that reason the rats were weaned at 14 days of age and fed for three days on a warm liquid ration of the composition shown in table 15.

Table 15. Composition of Ration V.

	Parts
Casein (neutralized by Na_2CO_3)	5.0
Osborne-Mendel salt mixture	1.0
Sugar (lactose or sucrose)	5.0
Lard	4.0
Warm water	84.5
Cod liver oil	0.5

The liquid was fed every four hours by dropper day and night. The composition of the ration is only approximate and after the first day the water was gradually decreased. On the third day the water was decreased by half. The animals were then transferred to ration VI. The rats gained almost no weight the first week which shows that this ration was not satisfactory.

Butter oil was replaced by lard because lard is cheaper and contains no galactose, while butter oil may contain a trace of lactose if there is not a complete separation between the curd and the oil. The growth, rates showed, that with lard in the ration the lactose animals did not grow as rapidly as the sucrose animals. Lard was discontinued in the next experiment and butter oil was used again.

There were 20 animals in each group. There was almost no change in weight the first week. For the whole experiment the average daily gain was 3.8 grams on the sucrose ration and 2.82 grams on the lactose ration.

Table 16. Composition of Ration VI.

	Parts
Casein	20
Sugar (sucrose or lactose)	20
Lard	10
Yeast, dry	5
Cornstarch	37
Osborne-Mendel salt mixture	4
NaCl	1
Cod liver oil	3
Total	100

Table 17. Weights of Rats in Experiment VI on Ration V.

	Age in days				
	14	32	44	51	77
Sucrose	24.1	62.6	70.0	143.5	257.0
Lactose	21.7	44.9	44.5	113.3	199.4

Table 18. Hemoglobin Readings of Rats in Experiment VI on Ration V.

	Age in days				
	26	32	42	51	72
Sucrose	85.3	86.1	94.2	98	116.9
Lactose	113.1	104.0	111.8	104.0	118.0

Table 19. Chemical Analysis of Rat Brains (males) in Experiment VI.

	Lactose	Sucrose
Average body weight in grams	163	191
Average brain weight in grams	1.65	1.70
Percent cholesterol	1.77±0.30	1.60±0.21
Percent unsaturated cerebrosides	0.36±0.16	0.37±0.18
Percent unsaturated phosphatides	1.99±0.46	1.95±0.26
Number of animals analyzed	9	14
Age at analysis, days	108-117	108-117

Table 20. Learning Scores of Male Rats in Experiment VI.

	Lactose	Sucrose	Std. Dev.	Critical ratio
Errors, average number				
Trials 1-70	333+28	358+154	42	0.55
Trials 7-70	247±74	268±148	46	0.45
Time, average seconds:				
Trials 1-70	536+161	543+178	70	0.10
Trials 7-70	281±72	248±102	35	0.90
Number of animals	10	14		

The sucrose fed animals gained weight faster than the lactose in experiment VI. This difference in growth makes the intelligence test of questionable value, for speed in running the maze is affected largely by the hunger incentive and hunger in groups of animals growing at different rates may not be the same.

It is evident that to raise animals with the same hunger, the animals should be raised on rations differing in only a minute quantity, if this added factor causes a difference in metabolism. The author would recommend that the lactose be decreased to 2-5 percent in a future experiment.

Summary of experiment VI: The learning scores of experiment VI show that there was no significant difference in learning between the two groups. There was also no appreciable difference in brain composition. Again the lactose fed rats had higher hemoglobin. The growth rates of experiments V and VI show that lard may not be as satisfactory as butter oil, for on ration IV with 20 percent butter oil the average weight of the lactose group at 71 days of age was 219 grams compared with 118 grams for the lactose groups at 72 days of age on ration VI containing 10 percent lard.

Experiment VII. Study of a Ration Containing Fifteen Percent Butter Oil

The object of this experiment was to compare the growth, hemoglobin, intelligence, and brain composition of rats raised on the sucrose and lactose rations VI and VII.

Ration VI contained 10 percent lard and ration VII 15 percent butter oil. After the animals of this experiment had been on ration VI with 10 percent lard for 22 days it was decided that the lard ration was not satisfactory, judging from experiment VI, and accordingly the rats were placed on ration VII containing 15 percent butter oil.

The sucrose animals made an average daily gain of 3.90 grams, the lactose 3.10 grams. There were 16 rats in each group. The animals were placed on ration VI at 16 days of age.

The effect of the lactose ration on hemoglobin appeared within two days. It was difficult to clip the tail of the 16 day old rats without injury to the animals and no hemoglobin readings are available before 18 days.

Table 21. Composition of Ration VII.

	Parts
Casein	20
Sugar	20
Butter oil	15
Yeast, dry	5
Cornstarch	35
Salt mixture (OM)	4
Cod liver oil	1
Total	100

Table 22. Chemical Analysis of Rat Brains (males) in Experiment VII.

	Lactose	Sucrose
Average body weight (grams)	183	211
Average brain weight	1.73	1.8
Percent cholesterol	1.40±0.25	1.44±0.28
Percent unsaturated cerebrosides	0.19±0.18	0.23±0.10
Percent unsaturated phosphatides	1.92±0.40	2.04±0.36
No. of animals analyzed	10	12
Age at analysis	135-140	135-140

Table 23. Learning Scores of Male Rats in Experiment VII.

	Lactose	Sucrose	Std. Dev.	Critical ratio
Errors, average number				
Trials 1-70	301±96	379±108	43.5	1.8
Trials 7-70	213±91	284±101	41.0	1.7
Time, average seconds				
Trials 1-70	482±22	544±121	35.0	1.8
Trials 7-70	274±75	310± 81	33.0	1.1
No. of animals tested	10	12		

Table 24. Weights of Rats on Ration VII in Experiment VII.

	Age in days			
	16	24	43	70
Sucrose	22.5	34.1	105.2	233
Lactose	23.3	32	84.7	191

Table 25. Hemoglobin Readings of Rats on Rations VI and VII in Experiment VII.

	Age in days				
	18	24	34	43	64
Sucrose	84.4	80.4	82.7	89	113
Lactose	95.8	94.8	108	104.4	111.2

Experiment VIII. Feeding Rats on Ration VIII and Comparison of Intelligence, Brain Composition, and Hemoglobin of the Animals.

The object of this experiment was to feed groups of male rats on ration VIII containing lactose, sucrose, or starch; and to compare the intelligence, brain composition, and hemoglobin of the animals. The feed was limited to that of the lightest group to maintain uniform growth and was weighed in daily. No record was kept of feed consumption. The rats were placed on the rations at 16 days of age.

There were seven rats in each group. Only male animals were used. The sucrose fed group made an average daily gain of 1.77 grams; the lactose, 177 grams; and the starch, 1.64 grams. The hemoglobin was run only once, when the rats were 23 days of age. The sucrose rats were found to read 77.7; the lactose, 109; and the starch, 84.

Table 26. Composition of Ration VIII.

	Parts
Casein	20
Carbohydrate (lactose, sucrose or starch)	20
Cornstarch	30
Butter oil	20
Yeast, dry	5
Osborne-Mendel salt mixture	4
Cod liver oil	1
Total	100

Table 27. Weight of Animals on Ration VIII in Experiment VIII.

	Age in days									
	16	25	33	40	51	61	68	73	80	87
	(grams)									
Starch	26	45	74	94	137	146	159	161	156	154
Sucrose	26	59	82	104	123	175	161	180	164	161
Lactose	27	49	76	91	133	163	178	194	168	162

Table 28. Chemical Analysis of Rat Brains (males) in Experiment VIII.

	Starch	Lactose	Sucrose
Average body weight (grams)	175	180	180
Average brain weight (grams)	1.72	1.74	1.73
Percent cholesterol	1.50±0.22	1.43±0.17	1.47±0.20
Percent unsaturated cerebrosides	0.30±0.05	0.30±0.10	0.30±0.14
Percent unsaturated phosphatides	2.00±0.58	2.64±0.12	2.42±0.33
Number of animals analyzed	7	7	7

Table 29. Learning Scores of Male Rats on Ration VIII in Experiment VIII.

	Starch	Lactose	Sucrose
Errors, average number Trials 5-69	127±95	304±187	289±170
Time, average seconds Trials 5-69	248±70	312± 47	293±112
Number of animals	7	7	7

	Standard Deviations		Critical Ratio	
	Time	Errors	Time	Errors
Starch and lactose	32	78	2.0	2.3
Sucrose and lactose	46	91	0.5	0.2
Sucrose and starch	50	74	0.9	2.2

Summary of experiment VIII: The critical ratio of the comparison of time and errors for the maze test is in all cases less than four, showing that there is no significant difference in intelligence. Again the hemoglobin of the lactose fed rats was higher than that of the sucrose or starch fed animals. Growth was made uniform in all three groups by limiting the feed to that of the lightest group.

Experiment IX. Precocity of the Sucrose Fed Rats

It was observed that the vaginae of the sucrose fed rats were open before those of the lactose group and in the last two experiments records were kept of the time of opening. Record was also kept on a group of stock females. This experiment showed a significant difference in the maturity of sucrose fed female rats when compared with animals fed other sugars.

Table 30. Precocity and Sugars in Ration.

Ration VIII	Average age in days when vagina opened	Number of animals
Stock	54.7 ± 2.0	7
Sucrose	44.2 ± 6.4	8
Lactose	59.0 ± 9.2	3
Starch	47.3 ± 3.9	3

DISCUSSION AND CONCLUSIONS

Brain Composition

There was no appreciable difference in the brain composition of groups of sucrose and lactose fed rats. It cannot be concluded, however, that the animals synthesized galactose, for the ration may have contained 0.06 percent galactose.

Intelligence

Experiments IV and V indicated that the lactose animals had superior intelligence. In these experiments feeding was ad libitum and growth was uniform in both sucrose and lactose groups. In experiments VI and VII, the difference in intelligence was less significant, but in these two experiments the sucrose fed animals grew faster. The maze test depended for its accuracy on the uniformity of incentive (hunger) and so was rendered less reliable by this difference in growth.

In experiment VIII the feed was limited to maintain uniform growth. This in itself may have been enough to render the maze test unreliable, for animals must be treated alike to maintain similar hunger. In this experiment the sucrose fed animals were slightly more intelligent than the lactose fed animals.

It cannot be concluded that intelligence was affected by a lack of galactose in the ration for the number of animals in experiments IV and V was too few. And neither can it be concluded that intelligence was not affected by absence of galactose, for there was reason to believe that hunger was not the same in the groups compared in experiments VI, VII, and VIII, where only small differences in intelligence were noted.

Growth

In view of the literature on the utilization of glucose, galactose, and fructose, one may conclude that the sucrose fed animals grew faster in experiments VI and VII because of the presence in the lactose ration of galactose as compared with fructose in the sucrose ration.

The almost identical growth in experiments IV and V was more in line with the usual efficient utilization of lactose by young mammals. The growth rates of all animals are summarized in table 31.

Table 31. Summary of Daily Gain of All Rats.

Experiment number	Ration	Average daily gain in grams	Number of animals	Sex
IV	Sucrose IV	3.29	7	Male
IV	Lactose IV	3.33	8	Male
V	Sucrose IV	3.77	8	Mixed
V	Lactose IV	4.36	8	Mixed
VI	Sucrose V	3.80	20	Mixed
VI	Lactose V	2.82	20	Mixed
VII	Sucrose VI	3.90	16	Mixed
VII	Lactose VI	3.10	16	Mixed
VIII	Starch VII	1.64	7	Male
VIII	Sucrose VII	1.77	7	Male
VIII	Lactose VII	1.77	7	Male

Hemoglobin

It was found that young lactose fed rats had consistently a higher hemoglobin than the sucrose fed animals. In experiments V, VI, VII, and VIII the hemoglobin increased at about 50 days of age to that of the lactose fed rats. In experiment VII the hemoglobin of the sucrose fed group was 14 percent below that of the lactose group, after only two days on the artificial rations. This phase of the work should be studied further.

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