Some nutritional properties of unrefined sugar and its promotion of the survival of new-born rats

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1. The claims that rats fed on diets with 'brown sugar' (unrefined muscovado) perform better in a number of ways than do rats fed on refined white sugar (sucrose) have been examined.

2. Male Wistar rats were fed on purified diets from weaning, in which the carbohydrate component was either maize starch or unrefined sugar or sucrose. The sugars produced no differences in growth rate, body composition, or the weights of liver or kidneys. Compared with sucrose, unrefined sugar produced an increase in blood cholesterol and in the activity of hepatic fatty acid synthetase, and a greater increase in blood triglyceride. In confirmation of earlier results, rats fed on either sugar had heavier livers and kidneys, increased activity of hepatic glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) and a higher concentration of plasma triglyceride compared with rats fed on maize starch.

3. Female Sprague–Dawley rats were fed on the same three diets as the male rats, and mated when they weighed about 200 g. No difference was seen in their ability to mate, the progress of pregnancies, or the sizes of the litters. Does fed on unrefined sugar produced litters of higher viability than did does fed on starch or sucrose. Survival was between 85 and 100% with unrefined sugar and between 30 and 75% with starch or sucrose.

4. Unrefined muscovado sugar has thus been shown to contain a factor required by female rats for the proper viability of their pups. This may be the same 'Reproductive Factor R' as that described by Wiesner & Yudkin (1951). In certain circumstances, unrefined muscovado sugar might therefore contribute to the nutritional value of a human diet, although in what circumstances, in what respect and to what extent it might do so, is by no means clear.

The Soviet workers Brekhman & Nesterenko (1983) have compared the effect of feeding either white sugar or unrefined sugar to rats. Their results led them to believe that unrefined sugar increases the rate of growth, prolongs the lifespan, increases the resistance to physical stress, produces a smaller increase in the blood concentration of cholesterol and triglyceride, and improves reproductive performance. The white sugar in the diets was the virtually pure sucrose known to the public simply as sugar. What Brekhman & Nesterenko (1983) refer to as brown sugar was unrefined muscovado, one of the several sorts of brown sugar that are available; most brown sugars are made by the addition of molasses or of caramel to white sugar.

Among nutritionists, the conventional view is that no sort of brown sugar contains a sufficient concentration of vitamins or mineral elements, and certainly of protein, to confer any measurable nutritional advantage if it were to be substituted for the sugar in the diet of human subjects (Yudkin, 1972).

The results of a study of the comparative effects in rats of dietary maize starch, sucrose and muscovado unrefined sugar are reported here. The diets were similar to those used for many years, in which the effects of starch and sucrose, the two major carbohydrates in human diets, were compared (Table 1). For ethical reasons, experiments on the effects of the sugars on response to stress, as performed by Brekhman & Nesterenko (1983), have not been carried out.

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Table 1. *Composition of diet (g/kg)*

Carbohydrate	660·0
Casein	250·0
Mineral mix (QEC)*	40·0
Vitamin mix (QEC)†	20·0
Maize oil	9·0
Solkafloc	20·0

* Mineral mix (Queen Elizabeth College, London) (/kg diet): calcium 7 g, potassium 4·3 g, sodium 2·4 g, magnesium 0·5 g, manganese 44 mg, iron 29 mg, zinc 17 mg, copper 4 mg, iodine 0·8 mg.

† Vitamin mix (Queen Elizabeth College, London) (mg/kg diet): ascorbic acid 75, nicotinic acid 60, Ca-D-pantothenate 40, α-tocopherol 76, retinol 23, cholecalciferol 15, choline bitartrate 1800, thiamin hydrochloride 10, riboflavin 10, pyridoxine 10, folic acid 5, D-biotin 1, menaphthone 1, cyanocobalamin 0·05.

METHODS

Male rats of the Wistar strain (A. Tuck & Son Ltd, Beeches Road, Battlebridge, Essex) and female rats of the Sprague–Dawley strain (from Queen Elizabeth College Breeding Unit) were used. They were housed in individual cages and given unrestricted access to food and water. Groups of animals were made by distribution of littermates of approximately the same body-weights. The animals were weighed daily. For the metabolic experiments, the animals were killed by a blow on the head and blood collected from the beating heart into tubes containing EDTA. The liver and kidneys were rapidly removed, a portion of each chilled for enzyme assay, and the remainder frozen in liquid nitrogen and reserved for any further analysis that might be required. Glucose-6-phosphate dehydrogenase (G6PDH, *EC* 1.1.1.49 was assayed by the method of Löhr & Waller (1974), fructose-1,6-diphosphatase (F1,6DP, *EC* 3.1.3.11) by the method of Latzko & Gibbs (1974) and fatty acid synthetase (FAS) by the method of Gibson & Hubbard (1960). Plasma triglyceride was measured by the Boehringer Peridochrom Kit and cholesterol by the Boehringer test combination-C system kit. The carcasses were dried at 105 ° for determination of their water content, and fat extracted by the Soxhlet method.

Metabolic experiments

Expt 1. Nine groups, each of four male weanling Wistar rats, were used. Three groups of these were fed on each of three diets, in which the carbohydrate was either maize starch, sucrose or unrefined sugar. After 4, 6 and 8 weeks, three groups of rats were killed, one from each of the diets. Body composition, plasma lipids and liver and kidney enzymes were determined.

Expt 2. This was carried out in the same manner as Expt 1, except that no assay was made of enzyme activity. Three groups each of six male weanling rats were fed for 8 weeks.

Expt 3. Brekhman & Nesterenko (1983) had reported that the disturbance in carbohydrate metabolism produced with dietary sucrose was much less evident when the diet contained unrefined sugar instead. The effect in rats rendered diabetic with streptozotocin was therefore compared.

Six groups, each consisting of six male rats, were used. Two of the groups were given diets with either starch or white sugar or unrefined sugar, and the rats in one of each of these groups received an injection of streptozotocin in the tail vein, with a dose of 65 mg/kg body-weight. After 3 weeks on the diets the animals were killed and liver weight, plasma lipids and liver and kidney FAS determined.

Reproductive performance

In the remaining experiments we studied the effects of unrefined sugar on reproduction in female Sprague–Dawley rats. They were fed on diets with one of the three sources of carbohydrate from weaning through mating and pregnancy to the end of lactation. The number of pups born, mean litter size and survival of the pups were recorded.

Expt 4. Viability of litters. Three groups each of eight female Sprague–Dawley rats were mated when their body-weight reached 200 g.

Expt 5. Viability of second litters. The same eight mothers as in Expt 4 continued to receive the same diets, and were mated for the second time 2 weeks after they had completed their first lactation.

Expt 6. Viability of second generation. Six of the surviving pups from each of the groups in Expt 4 were weaned onto the same diets as their mothers had been given and they too were mated when their weight reached about 200 g.

Expt 7. Reversibility of non-viability of litters by unrefined sugar. Rats from Expt 6 which had been fed on either starch or sucrose were given the diets containing unrefined sugar after their pups had been weaned at 22 d from birth. They were mated again 2 weeks later.

Expt 8. Since all the previous experiments had been carried out on one group of rats and their progeny, a new batch of female Sprague–Dawley rats was used. These were divided into three groups, each of eight rats, fed from weaning on diets containing one of the three sources of carbohydrate and mated when their body-weight reached about 200 g.

Statistics

The results of the metabolic experiments were analysed by Duncan Multiple *F* tests (Duncan, 1955). For the experiments on reproductive performance, the χ^2 test was used.

RESULTS

Metabolic experiments

Expt 1. Most of the measurements revealed no significant differences between the groups fed on either sucrose or unrefined sugar; however, compared with white sugar, unrefined sugar produced an increase in blood cholesterol and triglyceride which reached statistical significance by week 8 (Table 2).

As expected, compared with rats fed on starch, those fed on either sugar showed differences similar to those that have been previously reported (Al-Nagdy *et al.* 1970; Bender *et al.* 1970; Kang *et al.* 1979). The unrefined-sugar-fed rats had heavier livers and kidneys, and increased activity of G6PDH in the liver and kidneys and a higher concentration in the blood of cholesterol and triglyceride.

Expt 2. The results showed again that the sugar-fed rats had heavier kidneys and livers. However, in this experiment the differences in the concentration of triglyceride and cholesterol between rats fed on unrefined sugar and sucrose were not significant (Table 3).

Expt 3. As in Expts 1 and 2, the non-diabetic animals fed on white sugar or unrefined sugar had larger livers and higher blood concentrations of cholesterol and triglyceride than did the animals fed on starch. There was also a higher activity of FAS in the liver (Table 4).

In the diabetic rats, there was also a difference in the effects of the two types of sugar. White sugar significantly increased the concentration of blood triglyceride but not of cholesterol nor of the activity of FAS in liver or kidney. On the other hand, unrefined sugar increased the concentration of cholesterol and of hepatic FAS, and increased still further the concentration of triglyceride.

Table 2. *Expt 1. Body composition, plasma lipids, and liver and kidney enzymes, in male rats fed on diets containing starch, sucrose or unrefined sugar for 8 weeks*

(Values are means with their standard errors for four rats per group)

	Diet						Significant differences ($P <$)		
	Unrefined sugar (a)		Sucrose (b)		Starch (c)				
	Mean	SE	Mean	SE	Mean	SE	(a) *v.* (c)	(b) *v.* (c)	(a) *v.* (b)
Wt gain (g)	396·0	21·5	379·0	7·3	370·0	13·0	NS	NS	NS
Water (g/kg body-wt)	60·8	1·25	61·8	0·63	60·5	1·80	NS	NS	NS
Fat (g/kg body-wt)	18·0	1·75	17·0	0·70	18·8	2·10	NS	NS	NS
Liver wt (g)	19·1	1·20	16·8	0·55	14·1	1·10	0·01	NS	NS
Kidney wt (g)	3·7	0·10	3·7	0·09	2·9	0·17	0·01	0·01	NS
Plasma triglyceride (mg/l)	2300	230	1470	100	800	51	0·01	0·05	0·01
Plasma cholesterol (mg/l)	870	40	600	48	580	60	0·01	NS	0·01
Liver G6PDH (units/g)	49·9	4·4	42·7	1·5	31·3	3·3	0·01	0·05	NS
Kidney G6PDH (units/g)	6·59	0·31	5·89	0·24	4·85	0·08	0·01	0·05	NS
Liver F16DP (units/g)	13·5	0·7	9·1	1·7	13·0	2·2	NS	NS	NS

G6PDH, glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49); F16DP, fructose-1,6-diphosphatase (*EC* 3.1.3.11).
NS, not significant (Duncan Multiple *F* test).

Table 3. *Expt 2. Body-weight gain, liver and kidney weight and plasma lipids, in male rats fed on diets containing starch, sucrose or unrefined sugar for 8 weeks*

(Values are means with their standard errors for six rats per group)

	Diet						Significant differences ($P <$)		
	Unrefined sugar (a)		Sucrose (b)		Starch (c)				
	Mean	SE	Mean	SE	Mean	SE	(a) *v.* (c)	(b) *v.* (c)	(a) *v.* (b)
Wt gain (g)	449·0	—	438·0	—	437·0	—	NS	NS	NS
Liver wt (g)	21·0	1·5	18·9	1·2	15·6	1·2	0·05	NS	NS
Kidney wt (g)	3·89	0·22	3·56	0·19	3·05	0·22	0·05	NS	NS
Plasma triglyceride (mg/l)	1940	220	1880	240	1120	170	0·05	0·05	NS
Plasma cholesterol (mg/l)	1000	55	840	85	710	64	0·05	NS	NS

NS, not significant (Duncan Multiple *F* test).

Table 4. *Expt 3. Liver weight, plasma lipids and liver and kidney fatty acid synthetase (FAS) in male rats fed on diets containing starch, sucrose or unrefined sugar for 3 weeks*

(Values are means with their standard errors for six rats per group. Three groups were normal and three groups were made diabetic by injection with streptozotocin at the beginning of the experiment)

	Diet						Significant differences ($P <$)		
	Unrefined sugar (a)		Sucrose (b)		Starch (c)				
	Mean	SE	Mean	SE	Mean	SE	(a) *v.* (c)	(b) *v.* (c)	(a) *v.* (b)
Normal rats									
Liver wt (g)	20·0	1·5	17·6	1·3	14·8	0·6	0·01	NS	NS
Triglyceride (mg/l)	1930	15	1950	125	1620	62	0·05	0·05	NS
Cholesterol (mg/l)	1040	59	940	59	720	22	0·01	0·05	NS
Liver FAS (μmol NADPH/g)	3·50	0·13	3·66	0·14	2·88	0·14	0·01	0·01	NS
Kidney FAS (μmol NADPH/g)	0·47	0·05	0·47	0·03	0·50	0·01	NS	NS	NS
Diabetic rats									
Liver wt (g)	12·2	1·4	13·6	2·4	11·5	1·1	NS	NS	NS
Triglyceride (mg/l)	3060	420	2880	188	1470	143	0·01	0·01	NS
Cholesterol (mg/l)	1260	70	960	80	850	90	0·01	NS	0·01
Liver FAS (μmol NADPH/g)	2·25	0·23	1·36	0·08	1·35	0·23	0·01	NS	0·01
Kidney FAS (μmol NADPH/g)	0·62	0·04	0·44	0·02	0·36	0·03	0·01	NS	0·01

NS, not significant (Duncan Multiple *F* test).

Table 5. *Expt 4. Survival of first-litter pups born to mother rats fed on diets containing starch, sucrose or unrefined sugar from weaning and up to the end of lactation*

(Values are for eight rats per group)

	Diet		
	Unrefined sugar	Sucrose	Starch
No. of pups born	86	91	80
Mean size of litter	10·8	11·4	10·0
Survival of pups (no.)	69	26	30
Survival of pups (%)	80	28	37

These differences between unrefined and refined sugar are the opposite of what would have been expected if, as Brekhman (1980) suggests, unrefined sugar mitigates the effects of white sugar on carbohydrate metabolism.

Reproductive performance

Expt 4. Viability of litters. There was no significant difference in the interval before conception, or in the number of pups born to the does in each group. However, most of the pups born to the does fed on either white sugar or starch developed a distended abdomen. The distension appeared to be caused by an accumulation of clotted milk in the stomach, but the pathology of the condition has not yet been fully investigated. More importantly, this abdominal distension was associated with a considerable mortality (Table 5). By 21 d from the date of birth, when they would have been ready for weaning, fewer than half the rats from does fed on starch or sucrose had survived, compared with 80% of those from does fed on unrefined sugar.

Expt 5. Viability of second litters. The pattern of survival of the pups was similar to that seen in the first litters (Expt 4), the best viability being in the litters of mothers fed on unrefined sugar. It was difficult to determine the precise numbers that died during the first 3 d; for example, pups are sometimes killed intentionally or inadvertently by the mother, and may then be cannibalized. In this and in later experiments, therefore, the percentage of pups that have survived from the 3rd day after birth was recorded (Fig. 1, Table 6). The different viability of pups from does fed on the different diets became evident 1 week or more after their birth; little or no difference was seen during the first 7 d or so. It was also evident that from the 3rd day the diet with unrefined sugar gave the greatest proportion of surviving pups, and the diet with starch the smallest proportion.

Expt 6. Viability of second generation. The growth of the three groups was not significantly different. This time, however, the litters were significantly smaller from the rats fed on starch or white sugar than from those fed on unrefined sugar (Table 7). Most of the pups again survived the first 7 d, but thereafter there was considerable mortality among those from does fed on sucrose, and especially from does fed on starch.

Expt 7. Reversibility of non-viability of litters by unrefined sugar. Rats from Expt 6 in due course produced litters in which almost all the pups survived to the age of weaning (Table 8). No abdominal distension occurred in any of them.

Expt 8. The viability of the pups was similar to that seen in previous experiments; all but one rat survived in fifty-six pups born to does fed on unrefined sugar, three-quarters survived from those fed on white sugar, and half from those fed on starch (Table 9).

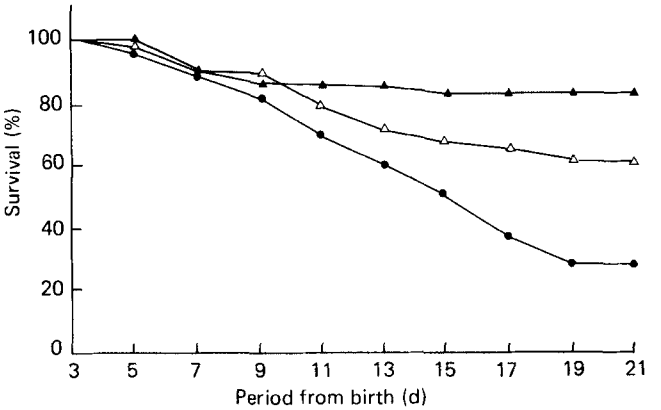

Fig. 1. Expt 5. Survival of pups from day 3 after birth (see Table 6). Diets: (▲), unrefined sugar; (△), sucrose; (●), starch.

Table 6. *Expt 5. Survival of second-litter pups born to mother rats fed on diets containing starch, sucrose or unrefined sugar from weaning and up to the end of the second lactation*

(Values are for eight rats per group. Mother rats are those used in Expt 4 (see Table 5). They were remated 14 d after their first litter was weaned)

	Diet		
	Unrefined sugar	Sucrose	Starch
No. of pups born	101	98	100
Mean size of litter	12·6	12·3	13·5
Survival of pups at day 3 (no.)	76	93	89
Survival of pups from day 3 (no.)	64	58***	26***
Survival of pups from day 3 (%)	84	62	29

Values significantly different compared with unrefined-sugar group (χ^2 test): *** $P < 0{\cdot}001$.

Table 7. *Expt 6. Survival of first-litter pups to weaning at 21 d born to the female progeny of rats from Expt 4 fed on diets containing starch, sucrose or unrefined sugar from weaning and up to the end of lactation*†

(Values are for six rats per group)

	Diet		
	Unrefined sugar	Sucrose	Starch
No. of pups born	86	43	56
Mean size of litter	14·3	7·2	9·3
Survival of pups at day 3 (no.)	75	39	53
Survival of pups from day 3 (no.)	70	20***	20***
Survival of pups from day 3 (%)	93	51	38

Values significantly different compared with unrefined-sugar group (χ^2 test): *** $P < 0{\cdot}001$.

† For details, see p. 595.

Table 8. *Expt 7. Survival of second-litter pups to weaning at 21 d born to the female progeny of rats from Expt 6 and fed on diets containing starch, sucrose or unrefined sugar from weaning to the end of the first lactation and then fed on the diet containing unrefined sugar only**

(Values are for six rats per group)

	Diet	
	Sucrose–unrefined sugar	Starch–unrefined sugar
No. of pups born	45	74
Mean size of litter	7·5	12·3
Survival of pups at day 3 (no.)	45	74
Survival of pups from day 3 (no.)	45	71
Survival of pups from day 3 (%)	100	96

* For details, see p. 595.

Table 9. *Expt 8 (repeat of Expt 4 with new animals). Survival of first-litter pups born to mother rats fed on diets containing starch, sucrose or unrefined sugar from weaning and up to the end of lactation*

(Values are for seven rats per group for the starch and sucrose diets and six rats per group for the unrefined-sugar diet)

	Diet		
	Unrefined sugar	Sucrose	Starch
No. of pups born	43	66	58
Mean size of litter	7·2	9·4	8·3
Survival of pups at day 3 (no.)	43	65	52
Survival of pups from day 3 (no.)	42	48***	25***
Survival of pups from day 3 (%)	98	74	48

Values significantly different compared with unrefined-sugar group (χ^2 test): *** $P < 0{\cdot}001$.

DISCUSSION

The experiments with male rats showed the usual difference between the effects of sucrose and of starch on such items as the weight of the kidneys and liver, the activities of enzymes in the kidney and liver, and the concentration in the blood of cholesterol and triglyceride.

However, unlike the results of Brekhman & Nesterenko (1983), the substitution of unrefined sugar for sucrose did not eliminate or reduce these effects; in some respects, as with the concentraton of blood triglyceride, the effects of the unrefined sugar were greater than those of sucrose. It was only in reproductive experiments with female rats that unrefined sugar demonstrated a significant superiority over sucrose. However, the considerable fetal mortality that the Soviet workers (Brekhman & Nesterenko, 1983) reported with sucrose, and its prevention by unrefined sugar, was not observed here. What was found was a significant improvement in survival in the litters born to does fed on unrefined sugar compared with the survival in litters from does fed on sucrose or starch.

The experimental methods described by Brekhman (1980) and his presentation of results, are not easy to understand. The simplest account of the method is given in two short books that summarize their work (Brekhman, 1980; Brekhman & Nesterenko, 1983), and describe in some detail its interpretation in the light of their views on 'biologically-active substances'. We have also been able to see a translation of a more detailed account of this work in the form of seventeen research reports carried out by forty-three Soviet scientists, both on sugar and on a variety of 'unrefined' plant extracts; eleven of the seventeen projects related to unrefined sugar.

One possible reason for our failure to confirm the nutritional work of Brekhman & Nesterenko (1983) is that they administered the sugar to their rats in one of two ways, both of which were unconventional, not to say idiosyncratic, in nutritional research. One way was to feed their standard diet, and to administer the sugar each day by stomach tube. The amounts were proportional to body-weight, and were usually either 2 or 50 g/kg. From their publication, it appears that their rats thus received either between 0·5 and 1 g sugar/d, or between 5 and 10 g/d. It is difficult to imagine that as little as 1 g sugar could have any considerable effect; on the other hand, rats receiving more than 5 g sugar must have eaten relatively little of their basal diet, since the total amount consumed by these rats was likely to have been between 10 and 15 g. The second method by which the sugars were administered was by mixing them with the basal diet in amounts that were sometimes as high as 90% of its total energy. With either method, therefore, the amounts of protein, vitamins and mineral elements from the basal diet might well have been inadequate.

The authors give no explanation why they used these methods in constructing the diets. The basal diet is described as a mixture of foodstuffs including a grain mix, mixed feed, bread, groats, whale meat and root crops. Occasionally, instead of this, a diet comprising 90 g/kg of one of the sugars had added to it 100 g boiled egg/kg.

As the amount of sugar in the diet was increased, the amount of other dietary components clearly decreased in proportion. Thus, in a mixture in which a large proportion of sugar is added to an otherwise adequate basal diet, nutrients in unrefined sugar can, at least to some extent, make up for those that are diluted to an extent that makes the basal diet nutritionally inadequate. In other words, diets diluted with a high proportion of refined white sugar (sucrose) would have been deficient in several nutrients, and the substitution of the refined sugar by unrefined sugar would have corrected or reduced a deficiency of some of them.

In our experiments, we gave diets that, as far as we knew, contained all the essential nutrients in adequate quantities, so that no benefit could be expected when unrefined sugar was added. Nevertheless, since unrefined sugar fed to does improved the viability of their young, we must conclude that our own basal diet was itself lacking in some nutrient essential for successful reproductive performance. We have no reason to suppose that it is inadequate for the proper growth of rats from the age of weaning, but it does appear to be deficient in what has previously been called Factor R, or some similar nutrient required for normal development before weaning (Wiesner & Yudkin, 1951, 1952, 1958). In those experiments, dams that were fed on purified diets from weaning produced pups that did not survive the usual 3-week period of lactation. The addition of liver significantly repaired this deficiency, and it was to the unidentified principle in the liver that the name 'Reproductive Factor R' was given.

Folley *et al.* (1947) had shown that smaller litters were born to mothers fed on a purified diet from pairing, and fewer of the pups survived; continuation of the diet through successive generations resulted in further deterioration of reproductive performance. Dryden *et al.* (1952) found that lactation appeared normal in dams fed on a purified diet

after parturition, but if the diet were continued, the proportion of surviving pups from successive matings decreased.

Greenfield *et al.* (1969) reviewed the composition of purified diets used in nutrition research. Their survey revealed insufficiencies in some of the diet formulae used. Guided by the (US) National Research Council (1962) recommendations for the requirements of the rat, they formulated a base for purified diets that can fulfil the nutritional requirements within the sphere of available nutritional knowledge, support growth and reproduction and present all the nutritional components in one complete formula. Their mineral salt and vitamin mix formulae were used to make up the experimental diets used in the present study. The better performance of animals fed on our current purified diet could be due to the fact that it contains a more complete range of mineral elements and vitamins, although an alternative explanation is that the rats we have now been using are less susceptible to deficiency of the reproductive factor. However, in whatever ways the current Queen Elizabeth College diets are better than those used more than 25 years ago, they still appear to lack Factor R. It seems that Factor R is present in unrefined sugar, and we propose to renew the early attempts to identify it. Preliminary investigations suggest that it is likely to be one or more of the trace elements rather than one or more organic substance.

We have no explanation for one unexpected result. This was that pups born to rats fed on sucrose were more likely to survive than those born to rats fed on starch.

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