CL. ON GALACTOSE AS THE DIETARY CARBOHYDRATE.

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IN course of an investigation on a possible relationship between the requirement of vitamin B_1 and the nature of the dietary carbohydrate [Guha, 1931] it was observed that if galactose formed the sole carbohydrate in a fat-free diet the rats rapidly declined in weight and died. When young rats of about 50 g. in weight were used, death occurred within 3–4 days. Uniform results were obtained with galactose (extra pure) of the British Drug Houses and with Kahlbaum's product. Though the utilisability of galactose is known to be relatively poor, this unique behaviour of galactose among the carbohydrates studied merited further attention, especially as such studies might have some relation to the still unsolved problem of the significance of the occurrence of lactose in milk. In the present investigation the effect of a galactose diet unsupplemented by any other adequate source of non-protein energy on young and adult rats has been studied. Experiments have also been carried out to find how far the ill-effects observed could be averted by the presence of varying amounts of glucose, fat and protein in the diet. The behaviour of mice under similar circumstances has also been studied in a few preliminary experiments. The results of the work, though incomplete, are published at this stage as the investigation cannot be pursued further at the moment.

EXPERIMENTAL.

The rats used were mostly of the black and white strain, a few being albinos. For comparative experiments litter-mates were employed. As usual, the animals were kept in separate cages and all vitamin supplements were fed separately from the basal diet except when yeast was used. The vitamin B_1 and B_2 preparations used were the same as employed in the work described in the preceding communication [Guha, 1931]. Each animal received 2 drops of cod-liver oil daily. In several cases records of the food and water intake and of the urinary output were kept.

The following diets were used. The caseinogen used was the "light, white" variety of the British Drug Houses.

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Diet 19

	Parts
Galactose	75
Caseinogen	21
Salt mixture (McCollum)	4

Diet 19 (*a*)

	Parts
Galactose	37·5
Glucose	37·5
Caseinogen	21
Salt mixture	4

Diet 19 (*b*)

	Parts
Galactose	37·5
Palm kernel oil	37·5
Caseinogen	21
Salt mixture	4

Diet 19 (*c*)

Galactose	70
Caseinogen	21
Dried yeast	5
Salt mixture	4

Diet 19 (*d*)

Galactose	35
Glucose	35
Caseinogen	21
Dried yeast	5
Salt mixture	4

Diet 19 (*f*)

Glucose	70
Caseinogen	21
Dried yeast	5
Salt mixture	4

Diet 19 (*g*)

Galactose	60
Palm kernel oil	16
Caseinogen	21
Salt mixture	4

Diet 19 (*h*)

Galactose	35
Caseinogen	56
Dried yeast	5
Salt mixture	4

Diet 19 (*j*)

Galactose	70
Caseinogen	19
Sodium dihydrogen phosphate	2
Dried yeast	5
Salt mixture	4

Diet 19 (*k*)

Galactose	70
Palm kernel oil	10
Caseinogen	21
Salt mixture	4

Diet 19 (*l*)

Galactose	70
Palm kernel oil	5
Caseinogen	21
Salt mixture	4

Diet 1

Glucose	75
Caseinogen	21
Salt mixture	4

A. *Experiments with adult rats.*

Rats which had been growing for a considerable period on diet 1, containing glucose supplemented by vitamin B_1 and B_2 preparations and cod-liver oil, were fed on diet 19 with the same supplements. The rats rapidly declined and died (Fig. 1).

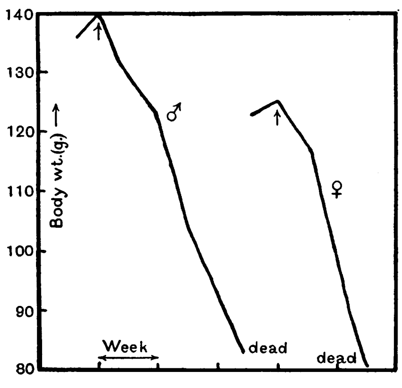

Fig. 1. ↑ Diet 19 supplemented with vitamin B_1 and B_2 preparations.

B. *Experiments with young rats.*

Litters of young rats were broken up into groups of two. The behaviour of these groups on diets 19 (*c*) containing galactose, 19 (*f*) containing glucose, 19 (*d*) containing equal parts of glucose and galactose, 19 (*j*) containing galactose with extra phosphate and 19 (*h*) containing galactose with extra caseinogen is illustrated in Figs. 2 and 3. All the diets contained 5 % yeast as the source of "vitamin B," which contributed to a small extent to the energy value of

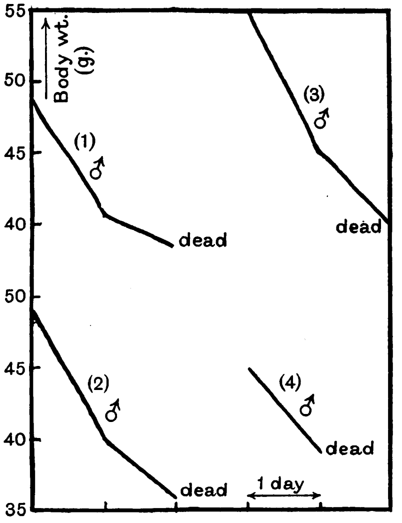

Fig. 2.

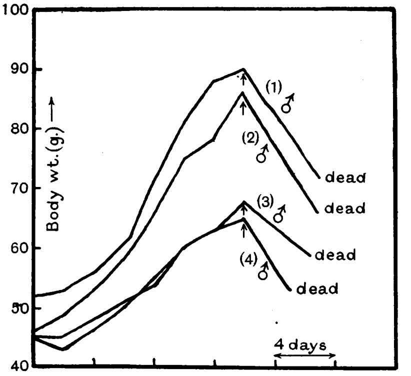

Fig. 3.

Fig. 2. Curve (1). Diet 19 (*c*). Curve (2). Diet 19 (*c*). Curve (3). Diet 19 (*h*). Curve (4). Diet 19 (*h*).

Fig. 3. Curve (1). Diet 19 (*f*) from beginning of experiment; ↑ diet 19 (*c*).
Curve (2). Diet 19 (*f*) from beginning of experiment; ↑ diet 19 (*c*).
Curve (3). Diet 19 (*d*) from beginning of experiment; ↑ diet 19 (*j*).
Curve (4). Diet 19 (*d*) from beginning of experiment; ↑ diet 19 (*j*).

the diets. In diet 19 (*j*) extra phosphate was incorporated to find if it had any influence on the utilisation of galactose. Diets 19 (*c*), 19 (*j*) and 19 (*h*) were unable to support life, while the animals receiving diet 19 (*d*) grew at a markedly slower rate than those receiving diet 19 (*f*). This last case shows that 35 °/₀ glucose cannot support the normal growth of young rats and that the same quantity of galactose cannot replace it in the diet.

(*a*) *Food and water intake.* In Table I are given the figures for the food and water intakes of some animals receiving diets 19 (*f*) and 19 (*d*). Although the animals receiving diet 19 (*d*) were growing more slowly than those receiving diet 19 (*f*), the former were actually consuming more food than the latter. The water intake was also considerably higher.

Table I.

Rat No.	Sex	Diet	Period over which food intake records were kept (days)	Average daily food intake (g.)	Average daily water intake (cc.)
1	♂	19 (*f*)	10	9·9	22·6
2	♂	19 (*f*)	15	10·6	17·8
3	♂	19 (*d*)	10	12·6	31·2
4	♂	19 (*d*)	15	12·8	34·8

The animals receiving diet 19 (*c*) consumed larger quantities of food and took much more water than those of any other group. It was difficult, however, to keep accurate food intake records owing to the scattering of the diet. The urinary output of these animals was also considerably higher, reaching as much as 40–45 cc. daily.

(*b*) *Condition of the animals.* The rats on diet 19 (*c*) soon lost the lustre of the eye and became hunched. The fur became ruffled and other signs of weakness resembling those observed in vitamin B_1-deficient rats were also present. The animals lay sometimes in a comatose condition with extended limbs for 20 hours before death. In this condition there was no appreciable response to two injections of a total volume of 1·2 cc. of 10 % glucose.

Post mortem examination, kindly carried out by Mr J. R. M. Innes, revealed no organic lesions. The intestinal tract had an unhealthy appearance.

C. *Experiments with young rats.*

In Fig. 4 are illustrated the growth curves of young rats on diets 1, 19, 19 (*a*), 19 (*b*), 19 (*g*), 19 (*k*), 19 (*l*) and 19 (*c*). Of these diets all except diet

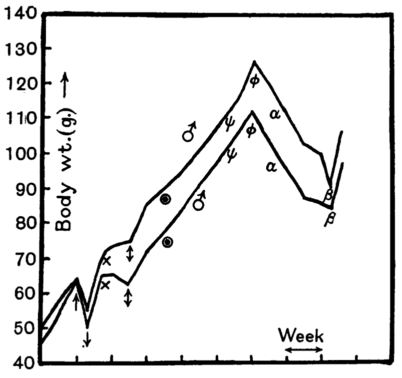

Fig. 4.

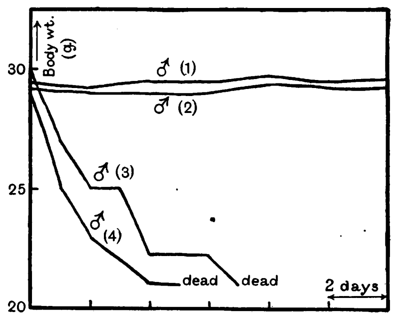

Fig. 5.

Fig. 4. Diet 1 from beginning of experiment; ↑ diet 19; ↓ diet I; × diet 19 (*a*); ↕ diet 19 (*b*); ⊙ diet 19 (*g*); ψ diet 19 (*k*); ϕ diet 19 (*l*); α diet 19 (*c*); β "complete" synthetic diet.

Fig. 5. Experiments with mice. Curve (1). Diet 19 (*f*). Curve (2). Diet 19 (*f*). Curve (3). Diet 19 (*c*). Curve (4). Diet 19 (*c*).

19 (*c*), which contained yeast, were supplemented daily by 1 cc. of a vitamin B_1 concentrate and 1 cc. of a vitamin B_2 preparation, so as to eliminate as far as possible the source of energy provided by the glycogen, fat and protein of yeast. Diet 19 (*g*) was made up so as to contain an amount of palm kernel oil approximately isodynamic with 37·5 g. glucose. This was done in order to compare the effects of this diet and of diet 19 (*a*) which are calorifically approximately equivalent, neglecting galactose as a source of energy. It will be observed that diet 19 (*g*) has a distinctly better effect. It will also be noticed that, by gradually lowering the content of fat in the galactose diet, growth can be progressively inhibited. (Compare diets 19 (*b*), 19 (*g*), 19 (*k*)

and 19 (*l*).) Food intake records were kept throughout this set of experiments, which show that the greatest food consumption occurred with diet 19 (*c*), approaching sometimes 23 g. daily.

D. *Experiments with mice.*

Only a few preliminary experiments could be carried out with mice[1]. These have yielded results similar to those obtained with rats. These animals, which were nearly adult, were fed on diets 19 (*f*) and 19 (*c*). The results are illustrated in Fig. 5. It will be observed that, while the glucose diet was apparently adequate, the galactose diet failed to support life.

The striking fact about the mice receiving diet 19 (*c*) was that they exhibited all the symptoms typical of "beriberi" in the rat in addition to those observed in the rats on the same diet. Thus, after 5–7 days on this diet there was loss of muscular co-ordination and they went into convulsions when swung, which were indistinguishable from "beriberi" convulsions in the rat. The animals could remain in the moribund condition for over 40 hours. It has to be mentioned that they were eating considerable quantities of food for the first three days and were, therefore, receiving ample quantities of yeast from the diet.

Discussion.

The results described indicate that the degree to which galactose can be utilised by the rat is singularly low. Death can be prevented by replacing part of the galactose by glucose or by fat but not by increasing the protein content of the diet. That the limiting factor in the galactose diet is its inability to supply sufficient energy to the rat is also indicated by the proportionate growth responses to graded amounts of fat in the galactose diet. The increased water intake and diuresis on the galactose diet is readily intelligible in the light of our knowledge about the rapid absorption of galactose through the intestinal wall [Cori, 1925; McCance and Madders, 1930] and of its low renal threshold [Folin and Berglund, 1922]. In fact, this appears to be the reason why death is so quick in young animals on the galactose diet. As has been pointed out, the food intake on this diet is greater than on any other, which indicates that the low utilisability of galactose prompts the consumption of more food and, as galactose is quickly absorbed and cannot be readily oxidised or converted into glycogen [Lusk, 1915; Weinland, 1899; Ishimori, 1912–13], it has to be excreted. Thus, the whole process of digestion, absorption and excretion involves a greater strain than would have been the case if the animal were only starving and, therefore, apparently precipitates death. In this matter these animals behave differently from vitamin B_1-deficient rats, because, while the former continue to consume food till shortly before death and undergo virtual but not actual starvation, the vitamin B_1-deficient animals, on the other hand, starve themselves voluntarily. Attention has to

[1] I am indebted to Miss E. Turner for supplying me with these animals.

be drawn to the fact that many of the symptoms are common to both groups of animals, which, again, supports the contention that in vitamin B_1 deficiency the usual symptoms are simply those of starvation, consequent on anorexia. The typical symptoms of "beriberi" observed in mice subsisting on the galactose diet and receiving ample "vitamin B" also indicate the non-specificity of the so-called "beriberi" symptoms.

These experiments make the question of the function of lactose in nutrition still more puzzling. While Folin and Berglund [1922] have shown that the utilisation of galactose is apparently increased in presence of glucose, the present investigation indicates the desirability of the study of the rôle of fat in this connection. It appears from this study that the energy value of the fat in milk might be nearly as important for the nutrition of the young as are the vitamins it carries.

SUMMARY.

Young and adult rats on a fat-free diet containing galactose as the sole carbohydrate rapidly decline in weight and die. The symptoms of these animals are described. Mice behave similarly under the same conditions and develop typical "beriberi" symptoms although receiving ample quantities of "vitamin B." How far growth can be supported in young rats by incorporating varying quantities of glucose, fat and protein in the galactose diet has been studied.

My thanks are due to Sir F. G. Hopkins for criticism and advice.

REFERENCES.

Cori (1925). *J. Biol. Chem.* **66**, 691.
Folin and Berglund (1922). *J. Biol. Chem.* **51**, 213.
Guha (1931). *Biochem. J.* **25**, 1367.
Ishimori (1912–13). *Biochem. Z.* **48**, 332.
Lusk (1915). *J. Biol. Chem.* **20**, 555.
McCance and Madders (1930). *Biochem. J.* **24**, 795.
Weinland (1899). *Z. Biol.* **38**, 16.