# THE EFFECT OF PROLONGED ADMINISTRATION OF 1-AS-CORBIC AND d-GLUCONIC ACID ON THE BODY-WEIGHT, FOOD, WATER AND SALT-SOLUTION CONSUMPTION OF ALBINO RATS.\*

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(With 3 Figures and 2 Tables in the text)

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It is more and more generally accepted (BEZNÁK, BEZNÁK, GÁSPÁR-RÁDY 1941; BACKMAN, 1931) that the physiological and biochemical mechanism of growth is not the same in its whole course, but varies according to age.

Sachs (1874) and Minot (1907) observed that the general property of the absolute growth velocity<sup> $\circ \circ$ </sup> curve is: that it is divided into three periods. The growth velocity increases during the first period, maintains its highest level during the second, and decreases during the third. According to BACKMAN these periods result from the different combinations of three processes: steady growth, acceleration, retardation. In trying to work out an equation for the growth of albino rats, DONALDSON (1924) found that he got the measured and estimated values to tally only if he established two equations for the growth period; one for the 10-80th day and one for the 80-365th day. Consequently, according to DONALDSON, growth takes place in two periods (a faster and a slower).

One of the chief aims of our work was to examine experimentally — by measuring the food and water consumption during the whole growth period — whether 'the physiological and biochemical mechanism of growth changes during the different stages of life.

The effect of an indispensable nutritional factor has chiefly been

\* Part of the results of this work was published at the meeting of the Hungarian Physiological Society, May 22nd, 1941. (BEZNÁK et al. 1941)

<sup>oo</sup> The absolute growth velocity means the percentual relation between the increase in growth during a given period and the initial-weight at the beginning of the same period.

tested on young animals (by totally eliminating the factor in question). In reality nutrition differs from experimental conditions in these two points: firstly, whatever the mode of nutrition may be, it generally covers the whole life span; secondly, small disequilibria in nutrition take place rather than a total lack of a definite nutritional factor. Recently great quantities of vitamins  $B_1$  and C have been taken, though their effect is known only through relatively short experimental periods.

Our experiments answer the question: What is the effect of prolonged and greatly over-dosed administration of a single nutritional factor (vitamin C) if the diet is otherwise balanced?

The symptoms of C-hyper-vitaminosis have been studied only in the case of such organisms as are unable to synthetise vitamin C (man, guinea pig) and only the acute effect of relatively short experimental periods has been observed. RIET-SCHEL (1939) and CENTAMINI (1938) administered vitamin C to rats for longer periods but did not observe its effects.

Apart from observing the effects of the prolonged over-dose of vitamin C, we are also able to account to some extent for their physiological mechanisms and can partly determine which part of the vitamin C molecule is responsible for the effect brought about. Dgluconic acid has many qualities in common with vitamin C (especially that it is also a fairly strong water-soluble hexuronic acid) but it has no anti-scorbutic effect. The effects brought about by vitamin C only may be attributed to the anti-scorbutic redox-group of the molecule, whereas the common effects are caused by the identical qualities of the two compounds, especially their acidity.

#### METHODS.

Our work was done on male albino rats. The composition of their diet was the following: 25-25% wheat, rye and corn, 15% casein, 3.5% butter, 4% McCollum salt mixture, 1.3% cod liver oil. Our experimental animals were the offspring of mothers living on a complete diet. During the gestation and suckling period the mothers got the above experimental diet, which was also fed to the experimental animals straight after the weaning.

All rats lived in individual cages which made refecation impossible. The food consumption was measured daily with an accuracy of  $\pm$  0.1 g. The rats were supplied with measured quantities of tap water and of a 1% solution of NaCl, given to them in suitable vessels. The remaining fluids were measured each day with an accuracy of 1 ml. From time to time we changed the contents of the vessels and their places in the cage also (the salt where the water had been and vice versa). We tried thus to exclude the possibility of the rats being

influenced by the shape of the vessels or their places, rather than seeking the composition of the fluid itself.

The rats were divided into three groups. The first one received each day through a stomach tube 20 mg ascorbic acid in 0.2 ml distilled water, the second 20 mg d-gluconic acid\* in 0.2 ml distilled water, the third one only 0.2 ml distilled water. Each group consisted of ten rats. The experimental period lasted from the 18th of March, 1941, to the 6th of November, 1941, that is, from the 48th day to the 256th day of the rats' life. We did not notice any difference in the behaviour and condition of the three groups. Intercurrent death took place five times, twice in the ascorbic, once in the gluconic acid and twice in the normal control group.

The rats were weighed with an accuracy of  $\pm 1$  g.

Our paper contains the following mathematical formulae: n = the totalnumber of the members of the different groups.  $M = \text{mean value} = \sum_{n=1}^{\infty} \frac{x}{n}; \mu = \frac{1}{2} = \frac{1}{2} \frac{1$ 

coefficient (the magnitude of change of one value corresponding to unit change of the other.)  $0_1$ 

## EXPERIMENTAL RESULTS AND THEIR DISCUSSION.

### a) The growth curve.

Figurel. shows the mean values of our growth curves as well as those of DONALDSON (1924.) which we transcribed for the sake of comparison.

As is seen from the Figure, the final mean body weight of the animals receiving ascorbic or gluconic acid is 11.5% higher than that of the normal animals. The mean values of our normal animals are almost identical with those given by the DONALDSON curve.

The growth velocity curves (SACHS, 1874) of the three groups are also represented in Figure 1. that is, it shows the percentual correlation between the gain in weight during a 10 days' period and the weight at the beginning of the same period. As Figure 1 shows, the growth velocity curves of the three groups are almost identical.

\* Part of the gluconic acid was furnished by Professor GÉZA ZEMPLÉN, for whose kindness we are glad to express our thanks.

Our experiments do not cover the beginning of the growth curves. The extrauterine growth (growth velocity according to SACHS) of the albino rat takes place in three phases: 1.) The quickening of the growth velocity from the 10th day (12 g) to the 30th (28 g). 2.) The attainment of a peak during the 30th to 40th day (28-38 g of body weight). 3.) The slowing down of the growth velocity from the 35-40th day to the 160th day (240 g). To these could be added as a fourth period the phase of steady body weight until the decline of old age.

To decide the question whether ascorbic or gluconic acid had any influence on body weight, we calculated in 10 days' intervals the mean



d) The long vertical lines indicate the limits of the phases of growth according to Sachs, respectively according to our experiments.

body weights of the three groups and their significant differences. Figure 1. shows these differences in per cent. According to these calculations the mean body weights of the groups receiving acid was only 3.5% higher than that of the normal groups till the 70th day (k = 0.7-1.1. After this 'the difference becomes suddenly greater (9-11.5%) and remains on this level till the end of the experiment.

The third period of the growth curve as devised by SACHS should — according to our experiments — be divided in two: in the first the decrease of the growth velocity takes place quickly, in the second more slowly. The first period extends in our case to the 70th day, in that of DONALDSON to the 80th day. We might call the first period the 3a phase and the second the 3b phase. As was mentioned introductorily DONALDSON expresses growth of rats with two equations, the first covering growth in the first 80 days, and the second, growth from the 80th day onwards. Accordingly, DONALDSON'S first period includes SACH'S first, second and our 3a phase, his second period our 3b and 4th phase.

The fact that there was no difference in the mean values of body weights in normal and acid-receiving groups till the 70th day, when there appeared suddenly a difference of 9%, which increased gradually to 11.5%, admits of two explanations. It is possible that at this juncture some change in the metabolism of growing rats takes place, in consequence of which some rats attain a greater body weight. The other possiblity is that the difference is caused by the acid administration. In the first instance the greater mean body weight of the groups receiving acid must be attributed to haphazard group formation. The significant difference of the mean body weight values is only 2, so that we cannot exclude with an absolute certainty the possibility of haphazard group formation. Still, if we take into consideration a) that the two acid-receiving groups grew suddenly and simultaneously, from the same time onwards, better than the normal group and b) that, in spite of the fact that the body weight of the gluconic acid-receiving groups was the smallest in the beginning, yet they grew better than normal and better even than the ascorbic acid animals, we must consider it more likely that the difference in weight was caused by the acid administration and not by haphazard group formation. Again, the acid-administration might cause better growth just from that point onwards in two ways: a) either the acid must be given during several weeks to develop its influence, or b) the changed metabolism reacts differently to acids. The circumstance that

growth of normal rats slows down considerably from that point, argues in favour of the second hypothesis.

Thus C hyper-vitaminosis has no specific influence on growth, and the small effect observed is the same in the case of another non anti-scorbutic hexuronic acid. Contrary to this,  $B_1$  hyper-vitaminosis on a SHERMAN CHASE 515 complete diet has a definite, strong influence on growth, so that the  $B_1$  hyper-vitaminotic animals grew to 450 g, 25% more than the final weight of our biggest rats (360g) (WATER-MAN and AMMERMAN 1938).

b) The food, water, and 1% salt-solution consumption.

Growth consists of retaining in the body some elements of the food and water consumed. The next question to be considered therefore is the following: What is the connection during the different phases of growth between the velocity of growth and the quantity of food, salt and water consumed. Though the greater part of the body is composed of salt solution, attention in the past was mainly directed towards food consumption rather than water or salt solution consumption.

As regards the relation between growth and food consumption, RUBNER's law of continuous energy-loss (1908) and the LUSK-WILSON rule (1902) were generally accepted. According to the first, the energy quantity (for which the quantity of food consumed may be substituted) needed for the new-born to double its weight is the same whether it grows slowly or quickly. In other words, the plus body weight during growth is always the same percentage of the food consumed — independent of growth's being a slow or a quick process. The LUSK-WILSON rule is substantially the same as RUBNER's law — though it is more accurate: "Animals of the same breed and age reltain a definite and constant quantity of food consumed during the normal development of the young" (1. c.). According to these two rules, bigger and faster growing animals do not accomplish this (faster and greater growth) by greater retention but by eating more.

Our experiments are capable of controlling these two rules with the aid of probablity calculation. Figure 2 shows the food consumption. The mean consumption counted per 10 days and per animal increases rapidly during the first 90 days, then after a slight elevation remains on a constant level. Accordingly, the food consumption shows a slight elevation parallel with the relatively quicker growth during the 3a period. During the 3b period food consumption hardly changes, though growth still continues. During the 4th period of the growth

curve, when body weight is constant, food consumption diminishes. It is seen from this F i g u r e that the food consumption of the animals receiving either ascorbic or gluconic acid is practically the same, during the whole growth curve, as that of the normal group. There





is nowhere a significant difference in the food consumption of the animals belonging to different groups.

That RUBNER's rule is not valid for all parts of the growth curve can be shown by simple calculation. During the 3a period of growth,

that is from the 48th to the 68th day, the animals grow from 55 to 115 gs; in other words, they somewhat more than double their weight. During that time they consume 260 gs. of food. Therefore, to produce 1 kg. of growth, 4270 gs. of food are needed; that is, the 61 gs. of plus body weight represents 23% of the food consumed. In RUBNER's own experiment this was 36%. However, during the 3b and the following 4th period of growth, that is, from the 68th to the 208th day, the animals grow from 140 gs. to 269 gs. (that is, 129 gs, almost doubling their weight) and consume during that time 2260 gs. of food. For 1 kg. of plus body weight the food consumption is 17,520 gs, so that growth represents only 5.8% of the food consumed. The RUBNER rule can therefore not be valid for the whole length of the growth period, but (if it is valid at all) only for a small part of it, that is, the 3a sub-division.

As growth continues, food consumption remaining the same, it is evident that food consumption per unit weight and unit time, must diminish. The part of food consumption utilised by growth during the different periods is shown in F i g u r e 3. It is apparent from this Figure that growth has to be divided here too, into two parts .During the first (till the end of the 3a period) the part utilised by growth, counted per kg and day from the food consumed, per kg and day, diminishes quickly, during the second (in the 3b and 4th period) slowly.

Whether the LUSK-WILSON rule is valid or not, can also be determined by our experiments. If the food consumed is built up in the body at the same rate by quickly and by slowly growing animals, then there should be no correlation coefficient between the growth in gs during identical periods and the precentual relation between growth and food consumption (the latter is called efficiency of growth). In our 30 animals the growth from the 48th to the 60th day was 65 gs ( $\mu = \pm$ 11.7) and growth as percentage of the food consumption was 25% $(\mu = \pm 3.3)$ . In Wilson's experiment this was 18–19%. The correlation coefficient (r) between the two groups of data is  $0.9, \pm 0.034$ , almost the maximum. (The same is obtained by using for calculation only the third group). Therefore WILSONS' rule is not valid even during the 3a period of growth. From this again it follows that the RUBNER rule cannot be valid either. The faster growing animals grow faster also because they build up in their bodies a greater part of the consumed food than do the more slowly growing ones. If a rat grows more quickly by one g each 20 days that means a corresponding greater retention of 25% of the food consumed. The contradiction between RUBNER, WILSON and our

results is probably caused by the circumstance that they experimented on small numbers of animals and only for a short time. (For example, WILSON observed 5 pigs from the same litter for 14—16 days.)

As is seen from F i g u r e 2, the increase in food consumption stops sooner than the increase in body weight. During the period in which food consumption remains unchanged growth can evidently only be





caused by a greater growth efficiency. In attaining a higher final body weight a third circumstance may also play a role: that is, the point on the growth curve from which the increase in food consumption

stops. If this point is reached earlier by faster growing animals (possessing greater efficiency of growth), then the quantity of food the animal is capable of building up in his body plays a more important role even during the 3a period than the increase in food consumption, in the attainment of the final body weight. This can also be determined by employing correlation calculation. The value of r between the efficiency of growth and the length of the period of increased food consumption is  $0.51 \pm 0.05$ . That means [that,admitting great individual variability, the period of increase in food consumption is shorter in the faster growing animals possessing greater efficiency of growth. This shows the great significance of growth efficiency. The sooner the growing animal attains the period of constant food consumption, the sooner begins, and the longer will be the period during which it grows only because it retains and builds up in its body a greater percentage of the food consumed.

Though from our experiments it seems indubitably proved that the retention of a greater percentage of the food consumed plays an important role in the efficiency of growth, it is nonetheless possible that greater food consumption also may be important. HOPKINS (1912) published results showing that those groups of rats that ate more per unit weight grew better. Nevertheless these better-growing rats used up, according to our experiments, a greater percentage of the food consumed for growth. To decide this question we calculated the correlation coefficient between velocity of growth and the amount of food consumed. The correlation coefficient (r) was found to be 0.55,  $=\pm 0.13$ ; that is, there is a certain tendency in the faster growing animals to eat more, but the correlation between velocity of growth and amount of food consumed is not so close as between velocity of growth and efficiency of growth. This latter is, therefore, the more important factor in determining the velocity of growth. Our conclusion is in agreement with that of PALMER and KENNEDY 1930, who with different experiments came to the conclusion, that "Individual variation in efficiency quotient, is a major, if not the controlling cause of individual variation in gain in weight of animals on the same diet."

Animals receiving acid attain an 11.5% higher body weight than the normal ones. How does this difference in body weight originate? One possibility is that hexuronic acids increase the efficiency of growth; the second, that, not influencing efficiency of growth, they increase the food consumption; the third, that, having no influence on either, they increase water retention. Table 1. shows the results calculated

from all the four periods of growth. According to this, the animals that receive acid (and grow 11.5% more) consume 5% less than normal ones to build up the same body weight. Therefore the increase of growth in dry weight is occasioned in some rats by the fact that acid increases efficiency of growth. As the value of k is low — 1.6 — in other animals this mechanism does not work. In these the food consumption has to increase, leaving efficiency of growth unchanged. The fact that there is a tendency — also with a low value of k — in the acid-

#### TABLE I.

No. Mean value Treatment μ Hx. Ascorbie acid 9 Ma = 7599 Gluconic acid 10  $M_{g.} = 8818$ Ascorbic ÷ gluconic  $M_{a, g} = 8709 \pm 185$ 19 42 acid 225 Normal 10 M<sub>n</sub>. = 8016 + 711Differences g 0/0  $M_{a,g} - M_{n} - 368 - 4.5$ 1.6

Food consumed in g. -s. necessary to build up 1000 g. -s of living weight, calculated from all the data obtained during the 3a, 3b and 4-th phases of growth

receiving (bigger) animals to eat more (see Figure 2.) is in accordance with this hypothesis.

Our results regarding the consumption of 1% salt-solution and of water, are also shown in F i g u r e 2. From these results the following conclusions can be drawn: the rats on this diet seek salt-solution and drink only small amounts of water. During the experimental period there were some days when they drank no water at all, but they always consumed, though in changing quantity, the salt-solution. The other important difference we observed in their consumption of salt-solution and of water is that water consumption is almost constant, independent of the growth of the rat. Whereas the rat quadruples its weight from the 48th to the 120th day, its water consumption is almost constant, independent of the growth of the rat. Contrary to this, salt-solution consumption runs somewhat parallel to the growth curve. From the 48th to the 60—70th day, during the 3a period, salt-solution consumption

increases with growth; from there on it remains constant to the 150th day, although body weight is doubled during that time. After this satsolution consumption attains a higher level, although growth has stopped.

The effect of acid administration on salt-solution and water consumption is to be seen in F i g u r e 2. Acid administration has no effect on watter consumption during the 3a and 3b period, but after the completion of growth, during the 4th period (constant body weight of 240— 250 gs) the water consumption of acid-receiving animals increases by 50%. The acid-receiving animals consume salt solution at a greater rate during the 3a period than the normal animals do. During the 3b period salt-solution consumption is constant although growth continues. During this period there is no difference in salt-solution consumption between acid-receiving and normal groups. The increase in salt solution consumption after the 3b period is greater in normal than in acid-receiving animals. Salt consumption apparently remains on this higher level during the whole fourth period.

As the mass of the body is water for the most part, growth can be interpreted, as the building up of such molecules as are capable of more and more water retention. The answer to the question whether the 11.5%higher body weight is caused by greater fluid retention or greater fluid consumption, is given in Table II. From this it is apparent that to build up 1 kg living weight, acid-receiving animals take up 15% more fluid than normal ones; that is, influenced by acids, the animals retain less fluid. As water consumption is almost the same in all groups, the difference refers primarily to salt consumption.

Table II. shows these results during all four periods of growth. To form a picture of the influence of acids during each period we calculated on 6 day averages the salt-solution consumption per day and per kg body weight. Acid-receiving animals have consumed at the end of the 3a period 33% more (k = 2), at the beginning of the 3b period 42% more (k = 2.3), at the end of the 3b period 12% more (k = 1.1) and at the beginning of the 4th period 3% more (k = 0.2) salt-solution than normal ones. Taking into account the results of the food consumption, we come to the conclusion that the higher final body weight is caused during the 3a period by greater salt-solution consumption and unchanged retention, and during the later periods by greater salt-solution retention.

There are some observations which indicate that there is a greater need of salt in organisms with suprarenal insufficiency. RICHTER (1938, 1939). We calculated, therefore, the correlation coefficient between the

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weight of the suprarenals and the average daily salt consumption and found  $r = \pm 0.185 \pm 0.13$  This means that among the animals with large suprarenals there are some which drink less salt than others with small suprarenals, but that this tendency is influenced by many different circumstances.

It is well known that the decreased functioning of the suprarenal cortex is accompanied by cessation of growth, and in adult rats by the diminishing of the restitutional metabolism. We calculated, therefore, the correlation coefficient between the suprarenal weight and the efficiency of growth in individual rats and found  $r = 0.7 \pm 0.01$ . This value is rather high and it means that 0.28% increase in the efficiency

#### TABLE II.

Fluid intake (1% salt solution + water) in g-s, necessary to build up 1000 g-s of living weight, calculated from all the data obtained during the 3a, 3b and 4-th phases of growth

Treaîtment	No.	Mean value µ	μ
Ascorbic acid	9	M <sub>a.</sub> = 8600	
Gluconic acid	19	M <sub>g.</sub> = 8818	
$\begin{array}{rllllllllllllllllllllllllllllllllllll$	19	$M_{a. g.} = 8709 \pm 1852$	426
Normal	10	$M_{n_{.}} = 7521 \pm 1478$	468
Differer	nces	g. g 0/0 k	
$M_{a.g.} - M_{n.} + 1188 $ %015 1.9			

of growth corresponds to 1 mg of suprarenal plus, or 1.7 mg greater suprarenal corresponds to 1% increase in the efficiency of growth. The biochemical mechanism of this definite correlation may be the following: 1.). Either the greater suprarenal corresponds to greater hormone secretion and that to greater efficiency in growth, or 2.) as we know from DONALDSON'S observations that suprarenal weight varies according to the body weight, this rule may also hold good for the suprarenal as part of the body (that is it attains greater growth by greater efficiency). The first mechanism is the more probable, as the correlation coefficient between the body weight and efficiency is 0.9, whereas between the efficiency of growth and suprarenal weight only

0.7. The fact that growth is caused by greater retention is more evident in the case of the body and its tissues (connective tissue, muscle) than in the suprarenal. Moreover, if the suprarenal is affected by illness growth stops; that is, the efficiency of restitution diminishes.

### SUMMARY.

1. A comparison of the alteration in the growth curve caused by administration of hexuronic acids with the curve showing food, saltsolution and water consumption supports the hypothesis that the physiological and cell biochemical mechanism of growth in mammals changes in the different phases of growth. In the growth curves of the mammals one can differentiate at least five such phases.

2. Neither the RUBNER nor the LUSK-WILSON rule is valid for the velocity of growth. The velocity of growth depends chiefly on the direct correlation between velocity and efficiency of growth and the indirect relation between the length of the period of increased food consumption and the efficiency of growth.

3. Among the animals with large suprarenals, there are more that drink less salt solution than among those with small suprarenals. There is a strong positive correlation between the size of the suprarenal and the efficiency of growth. The variations in consumption of salt-solution and of water bear no relation to one another.

4. The body weight of those rats which receive from their 48th to their 250th day, a daily dose of roughly 200 mg/kg 1-ascorbic or dgluconic acid, is 11.5% greater than that of normal rats. Statistical calculations and the careful weighing of the experimental elements make it likely that the difference is caused by the administration of the acids. Besides stimulating growth, ascorbic and gluconic acids act in identical ways on the consumption of food, salt-solution and water. These effects, therefore, are not due to the anti-scorbutic radical of the ascorbic acid but probably to the acid group present in both acids.

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