

ON VARIATION IN WEIGHT OF NORMAL GUINEA-PIGS IN
RELATION TO THE ESTIMATION OF FREE DIPHTHERIA
TOXIN.

BY J. A. CRAW,

Grocers' Company Research Scholar,

Hon. Demonstrator in Physiology, London Hospital Medical College.

(From the Lister Institute of Preventive Medicine.)

IN the foregoing paper (p. 512) relationships have been established between loss of weight in test guinea-pigs and dose of diphtheria toxin injected. In connection with the above, the normal increase of weight during the period of observation, the effect of the animal ceasing to eat, or any change of diet, had to be taken into consideration. The following note records observations on normal increase of weight, with constant and variable diet, on healthy animals.

Method.

Ninety-four guinea-pigs were sorted out into batches of five individuals (one batch consisting of 4), so that the companion pigs did not differ from one another by more than 5 grams. Each batch was placed in a separate and similar cage and all were fed regularly at 7 A.M. and 4 P.M. daily. The pigs were weighed regularly between 11 A.M. and noon, and the weight of each individual noted daily, a spring balance being used, the divisions on the scale of which would indicate a difference of 5 grams.

Normal Increase in Weight.

All the pigs were fed with oats at 7 A.M. and with cabbage and oats at 4 P.M. for a week. The number of pigs and the mean values for the normal increase are given in Table I for five days, two days being allowed for the animals to become acclimatised to their surroundings,

diet, the relatively high temperature prevailing, viz. 16° C. at midnight to about 30° C. at midday, and to recover from a railway journey, diet of unknown character, etc. From the figures it is probable that, for guinea-pigs of 160 to 210 grams initial weight, the rate of increase in weight is approximately the same and is nearly a straight line relation, *i.e.* the increment is proportional to the time. The absolute value of the increment per diem, under the above circumstances, was 7 to 9 grams in the two most divergent series, with the exception of one set, in which only four pigs were classed, where the value is approximately 6 grams. I conclude, therefore, that for guinea-pigs used as indicators of free diphtheria toxin the normal increase in weight will be practically independent of the difference in absolute weight of the test animals, provided this difference does not exceed 60 grams, and further that, as the daily increment probably decreases slowly with increasing absolute weight of the test animals in an hyperbolic relation, the value for guinea-pigs of 250 to 260 gms. will be in the neighbourhood of 6 gms. per diem.

TABLE I.

Average weights of guinea-pigs showing increment during 5 days.

No. of pigs	0	1	3	4	5
7	210	216	234	243	245
23	202	210	229	235	241
13	191	200	219	224	228
19	183	190	209	215	219
16	173	180	200	204	209
9	163	170	186	194	203
4	150	151	168	180	180

Effect of Temporary Change of Diet.

Of the entire series of pigs, 29 were retained on the diet above described and 65 pigs were given a single meal in which grass was substituted for cabbage, and then the original food continued for four days. The pigs would not at first eat the grass so that the effect was striking, for the average per pig of the cabbage group increased by 7 gms., 7 gms., 7 gms. and 5 gms. on succeeding days, whereas the grass group decreased by 10 gms. per pig on the first day and on substituting cabbage, increased on the succeeding days by 12 gms., 6 gms. and 6 gms.; being at the end of this period 6 gms. lower in average weight than the pigs of the cabbage group. Unless a constant diet be employed during the test period of diphtheria toxin any relation between lethal dose and weight will be seriously affected.

Effect of Continued Change of Diet.

Many of the pigs originally closely approximating in weight had increased in weight to a much greater extent than others, although subject to the same food conditions, and consequently it was considered advisable to sort out the animals into groups of five, the individuals of each batch differing, as before, by not more than 5 gms. This selection necessitated, for many pigs, a change of cage and of companions, and the effect was that the majority of these pigs lost an average of 5 gms., and in the case of certain animals 10 gms. It will be seen from this that very slight changes in the conditions may interfere with the dose-weight relation. Other slight changes produce equally marked effects. In order to obtain a fairly uniform condition, all the pigs were now kept on cabbage and oats for a week. The cages were then separated into two groups, each containing batches of pigs corresponding closely in weight, the total number of pigs selected for each series being 36. One group was fed on oats and cabbage and the other on oats and grass for seven days. The average weight of each group at the initial weighing being taken as the origin, the total changes in weight for this period are given in Table II.

TABLE II.

Change in weight during 9 days on diet.

A=Cabbage and Oats; B=Grass and Oats.

1	2	3	4	5	6	7	8	9
A 0·5	A 5	A 10·5	A 17	A —	A 21	B 27	B 21	22
B 4	B -4	B -1	B 5·5	B —	B 12	A 13·5	A 24	35·5

The figures (Table II) show that the effect of substitution of grass for cabbage is attended by an initial marked diminution in the weight of the animals. After two days the pigs fed on grass begin to increase in weight at the same rate as those fed on cabbage, although owing to the initial depression they show no tendency to attain an equal absolute weight. On the seventh and eighth days the grass group was given cabbage and the cabbage group was placed on a grass diet. The change from cabbage to grass produced a fall on the eighth day, followed by a slow rise on the ninth, whereas the change from grass to cabbage gave rise to a very great increment in weight on the eighth and ninth days, the original grass series being at the end of this period 13·5 gms. heavier than the original cabbage series.

Effect of other Changes in Diet for One Day.

Ten guinea-pigs which had been fed on grass for a week were given dry grass and oats and showed on the following day an increment in weight of 4 gms. per pig.

Ten pigs of similar weights and also accustomed to a grass diet were supplied with dry grass, oats and a liberal supply of water in separate troughs; in this case the average increment was 9.5 gms.

Under the same conditions, dry grass, oats and minced beef gave a rise in weight of only 4 gms.

The pigs did not eat the beef.

Further experiments with beef alone led to a loss in weight of 21 gms., as however it was found that the pigs had not eaten the beef, the result is obviously due to no food ingested.

This decrease of 21 gms. per day is approximately of the same magnitude as the decrements obtained on the injection of lethal doses of solutions containing free diphtheria toxin, which lends support to the view that the latter largely represent starvation curves.

In conclusion, the normal variation of the guinea-pig as regards weight seems, to me, to give an experimental error of at least 10% in the estimation of lethal doses from decrement in weight under favourable circumstances, and under ordinary conditions the error is probably two or three times this magnitude.