LXXXVI. VARIATION IN GROWTH RESPONSE OF RATS IN VITAMIN A TESTS COMPARED WITH THE VARIATION IN RATS GROWING NORMALLY.

BY KATHARINE HOPE COWARD.

*From the Pharmacological Laboratories, Pharmaceutical Society
of Great Britain.*

(Received April 26th, 1932.)

ROUTINE tests for vitamin A in foodstuffs carried out in these laboratories are based on the power of the substance under test to restore growth in animals which have ceased to grow on a diet considered deficient only in vitamin A. The test is made in direct comparison either with some other foodstuff convenient for comparison, or with a particular sample of cod-liver oil which we have for some time used as a standard of reference [Morgan and Coward, 1931; Coward, Dyer, Morton and Gaddum, 1931]. The method of comparison is as follows. About 10 rats which have become steady in weight on a diet deficient in vitamin A are all given equal daily doses of the substance under test for 5 weeks, and about 10 similar rats are all given equal daily doses of the substance with which comparison is being made. The average increase in weight for the period is determined for each group, and the comparison between the vitamin A contents of the two doses is drawn by reference to the curves described by Coward *et al.* [1930]. That is, the relative amounts of the standard cod-liver oil which would have brought about these mean increases are determined, and from the figures so obtained are calculated the relative potencies of the two substances compared. Greater accuracy is obtained by comparing bucks with bucks and does with does according to the curves described by the same writers [1931].

The problem then presented itself of how far small differences in average response were significant, and calculations were made for each test from the formula

$$\frac{x_1 - x_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}},$$

in which x_1 and x_2 are the mean increases in weight of the two groups of rats in 5 weeks, and ϵ_1 and ϵ_2 are the standard errors of the two groups;

$$\epsilon = \sqrt{\frac{\sum d^2}{n(n-1)}}.$$

It was tentatively accepted that if the value so obtained were greater than 2, the difference was significant.

This, however, appeared to be unsatisfactory for sometimes ϵ was small and sometimes large. It became evident that it would be sounder to obtain the average standard deviation and apply it to all tests. Dr J. H. Gaddum, when consulted on this matter, suggested the finding of the average (weighted) value of $\sigma^2 \left[= \frac{\Sigma d^2}{n-1} \right]$ for as many tests as possible and the use of it in comparisons in the formula

$$\frac{x_1 - x_2}{\sqrt{\frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2}}} \left[= \frac{x_1 - x_2}{\sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \right],$$

which would have a value greater than 2 if $x_1 - x_2$ were significant. This has been done and also, for comparison, the standard deviation of the mean increases in weight in 5 weeks of rats from our normal colony has been calculated.

Table I. *The standard deviation of 5 weeks' increase in weight of normal rats according to the age and weight at the beginning of the period.*

Standard deviation (S.D.) calculated as $\sqrt{\frac{\sum d^2}{n-1}}$; all weights in g.						
		Periods of 5 weeks beginning at the				
	No. of rats	4th week of life	5th week of life	6th week of life	7th week of life	8th week of life
Bucks—1st generation	54					
Av. initial weight		46.7	61.8	84.0	104.3	121.5
Mean increase		92.2	91.1	81.7	73.9	65.3
S.D. of mean increase		17.31	17.46	15.89	15.97	16.71
S.D. as % of mean increase		18.8	19.2	19.5	21.6	25.6
Bucks—2nd generation	41					
Av. initial weight		44.2	60.6	84.9	103.1	121.3
Mean increase		92.5	88.0	78.7	71.0	64.0
S.D. of mean increase		18.99	20.15	20.49	16.18	13.556
S.D. as % of mean increase		20.6	22.9	26.0	22.8	21.2
Bucks—3rd generation	5					
Av. initial weight		—	61.8	84.0	104.2	—
Mean increase		—	92.2	81.2	75.2	—
S.D. of mean increase		—	23.68	23.82	27.88	—
S.D. as % of mean increase		—	25.7	29.3	37.1	—
Does—1st generation	204					
Av. initial weight		42.4	55.9	71.7	84.4	95.9
Mean increase		63.6	59.2	52.0	45.9	40.1
S.D. of mean increase		11.94	11.62	10.83	9.59	8.91
S.D. as % of mean increase		18.8	19.6	20.8	20.1	22.2
Does—2nd generation	143					
Av. initial weight		40.3	54.4	70.2	83.4	93.7
Mean increase		63.6	59.1	50.8	44.9	41.0
S.D. of mean increase		13.38	12.51	12.15	11.12	10.35
S.D. as % of mean increase		21.0	21.2	23.9	24.8	25.2
Does—3rd generation	22					
Av. initial weight		41.9	58.6	76.5	90.9	102.6
Mean increase		69.7	62.9	52.7	44.7	37.5
S.D. of mean increase		11.26	9.42	8.41	6.64	6.66
S.D. as % of mean increase		16.2	15.0	15.95	14.85	17.8

The standard deviation (σ) of a single determination of the increase in weight of normal rats in 5 weeks.

In case the standard deviation should depend on the initial weight of the group of rats, we calculated this function for periods beginning at the 4th, 5th, 6th, 7th and 8th weeks respectively, of the rats' lives. The results of this calculation are collected in Table I.

It is interesting to note that there is a tendency in the standard deviation of the mean increase to decrease as the initial weight of the group increases, but when expressed as a percentage of the mean increase, this figure increases as the initial weight of the group increases. Perhaps the figures that are most useful for comparison with the results in the vitamin A tests are the standard deviations of the increases of those rats which were about the same weight at the beginning of some selected 5 weeks' period as the rats at the beginning of the 5 weeks' tests. That is $\sigma = 17$ (approx.) for bucks (the period 6–11 weeks) and 10 (approx.) for does (the period 7–12 weeks). (These numbers also happen to agree very well with the averages of all the standard deviations of the normal bucks and does respectively.) Thus the normal standard deviation of the mean increase in weight during 5 weeks' growth may be accepted as about 17 for bucks (initial weight, 84.5 g.) and about 10 for does (initial weight, 81.4 g.).

The standard deviation (σ) of a single determination of the increase in weight of rats in 5 weeks in vitamin A tests.

The results of 108 tests for vitamin A are summarised in Table II. They are all comparable in so far as they have all been carried out under similar conditions of basal diet, housing, etc. In each test all the animals received

Table II. *The standard deviation in the increase in weight of 108 groups of experimental rats (bucks and does calculated separately) which have become steady in weight on a diet deficient in vitamin A and have then been given a daily dose for 5 weeks of some substance to be tested for vitamin A.*

No. of test	No. of rats		Mean increase g.		$\sigma = \sqrt{\frac{\sum d^2}{n-1}}$	
	♂	♀	♂	♀	♂	♀
1	7	7	-19.3	-4.0	13.13	11.58
2	17	7	12.5	13.3	9.70	5.45
3	10	17	18.8	16.1	14.48	15.79
4	19	11	30.6	22.6	10.35	10.02
5	18	12	55.7	36.9	11.67	10.93
6	15	16	53.0	37.2	14.38	9.21
7	10	9	4.2	11.8	10.72	12.75
8	4	5	35.7	24.6	14.80	8.12
9	7	10	-0.9	-0.8	8.07	12.17
10	6	13	27.7	20.2	12.44	5.27
11	13	4	61.4	37.2	19.09	13.67
12	7	11	54.9	36.2	19.67	12.12
13	8	12	2.5	6.3	8.16	8.17
14	8	11	15.0	17.0	14.71	15.22
15	10	16	19.2	9.4	12.66	15.61

Table II (*cont.*)

No. of test	No. of rats		Mean increase g.		$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$	
	♂	♀	♂	♀	♂	♀
16	10	11	18.0	14.9	11.75	8.91
17	10	11	1.7	9.8	7.41	17.27
18	8	12	40.4	27.2	19.59	9.15
19	14	6	42.4	30.3	10.05	17.22
20	8	8	18.4	18.5	12.98	4.72
21	10	9	9.7	7.0	12.76	12.62
22	10	12	14.6	15.3	12.02	7.28
23	2	8	31.5	22.8	0.71	9.32
24	7	7	13.7	12.0	10.95	8.26
25	6	4	-4.0	-2.0	19.13	8.04
26	5	5	1.8	6.2	6.83	15.77
27	7	3	16.4	11.0	16.64	3.46
28	4	5	27.0	22.6	12.68	6.60
29	5	3	-0.4	6.0	16.29	5.29
30	5	5	18.2	24.8	14.53	6.94
31	6	6	7.8	6.7	11.99	11.70
32	7	7	16.1	13.7	14.46	11.73
33	6	6	9.3	14.5	13.62	16.71
34	9	4	11.4	9.2	18.19	19.97
35	5	5	32.4	30.0	8.97	7.18
36	4	8	2.2	9.9	32.29	12.34
37	6	5	35.3	37.8	32.62	8.98
38	4	6	37.2	25.5	18.47	6.37
39	3	6	27.0	18.0	21.63	7.40
40	6	4	15.8	19.2	20.82	8.93
41	4	5	-20.2	5.6	5.63	14.02
42	4	5	44.2	36.8	16.52	3.77
43	5	6	13.4	13.7	16.30	17.95
44	4	6	46.0	37.7	6.48	17.16
45	4	6	-2.5	-2.8	7.05	9.83
46	8	8	-3.6	4.5	19.73	8.85
47	5	5	18.0	11.4	13.62	7.60
48	4	5	27.7	18.4	8.42	7.37
49	6	5	-0.3	0.6	7.54	9.08
50	5	5	24.8	28.2	18.67	4.09
51	4	3	47.0	27.3	12.30	11.73
52	6	4	24.8	13.2	22.62	7.42
53	3	3	45.0	20.0	10.58	8.54
54	5	4	34.6	26.5	2.88	5.07
55	8	2	25.0	30.5	18.14	13.45
56	5	5	32.8	35.0	3.96	6.36
57	4	6	42.2	27.7	21.12	6.07
58	3	5	14.7	11.4	6.04	14.16
59	5	5	27.0	29.4	13.10	12.12
60	4	6	25.7	26.5	10.78	8.12
61	8	12	19.1	15.8	25.28	13.96
62	5	4	29.6	30.7	19.77	4.93
63	5	5	41.4	30.4	12.83	3.87
64	6	5	66.8	39.0	10.54	11.49
65	5	5	30.8	35.0	13.06	6.33
66	4	6	10.7	16.0	13.60	17.02
67	5	4	16.0	13.5	23.37	14.40
68	7	3	7.0	10.0	10.45	13.75
69	3	3	26.3	32.7	2.12	17.01
70	3	6	26.3	16.0	5.86	15.82
71	2	5	15.5	6.4	10.63	12.82
72	4	6	16.0	12.5	12.68	13.10
73	7	4	-0.1	12.2	19.39	16.28
74	4	6	16.5	7.5	11.75	8.16
75	4	6	32.0	17.0	8.08	4.90

Table II (cont.)

No. of test	No. of rats		Mean increase g.		$\sigma = \sqrt{\frac{\sum d^2}{n-1}}$	
	♂	♀	♂	♀	♂	♀
76	4	6	28.5	22.7	10.30	3.22
77	5	5	46.8	34.2	14.77	5.40
78	2	7	34.5	36.7	55.86	11.58
79	6	4	3.0	16.7	18.41	12.58
80	4	7	7.0	21.3	11.78	16.19
81	7	3	34.0	14.7	18.33	9.82
82	2	7	27.5	25.1	20.50	10.73
83	6	4	45.8	29.5	16.67	7.79
84	5	5	25.0	21.8	13.58	10.75
85	3	5	15.3	20.4	10.02	4.30
86	5	5	44.6	21.6	17.13	4.00
87	4	6	12.7	30.3	11.09	6.63
88	4	4	38.5	23.5	10.52	7.57
89	4	5	38.7	33.4	16.28	8.57
90	3	7	41.0	30.6	2.00	11.69
91	3	5	34.3	17.4	18.15	6.32
92	4	5	34.5	20.4	14.25	5.29
93	4	6	83.0	50.2	12.11	5.31
94	4	5	77.0	45.4	5.26	11.85
95	5	5	7.0	9.0	6.67	7.97
96	5	5	31.4	20.6	3.74	6.82
97	5	4	-4.8	0.7	19.83	3.70
98	6	4	9.3	8.5	17.49	7.53
99	2	8	51.5	30.6	13.45	10.52
100	2	8	49.5	36.6	0.71	12.09
101	4	6	41.0	14.5	3.56	7.86
102	4	3	48.5	34.7	14.26	11.02
103	5	3	17.0	28.7	7.60	4.36
104	5	4	27.4	29.7	11.47	16.40
105	7	2	19.9	13.5	15.69	0.71
106	4	6	56.2	44.5	19.62	10.72
107	2	6	30.0	18.8	22.63	13.62
108	6	2	29.0	3.5	8.85	23.33

Total number of tests = 108
 „ bucks = 635 } = 1307 rats
 „ does = 672 }

Average initial weight of bucks = 86.9 g.
 „ „ does = 83.6 g.

Average variance = $\sigma^2 = \frac{\sum d^2}{N - m}$

where N = the total number of observations and m = the number of means (i.e. N = the number of rats, m = the number of tests);

for bucks = 216.74
 for does = 121.44.

Therefore the standard deviation of a single determination

for bucks = 14.72
 for does = 11.02.

the same daily dose. The substances tested and their doses are immaterial in the present investigation and are therefore not included. The number of rats (bucks and does respectively), average increase in weight in 5 weeks and the

standard deviation for a single determination $\left[\sigma = \sqrt{\frac{\sum d^2}{n-1}}\right]$ of the increase in weight, have been tabulated for each of the tests.

The average initial weights of the groups of bucks and does respectively are indicative of the weights at which the rats of our colony cease to grow when given a diet deficient in vitamin A. Occasional rats have not ceased to grow at a weight of 120 g. (bucks) or 110 g. (does) and are, therefore, not used for tests. It is interesting to note that the average weight at which 635 bucks ceased to grow on the vitamin A-free diet was 86.9 g. and that at which 672 does ceased to grow was 83.6 g. which is not much less than that of the bucks. This would appear to indicate that the reserves of vitamin A of the young bucks when first given the vitamin A-free diet are not appreciably greater than those of the young does.

The mean increases in weight of the bucks and does in 5 weeks necessarily varied greatly as the doses of substances tested varied greatly in their vitamin A contents. The final average for the whole number of 108 tests is not of interest except perhaps in supplying an average figure when correlating different standard deviations with different mean increases (see later part of paper).

The standard deviations of single determinations show a wide range among the various tests. The average standard deviation is 14.72 for the bucks and 11.02 for the does.

This result means that about 1 of every 3 bucks used in a test may be expected to give an increase in weight of 14.72 g. more or less than the average increase in weight. Similarly 1 of every 3 does may be expected to give an increase in weight of 11.02 g. more or less than the average increase. This would appear to show that it is more accurate to use does for these tests than bucks, but actually this is not so, for the curve of response to doses of vitamin A is steeper for bucks than for does, which more than compensates in accuracy for the greater standard deviation (see later part of paper).

Variation in the standard deviation.

Before the figures 14.72 (bucks) and 11.02 (does) were accepted as applicable to all tests, an analysis was made to see whether the value of σ varied (a) according to the average initial weight of the group, *i.e.* the weight at which the rats became steady in weight and the 5 weeks' test period was begun, and (b) according to the mean increase in weight of the group. The result of the first analysis is shown in Table III. The tests have been grouped according to the average initial weights of the rats and the different values of the standard deviations for the various tests in each group have been averaged. The numbers of tests in each group are also recorded. It may be seen that there is no correlation between standard deviation and average initial weight of the group of rats on test. Similarly, in Table IV, in which the tests have been grouped according to the mean increase in weight of each group, it may be

Table III.

Average values of σ when the average initial weights of the groups are:											
	Up to 75 g.	75-80 g.	80-85 g.	85-90 g.	90-95 g.	95-100 g.	>95 g.	>100 g.			
	11.98	14.24	11.70	14.03	10.94	15.19	11.28	18.07	9.86	14.51	7.70
No. of tests	22	26	17	18	20	19	20	18	18	15	11
											12

Table IV.

Average values of σ when the mean increases of the groups are:							
	Up to 10 g.	10-20 g.	20-30 g.	More than 30 g.			
	14.63	12.57	14.58	11.43	15.26	8.60	16.44
No. of tests	23	23	26	32	18	27	41
							26

seen that the standard deviation does not vary according to the mean increase in weight of the groups.

Hence it would appear to be safe to adopt 14.72 for bucks and 11.02 for does as the standard deviation of a single determination of the increase in weight in 5 weeks whatever the average initial weight of the group and whatever the mean increase in weight in 5 weeks.

This raises the question of how far the accuracy of the final result of a test is affected by the general acceptance of these standard deviations for all mean increases in weight. In particular, how far does the range of results (2 out of 3 times) differ in a test where the mean increase is, say, 0, from the range in one where the mean increase is, say, 30 g.? This has been calculated in Table V for mean increases of 0, 10, 20, 30 g. respectively for both bucks and does. From the curves relating mean increase in a group of rats to the dose of vitamin A given [Coward, Key, Dyer and Morgan, 1930, 1931], the

Table V. *To show that the degree of accuracy of the test is the same, however the mean increases of the groups may vary.*

Mean increase, say:		Equiv. dose of oil A		Range of increase		Range of equiv. dose of oil A		Percentage range of equiv. doses	
		♂ g.	♀ g.	♂ g.	♀ g.	♂ mg.	♀ mg.	♂	♀
0	0	0.5962	0.3528	+14.72 -14.72	+11.02 -11.02	1.1695 0.3039	0.8904 0.1397	196.2 51.0	252.4 39.6
10	10	0.9423	0.8173	+24.72 -4.72	+21.02 -1.02	1.8484 0.4803	2.0635 0.3237	196.2 51.0	252.5 39.6
20	20	1.4894	1.8940	+34.72 +5.28	+31.02 +8.98	2.9214 0.7591	4.7818 0.7502	196.1 51.0	252.5 39.6
30	30	2.3540	4.3883	+44.72 +15.28	+41.02 +18.98	4.6175 1.1998	11.0790 1.7382	196.1 51.0	252.5 39.6

dose of a cod-liver oil that would have brought about each of these mean increases has been calculated. Similarly, the doses corresponding to mean increases in weight of 0 ± 14.72 g., 10 ± 14.72 g., 20 ± 14.72 g., 30 ± 14.72 g. (bucks) and also 0 ± 11.02 g., 10 ± 11.02 g., 20 ± 11.02 g., 30 ± 11.02 g. (does) have been calculated. Then each of these doses has been further calculated as a percentage of the dose corresponding to the mean increase. It may be seen in the last columns of Table V that the range of doses is about 50–200 % of the mean according to the bucks, and about 40–250 % according to the does, no matter what the mean increase in weight of the group. Thus it is shown that the degree of accuracy obtained by the use of the same values of the standard deviation is the same whatever the mean increase in weight from which the calculation is made. The fact that the does give a wider range than the bucks, *i.e.* a less accurate result in spite of their having a smaller standard deviation, is due to the growth curve of the does being less steep than that of the bucks.

The standard error (ϵ) of the mean.

The foregoing determinations have dealt with the standard deviation of a single determination. It is necessary to find how far the accuracy of an average result is affected by the number of animals in the group used for the test. In other words, the standard deviation of the mean (*i.e.* $\sigma/\sqrt{n}$) with different values for n must be determined. This is shown in some detail in Tables VI and VII. In Table VI a mean increase of 0 g. for bucks and does

Table VI. *To show that when 10 rats (bucks or does, but not a mixture) are used in a test, the vitamin value of the dose will be within 25 % above and 21 % below (according to the bucks), and within 35 % above and 25 % below (according to the does) the true value, two out of three times of performing the test. Similarly the result will be within 56 % above and 37 % below (according to the bucks), and within 80 % above and 44 % below (according to the does) the true value, 21 out of 22 times of performing the test.*

Standard error of the mean ($\epsilon = \frac{\sigma}{\sqrt{n}}$).									
When the number of rats is 10, $\epsilon = 4.66$ (bucks) and $\epsilon = 3.48$ (does).									
Mean increase		Equiv. dose of oil A		Range of mean increase when $n=10$		Range of equiv. dose of oil A		Percentage range of equiv. doses	
♂ g.	♀ g.	♂ mg.	♀ mg.	♂ g.	♀ g.	♂ mg.	♀ mg.	♂	♀
(a) $\pm \epsilon$ where $\epsilon = \frac{\sigma}{\sqrt{n}}$.									
0	0	0.5962	0.3528	+4.66 -4.66	+3.48 -3.48	0.7379 0.4817	0.4726 0.2633	123.8 80.8	134.0 74.6
(b) $\pm 2\epsilon$.									
				+9.32 -9.32	+6.96 -6.96	0.9129 0.3891	0.6331 0.1965	153.1 65.3	179.5 55.7

Table VII. *The degrees of accuracy obtainable with different numbers of rats.*

No. of rats	Mean increase		Equiv. dose of oil A		Value of $\epsilon = \frac{\sigma}{\sqrt{n}}$		Value of 2ϵ		Range of equiv. doses of oil A (i.e. mean $\pm 2\epsilon$)		Percentage range of equiv. doses	
	♂ g.	♀ g.	♂ mg.	♀ mg.	♂ g.	♀ g.	♂ g.	♀ g.	♂ mg.	♀ mg.	♂	♀
2	20	20	1.4894	1.8940	10.48	7.93	20.96	15.86	3.8877 0.5705	7.1812 0.4994	261.0 38.3	379.1 26.4
3	—	—	—	—	8.50	6.36	17.00	12.72	3.2427 0.6839	5.5158 0.6503	217.7 45.9	291.2 34.3
5	—	—	—	—	6.58	4.93	13.16	9.86	2.7202 0.8153	4.3371 0.8270	182.6 54.7	229.0 43.7
10	—	—	—	—	4.65	3.48	9.30	6.96	2.2793 0.9734	3.3995 1.0554	153.0 65.4	179.5 55.7
15	—	—	—	—	3.80	2.84	7.60	5.68	2.1086 1.0517	3.0549 1.1752	141.6 70.6	161.3 62.0
20	—	—	—	—	3.29	2.46	6.58	4.92	2.0128 1.1018	2.8635 1.2526	135.2 74.0	151.2 66.1
25	—	—	—	—	2.94	2.20	5.88	4.40	1.9494 1.1384	2.7416 1.3086	130.9 76.4	144.8 69.1
36	—	—	—	—	2.45	1.84	4.90	3.68	1.8638 1.1899	2.5805 1.3785	125.1 79.9	136.2 72.8

has been selected (*i.e.* maintenance of weight). The doses of the standard oil which would just maintain weight in bucks and does respectively have been determined from the curves already described in previous papers. The ranges of the mean increases in weight when the number of animals in the group is 10 have been calculated from the formula $\epsilon = (\sigma/\sqrt{10})$. The range of doses of the standard oil corresponding to the range of mean increases has been calculated from the curves and expressed as percentages of the actual doses in the last column. This shows that 2 out of 3 tests with 10 animals in each test will give a value within 24 % above and 20 % below the true value according to the bucks, and within 34 % above and 26 % below, according to the does.

Similarly values have been calculated for ranges of $\pm 2\epsilon$, and from these it is seen that in 21 out of 22 tests with 10 animals in each test the values found will lie within 54 % above and 35 % below the true value according to the bucks, and within 80 % above and 45 % below according to the does.

The degree of accuracy obtainable with different numbers of rats.

In Table VII are calculated in the same way the degrees of accuracy obtainable from different numbers of animals. They are most easily estimated by reference to Fig. 1 in which the percentage range of accuracy has been plotted against the number of animals used in the test. The uppermost and lowest curves represent the results obtainable with does, the middle ones represent those obtainable with bucks. It is easily seen that an increase in the number of animals gives a greater degree of accuracy but it is evident that the higher numbers (say 20–36) would bring about so small an increase in accuracy over that obtained with, say, 10 rats, that it would be disproportionate to the extra work involved in handling 20–36 rats rather than 10. To obtain a result 21 out of 22 times within 50 % above and 33 % below, 11 bucks

or 21 does would be required. To obtain a result within 60 % above and 38 % below, 8 bucks or 15 does would be required. Smaller numbers of animals than these would give disproportionately greater degrees of inaccuracy, and it would appear to be a practical outcome of the work to suggest the use of 8 bucks or 15 does for a vitamin A test. This would give a 21/22 probability of being within 60 % above and 38 % below the true value and a 2/3 probability of being within 27 % above and 21 % below the true value.

Fig. 1. To show the range of values, calculated as a percentage of the true value, obtainable with different numbers of rats, the range being given as a 21/22 probability of the value lying within $\pm 2\epsilon$ where ϵ is the standard error of the determination made with the stated number of animals.

The ratio of bucks to does giving a particular degree of accuracy is shown by the curves to be the same, 1:2 (approx.) at all percentages of accuracy. This is determined partly by the different standard deviations of the single determination, 14.72 for bucks, 11.02 for does, and partly by the fact that the slope of the curve of growth for bucks is greater than that for does.

The standard deviation of the logarithm of the result (λ).

The standard deviation of the logarithm of the result is obtained by dividing σ by the slope of the line connecting the response with the log (to base 10) of the dose [Coward, Dyer, Morton and Gaddum, 1931]. Since the curve relating the response of bucks to the log of the dose is $y = 11.3 + 50.3x$ (where x = the log of the dose) and that for does is $y = 12.4 + 27.4x$, then the standard deviation of the logarithm of the result is

$$\frac{14.72}{50.3} = 0.293 \text{ for bucks } (\lambda_1),$$

and $\frac{11.02}{27.4} = 0.402 \text{ for does } (\lambda_2).$

The error of the test is then $\frac{\lambda_1}{\sqrt{n}}$ for the bucks and $\frac{\lambda_2}{\sqrt{n}}$ for does. (λ^2 has been termed the "logarithmic variance" of the test.)

From this λ_1^2 is seen to be 0.085849 and λ_2^2 is seen to be 0.161604.

That is λ_1^2 is about half of λ_2^2 , which agrees with the previous finding that about twice as many does as bucks are required for any given degree of accuracy.

Significant differences in mean increases in weight of two groups of rats.

To determine significant differences in mean increases in weight of any two groups of rats, the formula

$$\frac{x_1 - x_2}{\sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

has been used.

x_1 and x_2 = mean increases of the two groups respectively,

σ = 14.72 (bucks),

= 11.02 (does),

n_1 and n_2 = the number of animals in the two groups respectively.

If the value so found is equal to or greater than 2, the difference may be considered significant. Obviously the greater the value of n , the smaller need be the value of $x_1 - x_2$ for the difference to be considered significant. In Table VIII (column 2) values of $x_1 - x_2$ have been worked out for various values of n (n_1 and n_2 being made equal in each calculation for the sake of simplicity). Further, by taking a mean increase of 10 g. for one test and 10 *plus* each value of $x_1 - x_2$ so found for the other (column 3), and determining the dose of the standard oil (column 4) which would bring about these mean increases, there have been determined the percentage differences (column 5) in the vitamin values of the doses of substance tested, which were indicated by the various values of $x_1 - x_2$. Thus, if only 7 bucks were used for testing each of two samples, the difference between the mean increases of the groups would have to be at least 15.75 g. to show that the dose of one substance tested differed significantly from the other and then it would appear to have 204.2 % of the value of the other dose. Similarly the use of 25 bucks on each dose would have to give a difference between the mean increases of at least 8.33 g. to allow the conclusion that the dose of one substance had 145.3 % the value of the other. Figures for the does have also been calculated.

Dr J. H. Gaddum has suggested that when a comparison between two substances is made by using two groups of animals, each of which contains both bucks and does, the relative potency of the substances as indicated by the bucks and the relative potency as indicated by the does may best be averaged by the use of the formula:

$$\frac{\frac{n_1 x_1}{\lambda_1^2} + \frac{n_2 x_2}{\lambda_2^2}}{\frac{n_1}{\lambda_1^2} + \frac{n_2}{\lambda_2^2}},$$

where n_1 = the total number of bucks used in the comparison; n_2 = the total number of does used in the comparison; x_1 = the log of the ratio of the potency of the two substances according to the bucks; x_2 = the log of the ratio of the potency of the two substances according to the does; λ_1^2 = the logarithmic variance of the bucks; λ_2^2 = the logarithmic variance of the does.

Table VIII. *To show (a) the least value that the difference ($x_1 - x_2$) between two mean increases must have in order to be considered significant when different numbers of animals are used for comparisons, and (b) the corresponding differences in vitamin A value which are indicated by these significant differences in mean increases.*

	No. of animals (1)	Least value of $x_1 - x_2$ to be significant (2) g.	Mean increases (say) (3) g.	Doses of standard oil which would have brought about these increases (4) mg.	Value of richer substance stated as a percentage of value of poorer substance (5)
♂	3	24.02	10 34.02	0.95 2.83	297.9
	5	18.61	10 28.61	0.95 2.21	232.6
	7	15.75	10 25.75	0.95 1.94	204.2
	9	13.87	10 23.87	0.95 1.78	188.0
	12	12.01	10 22.01	0.95 1.64	172.6
	16	10.42	10 20.42	0.95 1.52	160.0
	25	8.33	10 18.33	0.95 1.38	145.3
♀	3	17.98	10 27.98	0.82 3.70	451.2
	5	13.93	10 23.93	0.82 2.64	322.1
	7	11.79	10 21.79	0.82 2.20	268.3
	9	10.38	10 20.38	0.82 1.96	239.0
	12	8.99	10 18.99	0.82 1.74	212.2
	16	7.80	10 17.80	0.82 1.57	191.5
	25	6.23	10 16.23	0.82 1.38	168.3

If we accept the finding that twice as many does as bucks are required to obtain the same degree of accuracy, then the formula reduces to $\frac{2n_1x_1 + n_2x_2}{2n_1 + n_2}$ of which the logarithmic variance is given by the formula $\frac{1}{\lambda^2} = \frac{n_1}{\lambda_1^2} + \frac{n_2}{\lambda_2^2}$.

SUMMARY.

The results of 108 tests (involving 1307 rats) for vitamin A carried out in this laboratory under similar conditions of basal diet, housing *etc.* have been examined with a view to determining the degree of accuracy obtainable with reasonably small numbers of animals in general tests for vitamin A. The growth of 469 normal rats has been examined for comparison.

The standard deviation ($\sigma = \frac{\sum d^2}{n-1}$) of a single determination of the increase in weight of rats (bucks or does) in a 5 weeks' growth test for vitamin A is not different from that of the normal increase in weight of rats (bucks or does) for the same period and beginning at the same average weight.

The standard deviation ($\sigma = 14.72$ for bucks and 11.02 for does) of the experimental animals is the same whatever the mean increase in the test may be and whatever the average initial weight of the groups may be.

The standard deviation allows the same percentage range of accuracy whether the mean increase in weight and corresponding dose of standard oil are low or high. This has been shown by evaluating the vitamin A content of a dose of test substance in terms of the standard oil by means of the curves described by Coward, Key, Dyer and Morgan.

The degrees of accuracy obtainable with different numbers of rats (bucks or does) have been calculated. To get equal degrees of accuracy about twice as many does as bucks must be used in a test; this is due to the steeper curve relating the growth of bucks to the dose, which more than compensates for their greater standard deviation.

The difference between mean increases in weight of two groups of animals which must be obtained in order that the results may be considered significantly different has been calculated for different numbers of animals in the groups compared. The corresponding percentage differences in vitamin value of the doses compared have also been calculated.

REFERENCES.

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