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VARIATION IN SUSCEPTIBILITY OF GUINEA-PIGS TO DIPHTHERIA TOXIN.

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Introduction.

During the course of investigations which have been made during the past two years on the transmission of immunity to diphtheria toxin in guinea-pigs it was observed that a seasonal variation in the dose was necessary in order to cause the death of control animals. This variation was seen to follow somewhat the time of year at which the tests were made, so that during summer a larger dose was necessary than during the winter. We found also that in winter a guinea-pig took a longer time to come up to the standard weight of 250 grms. than in summer, and that the lethal dose varied directly with its age.

So far as we are aware there are no records at present in the literature of such variation in the case of diphtheria intoxication. Reid Hunt and Seidell have found that the lethal dose of acetonitril to mice is lower during the summer than during the winter months; the reverse of that which we have observed in the case of diphtheria toxin on guinea-pigs. In their paper no mention is made either of the ages of the mice used or of the variation if any in susceptibility dependent upon age apart from weight.

Method.

The toxin selected was a stable one, having been prepared in December 1900. On each occasion of testing guinea-pigs, the progeny of treated parents which had arrived at the standard weight of 250 grms., control tests were made on normal guinea-pigs of the same weight in order to guard against false conclusions arising from any unexpected deterioration. In this way results have accumulated from more than 100 normal guinea-pigs. As will readily be understood a large number of animals were not necessary as controls on each occasion, the tests

being repeated every few days. The results obtained therefore cannot be taken as giving exact quantitative differences between the minimal lethal doses but rather as indications of the extent of the differences. To obtain more accurate determinations it would be necessary to inject long series of guinea-pigs and definitely fix the lethal dose for each season of the year. This we are now carrying out.

Results.

Variation in Resistance.

The minimal lethal dose of toxin J90A which was used throughout these investigations is here given for different times from Jan. 1908 to July 1909. It is seen that in addition to the annual variation, there is an apparent increase in toxicity during this present year as compared with the previous. This we suggest may be due to differences in climatic conditions of the two years.

TABLE I.

Showing variation in the approximate m. l. d. of diphtheria toxin for guinea-pigs according to season.

Month	Fatal dose
January 1908	0·008 c.c.
May 1908	0·009
September 1908	0·010
November 1908	0·0075
January 1909	0·006
April 1909	0·0065
July 1909	0·009

These numbers when plotted out give the following curve.

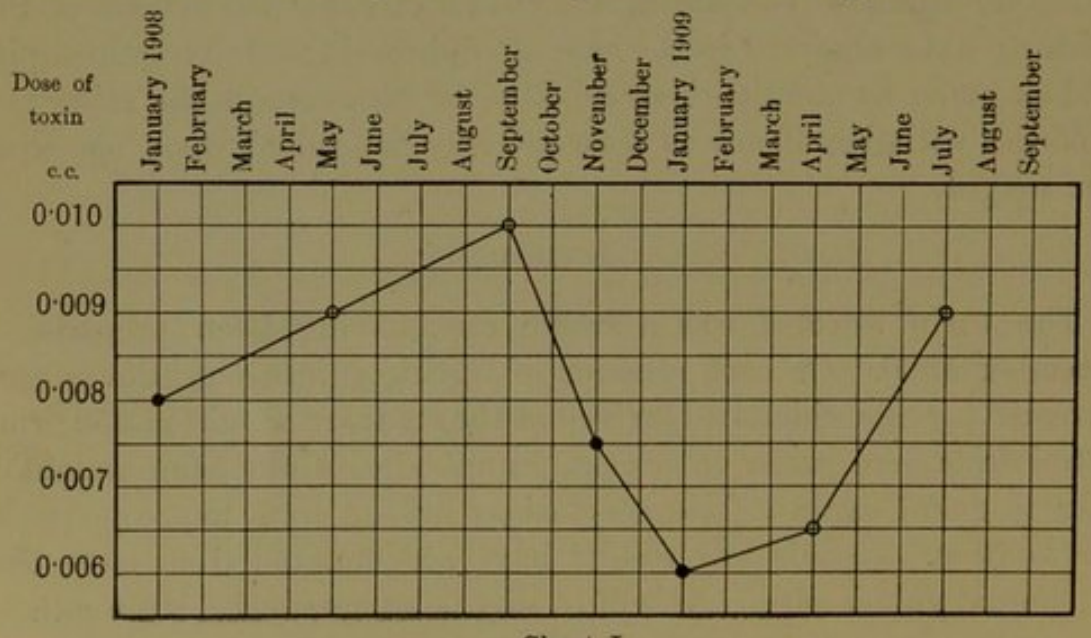

Chart I.

These figures as above stated are to be considered only as close approximations to the exact lethal doses. The most accurate indications are given by taking the percentage number of animals that survive beyond the 5th day when injected with a certain dose. For the purpose of Table II the results are divided into three sections according to the results at three different doses—0·006 c.c., 0·008 c.c. and 0·010 c.c. In forming the table any survivals at a higher dose are included among those at a lower dose on the assumption that a pig that survived a higher dose would naturally have survived a lower dose had that dose been given instead. In the same way any deaths at a low dose are included among the deaths at a high dose.

TABLE II.

Showing number and percentages of survivals and deaths of guinea-pigs injected with certain doses of diphtheria toxin at different seasons.

Dose :—	0·006 c.c.		0·008 c.c.		0·010 c.c.		Percentage survivals at		
Months	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	0·006 c.c.	0·008 c.c.	0·010 c.c.
Jan. 1908	0	4	5	0	—	—	100	0	—
Feb.	—	—	1	2	—	—	—	66	—
Mar.	—	—	1	0	—	—	—	—	—
April	—	—	—	—	8	0	—	—	0
May	—	—	1	6	11	0	—	85	0
June	—	—	0	5	1	4	—	100	80
July	—	—	—	—	0	3	—	—	100
Aug.	—	—	—	—	0	1	—	—	—
Sept.	—	—	0	3	4	1	—	100	20
Oct.	—	—	8	1	13	0	—	11	0
Nov.	2	6	11	0	—	—	75	0	—
Dec.	5	2	8	0	—	—	28	0	—
Jan. 1909	11	0	—	—	—	—	0	—	—
Feb.	3	1	—	—	—	—	25	—	—
Mar.	4	2	—	—	—	—	33	—	—
April	1	6	3	0	—	—	85	0	—
May	—	—	3	3	12	0	—	50	0
June	0	4	11	3	—	—	100	21	—
July	0	4	13	3	14	0	100	18	0
Aug.	0	10	12	7	20	1	100	36	4
Sept.	—	—	5	0	—	—	—	0	—

These results are expressed in graphic form in Chart II. This consists of three curves—the lowest depicting the percentage number of survivals at 0·006, the middle curve at 0·008 and the top curve at 0·010 c.c.

Within the limits of a third to sixth day death the product of toxin dose and lethal time appears to be a constant when the average of a number of results is taken (Dean and Craw, *Journ. of Hygiene*, 1907). Table III shows that the value of this product varies with the time of year.

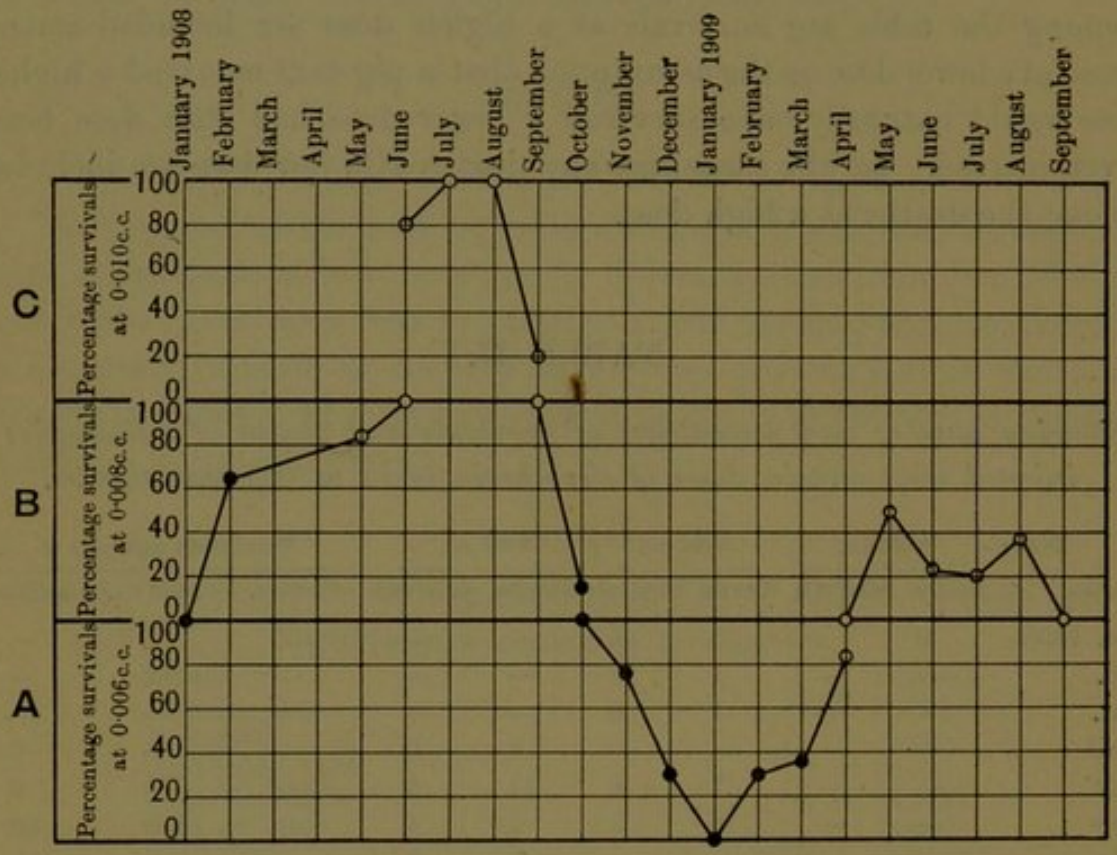

Chart II.

TABLE III.

Showing that the product of lethal dose and lethal time varies according to the season.

Date	Product of Toxin dose (in c.c.) and lethal time (in days)	No. of observations
January—March 1908	·0249	9
April—June ,,	·0496	28
July—September ,,	·0460	32
October—December ,,	·0323	29
January—March 1909	·0284	15
April—June ,,	·0403	20
July—September ,,	·0444	22

These numbers when plotted out give the following curve.

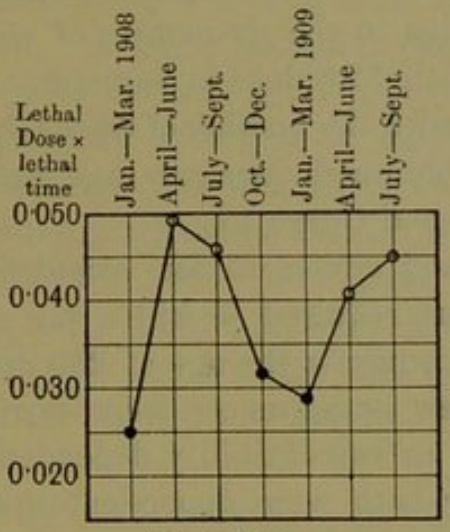

Chart III.

Rate of Growth.

It was at first thought that the variations in age of guinea-pigs of 250 grams weight in summer and in winter might account for the difference in susceptibility. At different times a number of animals

TABLE IV.

Showing average ages of 250 gram weight guinea-pigs at different seasons of the year.

Date	No. of animals	Average age
October—December 1908	71	45·2 days
January—March 1909	71	44·0 ,,
April—June 1909	57	34·6 ,,
July—September 1909	33	29·8 ,,

These numbers when plotted out give the following curve.

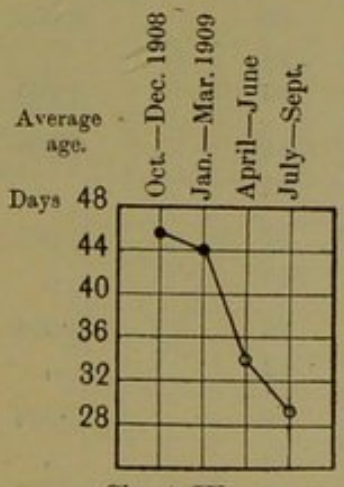

Chart IV.

were put aside and the times taken to reach 250 grams weight were noted. From these records it has been calculated that the average age of 250 gram guinea-pigs in the six months of winter (Oct.—March) is 44·6 days compared with 32·8 days in summer. Individual variations are so great compared with the number of cases taken that it is necessary to divide the results obtained quarterly and not monthly. This has been done in Table IV and Curve IV.

From the tables and curves so far recorded it appears that in summer larger doses are required to kill a pig of 250 grams than in winter and also that in summer the age of 250 gram pigs is much less than in winter. A few experiments were carried out to determine whether the variation in susceptibility depended upon the age of the guinea-pigs or whether both were dependent upon some third factor. In May, June and July 1909 pigs of known age were whenever possible used as control pigs and the results are all recorded in Table V.

TABLE V.

Showing that susceptibility of guinea-pigs to diphtheria toxin varies inversely as the age of the animal.

Date of experiment	Age of guinea-pig in days	Dose of toxin injected, in c.c.	Number of days till death
3 May 1909	41	0·008	4
	49	,,	7
10 May	27	0·009	5½
	38	,,	4
18 May	46	0·009	4
	57	,,	5
21 May	43	0·009	3
	49	,,	3
25 May	20	0·008	3
	28	,,	3
	35	0·009	4
15 June	31	0·008	3
	31	,,	3
25 June	27	0·008	3
	43	,,	11
13 July	26	0·008	4
	32	,,	6
20 July	29	0·008	4
	35	,,	6
	68	0·009	4

From a consideration of this table it appears in general that the fatal dose for an old guinea-pig is greater than that for a young guinea-pig of the same weight. A single exception (for which we can suggest no explanation) is seen in the case of two guinea-pigs injected on May 10th. In one case where no difference in date of death is noted the two pigs are of the same age. In the other two cases the difference in age is very small and it is quite possible that a difference would have been observed had observations of death been more frequently taken than morning and evening. If, then, age were the only factor causing variation in susceptibility, it would follow that the fatal dose of a toxin would be higher in winter than in summer, but the reverse is the case.

Relationship of Loss in Weight to Time of Death.

The loss in weight of guinea-pigs in the first few days after an injection may often be taken as an indication of the day upon which the animal will die. The ratio between the loss in weight and day of death varies with the time of year.

TABLE VI.

Showing the loss in weight in grams during the first 24 hours of guinea-pigs injected with diphtheria toxin which died on either the fourth or fifth day after injection.

Time of year	No. of experiments	Loss of weight
April—June 1908	27	11·1 gms.
July—Sept. ,,	19	8·1 ,,
Oct.—Dec. ,,	56	13·2 ,,
Jan.—Mar. 1909	47	14·9 ,,
April—June ,,	78	5·1 ,,
July—Sept. ,,	138	4·0 ,,

These numbers when plotted out give the following curve.

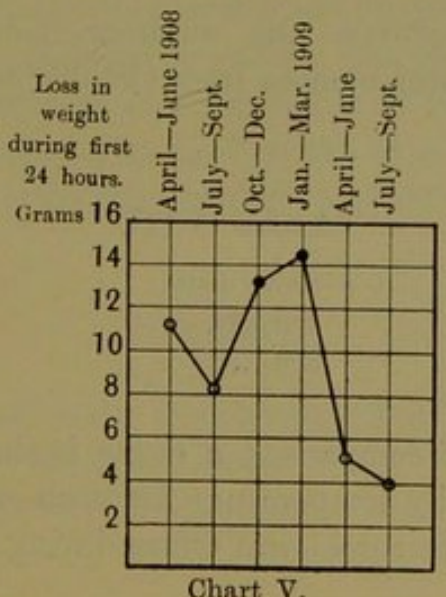

Chart V.

TABLE VII.

Showing the loss in weight in grams during the first 24 hours of guinea-pigs injected with diphtheria toxin, which died between the 6th and 10th day.

Time of year	No. of experiments	Loss of weight
April—June 1908	13	6·1 gms.
July—Sept. ,,	9	5·5 ,,
Oct.—Dec. ,,	11	11·8 ,,
Jan.—Mar. 1909	25	10·8 ,,
April—June ,,	144	5·5 ,,
July—Sept. ,,	61	2·8 ,,

These numbers when plotted out give the following curve.

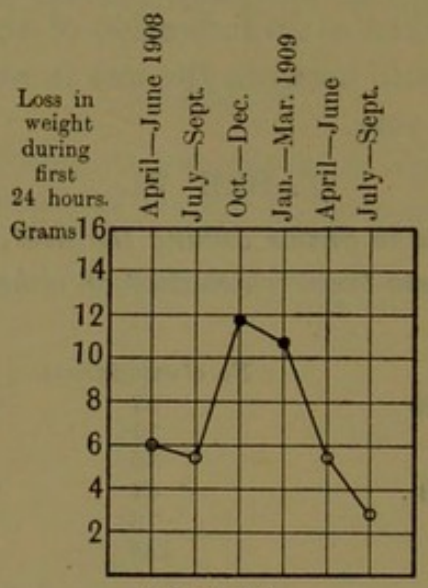

Chart VI.

The comparison between the summer months from April to September and the winter months from October to March is shown in Table VIII.

TABLE VIII.

	4—5th day death	6—10th day death
Summer	5·3 gms.	4·4 gms.
Winter	14·0 ,,	11·1 ,,

For the purpose of comparison a curve is shown on Chart VII for the average of the daily temperature for each month as given in the official reports of the Meteorological Office during the period covered by the results.

If we compare the number of months when the average temperature was below 40° F. during the winter of 1907—8 with that of 1908—9, and also the number of months when the average temperature in summer was above 56° F. or 60° F., it is obvious that distinctly more wintry conditions have prevailed during this latter season. This may

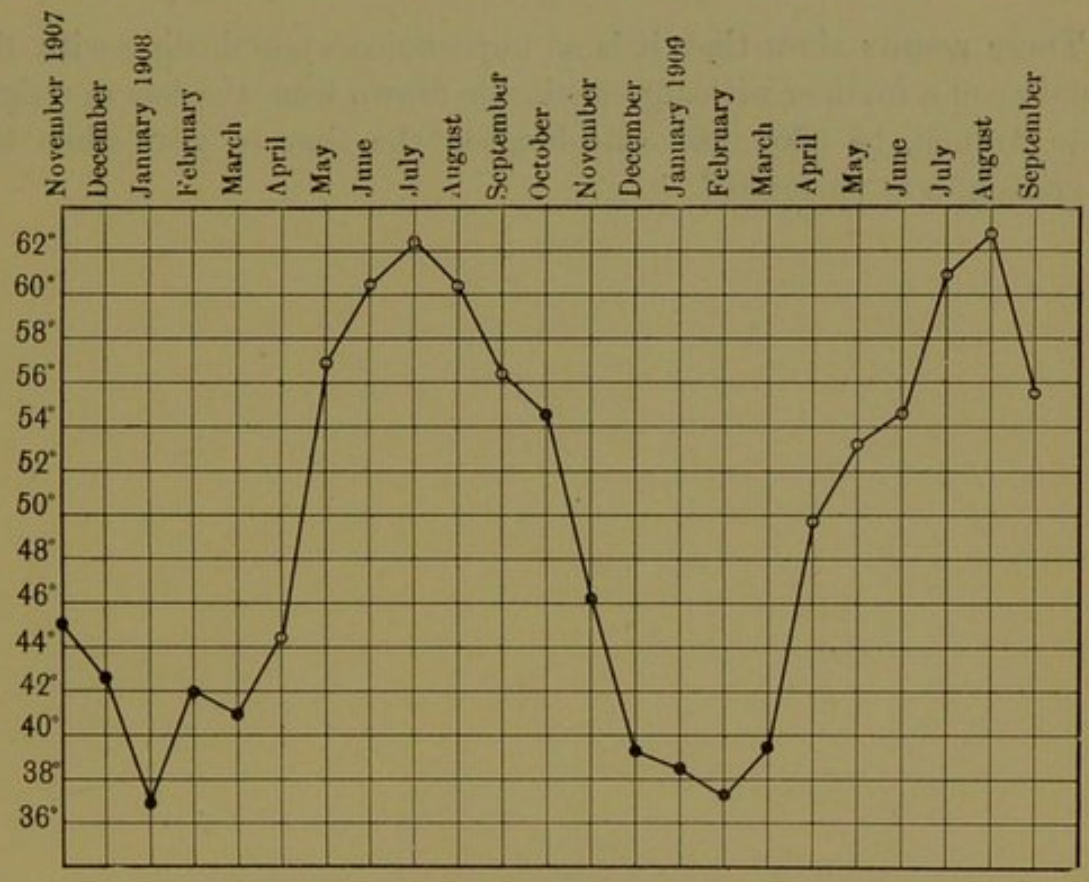

Chart VII. Curve showing average monthly temperatures.

help to explain the fact above recorded and shown in Curve No. 1 that our guinea-pigs have exhibited a greater susceptibility to diphtheria toxin during the present year as compared with last year. It should be pointed out that the guinea-pigs used in the experiments were kept in a special animal house artificially heated in winter.

Summary.

1. A larger dose of toxin is necessary to kill a guinea-pig of the same weight in summer than in winter.

2. The rate of growth of guinea-pigs is more rapid in the summer months.

3. The weights of guinea-pigs are least affected by lethal doses in summer.

4. For guinea-pigs of the same weight the fatal dose increases with the age.

Conclusion.

These results show that it is of importance when dealing with the fatal dose of a toxin or with any conclusion drawn from the loss in weight of an animal, to take into consideration the time of year when the experiment was performed.