ON THE INFLUENCE OF NUCLEIC ACIDS OF VARIOUS ORIGIN UPON THE GROWTH AND LONGEVITY OF THE WHITE MOUSE

by

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(Submitted for publication 2nd February, 1928.)

STATEMENT OF THE PROBLEM AND OUTLINE OF THE RESULTS.

In 1922 it had begun to appear probable to the author that the course of growth and the onset of senescence in animals and plants are determined by the ratio of nuclear (chromosomal) materials to the cytoplasm (protein) of the cells composing the organism (the nucleo-cytoplasmic ratio). This conception has been elaborated in other publications (6, 7), and need not further detain us here. It is sufficient to state that, guided by this conception, it appeared possible that if the nuclei in animal tissues could be supplied with nutritive materials in excessive abundance in comparison with the cytoplasm, and if by this means nuclear growth could be in the slightest degree facilitated disproportionately to the growth of cytoplasm, the growth of the animal should be modified and senescence delayed. While it was hardly probable *a priori* that administration of nucleic acids by mouth could accomplish such a result, it nevertheless appeared of importance to ascertain the effects of prolonged oral administration of nucleic acids to animals in great excess of their normal intake.

Although the nuclear substances (chromosomes), which have been shown by modern genetic research to determine the development of organisms are invariably associated with nucleic acid (for this is the substance which is actually stained by nuclear dyes (5)), it is by no means certain that genetic factors are actually nucleic acids. Accordingly, in the first instance, thymus nuclei were

[1] These experiments were initiated in 1922 and the expenses of the investigation were defrayed until February, 1927, by the Animal Products Research Foundation of the University of Adelaide. The manufacture of the large amounts of Spleen Nucleic Acid employed in some of the experiments was rendered possible by a grant from the Commonwealth Department of Health. Since February, 1927, the expenses of the investigation have been defrayed by the Commonwealth Council for Scientific and Industrial Research as part of its programme of investigation of factors which determine the growth and nutrition of animals.

employed, prepared from calf-thymus by digesting the protoplasm off the nuclei with pepsin-hydrochloric acid. Thus not only nucleic acid, but presumably some other nuclear constituents as well, were simultaneously exhibited to the animals.

The results of this experiment (figures 1 and 2, Tables 1 to 4) showed that while thymus nuclei exert little effect upon the growth of the white mouse (when superadded to a varied and abundant diet of rice, milk, maize, and egg), yet longevity is very decidedly enhanced by the administration of an amount of thymus nuclei corresponding to 0·5 grammes of fresh thymus tissue daily, the average duration of the life of male mice being increased eleven weeks, which is 12·6 per cent. in excess of the normal life-duration, while the life-duration of female mice was increased fifteen weeks, which is 17 per cent. in excess of the normal life-duration. These effects, as the diagrams show, are far in excess of the combined probable error of the estimations.

The effect thus obtained might have been attributable to nucleic acid, or, on the contrary, to some other unknown constituent of the nuclei which is not soluble in pepsin-hydrochloric acid. Again, it might have been attributable to the correction of some dietary deficiency which was supplied by the cleavage-products of nucleic acid, such as phosphoric acid or purine or pyrimidine bases. The varied character of the basal diet, which was supplied *ad libitum*, however, and the presence in it of milk and egg, rendered any shortage of either phosphoric acid or the raw material for the manufacture of purines (Histidine) very unlikely. However, in order to throw further light upon these possibilities, it was considered advisable, in 1924, to control the investigation by an experiment in which the nucleic acid administered was of very different structure and origin, namely, yeast nucleic acid. The fragments into which this substance would split upon complete hydrolysis would correspond very closely in nutritive value with the fragments resulting from the complete hydrolysis of thymus nucleic acid (guanine, adenine, cytosine, uracil, pentose, and phosphoric acid, as compared with guanine, adenine, cytosine, thymine, hexose, and phosphoric acid), but the intact nucleic acid, or the nucleotides resulting from its partial hydrolysis, would differ very essentially from thymus nucleic acid, the conversion of the one into the other involving the substitution of hexose for pentose in the interior of the nucleotide chain.

Fresh control (normal) animals were employed, litter brothers of the experimental animals, because the growth-curve of every generation of animals has been found, in our experience, to differ somewhat from the growth-curve of any other generation. The result of this experiment was to show that the effect of the daily administration of 25 milligrammes of yeast nucleic acid is almost identical with the effect of administering thymus nuclei. Growth itself is but slightly affected, if at all, but the duration of life is greatly prolonged (figure 3, Tables 5 and 6). Male animals were employed, and the mean duration of life

was prolonged by fourteen weeks, which was 16 per cent. of the normal life-duration, the effect being again far in excess of the combined probable errors of the estimations.

Up to this point the results obtained were consistent with the view that the effect of nucleic acids upon longevity might be attributable to the correction of a dietary deficiency (purine, pyrimidine, or phosphoric acid). Two widely differing sources of nucleic acid had been found to be identical in their effects, and it appeared unlikely (but not impossible) that the products of the incomplete hydrolysis of yeast nucleic acid could be utilized directly in nuclear metabolism, without preliminary splitting into their constituent parts. Further experiments showed, however, that a nucleic acid of yet other origin, and at first sight much more closely related to thymus nucleic acid, could exert totally different effects upon growth and longevity.

Towards the end of 1924 an experiment was undertaken in which animals received 15 milligrammes daily of spleen nucleic acid. Fresh controls were instituted, as before, belonging to the same generation but not to the same litters as the experimental animals. Female animals were employed. In this case growth was very greatly affected (figure 4, Tables 7 and 8), being accelerated, subsequently to the twentieth week of age, to such an extent that at seventy weeks of age the treated animals exceeded the weight of the normal animals by over 4·5 grammes, a quantity far exceeding the combined experimental errors of the estimations. As stated, the control animals were not litter sisters of the experimental animals, but we have not hitherto in our experience found groups of 36 animals of the same generation differing from one another to anything like the extent observed in this experiment. Moreover, the form (contour) of the growth-curve of the experimental animals does not at all resemble the form of the growth-curve of normal animals. The effect was progressive and sustained, the treated animals retaining superiority to the controls for 70 weeks of their lives (from the twentieth to the ninetieth week). On the other hand, the effect upon longevity was slight and uncertain. It did not exceed, as the diagram shows, the combined experimental errors of the estimates. We cannot therefore be certain of the reality of the observed effect upon longevity, but, such as it was (5 per cent. of the normal life-duration), it was in the same direction (prolongation of life) as that exerted by thymus nuclei and by vegetable nucleic acid.

The spleen nucleic acid was certainly absorbed, otherwise it could not have exerted such a pronounced effect upon growth. Yet its effect upon longevity was far inferior to the effects of thymus nuclei and yeast nucleic acid, which on the other hand exerted no effect upon growth. If the spleen nucleic acid was split into its constituents before absorption, and yeast nucleic acid and thymus nucleic acid were similarly subjected to complete hydrolysis, then it is difficult to see why they should have exerted such widely differing effects upon growth and longevity. It appears unavoidable to conclude that spleen nucleic

acid, on the one hand, and thymus nucleic acid and yeast nucleic acid on the other, were employed differently and probably, therefore, in different stages of hydrolysis, in the metabolism of the animals. Our previously published study of the metabolism of yeast and spleen nucleic acids in man (8) suggests that spleen nucleic acid is less readily split into its constituent parts in the animal body than yeast nucleic acid. It is probable, therefore, that spleen nucleic acid was, partially at least, utilized in some less completely hydrolyzed form than yeast nucleic acid. Why thymus nucleic acid should resemble yeast nucleic acid in this respect, rather than spleen nucleic acid, is not at all clear, for chemical investigation has not yet revealed any difference between these two nucleic acids of animal origin (4).

On the other hand, the method employed for the preparation of the thymus nuclei would not have removed guanylic acid, which is present in thymus tissue, and is probably derived from vegetable nucleic acids by partial hydrolysis (2). It is possible, therefore, that the observed effects of thymus nucleic acid were partially attributable to guanylic acid, which would constitute one of the four nucleotides derivable from yeast nucleic acid. The spleen nucleic acid was a coarsely granular preparation, not easy to incorporate uniformly with the egg in which it was administered to the animals. This may have rendered it less easy to absorb from the alimentary canal, which would account readily enough for its comparative lack of effect upon longevity. It would not account at all, however, for its superior effect upon growth. Furthermore, our investigations upon the metabolism of yeast and spleen nucleic acids in man, to which reference has been made, showed that although this preparation of spleen nucleic acid was slightly less readily absorbed than the yeast nucleic acid, yet the difference of absorption was in no way comparable with the difference of effect upon longevity which was noted in these experiments upon mice. The interpretation of these peculiar differences in the effects of nucleic acids of various origin must therefore await the outcome of further investigation.

METHODS EMPLOYED.

Thymus Nuclei. The method of preparing thymus nuclei was that of Abderhalden and Kashiwado (1), slightly modified as follows:

Thymus glands from calves were thoroughly freed from adherent fat and then finely minced. Two and a half kilos of minced tissue were suspended in 6·5 litres of tenth normal hydrochloric acid in $\frac{M}{15}$ sodium chloride (total molecularity of the solution $= \frac{M}{6}$). To this was then added 5·5 litres of $\frac{M}{6}$ sodium chloride in which had been dissolved 10 grammes of pepsin scales. Sixty cubic centimetres of chloroform were added to the mixture, which was kept at 34°C.

to 37°C. for a week, shaking thoroughly twice a day. At the end of the week 10 grammes more of pepsin scales and 60 cc. more of chloroform were added, and the mixture digested, as before, for another week. The supernatant fluid was then syphoned off, and the residue centrifuged and suspended repeatedly in $\frac{M}{6}$ sodium chloride until the washings were neutral. The residue was then dried upon a boiling water-bath, taken up in about 600 cc. of ether containing 50 cc. of alcohol, kept for a few days standing under this mixture, and then again dried on the water-bath, together with the ether. This procedure was adopted to secure the complete disintegration of the nuclei, which, it was thought, might otherwise partially escape digestion when administered to animals. Microscopic examination of the nuclei before drying showed that they were intact, and that only a very small proportion of them retained small adherent shreds of cytoplasm.

The yield of thymus nuclei was determined by weighing each sample, and the material was administered to the animals in such proportion as to correspond to ½ gramme of fresh thymus tissue per day. The yield per gramme of thymus varied in different lots between 0·07 and 0·08 grammes. According to P. A. Levene (3), 150 grammes of nucleic acid are obtainable from 10 lbs. (4,500 grammes) of thymus. This yield would correspond to 33 milligrammes of nucleic acid per gramme of thymus, or 16 milligrammes per day in the dose administered to the mice.

Yeast Nucleic Acid. A commercial product was used, purchased from The British Drug Houses Ltd.

Spleen Nucleic Acid. The spleen nucleic acid was prepared by a modification of the method of Levene (3), as follows:

The spleens, in 10-lb. lots, were stripped of their connective tissue covering, ground in a mincer, and then transferred to 5 litres of water, containing 250 grammes of sodium hydroxide. The mixture was heated to boiling in enamel buckets, being mechanically stirred meanwhile. Boiling was continued for 35 minutes. The mixture was then neutralized with glacial acetic acid, the neutral point after a little experience being recognizable by the change of colour of the mixture. Fifty cc. of colloidal iron solution (British Drug Houses Ltd.) were then added, and the mixture filtered. A small amount of $CaCl_2$ solution was added to the filtrate to remove excess of colloidal iron, and the solution again filtered after allowing to stand overnight. To 3 litres of this filtrate were added 500 cc. of concentrated HCl and 8 litres of 98% ethyl alcohol, containing 5% of methyl alcohol. The precipitate was collected and washed with ethyl-methyl alcohol mixture until free from HCl, the final washing being with pure ethyl alcohol. The precipitate was dried at 37°C., and used without further purification. It contained 13·97% of nitrogen (theoretical 14·79%) and 9·23% of phosphorus (theoretical 8·73%). It contained a very appreciable but undetermined amount of iron, which, however, could not have been responsible for the

effects of the preparation upon growth, since unpublished experiments conducted in the author's laboratory in the University of California in the years 1916-1918 showed that the administration of iron (ferric chloride) in non-toxic amounts to animals already in receipt of a varied and abundant diet is unaccompanied by effects of any kind upon their growth.

Feeding and Care of the Animals. The technique employed in housing and caring for the animals has been fully described in previous publications (9, 10). Epidemic disease has been totally eradicated from our present stock of animals by the rigorous sacrifice in the past of all infected animals, so that infections, other than occasional local infection of males through injuries received in fighting, did not affect the results in any way. Deaths attributable to infections following injury were recorded as "accidental."

The diet consisted of rice cooked in a double-walled boiler with a mixture of equal parts of milk and water. The rice was cooked until the grains were soft and yet separated from one another. This was administered to the animals every morning (except Sundays), and a handful of maize (dipped in boiling water to prevent possible introduction of favus) every evening. Each compartment of six animals received 6 cc. of mixed and strained white and yolk of egg every morning before any other food was administered. This was shared between them, and was almost invariably consumed completely during the 24 hours, except by the very youngest animals and extremely aged animals (over 800 days), which occasionally left a portion of it unconsumed. The allowance of egg was reduced in proportion as deaths reduced the populations of the compartments. The various nucleic acids were incorporated in the egg in such proportion that the required daily dose for each mouse was contained in 1 cc.

Graphic Method of Recording Results. The diagrams in figures 1 to 4 were constructed in accordance with principles which have been discussed in previous communications (7, 10, 11). The growth of each group of animals is represented, not, as usual, by a line connecting the observed averages, but by a shaded ribbon-like area, of which the centre at any age is the observed average weight and the width is twice the "probable error" of the average. The average weight and the degree of certainty attaching to the average are thus simultaneously displayed, and we can say that any similar group of animals would, more probably than not, yield a curve of growth lying wholly within the shaded area. For equal numbers of animals also the width of the area is proportional to their variability. Where two such shaded areas overlap they are to be regarded as "possibly identical." Overlapping portions of the areas, therefore, represent averages which cannot be certainly stated to differ significantly from one another. When only five survivors remain the computation of the probable error of the average becomes meaningless, and the curve is continued as a broken line, which represents, in the usual way, the average weights of the survivors.

The mean durations of life are similarly represented by circles, of which

the centres are situated at the observed average age of death, and the diameters are twice the probable errors of the averages. When two such circles intersect it means that the life durations of the two groups of animals concerned cannot certainly be said to differ. The diameters of the circles also display the relative variabilities of life-duration.

Curves are also inset in each diagram, which display the percentages of survivors of each class of animals at successive intervals of 50 days. Accidental deaths are excluded in the computation of the survival curves and the life-durations of the experimental and control groups of animals.

DETAILED ANALYSIS OF RESULTS.

Comparison of Mice Receiving Thymus Nuclei with Normal Mice.

(a) *Males* (Figure 1, Tables 1 and 3). The curves of growth are identical until the eightieth week, when the normals begin to fall and the treated animals to rise in weight, leading to a slight separation of the curves. The age at which only five individuals survive is twenty-two weeks later in the treated animals than in the controls. The average duration of life is eleven weeks longer in the treated than in the control animals. The survival-curve of the treated animals is widely separated from that of the normals, and is consistently superior to it, except at 50% (about 650 days), when the two curves temporarily approach one another closely. The variability of treated animals is increased in comparison with the normals.

(b) *Females* (Figure 2, Tables 2 and 4). The curves of growth are virtually identical until the seventieth week, when they diverge, owing to the continued ascent of the curve of growth of the treated animals and the descent of the curve of growth of the controls. The separation is more distinct than in the case of the males. The age at which only five individuals survive is sixteen weeks later in the treated animals than in the controls. The average duration of life is fifteen weeks longer in the treated than in the control animals. The survival curve of the treated animals is widely separated from that of the normals and consistently superior to it. The variability of treated animals is decidedly increased in comparison with the normals.

Comparison of Mice Receiving Yeast Nucleic Acid with Normal Mice. Only males were employed in this experiment (figure 3, Tables 5 and 6). Growth is slightly but definitely retarded from the tenth to the thirtieth week. Thereafter the curves virtually coincide, but from the seventieth to the ninetieth weeks the curve for the treated animals only just touches the upper margin of the curve for normals. In other words, there is a detectable tendency for the curves to separate at this age. The age at which only five individuals survive is fourteen weeks later in the treated animals than the controls. The average duration of life is also fourteen weeks longer in the treated than in the control animals. The

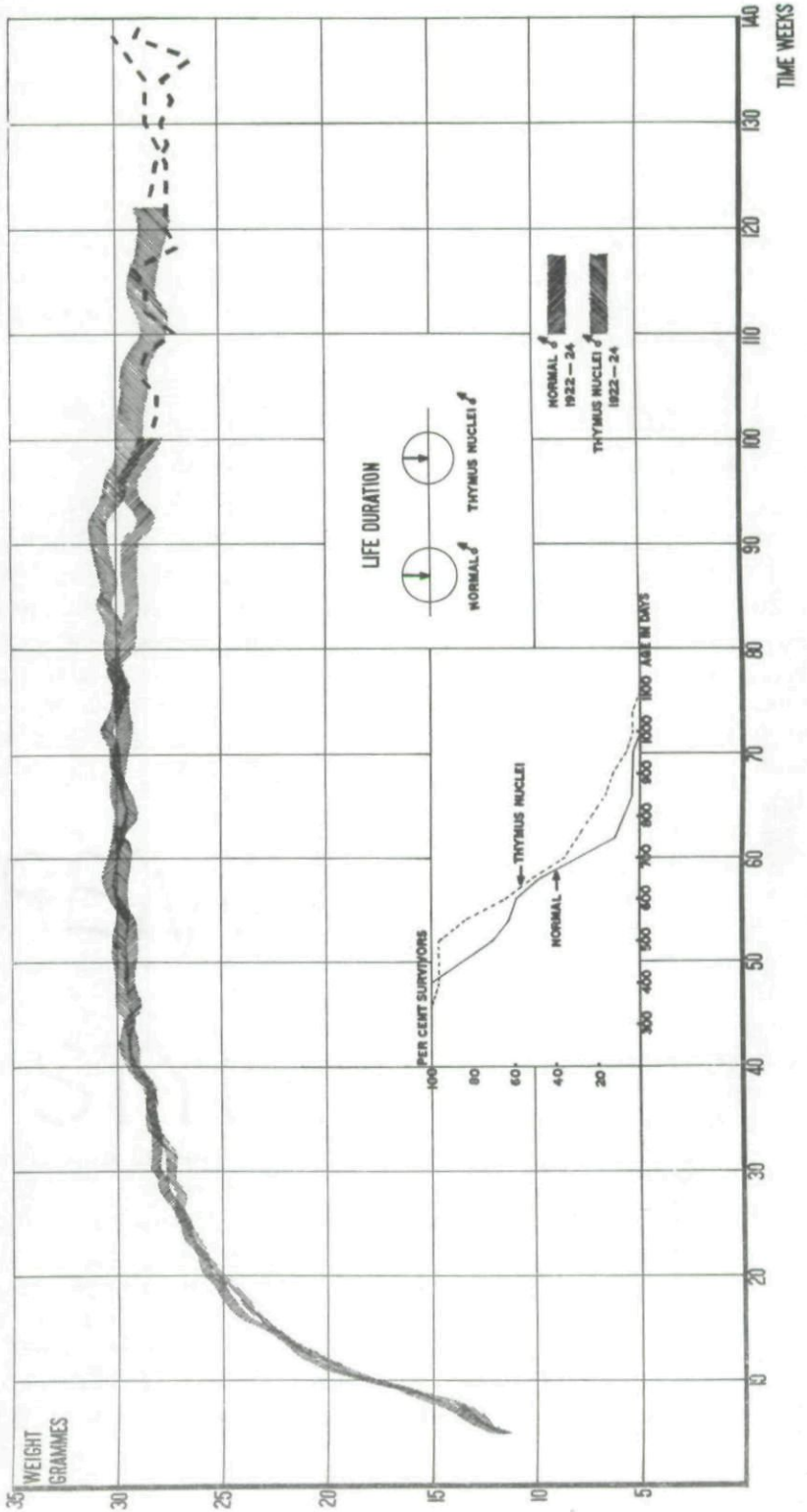

Figure 1. Comparison of the growth and longevity of normal male mice and male mice in receipt of thymus nuclei corresponding to 0.5 grammes of fresh thymus tissue daily.

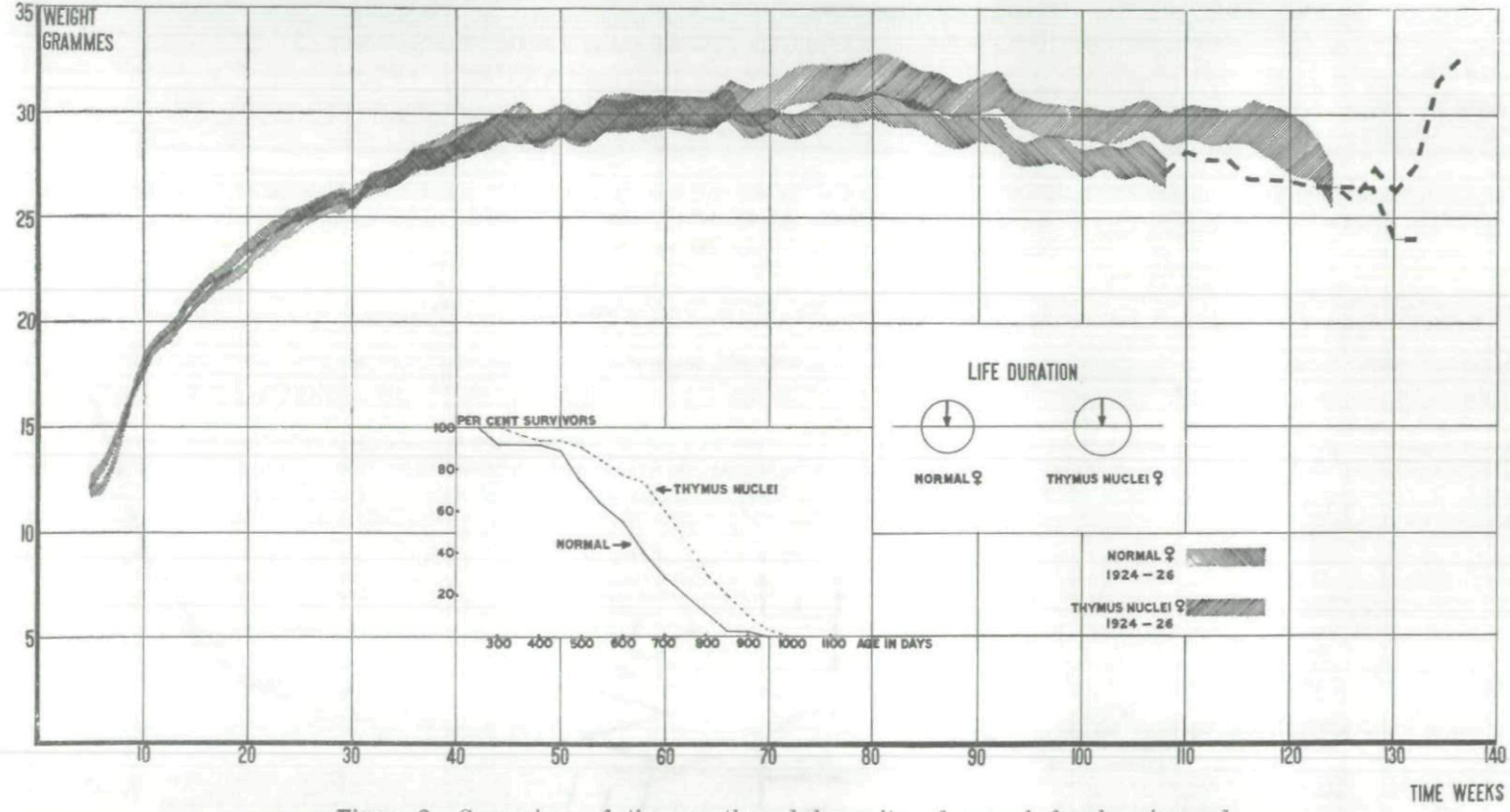

Figure 2. Comparison of the growth and longevity of normal female mice and female mice in receipt of thymus nuclei corresponding to 0·5 grammes of fresh thymus tissue daily.

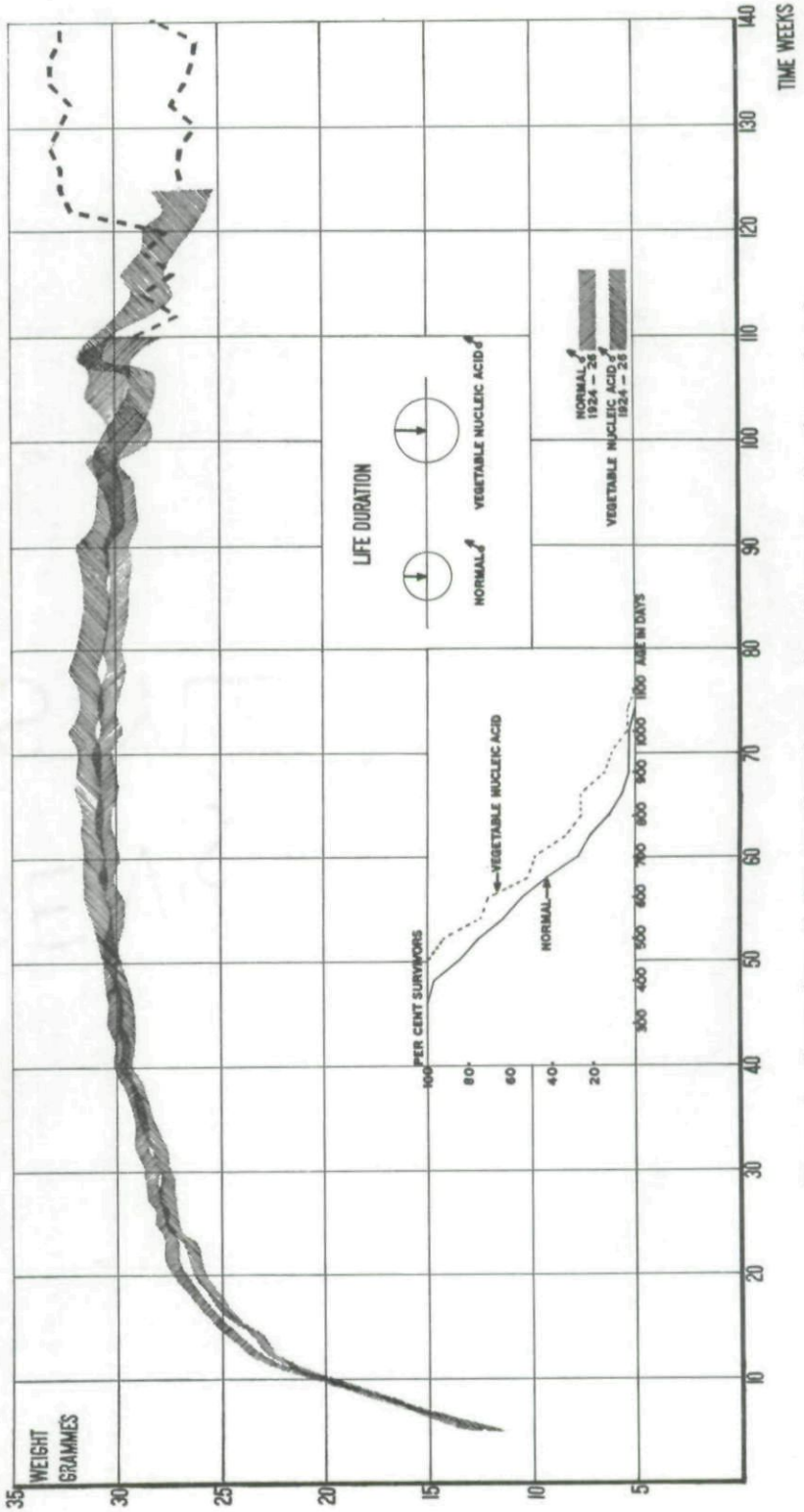

Figure 3. Comparison of the growth and longevity of normal male mice and male mice in receipt of 25 milligrammes of yeast nucleic acid daily.

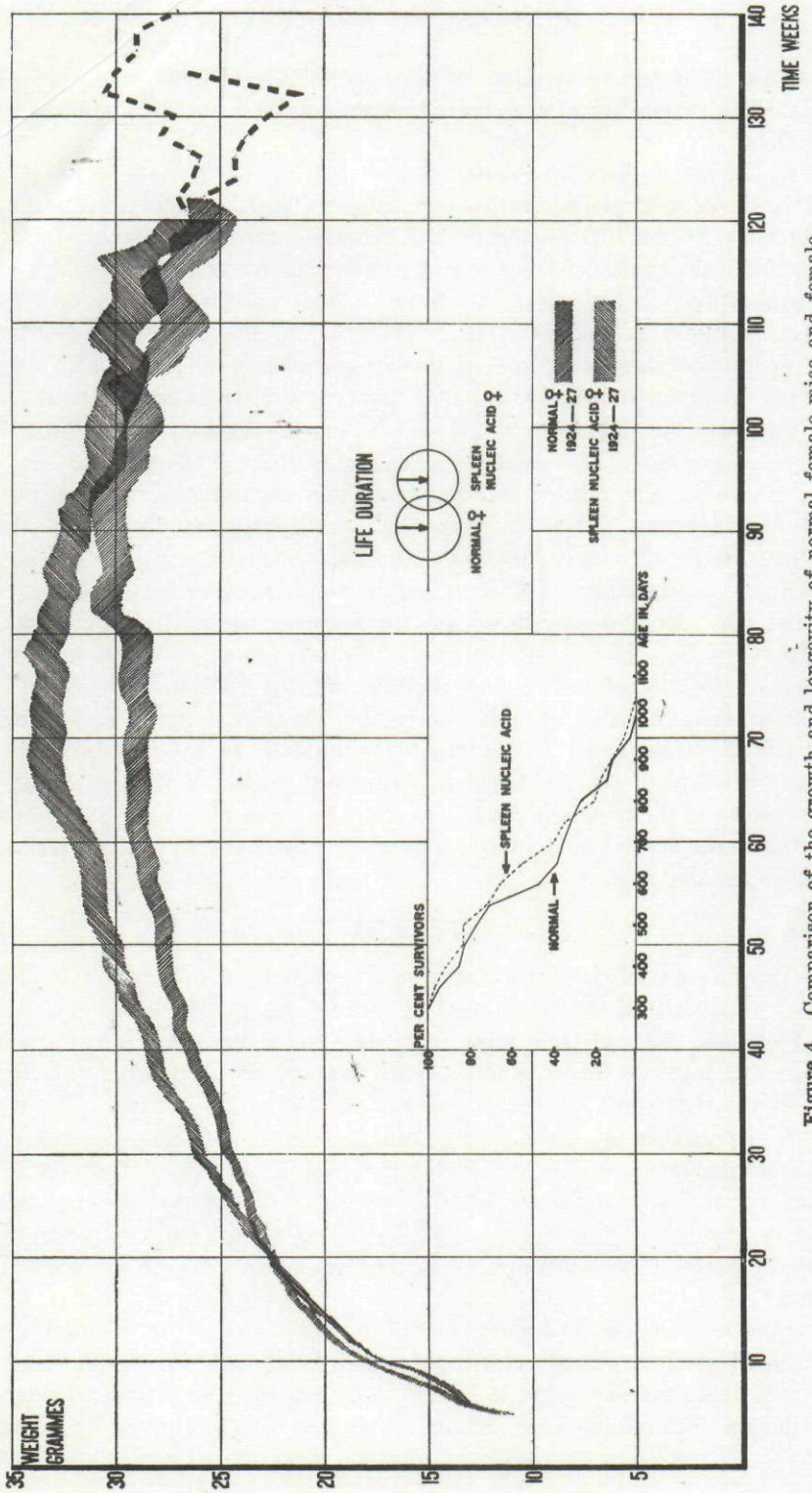

Figure 4. Comparison of the growth and longevity of normal female mice and female mice in receipt of 15 milligrammes of spleen nucleic acid daily.

survival curve of the treated animals is consistently superior to that of the normals. The variability of the treated animals is increased in comparison with the controls.

Comparison of Mice Receiving Spleen Nucleic Acid with Normal Mice. Only females were employed in this experiment (figure 4, Tables 7 and 8), and the controls were not litter-sisters of the experimental group, having been born some two months earlier. They were produced, however, from females of the same generation (litter-sisters of those which produced the experimental animals) by the same fathers. The relationship of the controls to the experimental group was therefore closer than first-cousinship, since not only were the female parents litter-sisters, but the male parents were the same for both groups.

After slight but definite retardation between the sixth and eighteenth weeks the curve of growth of the treated animals cuts that of the normals and rises rapidly above it, the mid-points of the curves becoming separated by 4·65 grammes at 72 weeks. After 78 weeks the growth curve of the treated animals falls almost as rapidly as it rose, while the normal curve continues to rise with the slight, steady incline which is characteristic of the normal growth-curve in the mouse (7). In consequence the two curves intersect again at 92 weeks, and thereafter coincide. The variability of the treated animals is consistently greater than that of the controls. The age at which only five individuals survive is two weeks later in the normal than in the treated animals, but the mean duration of life is 4·5 weeks longer in the treated animals than in the controls. This difference lies within the combined experimental errors of the estimates. The survival curve of the treated animals is definitely superior to that of the controls only between the 600th and 700th days of age. At other ages the survival curves virtually coincide

SUMMARY.

1. Thymus nuclei, in daily doses corresponding to 0·5 grammes of fresh thymus tissue, and yeast nucleic acid in daily doses of 25 milligrammes, exert little effect upon the growth of mice until after the seventieth week of age, when the senescent loss of weight which occurs in normals is delayed, so that the weight of treated animals becomes superior to that of the controls. Longevity, however, is considerably enhanced by both thymus nuclei and vegetable nucleic acid, the prolongation of life being eleven weeks, or 12·6% of the normal life-duration for males, and fifteen weeks, or 17% of the normal life-duration for females in receipt of thymus nuclei, while for males in receipt of yeast nucleic acid the increase of life-duration is fourteen weeks, or 16% of the normal life-duration.

2. Spleen nucleic acid, in dosage of 15 milligrammes per mouse daily, causes very decided acceleration of growth subsequently to eighteen weeks of age, the two curves becoming separated at 72 weeks of age by 4·65 grammes. After 78 weeks the treated animals lose weight rather rapidly, so that at 92 weeks the

curves again coincide. On the other hand, duration of life is only increased by 4·5 weeks, an increase which lies within the combined experimental errors of the estimates.

3. On the whole the effect of nucleic acid appears to consist essentially in a tendency to promote late (post-adolescent) growth and to enhance longevity. These two effects would appear, however, to be mutually opposed, so that when, as with spleen nucleic acid, the growth effect predominates, the effect upon longevity is at a minimum. If we suppose that the effects of nucleic acid are attributable to the sustainment of the mass of nuclear material in the cell, or its enhancement, then provided the cytoplasmic mass does not increase beyond the normal rate, i.e., provided excess of growth does not occur, the nucleo-cytoplasmic ratio should increase in comparison with the normals, and longevity should be enhanced. If, however, excess growth does occur then, to that extent, the nucleo-cytoplasmic ratio must be diminished and increased longevity should not occur. This obviously corresponds with the facts observed, and to this extent the results are consistent with the conception that life-duration is dependent upon the magnitude of the nucleo-cytoplasmic ratio.

4. In every instance administration of nucleic acid or of materials rich in nucleic acid (thymus nuclei) enhanced the variability of the animals. This may be related to inherent differences among the animals in their capacity to absorb nucleic acid from the alimentary canal, either arising directly from differences of absorptive capacity or indirectly out of differences in the rate of decomposition of nucleic acid in the intestine.

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TABLE 1.

Growth of Normal Male White Mice, 1922–24.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.	Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
35	5	11.77	±0.31	27	60	30.17	±0.25
35	6	12.94	±0.32	25	62	29.88	±0.30
35	7	13.64	±0.35	23	64	29.72	±0.31
35	8	14.93	±0.42	23	66	29.93	±0.30
35	9	16.16	±0.40	22	68	29.82	±0.30
35	10	18.00	±0.35	19	70	29.71	±0.37
35	11	19.73	±0.27	17	72	29.50	±0.34
35	12	20.79	±0.26	17	74	29.68	±0.37
35	13	21.46	±0.25	16	76	29.66	±0.37
35	14	22.06	±0.27	15	78	30.03	±0.37
35	15	22.91	±0.27	15	80	29.43	±0.37
35	16	23.91	±0.28	15	82	29.37	±0.37
35	17	24.40	±0.27	15	84	29.17	±0.34
35	18	24.76	±0.25	15	86	29.27	±0.37
35	19	25.07	±0.25	15	88	29.33	±0.40
35	20	25.61	±0.25	13	90	29.31	±0.42
35	21	25.84	±0.27	11	92	28.50	±0.43
35	22	25.96	±0.25	11	94	29.14	±0.39
35	23	26.40	±0.25	11	96	29.36	±0.52
35	24	26.66	±0.25	9	98	28.78	±0.58
35	25	26.70	±0.27	5	100	28.30	±0.57
35	26	26.96	±0.26	4	102	28.00	—
35	27	27.33	±0.25	3	104	28.00	—
35	28	27.73	±0.27	3	106	28.83	—
35	29	27.80	±0.25	3	108	28.50	—
35	30	28.06	±0.25	3	110	27.17	—
35	32	28.06	±0.25	2	112	28.50	—
35	34	28.11	±0.24	2	114	28.5	—
35	36	28.36	±0.23	2	116	29.0	—
35	38	28.33	±0.24	2	118	27.0	—
35	40	29.07	±0.25	1	120	27.5	—
35	42	29.17	±0.24	1	122	27.5	—
35	44	29.40	±0.27	1	124	27.5	—
35	46	29.06	±0.26	1	126	27.5	—
35	48	29.40	±0.26	1	128	28.0	—
35	50	29.30	±0.28	1	130	28.5	—
35	52	29.23	±0.26	1	132	28.5	—
34	54	29.34	±0.26	1	134	28.5	—
34	56	30.06	±0.26	1	136	not weighed	—
31	58	30.37	±0.31	1	138	30.0	—

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	650 days	48.1
300 „	100	700 „	29.6
350 „	100	750 „	11.1
400 „	100	800 „	7.4
450 „	85.2	850 „	3.7
500 „	70.4	900 „	3.7
550 „	63.0	950 „	3.7
600 „	59.3	1,000 „	0.0

Mean duration of life, 610 ± 18 days.

TABLE 2.

Growth of Normal Female White Mice, 1922–24.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.	Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
36	5	12.39	±0.33	32	58	30.06	±0.77
36	6	12.93	±0.32	32	60	30.08	±0.72
36	7	13.89	±0.30	32	62	30.05	±0.72
36	8	15.18	±0.31	31	64	29.98	±0.74
36	9	16.71	±0.36	30	66	30.33	±0.72
36	10	18.07	±0.30	28	68	29.39	±0.64
36	11	18.97	±0.25	27	70	29.67	±0.58
36	12	19.43	±0.24	25	72	29.32	±0.59
35	13	19.93	±0.27	24	74	29.63	±0.65
35	14	20.69	±0.27	22	76	30.32	±0.63
35	15	21.20	±0.27	22	78	30.20	±0.66
35	16	21.86	±0.27	22	80	30.39	±0.63
35	17	22.21	±0.27	20	82	30.03	±0.69
35	18	22.60	±0.30	20	84	29.93	±0.69
35	19	23.10	±0.32	19	86	29.29	±0.69
35	20	23.51	±0.32	19	88	29.00	±0.72
35	21	23.94	±0.33	16	90	29.22	±0.61
35	22	24.33	±0.34	14	92	29.14	±0.66
35	23	24.59	±0.33	12	94	28.04	±0.56
35	24	24.99	±0.37	11	96	28.23	±0.67
35	25	25.10	±0.35	10	98	28.20	±0.63
35	26	25.39	±0.34	10	100	27.65	±0.63
35	27	25.64	±0.39	8	102	27.94	±0.54
35	28	25.80	±0.40	6	104	27.58	±0.75
35	29	26.01	±0.37	6	106	27.83	±0.87
34	30	25.63	±0.31	6	108	27.17	±0.72
35	32	26.73	±0.42	4	110	28.13	—
35	34	26.90	±0.46	4	112	27.78	—
35	36	27.70	±0.48	4	114	27.78	—
35	38	27.79	±0.52	3	116	26.83	—
33	40	28.21	±0.58	2	118	26.75	—
33	42	28.67	±0.62	2	120	26.75	—
32	44	29.31	±0.64	1	122	26.50	—
32	46	29.16	±0.68	1	124	26.50	—
32	48	29.27	±0.72	1	126	26.50	—
32	50	29.58	±0.78	1	128	26.50	—
32	52	29.33	±0.77	1	130	24.00	—
32	54	29.83	±0.80	1	132	24.00	—
32	56	29.80	±0.79				

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	650 days	40.0
300 „	91.4	700 „	28.6
350 „	91.4	750 „	20.0
400 „	91.4	800 „	11.4
450 „	88.6	850 „	2.9
500 „	74.3	900 „	2.9
550 „	62.9	950 „	0.0
600 „	54.3		

Mean duration of life, 610 ± 18 days.

TABLE 3.

Growth of Male White Mice Receiving Thymus Nuclei, 1922–24.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.	Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
36	5	11.49	±0.27	31	62	29.79	±0.25
36	6	12.43	±0.25	31	64	29.24	±0.27
36	7	12.96	±0.30	31	66	29.48	±0.25
36	8	14.11	±0.31	31	68	29.97	±0.23
36	9	15.86	±0.32	31	70	29.98	±0.27
36	10	17.72	±0.33	28	72	30.43	±0.28
36	11	19.01	±0.29	28	74	29.95	±0.27
36	12	20.14	±0.23	28	76	29.79	±0.31
36	13	21.13	±0.22	27	78	30.00	±0.31
36	14	21.93	±0.22	25	80	30.20	±0.33
36	15	22.57	±0.24	24	82	30.10	±0.36
36	16	23.17	±0.22	21	84	30.52	±0.34
36	17	23.75	±0.24	19	86	30.26	±0.41
36	18	24.00	±0.23	18	88	30.50	±0.43
36	19	24.56	±0.22	17	90	30.71	±0.45
36	20	25.13	±0.23	16	92	30.84	±0.45
36	21	25.51	±0.23	15	94	30.17	±0.56
36	22	25.90	±0.23	15	96	29.97	±0.53
36	23	26.13	±0.23	12	98	29.38	±0.58
36	24	26.44	±0.23	10	100	29.30	±0.65
36	25	26.86	±0.24	10	102	29.25	±0.63
36	26	27.06	±0.23	10	104	29.05	±0.68
36	27	26.92	±0.22	10	106	29.10	±0.66
36	28	26.99	±0.22	10	108	28.75	±0.65
36	29	27.40	±0.22	9	110	28.11	±0.63
36	30	27.40	±0.21	7	112	28.29	±0.82
36	32	27.46	±0.25	6	114	28.75	±0.66
35	34	28.30	±0.26	6	116	28.58	±0.66
35	36	28.31	±0.22	6	118	28.33	±0.68
35	38	28.50	±0.27	6	120	28.08	±0.74
34	40	29.25	±0.26	5	122	28.40	±0.72
33	42	29.61	±0.28	4	124	28.13	—
34	44	29.37	±0.27	4	126	28.00	—
34	46	29.75	±0.28	4	128	27.38	—
34	48	29.72	±0.28	2	130	27.75	—
33	50	29.86	±0.30	2	132	27.25	—
33	52	29.88	±0.30	2	134	27.75	—
33	54	29.64	±0.27	2	136	26.25	—
33	56	29.71	±0.27	1	138	29.00	—
33	58	29.74	±0.27	1	140	28.50	—
31	60	29.56	±0.28				

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	700 days	35.5
300 „	100	750 „	29.0
350 „	100	800 „	22.6
400 „	96.8	850 „	16.1
450 „	96.8	900 „	12.9
500 „	96.8	950 „	6.5
500 „	83.9	1,000 „	3.2
600 „	64.5	1,050 „	3.2
650 „	51.6	1,100 „	0.0

Mean duration of life, 687 ± 17 days.

TABLE 4.

Growth of Female White Mice Receiving Thymus Nuclei, 1922–24.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.	Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
36	5	11.94	±0.33	29	60	30.02	±0.83
36	6	12.07	±0.29	29	62	29.86	±0.83
36	7	12.96	±0.31	29	64	29.86	±0.88
36	8	14.69	±0.31	29	66	30.67	±0.89
36	9	16.36	±0.29	29	68	30.24	±0.88
36	10	17.51	±0.26	29	70	30.53	±0.88
35	11	18.63	±0.25	28	72	31.21	±0.88
35	12	19.01	±0.23	27	74	31.39	±0.90
32	13	19.48	±0.25	27	76	31.46	±0.83
32	14	20.03	±0.24	26	78	31.48	±0.92
32	15	20.77	±0.26	25	80	31.82	±0.94
31	16	21.16	±0.26	23	82	31.72	±0.98
31	17	21.71	±0.29	23	84	31.11	±0.98
31	18	21.94	±0.28	23	86	31.02	±0.88
31	19	22.31	±0.30	22	88	30.57	±0.79
31	20	22.66	±0.30	22	90	30.84	±0.88
31	21	23.34	±0.35	21	92	31.12	±0.88
31	22	23.55	±0.36	20	94	30.08	±0.74
31	23	24.13	±0.37	20	96	29.75	±0.77
31	24	24.31	±0.37	18	98	29.64	±0.75
31	25	24.81	±0.43	17	100	29.50	±0.79
31	26	25.10	±0.45	16	102	29.34	±0.77
31	27	25.34	±0.45	15	104	29.43	±0.80
31	28	25.68	±0.48	13	106	29.88	±0.76
31	29	25.58	±0.48	12	108	29.25	±0.80
31	30	25.95	±0.45	11	110	29.55	±0.84
31	32	26.52	±0.48	10	112	29.35	±0.94
31	34	26.97	±0.52	9	114	29.28	±0.76
31	36	27.71	±0.61	7	116	29.50	±1.09
31	38	28.08	±0.62	7	118	29.07	±1.22
31	40	28.61	±0.67	6	120	28.50	±1.45
31	42	28.82	±0.70	6	122	28.00	±1.18
31	44	29.21	±0.70	6	124	26.50	±1.01
31	46	20.70	±0.79	4	126	25.75	—
31	48	29.00	±0.77	3	128	27.33	—
30	50	29.63	±0.80	2	130	26.25	—
30	52	29.10	±0.80	2	132	27.50	—
29	54	29.91	±0.87	1	134	31.50	—
29	56	30.05	±0.85	1	136	32.50	—
29	58	29.88	±0.87				

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	650 days	73.3
300 „	100	700 „	60.0
350 „	96.7	750 „	46.7
400 „	93.3	800 „	30.0
450 „	93.3	850 „	20.0
500 „	90.0	900 „	10.0
550 „	83.3	950 „	3.3
600 „	76.7	1,000 „	0.0

Mean duration of life, 714 ± 19 days.

TABLE 5.

Growth of Normal Male White Mice, 1924–26.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.	Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
31	5	12.77	±0.34	29	62	30.45	±0.39
33	6	14.38	±0.29	27	64	30.67	±0.39
33	7	15.67	±0.32	25	66	30.32	±0.43
33	8	17.35	±0.30	25	68	30.40	±0.49
33	9	18.77	±0.37	25	70	30.68	±0.49
33	10	20.24	±0.38	25	72	30.20	±0.56
33	11	21.95	±0.33	23	74	30.39	±0.45
33	12	23.14	±0.28	22	76	30.25	±0.53
33	13	23.80	±0.31	21	78	29.98	±0.48
33	14	24.30	±0.26	20	80	30.13	±0.45
33	15	24.98	±0.29	19	82	29.92	±0.49
33	16	25.39	±0.30	18	84	29.81	±0.50
33	17	25.85	±0.29	17	86	29.79	±0.45
33	18	26.20	±0.29	15	88	29.77	±0.63
33	19	26.67	±0.32	15	90	29.93	±0.62
33	20	26.98	±0.33	14	92	29.50	±0.64
33	21	27.26	±0.35	12	94	29.67	±0.69
33	22	27.38	±0.36	10	96	29.90	±0.84
33	23	27.35	±0.34	10	98	30.65	±0.78
33	24	27.53	±0.34	9	100	29.22	±1.02
33	25	27.88	±0.33	9	102	29.39	±1.10
33	26	27.80	±0.33	7	104	30.50	±0.91
33	27	28.17	±0.33	7	106	30.21	±0.99
33	28	28.23	±0.34	7	108	30.50	±1.30
33	29	28.35	±0.34	6	110	29.00	±1.30
31	30	28.29	±0.38	5	112	27.00	—
33	32	28.79	±0.33	4	114	28.63	—
33	34	28.79	±0.33	4	116	27.13	—
33	36	28.97	±0.32	3	118	28.50	—
33	38	29.33	±0.31	3	120	27.50	—
33	40	29.79	±0.33	1	122	32.00	—
33	42	29.65	±0.35	1	124	32.50	—
33	44	30.09	±0.36	1	126	32.50	—
33	46	30.03	±0.36	1	128	33.00	—
33	48	30.24	±0.31	1	130	32.50	—
32	50	30.30	±0.35	1	132	32.00	—
31	52	30.24	±0.38	1	134	33.00	—
31	54	29.94	±0.35	1	136	33.00	—
31	56	29.98	±0.39	1	138	32.50	—
30	58	30.50	±0.36	1	140	32.50	—
30	60	30.13	±0.38	1	142	33.50	—

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	700 days	27.3
300 „	100	750 „	21.2
350 „	100	800 „	12.1
400 „	97.0	850 „	6.1
450 „	84.8	900 „	3.0
500 „	75.8	950 „	3.0
550 „	63.6	1,000 „	3.0
600 „	54.5	1,050 „	0.0
650 „	42.4		

Mean duration of life, 609 ± 16 days.

TABLE 6.

Growth of Male White Mice Receiving Vegetable Nucleic Acid, 1924–26.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
29	5	11.79	±0.30
36	6	13.58	±0.27
36	7	15.43	±0.31
36	8	16.75	±0.31
36	9	18.26	±0.33
36	10	19.83	±0.32
36	11	21.22	±0.25
36	12	22.29	±0.28
36	13	22.81	±0.29
35	14	23.14	±0.31
34	15	23.87	±0.28
34	16	24.51	±0.29
34	17	24.93	±0.28
34	18	25.34	±0.27
34	19	25.74	±0.29
34	20	26.00	±0.29
34	21	26.19	±0.30
34	22	26.35	±0.31
34	23	26.44	±0.32
33	24	27.12	±0.33
33	25	27.33	±0.32
33	26	27.39	±0.31
32	27	27.50	±0.32
32	28	27.58	±0.33
32	29	27.52	±0.33
32	30	27.66	±0.31
32	32	27.92	±0.34
32	34	28.38	±0.36
32	36	28.72	±0.34
32	38	28.95	±0.33
32	40	29.61	±0.31
32	42	29.44	±0.33
32	44	29.50	±0.37
32	46	29.52	±0.35
31	48	29.77	±0.34
28	50	30.27	±0.42
28	52	30.46	±0.44
28	54	30.68	±0.50
27	56	31.00	±0.56
27	58	31.02	±0.54
27	60	31.06	±0.48
27	62	31.19	±0.47
26	64	31.12	±0.50
26	66	31.33	±0.57
26	68	31.31	±0.56
25	70	31.24	±0.55
24	72	31.60	±0.60
24	74	31.19	±0.57
21	76	31.17	±0.55
19	78	31.55	±0.67
19	80	31.24	±0.65
19	82	31.00	±0.66
19	84	30.84	±0.61
19	86	30.95	±0.57
18	88	30.94	±0.68
17	90	31.06	±0.75
15	92	30.30	±0.72
14	94	30.21	±0.63
13	96	30.23	±0.55
13	98	30.46	±0.53
13	100	30.04	±0.68
11	102	29.36	±0.74
11	104	28.77	±0.73
10	106	28.60	±0.65
8	108	31.19	±0.65
7	110	30.71	±0.39
7	112	29.21	±0.72
7	114	28.14	±0.95
7	116	28.57	±0.97
7	118	27.93	±0.81
7	120	27.43	±1.15
7	122	26.57	±1.00
7	124	26.64	±1.49
5	126	26.90	—
4	128	26.75	—
4	130	26.00	—
3	132	27.17	—
3	134	26.50	—
3	136	26.17	—
3	138	26.00	—
1	140	28.00	—
1	142	28.50	—
1	144	28.00	—
1	146	25.50	—

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	700 days	48.2
300 „	100	750 „	33.3
350 „	100	800 „	25.9
400 „	100	850 „	25.9
450 „	100	900 „	14.8
500 „	92.6	950 „	11.1
550 „	74.1	1,000 „	3.7
600 „	70.4	1,050 „	3.7
650 „	51.9	1,100 „	0.0

Mean duration of life, 706 ± 21 days.

TABLE 7.

Growth of Normal Female White Mice, 1924–27.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.	Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
32	5	11.14	±0.26	28	58	28.57	±0.50
36	6	13.36	±0.28	27	60	28.83	±0.52
36	7	14.28	±0.25	27	62	28.98	±0.55
36	8	15.49	±0.25	27	64	28.87	±0.56
36	9	16.83	±0.25	27	66	28.85	±0.58
36	10	17.90	±0.22	26	68	29.00	±0.60
36	11	18.89	±0.21	25	70	29.00	±0.64
35	12	19.50	±0.22	25	72	28.68	±0.67
35	13	20.01	±0.23	24	74	29.08	±0.65
35	14	20.46	±0.22	23	76	29.13	±0.73
35	15	20.91	±0.23	22	78	28.91	±0.75
35	16	21.47	±0.23	20	80	29.08	±0.84
34	17	21.66	±0.23	17	82	30.44	±0.74
34	18	21.99	±0.25	14	84	30.46	±0.81
34	19	22.37	±0.24	13	86	29.80	±0.82
34	20	22.41	±0.25	13	88	30.11	±0.80
34	21	22.74	±0.25	12	90	30.25	±0.82
34	22	22.97	±0.25	12	92	30.46	±0.94
34	23	23.04	±0.25	11	94	30.22	±0.99
34	24	23.50	±0.26	11	96	29.40	±0.99
33	25	23.58	±0.25	11	98	29.13	±0.91
33	26	23.65	±0.26	11	100	28.59	±0.99
33	27	23.92	±0.27	10	102	28.80	±0.90
33	28	24.12	±0.28	10	104	29.45	±0.96
33	29	24.26	±0.31	10	106	27.85	±0.93
32	30	24.67	±0.30	9	108	27.38	±1.13
33	32	24.86	±0.31	9	110	26.61	±1.18
33	34	25.06	±0.33	8	112	27.25	±1.09
33	36	25.48	±0.34	8	114	27.87	±0.96
33	38	26.09	±0.35	8	116	26.43	±0.96
33	40	26.38	±0.34	6	118	26.00	±1.41
33	42	26.94	±0.35	6	120	25.33	±1.23
32	44	26.92	±0.37	5	122	26.00	±0.58
31	46	27.40	±0.38	4	124	24.12	—
31	48	27.61	±0.39	4	126	24.25	—
31	50	27.73	±0.41	4	128	23.75	—
29	52	27.67	±0.44	3	130	22.66	—
29	54	28.03	±0.46	3	132	21.16	—
28	56	28.43	±0.48	1	134	26.50	—

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	650 days	38.2
300 „	100	700 „	35.3
350 „	94.1	750 „	32.4
400 „	85.3	800 „	26.5
450 „	82.4	850 „	13.9
500 „	76.5	900 „	11.1
550 „	70.6	950 „	2.8
600 „	47.1	1,000 „	0.0

Mean duration of life, 632 ± 22 days.

TABLE 8.

Growth of Female White Mice Receiving Spleen Nucleic Acid, 1924–27.

Mortality Statistics.

Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.	Number of Animals.	Age in weeks.	Mean weight in grammes.	Probable error of the mean.
27	5	11.67	±0.29	30	64	32.81	±0.79
36	6	13.26	±0.26	30	66	33.13	±0.82
36	7	13.71	±0.24	29	68	33.39	±0.85
36	8	14.49	±0.24	29	70	33.21	±0.83
36	9	15.42	±0.24	29	72	33.33	±0.82
36	10	17.10	±0.21	28	74	33.23	±0.80
36	11	17.99	±0.18	27	76	33.00	±0.81
36	12	18.63	±0.22	25	78	33.52	±0.84
36	13	19.18	±0.22	25	80	33.14	±0.82
36	14	19.78	±0.21	24	82	32.93	±0.76
36	15	20.31	±0.21	23	84	32.45	±0.80
36	16	20.82	±0.22	22	86	32.72	±0.80
36	17	21.29	±0.22	22	88	32.18	±0.84
36	18	21.76	±0.23	21	90	31.90	±0.76
36	19	22.25	±0.24	19	92	32.00	±0.79
36	20	22.57	±0.22	19	94	31.23	±0.74
36	21	22.97	±0.23	16	96	30.43	±0.87
36	22	23.54	±0.25	14	98	30.42	±1.01
36	23	23.78	±0.25	14	100	30.46	±1.03
36	24	24.15	±0.25	14	102	30.00	±1.06
36	25	24.47	±0.27	13	104	29.69	±1.10
36	26	24.68	±0.28	12	106	29.29	±0.98
36	27	25.13	±0.27	10	108	30.20	±1.15
36	28	25.31	±0.29	9	110	29.66	±1.26
36	29	25.81	±0.30	9	112	28.83	±1.41
35	30	26.01	±0.32	8	114	28.93	±1.20
36	32	26.29	±0.31	7	116	29.42	±1.45
36	34	26.78	±0.33	7	119	27.21	±1.25
36	36	27.57	±0.38	6	120	26.33	±1.45
36	38	28.07	±0.38	5	122	27.0	—
36	40	28.24	±0.40	5	124	26.3	—
36	42	28.50	±0.40	4	126	25.9	—
35	44	29.29	±0.48	3	128	27.8	—
35	46	29.51	±0.48	3	130	27.2	—
35	48	30.09	±0.54	2	132	30.5	—
35	50	30.15	±0.55	2	134	30.0	—
33	52	30.76	±0.60	2	136	29.0	—
33	54	31.02	±0.61	2	138	29.0	—
33	56	31.06	±0.65	2	140	27.0	—
33	58	31.20	±0.67	1	142	28.5	—
33	60	31.56	±0.69	1	144	29.0	—
31	62	32.16	±0.77				

Mortality Statistics.

(Accidental deaths excluded.)

At Age.	Per cent. of Survivors.	At Age.	Per cent. of Survivors.
250 days	100	700 days	40.0
300 „	100	750 „	34.3
350 „	97.1	800 „	20.0
400 „	91.4	850 „	17.1
450 „	82.9	900 „	11.4
500 „	82.9	950 „	5.7
550 „	71.4	1,000 „	2.9
600 „	65.7	1,050 „	0.0
650 „	54.3		

Mean duration of life, 664 ± 20 days.