The Effect of Yeast Nucleic Acid on the Survival Time of 600 Day Old Albino Mice

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THE INVESTIGATION OF THE EFFECT OF NUTRITIONAL FACTORS ON THE PROCESS OF AGING HAS ONLY RECENTLY BEEN GIVEN ATTENTION. IN THIS PAPER, DR. GARDNER REPORTS ON THE INCREASE WHICH YEAST NUCLEIC ACID PRODUCES IN THE LIFE SPAN OF 600 DAY OLD ALBINO MICE, AND CONTRASTS HIS RESULTS WITH THOSE OF AN EARLIER INVESTIGATOR WHO ADMINISTERED BOTH YEAST AND THYMUS NUCLEIC ACID IN FAR LARGER AMOUNTS TO MICE FROM BIRTH. DR. GARDNER DISCUSSES THREE POSSIBLE EXPLANATIONS FOR THE INCREASE IN LONGEVITY PRODUCED BY NUCLEIC ACIDS: MAINTENANCE OF A HIGH NUCLEOCYTOPLASMIC RATIO IN THE CELLS, OXIDATION OF INGESTED NUCLEIC ACIDS IN PLACE OF CELL NUCLEIC ACIDS, AND STIMULATION OF LEUKOCYTIC ACTIVITY.

THE importance of nucleic acids in cells in regard to longevity was early claimed by Minot (1). Yeast, or ribose nucleic acid is found in the cytoplasm, and thymus, or desoxyribose nucleic acid in the nucleus of animal cells (2). Until recently it was thought that yeast nucleic acid was found only in plant cells, and thymus nucleic acid, only in animal cells. The concentration of nucleic acids in cells is quite large in many cases. The cytoplasm of rye embryo cells contains 3 per cent (3), yeast cells contain 5–10 per cent (4), and the pancreas, up to 10 per cent (5) nucleic acid of the dry weight.

Wherever there is cell growth (6) or cell division there is a high concentration of nucleic acid, as shown by spectrum absorption measurements (7). Minot (1) observed that as long as the ratio of nuclear material to cytoplasm was high, cells exhibited all of the characteristics of young cells. Robertson and his co-workers carried on extensive investigations on the effect of various nucleic acids on growth and longevity. Preliminary investigations showed that growth was coincident with an increase of nuclear material (8). A method was developed for the measurement of the nucleocytoplasmic ratio by estimating guanine (9). It was found that as tissues grew older, the nucleocytoplasmic ratio decreased. This was found to be the case in wheat (10), the organs of sheep (11), and the organs of white mice (12). Robertson attempted to slow down the fall of the nucleocytoplasmic ratio by feeding large amounts of nucleic acids to his experimental animals (13). This, in effect, increased the survival time of his animals, and large increases of the mean life span were obtained as compared with the controls. The increase in the mean life span was attributed to a decrease in the rate of decline of the nucleocytoplasmic ratio or the promotion of postadolescent growth (14). Robertson's work will be discussed more fully later.

It has been shown that most ingested yeast nucleic acid is oxidized in the body (15). Sometimes retention exceeds three or four days (16) with only 14–30 per cent accounted for by acid excretion. The rate of turnover in rabbits was found to vary from organ to organ and was highest in the muscles (16). The turnover rate was higher in the cytoplasm than in the nucleus. Tracer phosphorus showed a two-thirds turnover in fifty days. The nucleus was found to be the stable element in the cell, and the nucleic acids, the stabilizing agent. The rate of nucleic acid synthesis was highest during growth (18). Tracer

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Dr. Charles N. Frey of the Fleischmann Laboratories supplied the yeast nucleic acid used in the experiments reported on in this paper.

experiments using isotopic nitrogen showed that nucleoproteins could not utilize purines nor pyrimidines for synthesis (19) but probably were able to utilize nucleic acids. Using radioactive phosphorus it was observed that there is a slow and steady turnover of thymonucleic and ribonucleic acids in nongrowing liver, but a rapid turnover in growing, regenerating liver, the yeast nucleic (ribo) acid having a greater rate of turnover than the thymonucleic acid (20). It was suggested that ribonucleic acid was necessary for protein synthesis (20). This supports Robertson's hypothesis that nucleic acids promote growth by affecting the nucleocytoplasmic ratio, and thus affect the aging rate.

Robertson's work on the effect of nucleic acids on longevity has been neglected, probably on the assumption that the more recently acquired knowledge of vitamins and the mineral elements in nutrition invalidates earlier work (21). However, this contention is not justified by an examination of the diets of Robertson's animals. They were nutritionally adequate according to modern standards. Therefore it is considered desirable to reexamine and place on a practical basis some of Robertson's work and modify it in the light of modern advances in the field of nutrition.

Up till now the investigation of nutritional factors for animals and man has been almost wholly confined to the growth and health aspects in early life. The problem of aging has only recently been emphasized. Any nutritional factor that would materially increase the average life of older animals would be of interest because of its possible application to man.

Materials and Methods

In my experiment, 600-day old mice were used since mice are beginning to get old at that age.

On the basis of commercial practicability, only yeast nucleic acid can be utilized. Robertson (13) used such large quantities (25 mg. per mouse per day, or about 55 gm. for an adult human being) that they were not practical from a pharmaceutical standpoint. He based these amounts on his hypothesis of excess ingested nucleic acid being necessary to affect the nucleocytoplasmic ratio. However, on the basis of two other just as probable hypotheses for the mode of action of yeast nucleic acid, to be described later, only 2.5 mg. per mouse per day were used in my experiments. The corresponding amount for human beings, 5.5 gm., is entirely practical for clinical testing on aging people.

Seventy-two 600 day old female albino mice were divided into two groups of equal appearance in regard to health, weight, and vitality, one group to serve as controls. Thirty-one 600 day old male albino mice were similarly divided into two groups, 15 to be used for control purposes and 16 for treatment. The mice were all caged separately in clean, dry cages and fed small Purina Dog Chow Checkers ad libitum. Water containing thiamin (vitamin B_1) was given ad libitum to both controls and treated mice. Purina Dog Chow Checkers have been reported to be deficient in this vitamin (22).

The approximate analysis of the yeast nucleic acid used (sample P-36, 3A-872, 10-4-43) was: solids—90–95 per cent; nitrogen—15.17–15.95 per cent (dry basis); phosphorus—8.78–9.4 per cent (dry basis); biuret test—negative. The yeast nucleic acid was a stable powder, slowly soluble in water. It was administered in water solution at the rate of 2.5 mg. per mouse per day.

Results

Table 1 contains the data on the length of life of all mice used in the experiment.

The treated mice retained vitality and vigor longer than the controls. Also, the treated mice did not exhibit a noticeable loss of weight before death as the controls did. Fewer of the treated mice went blind before death than did the controls. In all respects, the mice treated with the nucleic acid appeared healthier and exhibited greater activity than the controls.

Whereas the mice were 600 days old at the beginning of my experiment, Robertson fed his mice from birth to death. However for the purpose of comparison, the percentage survivals at different periods were recalculated from Robertson's data (13) on a 600 day basis for mice receiving both yeast nucleic acid and thymus nucleic acid. Table 2 summarizes these calculations.

An examination of table 2 shows that the strains of mice used by Robertson and in the present investigation had different vitality curves. However, the significant increase of percentage survivals at different age levels indicates a real improvement with the use of nucleic acids.

A comparison of the average duration of life obtained by Robertson, who fed nucleic acid from birth, and that obtained in the present investigation in which old mice were used, would seem to favor Robertson's procedure. However on recalculating Robertson's survival data, which he published in complete form, and using mice from his experimental data that survived until the 600 day level, it is seen that the large improvement noted by Robertson is the result of early ingestion of nucleic acids. The improvement obtained in the later periods by Robertson compares remarkably well with that obtained in the present

Table 1. Data on Length of Life of All Mice Tested

Each treated mouse received 2.5 mg. of yeast nucleic acid per day. Length of life is reported in days.

	Males		Females	
	Controls	Treated	Controls	Treated
1	610	608	609	611
2	612	613	629	625
3	613	616	630	635
4	617	621	635	640
5	621	644	640	648
6	635	675	642	648
7	636	692	642	648
8	666	728	646	650
9	682	740	650	655
10	698	744	667	670
11	723	787	667	681
12	782	866	669	682
13	840	913	671	695
14	894	968	677	704
15	962	995	683	720
16	—	1,030	684	734
17			687	740
18			690	755
19			694	768
20			700	782
21			700	788
22			707	793
23			715	801
24			723	814
25			725	835
26			735	845
27			748	850
28			752	865
29			783	890
30			785	902
31			788	915
32			836	935
33			874	955
34			930	980
35			945	992
36			1,015	1,007
Mean Durations of Life and Probable Errors of the Mean				
	706 ±20	765 ±25	721 ±10	774 ±14

TABLE 2. MORTALITY STATISTICS ON MICE RECEIVING NUCLEIC ACIDS
C = Controls T = Treated

Days	Robertson's Investigation						Present Investigation			
	Males*		Females*		Males†		Males‡		Females‡	
	C	T	C	T	C	T	C	T	C	T
600	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
650	81.1	80.0	73.5	95.6	77.8	73.7	53.3	68.8	75.0	77.7
700	50.0	55.0	52.7	78.2	50.0	68.4	33.3	56.2	44.4	63.9
750	18.7	45.0	36.9	60.9	38.9	47.3	26.7	37.5	25.0	52.8
800	12.5	35.0	20.9	39.1	22.2	36.8	20.0	31.2	13.9	38.9
850	6.3	26.5	5.5	26.1	11.2	36.8	13.3	31.2	11.1	25.0
900	6.3	20.0	5.5	13.0	5.5	21.0	6.7	25.0	8.3	19.4
950	6.3	10.1	0.0	4.3	5.5	15.7	6.7	18.8	2.8	11.1
1,000	0.0	5.0		0.0	5.5	5.2	0.0	6.3	2.8	2.8
1,050		5.0			0.0	5.2		0.0	0.0	0.0
1,100		0.0				0.0				

* Mice received thymus nucleic acid corresponding to 0.5 gm. per day of fresh thymus tissue six days a week.

† Mice received 25 mg. of yeast nucleic acid per day for six days a week.

‡ The mice in the present investigation received 2.5 mg. of yeast nucleic acid per day for seven days a week.

work. Table 3 significantly points this out.

An examination of table 3 shows that the lengthening of the life span by feeding nucleic acids is above experimental error and is not dependent upon, nor invalidated by, work performed before the more recent acquisition of knowledge concerning vitamins and mineral metabolism. There is a slight difference between the increase obtained by thymus and by yeast nucleic acids. This may be the result of a difference in digestibility. Fortunately the only one

TABLE 3. MEAN DURATION OF LIFE AND THE PROBABLE ERRORS OF THE MEAN OF MICE RECEIVING NUCLEIC ACIDS FROM BIRTH AND FROM THE 600 DAY LEVEL

	Nucleic Acid from Birth (Robertson)		600 Day Levels			
			Robertson's Recalculations		Present Investigations	
	Males	Females	Males	Females	Males	Famales
Controls	610 ± 18	610 ± 18	703 ± 13	700 ± 13		
Treated with thymonucleic acid	687 ± 17	714 ± 18	758 ± 19	767 ± 14		
% Increase	12.6	17.0	7.8	9.5		
Controls	609 ± 16		726 ± 15		706 ± 20	721 ± 10
Treated with yeast nucleic acid	706 ± 21		777 ± 23		765 ± 25	774 ± 14
% Increase	15.8		7.0		8.4	7.4

which can be produced in commercial quantities, yeast nucleic acid, results in a significant lengthening of the life span. It is evident that treatment with nucleic acids is beneficial at both early and later periods of life, and after the usually accepted growth period has been completed. The improvement in vitality and consequent increase in longevity may hold true for human beings as well as for mice.

Discussion

The work of Robertson and his coworkers on nucleic acids was based on the hypothesis (8-a, 13) that the feeding of large quantities of nucleic acids (55 gm. on an adult human being basis) would result in postadolescent growth, and would tend to lessen the rate of decrease of the nucleocytoplasmic ratio in cells. Thus the cells would retain longer the characteristics of youth, and the individual would have a longer life span. Robertson proved his thesis of postadolescent growth and a longer life span, but recent work (15, 20) tends to show that the nucleic acids are metabolized and probably used in more parts of the cell than in the preservation of the nucleus alone.

In the course of these later investigations two other hypotheses were developed which may partially or wholly explain the increase in longevity that results from the feeding of yeast nucleic acid. The fact that only one tenth of the nucleic acid used by Robertson materially increased the survival values for albino mice partially invalidated Robertson's thesis that the nucleic acid is used only as a food material for growth. Also, the fact that yeast nucleic acid, which is found not in the nucleus but in the cytoplasm, increases the life span as well as thymus nucleic acid, which is found in the nucleus, would seem to indicate other modes of operation of nucleic acids than the effect on the nucleocytoplasmic ratio alone.

One hypothesis that would partially fit into Robertson's nutrient thesis is that there are two ways of maintaining a high nucleocytoplasmic ratio. Robertson stressed the growth of the nucleus, but the other way is by slowing down the metabolism of the nucleus by giving to the cell, nucleic acids that can be utilized in metabolism without destroying the nucleus or cytoplasm. Thus the cells live longer at a higher energy level. The ingested nucleic acids would then have a sparing action on the cell nucleic acids and be oxidized in place of them. This would be analogous to but not necessarily the same as the sparing action of vitamin E on vitamin A (23). To contrast Robertson's theory with the newer hypothesis, Robertson emphasized anabolism, while the sparing action hypothesis stresses the slowing down of the catabolism of the essential nucleic acids.

The second hypothesis succeeding Robertson's is based on the experimental fact that in strains of mice in which the females live longer than the males, the females have a higher white blood count, while in other strains, those mice having the highest white blood count live the longest, strain for strain, and females over males respectively (24). This has also been suggested as the reason that women live longer than men. The injection of solutions of sodium yeast nucleinate has been used for some time to stimulate leukocytic growth and activity (25). Therefore, if the daily ingestion of small quantities of yeast nucleic acid should stimulate the white blood cells and tend to keep them at a slightly higher level than they would be otherwise, the organism would be able to fight better and to recover more quickly from pathogenic substances than it ordinarily would. It is a known fact that the leukocytic response to infection is slower in old people than in young people. The decrease in resistance to infection is undoubtedly a major factor in aging, and just as the anti-reticular cytotoxic serum (ARCS) developed in Russia (26) is claimed to increase the resistance to infection and to promote a longer, healthier life, so nucleic acids may stimu-

late in a similar manner. This hypothesis is submitted in spite of the fact that Robertson failed to find any increase in leukocytic activity in his human subjects (28). However, his subjects ingested the nucleic acids for only a few weeks, and it may take several months to obtain an increase in activity. The lengthening of the life span of mice is not apparent until after about fifty days of treatment, a period which would correspond to about five years in man. Therefore the increase in leukocytic activity may be slow.

Nucleic acids in relatively large amounts have been eaten for several weeks without ill effects. Robertson and his co-workers found that 15 gm. per day of yeast nucleic acid, and also other nucleic acids in varying quantities, had no effect on the basal metabolism (27) and urinary excretion of man (28). I have eaten 5 gm. of yeast nucleic acid per day for weeks without any ill effects. (Yeast nucleic acid is sour to the taste, and it was found convenient to dissolve the nucleic acid in approximately one third its weight of sodium bicarbonate in half a glass of water. In this form it is palatable. The total dosage was divided into two parts and taken at morning and at night, so as to keep the metabolism balanced. Yeast sodium nucleinate can also be used for animals in place of the acid.)

As Robertson tested with three times the amounts I have suggested for use, there is no reason known at the present time for fearing to use yeast nucleic acid freely for veterinary experimental purposes on cats, dogs, and various other mammalian pets for extending their life spans, as well as for experimental therapy on aging men and women for the same purpose. Fortunately, a recently published method may make it possible to detect any decrease in the aging rate within a period of about two years (32). If found beneficial, nucleic acid may not be advocated much before middle life, but the fact that it can aid at later ages is indicative of the body's capacity to respond to proper stimuli. There is some evidence that old mice (over 700 days of age) also respond to yeast nucleic acid (29).

The development of factors which will effectively slow down degenerative changes and which may be used like vitamins is a major objective of gerontotherapeutic work. At the present time only two such materials may be obtained in the United States—yeast nucleic acid and methyl testosterone (or testosterone propionate). Testosterone has shown evidence of affecting degenerative changes to some extent, (30), and it has recently been highly publicized in a popular book describing its effects (31).

The use of both yeast nucleic acid and methyl testosterone together, and the development and use in America of Russia's anti-reticular cytotoxic serum in conjunction with the other two agents have not been investigated at the present time.

The effect of yeast nucleic acid on the human leukocytic level will be reported on later.

Summary

1. The effect of yeast nucleic acid on the longevity of 600 day old albino mice was investigated.

2. The average life of the male albino mice was increased 8.4 per cent and that of the female mice, 7.4 per cent by the daily ingestion of 2.5 mg. of yeast nucleic acid, added to an already adequate diet.

3. The investigation was correlated with the work of Robertson and his co-workers on the observed increase in longevity of albino mice obtained by the dietary addition of large amounts of yeast and other nucleic acids of various origins.

4. Two hypotheses to explain this increased longevity were discussed in addition to Robertson's theory of a nucleocytoplasmic ratio level upheld by an excess of nutrient nucleic acid. One hypothesis suggested that the ingested nucleic acid may be metabolized in place of the cell

nucleic acids; the other hypothesis suggested that the nucleic acid may increase leukocytic levels and that consequently a greater resistance to infection may be maintained.

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