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Increase in lifespan of rats following polypeptide pineal extract treatment

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With 2 figures

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Summary

The 20 month-long treatment of female rats with daily doses of 0.1 or 0.5 mg of polypeptide pineal extract (PPE) per animal increased their lifespan by 10 and 25%, respectively, as compared with controls. At the age of 16—18 months, 38% of control rats exhibited persistent disturbances in estral function (constant estrus or repeated pseudogestations), whereas these disorders were observed in 7% of experimental animals only. After administration of PPE to 16—18 month-old female rats checked for sterility by a two-week mating, a second mating period resulted in gestation development in four out of 16 animals and deliveries, accordingly. While chronic treatment with PPE did not affect the rate of neoplasm incidence, the mean age of tumour detection in the control group was 697 days and in experimental groups it was 811 and 868 days, respectively. Certain aspects of the interrelationship of rate of ageing, lifespan and specific age pathology are discussed.

A large-scale search for means of extending the lifespan of humans is under way. However, it has been generally assumed that the mechanisms of physiological ageing, on the one hand, and formation of specific age pathology, on the other, are essentially different. Hence, it follows that annulment of basic ageing-associated diseases would not necessarily result in a longer lifespan (Hayflick 1976). However, some evidence points to the key-role of regulatory disturbances in the mechanisms of natural death of higher organisms and specific age pathology (Dilman 1977a, 1978a, 1978b). According to the theory based on this evidence, formation of specific age pathology is related to the law of deviation of homeostasis which assures the development of the organism. On completion of body growth, the programmed deviation of homeostasis brings about age pathology development and the latter factor appears to be the immediate cause of natural death in old age (Dilman 1978a, 1978b). In the terms of this theory, the inevitable deviation of homeostasis manifests itself in the ageing-associated changes in the threshold of sensitivity of the hypothalamus to regulatory stimuli. This phenomenon occurs in the four main hypothalamus-controlled homeostatic systems — energy, reproductive, adaptive and thyroid homeostats (Dilman 1978b). From the viewpoint of this theory, lifespan may be prolonged by influencing the mechanism of development of diseases of ageing. Since our study was based on the above concept, we chose polypeptide pineal extract (PPE) as a factor of influence on lifespan and the rate of age pathology incidence. This preparation includes a number of polypeptides which exert a specific effect on the neuroendocrine system. For instance, our previous investigations showed that PPE can raise the sensitivity of the hypothalamo-pituitary complex to inhibition by prednisolone and estrogens (Ostroumova and Dilman 1972; Anisimov et al.

1973), can cause the estral function to resume in old rats with constant estrus (ANISIMOV et al. 1973) and lower the levels of insulin and triglycerides in blood (OSTROUMOVA and VASILJEVA 1976), i.e. it can influence the functional condition of the three main homeostatic systems. In this study, the use of lifespan duration as a criterion of PPE effect on ageing processes was supplemented by application of such a parameter as the condition of the reproductive system, because the ageing-connected termination of the reproductive function is a specific manifestation of ageing. The rate of incidence of spontaneous tumours was employed as the indicator of development of specific ageing-associated pathological processes, because ageing and tumour incidence are connected by a correlation both in man (DOLL et al. 1970) and rats (ANISIMOV 1976).

Material and methods

Polypeptide pineal extract (PPE) was prepared from acetonic powder of bovine pineal glands by extraction with 0.1 N acetic acid with the presence of $ZnCl_2$ for 72 hrs; after centrifugation the supernatant was precipitated by 4—8 volumes of acetone. The acetone and ether-dried precipitate was lyophilized and sterilized. Fractionation of PPE with Biocarb carboxyl cationite yielded three fractions, the ratio being 74 : 16 : 10 in the total extract. Subsequent analysis showed that fractions I, II and III have the following parameters: molecular weight = 250, 11,000 and 12,000 dalton; alfa-amine nitrogen = 1.40×10^{-3}, 0.34×10^{-3} and 4.0×10^{-3} μM/mg, and isoelectric points = 3.05; 5.0 and 10.5, respectively. Total PPE preparation was used in all experiments.

Series 1 of experiments consisted of 147 female rats supplied from the Rappolovo Animal Farm of the U.S.S.R. Academy of Medical Sciences. The animals were kept in plastic cages, six in each, under natural lighting conditions, at constant temperature of 23 °C; they were fed natural food and given tap water ad libitum. Beginning from the age of 3.5 months, some animals were injected 0.2 ml of saline (control) other rats received either 0.1 mg or 0.5 mg of PPE per animal in the same volume of saline, five times a week for 20 months (experimental groups). At the age of 16—18 months, when rats exhibit ageing-associated disturbances in estral function (ASCHHEIM 1976), vaginal smears were taken daily. Dead animals were autopsied. All neoplasms were examined microscopially.

In the course of Series 2 of the experiments, 17 females, aged 16—18 months, with constant estrus, were mated for two weeks; subsequently these females were observed for another 21 days. The one rat that became pregnant and gave birth was discarded. The remaining rats were injected 2 mg PPE, subcutaneously, 5 times a week, for three weeks. 7 days after the first injection, females were mated for another two weeks and the rats which gave birth were counted again. Experimental results were treated statistically using Student's t-test and the χ^2 test. The curves of survival time rates were transformed into linear anamorphoses by means of probit analysis (STORM 1979). The method of direct standardization was employed in calculations of the incidence of tumours (STORM 1979). Therefore, to statistically estimate the tumour potentiality of the rats we adopted a "Life Table" technique according to SCHARDEIN et al. (1968).

Results and discussion

The data presented in table 1 demonstrate a direct correlation between the dose of PPE administered to female rats and their life-span. For instance, when 0.1 mg PPE was administered, survival time increased by 10%, whereas after the administration of 0.5 mg — by 25%. The decrease in the slope of linear anamorphoses of the survival time curves for PPE-treated rats (fig. 1) shows that the rates of ageing of these animals were actually slowed down. This conclusion was also corroborated by the results of our study of estral function in the 16—18 month-old controls and experimental animals. At this age, 23% of the control animals exhibited constant estrus, while 15% had repeated pseudopregnancies. In the rats chronically injected with PPE in the dose of 0.5 mg, estral cycle disturbances had been observed in 7% of the animals ($p < 0.05$) by that period. It is noteworthy that out of 16 old rats which remained sterile after the first mating, four females became pregnant and gave birth to 5—9 fetuses in a litter. Taking into account our previous data on the lowering of the hypothalamic threshold of sensitivity to inhibition by estrogens and resumption of estrous cycles in spontaneous constant-estrus rats as a result of PPE treatment (ANISIMOV et al. 1973), it may be inferred that such treatment has a normalizing effect on the tonic and cyclic areas of the sex centre in the hypothalamus. Hence, if an eval-

Table 1. Effect of pineal polypeptide extract (PPE) treatment on lifespan and spontaneous tumour incidence in female rats

Group	Daily dose, mg	No of rats	Lifespan, days			Rate of tumour incidence*, %	Mean age of tumour detection, days	No. of tumours	Incidence of malignant tumours*, %
			mean	median	maximal				
Control (saline)	—	75	681 ± 14.5	705	1054	30.7 (34.9)	694 ± 22.6	30***	9.3 (9.5)
Polypeptide pineal extract	0.1	39	749 ± 20.1**	788	936	33.3 (34.0)	811 ± 26.3**	22****	7.7 (7.9)
	0.5	33	852 ± 33.8**	873	1112	42.4 (36.7)	868 ± 42.3**	27*****	9.1 (5.9)

* — in parenthesis — standardized index.

** — difference to control is significant, $p < 0.05$.

*** — one fibroma and 3 fibroadenomas of mammary gland, adenocarcinoma and 6 adenomas of pituitary, 5 thyroid-cell and 3 light-cell adenomas of thyroid, 2 adenomas of adrenal cortex, 3 leukemias, 2 thymomas, lymphosarcoma, lung reticulosarcoma, uterine adenomyoma and sarcoma of subcutaneous tissue.

**** — fibroma and 7 fibroadenomas of mammary gland, 7 adenomas of pituitary, thyroid-cell and 2 light-cell adenomas of thyroid, 2 leukemias, thymoma and sarcoma of subcutaneous tissue.

***** — fibroma and 6 fibroadenomas of mammary gland, adenocarcinoma and 4 adenomas of pituitary, 4 thyroid-cell and 2 light-cell adenomas of thyroid, 3 polyps and one fibroadenoma of uterus, 2 thymomas, adenoma of adrenal cortex, basal-cell cutaneous cancer, neurinoma of small intestine.

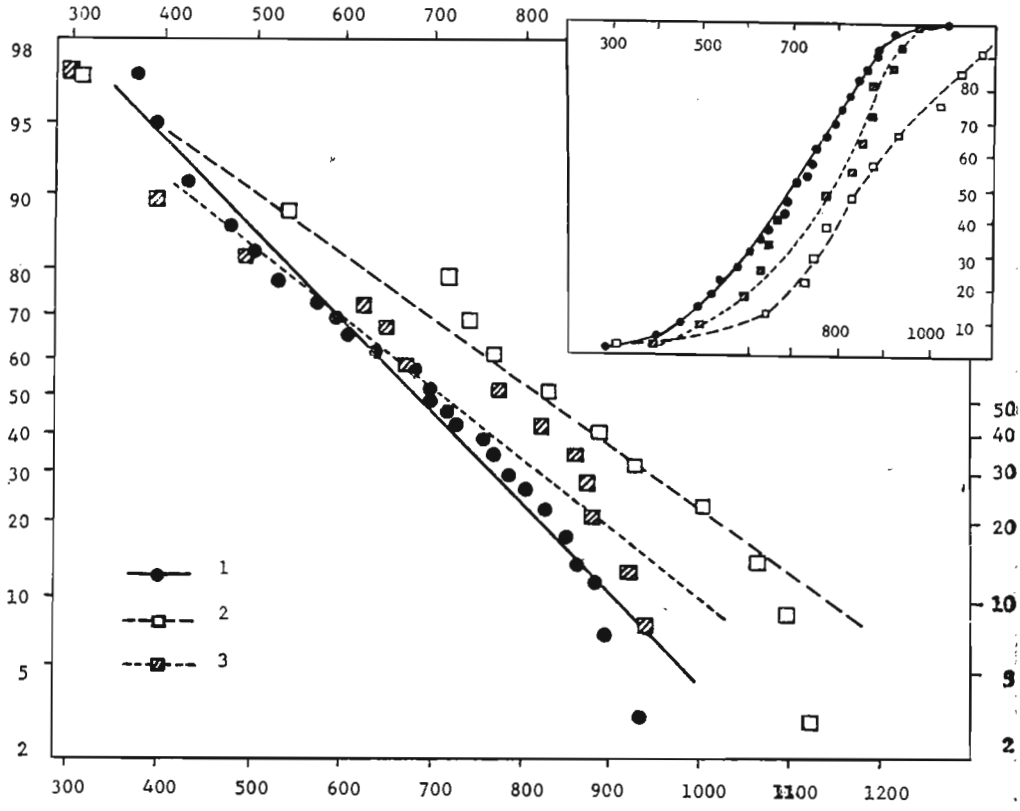

Fig. 1. Effect of polypeptide pineal extract (PPE) treatment on lifespan on female rats. Ordinate axis = percent (on the main diagram = probit scale), horizontal axis = age, days. Straight lines = survival; curves = mortality. 1: control (saline); 2: PPE, 0.5 mg per day; 3: PPE, 0.1 mg per day. Symbols show every third animal.

Table 2. Statistical estimation of the potentiality of rats treated with polypeptide pineal extract to develop tumours

	x*	lx	dx	cx	qx	px	Px	Qx
Control (saline)	180—400	75	5	72.5	0	100.00	100.00	0.00
	401—600	70	13	57.0	4	92.98	92.98	7.02
	601—800	53	21	41.5	10	75.90	70.57	29.43
	801—1000	22	12	16.0	9	43.75	30.87	69.13
	1001—1200	1	1	0.5	0	100.00	30.87	69.13
Polypeptide pineal extract, 0.1 mg	180—400	39	1	38.5	0	100.00	100.00	0.00
	401—600	38	7	34.5	0	100.00	100.00	0.00
	601—800	31	8	27.0	4	85.19	85.19	14.81
	801—1000	19	10	14.0	9	35.71	30.42	69.58
Polypeptide pineal extract, 0.5 mg	180—400	33	1	32.5	0	100.00	100.00	0.00
	401—600	32	1	31.5	1	96.83	96.83	3.17
	601—800	31	5	28.5	5	82.46	79.85	20.15
	801—1000	21	6	18.0	4	77.78	62.11	37.38
	1001—1200	10	6	7.0	4	42.86	26.62	73.38

x* — Interval of time in days
lx — No. of rats alive at beginning of period (lx + 1 = lx — dx — qx)
dx — No. of rats died during period without tumours
cx — Effective No. of rats during the period (lx — $^1/_2$ dx)
qx — No. of rats developing tumours during period
px — Estimated No. of rats not developing tumours during period (100(1 — qx/cx))
Px — Cumulative % of rats not developing tumours (Px x succeeding px)
Qx — Cumulative % of rats developing tumours (100 — Px)

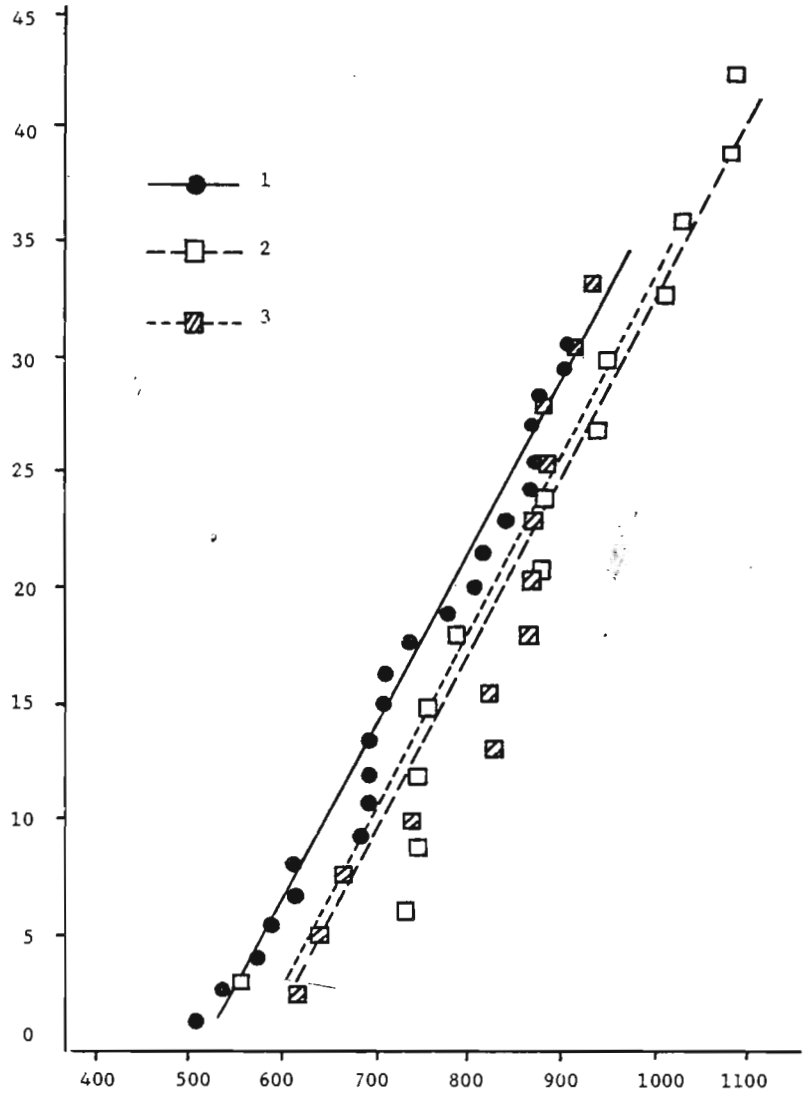

Fig. 2. Effect of polypeptide pineal extract (PPE) treatment on spontaneous tumour yield in female rats. Symbols show all tumour-bearing animals. Designations used as in fig. 1.

uation is made on the basis of the criterion of reproductive function, PPE treatment actually slows down the rate of ageing. In this connection, a problem arises in what manner the delay in ageing influences the incidence of pathological processes associated with ageing, in particular, the rate of tumour incidence.

Table 1 shows that PPE treatment results in slight increase in the relative rates of spontaneous tumour incidence, although this increase is statistically insignificant ($p > 0.05$). This increment seems to be due to the extension of the lifespan of the animals because the mean age tumour detection showed a statistically significant increase in the groups of PPE-treated rats. As can be seen from fig. 2, the rates of tumour incidence in the control group were much higher than in PPE-treated animals for each age group (e.g. 700, 800 or 900 days). This conclusion is also confirmed by the results of calculations of the standardized coefficients of tumour incidence (see table 1). Hence, the rise in tumour incidence rates in the

second experimental group should be accounted for by the prolongation of lifespan of these animals. The incidence rates, sites and histological types of the tumours in both control and experimental groups conformed to those usually observed in the rats of the strains used (ANISIMOV et al. 1978). It should be pointed out that the rates of incidence of malignant tumours were the same in all groups of the animals. The increment in the total number of tumours which arose in the PPE-treated rats was contributed to by benign tumours, chiefly, by pituitary adenomas and mammary fibroadenomas (see table 1). The mean age of detection of malignant tumours was 697 days in the control group and 800 and 973 days in the experimental groups, respectively.

Hence, the effect of PPE treatment is not limited to prolongation of lifespan of rats; also, it delays ageing as evidenced by the changes in the slope of linear anamorphoses of survival time curves and its effect on the reproductive system. Since the rates of tumour incidence for each age interval (e.g. 600, 800 days, fig. 2, table 2) were lower in experimental groups than in controls, it may be suggested that the delay of natural ageing results in retarding specific age pathology. Therefore, the solution of the problem of annulment of specific age pathology resulting in prolonging human lifespan depends to a considerable extent on the manner in which ageing-associated diseases will be eliminated. If we find drugs which will affect the mechanisms underlying body development, ageing and age pathology and, particularly, the regulatory mechanisms of these phenomena, elimination of age pathology may involve a simultaneous increment in lifespan duration. The effect of PPE demonstrates this possibility.

As far as the evaluation of PPE effect on the lifespan of animals and the rate of incidence of spontaneous tumours is concerned, it may be supposed that the antitumour effect of this preparation is related to its influence on the threshold of the sensitivity of the hypothalamo-pituitary complex, to inhibition by peripheral hormones (OSTROUMOVA and DILMAN 1972; ANISIMOV et al. 1973), improvement of carbohydrate tolerance and, therefore, decrease in blood-insulin and triglycerides (OSTROUMOVA and VASILJEVA 1976), i.e. influence on disturbances constituting one of the components of the syndrome of cancrophilia (DILMAN 1978b), influence on metabolic immunodepression (BELOKRYLOV et al. 1976; DILMAN 1978a, 1978b), inhibition of the respiratory circuit on mitochondrion level (KONDRASHOVA et al. 1977).

Literature

ANISIMOV, V. N., Spontaneous tumors in rats of different strains (Russ.). Vopr. Onkol. **22**, 8, 98—110 (1976).

— V. KH. KHAVINSON, V. G. MOROZOV and V. M. DILMAN, Pineal extract-induced decline in sensitivity of hypothalamo-pituitary system to estrogens in old female rats (Russ.). Dokl. Acad. Sci. USSR **213**, 483—485 (1973).

— V. A. ALEXANDROV, V. F. KLIMASHEVSKY, V. I. KOLODIN, A. YA. LIKHACHEV, V. B. OKULOV, K. M. POZHARISSKY and O. P. SAVELJEVA, Spontaneous tumors in rats bred at the "Rappolovo" nursery of the USSR Academy of Medical Sciences (Russ.). Vopr. Onkol. **24**, 1, 64—70 (1978).

ASCHHEIM, P., Aging in the hypothalamic-hypophyseal-ovarian axis in the rat. In: Hypothalamus, Pituitary and Aging. Ed. A. V. EVERITT and J. A. BURGES. Thomas Publ., Springfield 1976, pp. 376—418.

BELOKRYLOV, G. A., V. G. MOROZOV, V. KH. KHAVINSON and B. N. SOFRONOV, Effect of low-molecular extracts of heterologic thymus, pineal and hypothalamus on immune response in mice (Russ.). Bull. exper. Biol. Med. **81**, 202—204 (1976).

DILMAN, V. M., Metabolic immunodepression which increases the risk of cancer. Lancet **2**, 1207—1209 (1977a).

— Improvement of indices of cellular immunity after epithalamin treatment (Russ.). Vopr. Onkol. **23**, 6, 7—9 (1977b).

— Ageing, metabolic immunodepression and carcinogenesis. Mechan. Aging & Develop. **8**, 153—173 (1978a).

— Transformation of the developmental programme into the mechanism of age-associated pathology. Evaluation model of human specific age pathology and natural death (Russ.). Fiziologia Tcheloveka **4**, 579—595 (1978b).

DOLL, R., C. MUIR and J. WATERHOUSE, Cancer Incidence in Five Continents. UICC. 1970.

HAYFLICK, L., The cell biology of human aging. New Engl. J. Med. **295**, 1302—1308 (1976).
KONDRASHOVA, M. N., Y. V. EVTODIENKO, G. D. MIRONOVA et al., The norm and pathology in the light of mitochondrial energy processes. In: Biophysics of Complex Systems and Radiation Disorders. Ed. G. M. FRANK. Nauka, Moscow 1977, pp. 249—270.
OSTROUMOVA, M. N., and V. M. DILMAN, The influence of the pineal extract on the threshold of hypothalamic sensitivity to prednisolone suppression (Russ.). Vopr. Onkol. **18**, 11, 53—55 (1972).
— and I. A. VASILJEVA, Effect of polypeptide pineal extract on fat-carbohydrate metabolism. Probl. Endokrinol. (Mosk.) **22**, 3, 66—69 (1976).
SCHARDEIN, J. L., J. E. FITZGERALD and D. H. KAUMP, Spontaneous tumors in Holtzman-Source rats of various ages. Path. Vet. **5**, 238—252 (1968).
STORM, R., Theory of Probabilities. Mathematical Statistics. Statistical Control of Quality. MIR Moscow 1979.