Long-Term Treatment with Procaine (Gerovital H_3) in Albino Rats[1,2]

Anna Aslan, M.D., D.Sc.,[3] Alexandru Vrăbiescu, M.D.
Cornel Domilescu, M.V.D., Lucia Câmpeanu, M.D.
Mira Costiniu, M.D., and Stelian Stănescu, M.D.

RESEARCH carried out on white rats revealed improvement of general condition and increased resistance to experimental arthritis induced by formaldehyde after injections of procaine (Aslan, Nedler, & Todea, 1951). Later, increased resistance to sciatic nerve lesions induced by injection of croton oil was observed following injection of procaine (Aslan, 1962).

Besides the action exerted on psychic and general somatic conditions found in man during longitudinal studies on the same Ss (average age 83 years) undergoing procaine treatment for 12 years, a mortality of 4.1% in the procaine-treated group as against 16% in the controls was found (Aslan, 1956a; 1956b; 1957a; 1957b; 1960; Aslan, Vrăbiescu, & Câmpeanu, 1962; Aslan, David, & Câmpeanu, 1963; Parhon & Aslan, 1957).

However, it is difficult to assume a longer survival on the basis of clinical results obtained in a small number of individuals. Therefore an experiment was carried out with 1,840 white rats in order to gain information on the effects of procaine on behavior and morphological and physiological changes associated with age as well as life span.

MATERIALS AND METHODS

Animals.—White rats of the French Wistar strain (N = 1,840, unmated) were maintained in a closed colony for three years. The animals were divided in two groups of 920 each. Each of these groups was subdivided in four groups of about 230 animals—males and females, treated and controls. One-half of the first group was given procaine beginning at two months of age (procaine group α = Pr. α) while one-half of the second group received treatment beginning at the age of six months (procaine group β = Pr. β). The treatment was initiated at different ages to determine the period of the life cycle at which procaine administration would be most beneficial. The animals were kept in optimum breeding conditions, however without mating. All animals were maintained under similar environmental conditions at a temperature of 21 ± 1 C. and humidity of 60 ± 10%. No artificial control over daylight and darkness periods was employed. The diet contained 20% vegetable and animal proteins, 5% fats, the remainder as carbohydrate and roughage. The basal diet contained oats, whole wheat bread, whole milk, beef (once a week). Carrots, beet root (fodder), and white cabbage were included when in season. Germinated oats were given until the animals were six months old. Calcium salts were added periodically. All animals received identical diets.

Experimental design.—Each experimental animal received 4 mg. procaine/Kg. body weight three times per week for four weeks. This was followed by an intermission period of one month. This procedure was carried out for the remainder of the life span of the animals. The components of the solution administered in Gm./l. were: procaine, 20.0; benzoic acid, 1.2; potassium metadisulfite, 1.0; disodium phosphate, 0.1. Control animals received saline solution in a similar course of injections. The follow-up covered a period of 32 months.

[1] A preliminary report of this work was presented at the 6th International Congress of Gerontology, Copenhagen, Denmark, Aug. 11-16, 1963 (Excerpta Medica International Congress Series No. 57, pp. 38-39).

[2] Ministry of Health and Social Welfare, Bucharest Institute of Geriatrics.

[3] Director of the Bucharest Institute of Geriatrics, Bucharest, Rumania.

Psychological tests.—Fifty-five animals aged 24 months were selected at random from each of the three experimental female groups (18 controls; 17 Pr. α; 20 Pr. β) for learning and memory tests which followed the procedure described by Verzár and McDougall (1955). The maze consisted of a main passage 300 cm. long, which was provided with 13 choice points where errors could be made by the animals. All animals were fasted 20 hours before they were placed in the maze. During the first ten days the animals learned the maze. One trial was given each animal on each day and the reward was placed at gradually increasing distances in the maze. After these preliminary trials, the reward was placed at the end of the maze and time and errors were recorded for each animal for one trial each day for ten days. Mean errors per trial, mean running time per trial, mean shortest running time, and mean longest running time per trial were recorded.

Electrocardiograms.—Electrocardiograms were recorded in 20 randomly selected male rats from each of the three groups (Total N = 60). The records were made with the animals under ether narcosis in the supine position. Needle electrodes were placed subcutaneously in the extremities for the standard leads I and III and in the mediosternal and mid-axillary line for precordial leads (V. ant. and V. lat.) Calibration was 1 mV = 3 cm.; film speed was 100 mm./sec. Recordings were made in each animal at 24 months of age (during an intermission between two courses of injections).

Electrocardiographic changes due to anesthesia were disregarded (atrioventricular block, arrhythmia).

According to data from the literature, as well as from our comparative electrocardiographic findings in young animals (up to 12 months of age), the following criteria were used as evidence of myocardial dysfunction: a) high and

Table 1. Survival Curve of Male Rats.

Age (Mo.)	Control N=460	Procaine[a] N=230 % Survival	Procaine[b] N=239
2	97.6±0.70[c]	———	———
3	97.2±0.75	99.6±0.40	———
4	97.2±0.75	99.2±0.58	———
5	96.4±0.86	98.7±0.74	———
6	96.0±0.91	98.3±0.84	98.8±0.70
7	95.6±0.95	97.9±0.94	98.8±0.70
8	94.8±1.03	95.7±1.33	98.8±0.70
9	93.2±1.17	94.0±1.56	98.0±0.90
10	91.2±1.32	93.5±1.62	97.5±1.00
11	87.2±1.55	91.8±1.80	97.5±1.00
12	82.4±1.77	90.0±1.97	97.5±1.00
13	78.4±1.91	89.6±2.01	97.5±1.04
14	70.4±2.12	88.7±2.04	92.1±1.74
15	66.8±2.19	85.3±2.10	89.2±2.00
16	60.4±2.27	84.8±2.36	86.2±2.22
17	52.4±2.32	77.4±2.73	81.2±2.52
18	47.2±2.32	74.0±2.89	75.8±2.76
19	37.2±2.25	68.7±3.05	72.9±2.87
20	30.4±2.14	59.6±3.23	66.9±3.04
21	21.2±1.90	51.8±3.29	60.3±3.16
22	14.4±1.63	42.2±3.25	49.8±3.23
23	6.8±1.26	37.0±3.18	36.5±3.23
24	2.8±0.76	30.9±3.04	28.9±2.93
25	1.6±0.58	19.2±2.59	24.3±2.77
26	0.4±0.28	14.0±2.28	15.1±2.31
27	0	7.4±1.72	8.8±1.83
28	———	4.0±1.28	2.1±0.92
29	———	2.2	0.9±0.60
30	———	2.2	0.5±0.44
31	———	0	0.5
32	———	———	0
33	———	———	———

[a]2 months old at beginning of procaine treatment.
[b]6 months old at beginning of procaine treatment.
[c]Standard deviation calculated as $\sigma = \sqrt{\frac{PQ}{N}}$ where P=% alive, Q=100−P, N=number in group.

Table 2. Survival Curve of Female Rats.

Age (Mo.)	Control N=460	Procaine[a] N=200 % Survival	Procaine[b] N=217
2	99.6±0.28[c]	———	———
3	99.2±0.42	99.0±0.70	———
4	98.8±0.51	97.5±0.78	———
5	96.6±0.87	96.0±1.38	———
6	96.1±0.90	96.0±1.38	0
7	95.7±0.91	95.5±1.46	99.1±0.61
8	95.3±0.98	95.0±1.53	98.2±0.90
9	94.4±1.07	93.5±1.74	98.2±0.90
10	94.0±1.10	93.0±1.80	95.4±1.42
11	93.5±1.14	92.5±1.86	94.5±1.54
12	92.2±1.30	91.0±2.02	94.5±1.54
13	90.0±1.40	89.0±2.33	93.6±1.66
14	88.3±1.50	88.0±2.29	90.8±1.95
15	85.7±1.63	83.5±2.64	89.9±2.04
16	77.9±1.93	80.5±2.80	87.1±2.27
17	70.5±2.12	79.0±2.87	83.0±2.57
18	63.5±2.29	75.0±3.06	77.0±2.85
19	54.4±2.32	68.0±3.29	69.6±3.12
20	44.8±2.33	62.5±3.43	62.3±3.25
21	36.1±2.23	50.0±3.53	48.2±3.39
22	22.7±1.95	38.0±3.43	41.5±3.28
23	14.4±1.63	27.5±3.15	31.4±3.14
24	9.2±1.26	19.5±2.89	25.9±2.97
25	6.6±1.15	13.0±2.37	18.0±2.60
26	3.1±0.80	7.5±1.86	12.5±2.24
27	1.8±0.63	4.5±1.46	8.8±1.92
28	1.8±0.63	3.5±1.29	7.4±1.68
29	0	1.0±0.70	4.2±1.36
30	———	1.0±0.70	2.4±1.03
31	———	0	1.0±0.67
32	———	———	1.0±0.67
33	———	———	0

[a]2 months old at beginning of procaine treatment.
[b]6 months old at beginning of procaine treatment.
[c]Standard deviation, calculated as $\sigma = \sqrt{\frac{PQ}{N}}$ where P=% alive, Q=100−P. N=number in group.

peaked T in standard L (over 0.15 mV), b) high and peaked T in precordial L (over 0.33 mV), c) ST-T downward deflection in L_3 (over 0.066 mV), d) extensive and peaked P in standard L or precordials L (over 0.050 mV).

Cases presenting at least two of the above-mentioned aspects were considered as pathological. Cases with a deep (over 0.050 mV) and wide Q_3 wave were considered as myocardial infarctions. Q_3 was often accompanied by a less extensive Q_1 and usually at least one aspect of blood supply disorder (above-mentioned). An extended QRS over 0.018 sec. and delayed local negativity of over 0.015 sec. in one of the precordial leads were regarded as evidence of cardiac conduction disorders.

Histologic examinations.—An additional 20 female rats were selected from each group and histological examinations were made on sections from the myocardium at the level of the circumflex ramus, kidney at the level of the hilus, and the gastrocnemius muscle. Animals were sacrificed at the age of 24 months.

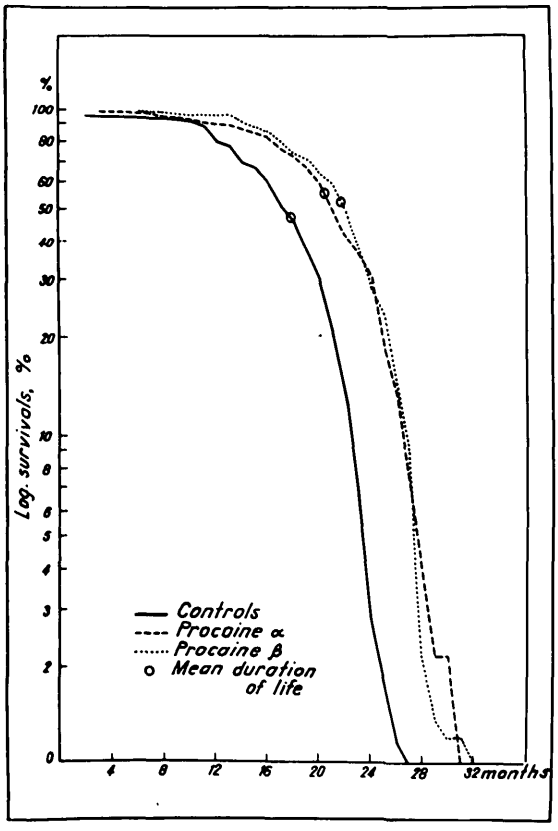

Fig. 1. Survival curves for males. ———— controls. - - - - - - - - Procaine α. . . . . Procaine β. o Mean duration of life.

After fixation in a 10% formalin solution buffered at pH 7.2, the frozen sections were stained with Sudan III, Sudan black, by the method of Schultz, paraffin embedded and stained with Mallory triple stain, Verhoeff, PAS, MacManus, Wilder impregnation, Mowry alcyan blue (Pearse, 1960).

Coronary lesions, the condition of muscle fibers, the ratio of ground substance and connective interstice tissue to myocardial tissue was observed in the myocardium; tubular degenerative lesions, interstitial and vessel changes in the kidney and myocyte lesions, and the ratio of ground substance and connective tissue to skeletal muscle were observed.

The evaluation of histological alterations was assessed on ten sections of each organ in each animal. Alterations were graded as follows: I, simple parenchymatous degenerative lesions; II, parenchymatous degenerative lesions and cell reaction with appearance of collagenous fibers; III, interstitial connective tissue with alterations of ground substance (accumulation of mucopolysaccharides); IV, myocardium with appearance of infarction and kidney with appearance of extensive tubular degenerative lesions.

Fig. 2. Control rat, age 20 months.

Fig. 3. Procaine-treated rat, age 20 months.

Table 3. Mortality Rate per 100 Animals According to Cause, Age, and Sex.

Anatomo-pathological diagnosis of death	0 to 6 Months			7 to 18 Months			Over 18 Months		
	Controls	Pr.α	Pr.β	Controls	Pr.α	Pr.β	Controls	Pr.α	Pr.β
A.—Male Rats									
Acute lung infections	1.6±0.58[a]	2.2±0.95	0.8±0.57	18.3±1.80	15.6±2.39	14.7±2.28	26.7±2.06	39.0±3.21	41.7±3.19
Chronic lung infections	—	—	—	3.1±0.80	3.4±1.19	4.0±1.09	3.1±0.80	5.3±1.47	3.7±1.03
Parenchymatous degenerative processes (heart, kidney, liver)	—	—	—	14.2±1.62	9.2±1.90	7.9±1.74	24.3±2.00	19.3±2.60	17.0±2.42
Tumors	—	—	—	3.5±0.85	1.7±0.85	2.5±0.77	2.8±0.76	1.5±0.80	1.8±0.86
Other causes	—	—	—	1.5±0.56	2.1±0.89	5.1±1.30	0.9±0.43	1.7±0.87	0.8±0.57
B.—Female Rats									
Acute lung infections	2.8±0.76	8.4±1.83	1.2±0.73	13.6±1.59	17.2±2.66	13.0±2.28	27.8±2.08	33.9±3.34	36.5±3.26
Chronic lung infections	—	—	—	1.2±0.5	0.4±0.43	1.2±0.73	5.4±1.03	2.5±1.10	6.7±1.69
Parenchymatous degenerative processes (heart, kidney, liver)	—	2.9±1.18	0.8±0.60	8.4±1.29	1.6±0.88	4.6±1.42	16.8±1.74	17.2±2.66	21.7±2.79
Tumors	—	—	—	3.3±0.83	2.3±1.05	0.9±0.64	3.5±0.85	1.9±0.96	2.1±0.96
Other causes	—	—	0.4±1.35	2.5±0.72	0.8±0.61	0.4±1.35	5.9±1.09	2.1±1.00	2.1±0.96
Sacrificed animals	—	—	—	—	—	—	8.0±1.26	8.4±1.95	8.4±1.88

[a] $\sigma = \sqrt{\frac{PQ}{N}}$ where P=% alive; Q=100−P; N=number in group.

Table 4. Body Weight (Gms.) of Rats.

Age (Mo.)	Controls Mn	Controls σ_m	Procaine α Mn	Procaine α σ_m	Procaine β Mn	Procaine β σ_m
Males						
3	178.9	1.52	189.6	1.73	182.7	1.22
6	265.9	2.10	278.4	2.00	239.6	1.73
9	279.6	1.98	293.9	2.00	273.3	2.02
12	310.3	2.14	317.3	2.24	275.4	2.12
15	331.4	2.85	331.3	2.65	279.0	2.24
18	346.6	3.05	348.6	4.01	289.0	2.84
21	331.3	3.20	358.1	5.38	305.8	2.79
24	300.7	3.02	343.8	5.13	281.2	2.48
27	——	——	293.7	5.20	263.5	2.41
30	——	——	——	——	——	——
Females						
3	152.5	1.61	163.5	1.28	159.3	1.60
6	185.7	1.83	208.3	2.60	179.1	1.80
9	196.9	2.00	207.1	3.08	188.3	1.75
12	207.7	2.17	222.4	3.18	194.9	1.80
15	228.9	2.27	246.5	3.40	199.3	2.10
18	244.8	2.68	247.5	3.56	212.5	2.43
21	237.8	3.07	245.2	3.62	212.5	2.65
24	236.2	3.06	232.7	3.80	208.9	3.04
27	——	——	214.8	3.72	212.6	3.15
30	——	——	——	——	184.0	3.20

Table 5. Maze Learning and Memory of Treated and Control Female Rats.

	Controls	Controls	Procaine α	Procaine β
Age in months	16	24	24	24
No. of animals	13	18	17	20
Mean errors made per trial	3.3± 0.2[a]	4.5± 0.1	3.0± 0.2	4.4± 0.2
Mean running time (sec.)	53.6± 2.6	116.7±14.5	56.6±15.0	53.9± 2.3
Mean shortest running time by trial (sec.)	20.3± 3.7	28.6± 4.5	18.4± 4.0	23.7± 3.2
Mean longest running time by trial (sec.)	95.4± 9.2	306.0±33.1	98.5±31.9	90.8±10.3

[a] Standard error of the mean.

Results

Life span.—The average life spans of the male animals (Table 1) were as follows: controls (N = 460), 537 ± 8.2 days; Pr. α (N = 230), 630 ± 11.0 days; Pr. β (N = 239), 651 ± 13.1 days. The increment in mean life span amounted to 17.3% in the Pr. α group and 21.2% in the Pr. β group over that of the controls ($P < 0.01$). In the females the average life spans were (Table 2): controls (N = 460), 605 ± 8.9 days; Pr. α (N = 200), 621 ± 10.7 days; Pr. β (N = 217), 652 ± 9.5 days. The life span of the treated groups of females was not significantly different from that of the controls (an increment of only

2.6% and 6.7% in Pr. α and Pr. β, respectively).

Survival curves (Fig. 1) showed that in male rats the procaine treatment was associated with an increase in life span of approximately four months under both conditions of procaine therapy.

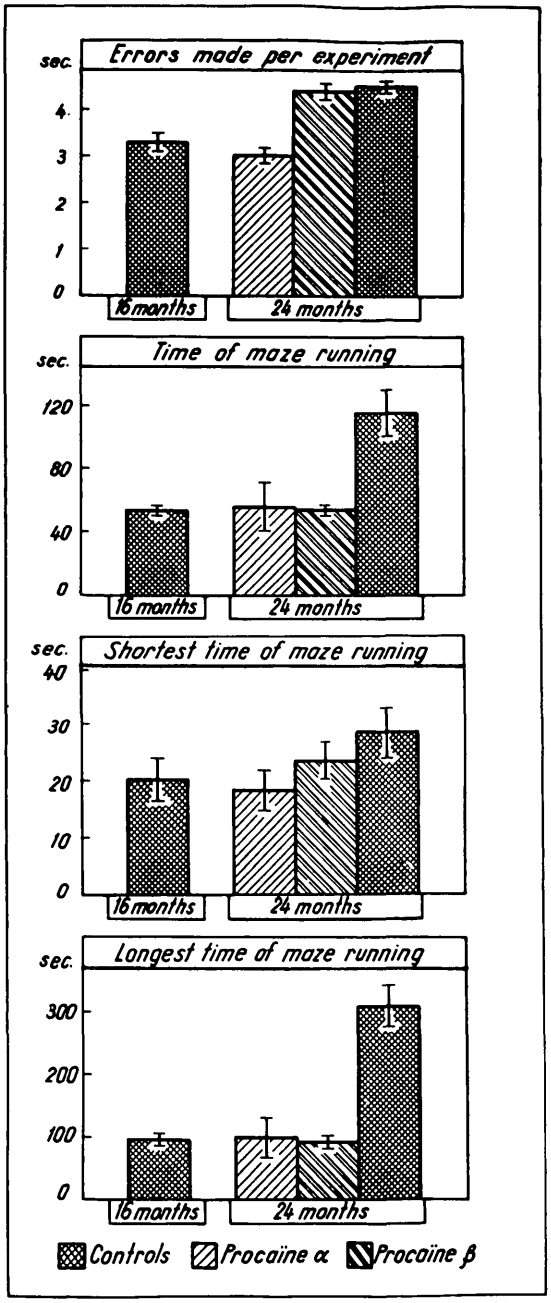

Fig. 4. Average time and error scores in treated and control female rats at 24 months of age compared with 16-month-old controls.

The highest death rate occurred in the control animals between the ages of 13 and 24 months and in the treated animals between the ages 19 and 32 months.

The causes of death in both male and female animals are shown in Table 3. Frequent causes of death among all animals were acute and chronic lung infections, parenchymatous degenerative processes, and tumors.

General appearance.—Incidence of skin infections and alopecia were much lower in treated than in control animals. Differences between the two groups were most conspicuous after 20 months of age. The effect of procaine

Table 6. Electrocardiogram of Rats. 24 Months of Age.

	Control	Procaine α	Procaine β
No. cases	20	20	20
Heart rate (per min.)	316±50.1	390±40.2	358±43.1
QRS axis (%)	R. 60±10.95 N. 35±10.67 L. 5± 4.84	R. 75± 9.72 N. 15± 7.96 L. 10± 6.70	R. 57.1±11.04 N. 38.1±10.81 L. 4.8± 4.65
A–V conduction (%)	35±10.63	0	42.5±10.72
Intra-V. conduction disorders (%)	10± 6.70	10± 6.70	19 ± 8.54
Arrhythmia (%)	5± 4.80	5± 4.81	4.75±4.47
Blood supply disorders (%)	80± 8.94	30±10.24	28.3± 9.79
Infarction (%)	20± 8.94	0	0
QRS/T discordance (%)	35±10.63	25± 9.67	28.6± 9.74

R. = Right deflection
N. = Normal
L. = Left deflection

$$\sigma = \sqrt{\frac{PQ}{N}}$$

P = % alive
Q = 100 − P
N = Number in group

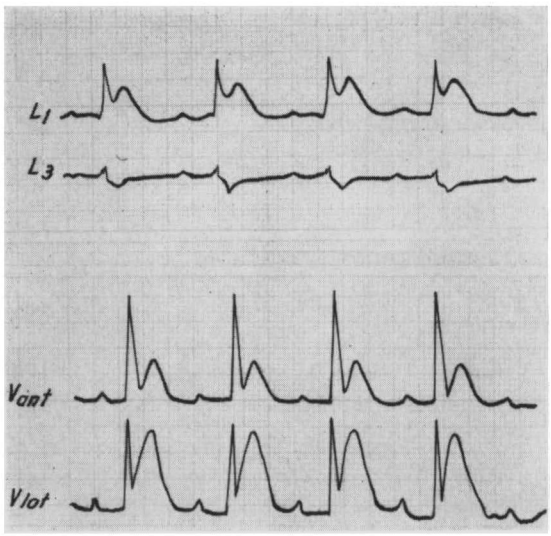

Fig. 5. Electrocardiogram from 24-month-old rat showing evidence of myocardial disease (High, peaked T in $V_{lat.}$, isoelectric T_3.

treatment on the general appearance of the animals is evident from a comparison of Figures 2 and 3 (Aslan et al., 1951). Changes in body weights throughout the experiment are shown in Table 4. The body weight decrement associated with senescence occurred later in the treated male rats than in the corresponding controls. No such difference was observed in female rats.

Learning and memory.—The results of the learning and memory tests are shown in Figure 4 and Table 5. Although the control groups and Pr. β groups made more errors at the age of 24 months than did the 16-month-old controls, the Pr. α group made significantly fewer errors at the age of 24 months than the Pr. β or the control animals. Both the Pr. α and Pr. β groups required less time to run the maze at 24 months of age than the control 24-month-old animals. In fact, the running time of the procaine-treated animals was the same as the 16-month-old controls. Similar relationships were observed for the mean shortest running time and the mean longest running time per trial.

Electrocardiograms.—The results from electrocardiograms recorded at 24 months of age in treated and control animals are summarized in Table 6. An example of an aberrant ECG is shown in Figure 5. There were no significant differences in heart rate between the treated and control groups. However, the incidence of pathological conditions was less in the treated than in the control animals. Almost 80% of the control animals showed some ECG evidence of impaired cardiac function, but only 30% of the Pr. α group and 28.3% of the Pr. β group showed such evidence. Twenty per cent of the control animals showed ECG evidence of myocardial infarction but none of the treated animals showed this. There were no significant differences in the incidence of arrythmia or conduction disorders.

Histological differences.—Figure 6 shows the accentuation of myocardial sclerosis often seen in the myocardium of the 24-month-old control animals. Figure 7 shows a comparable section of the left ventricle of a 24-month-old rat that had been treated with procaine. The myocardium shows less connective tissue in-

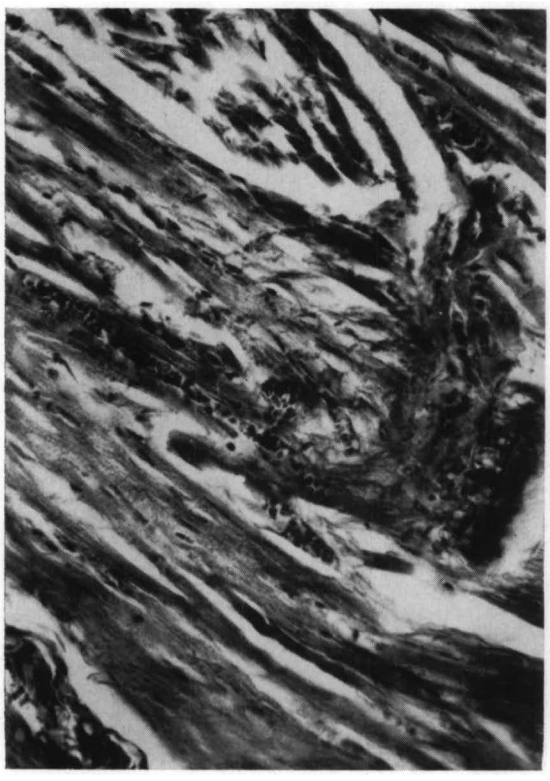

Fig. 6. Section of left ventricle of a 24-month-old control rat which shows abundant intermuscular connective tissue. PAS stain, 240 x.

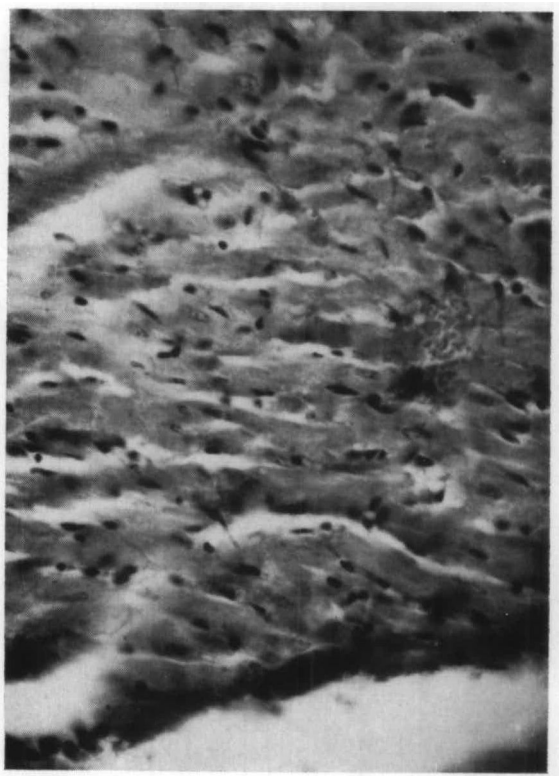

Fig. 7. Section of left ventricle of a 24-month-old animal from the Procaine β group. This section shows only slight interstitial cell infiltration with normal myoblasts. PAS stain, 240 x.

vasion in the treated than the control animal.

Kidney lesions found in both control and treated animals are shown in Figures 8 to 11. Although lesions were found in the kidneys of both groups of animals, tubular degenerative nephrosis was more evident in the controls than in the treated animals.

Discussion

The results obtained in life lengthening of males are in agreement with those of Berger (1960), who showed an increase in survival of five months and improvement in the general condition of 32 animals treated with procaine as compared to controls. The lack of any detrimental effect of procaine treatment in growing rats has been reported (Berger, 1960; Aslan, 1961). On the other hand, Verzár, (1959) who used a small number of rats, did not demonstrate any life lengthening or improvement of the general condition of the treated animals. However, Verzár employed a high dose level of procaine (25 mg./kg. body weight). Thus the discrepancy between the two studies may be caused by the difference in procaine dosage. Indeed, our own investigations (Aslan & Câmpeanu, 1958) showed that the oxygen consumption of a suspension of brewers' yeast was stimulated by low concentrations and inhibited by high concentrations of procaine.

Summary

Procaine was administered parenterally to Wistar rats from the ages of two or six months until death. This treatment resulted in an increase in the life span of male but not female rats. No differences significant in life span were observed between males treated at two months and those treated at six months of age. Procaine treated animals of both sexes were found to be more efficient in maze-running and showed less extensive morphological changes in kidney, skeletal muscle, and cardiac muscle than control animals.

References

Aslan, A.: Novocain als eutrophischer Faktor und die Möglichkeit einer Verlängerung der Lebensdauer. *Ther. Umschau., Bern, 9:* 165-173, 1956. (a)

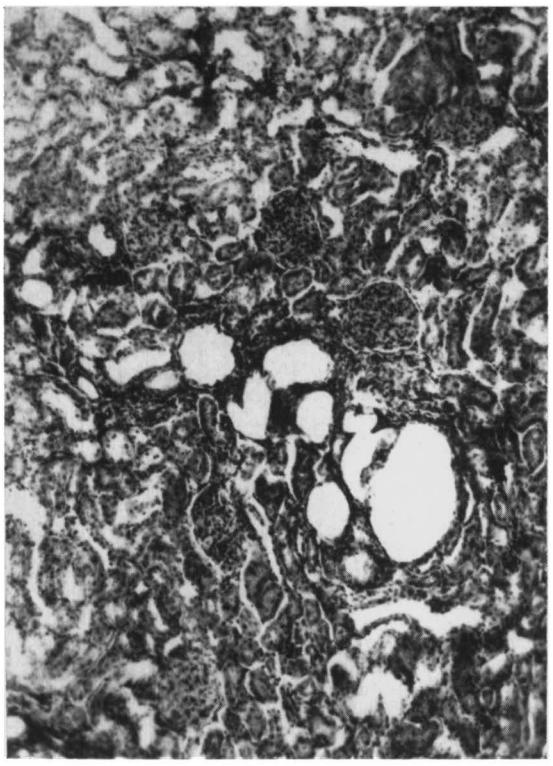

Fig. 8. Section from kidney of 24-month-old control rat which shows tubular nephritis PAS stain, 160 x.

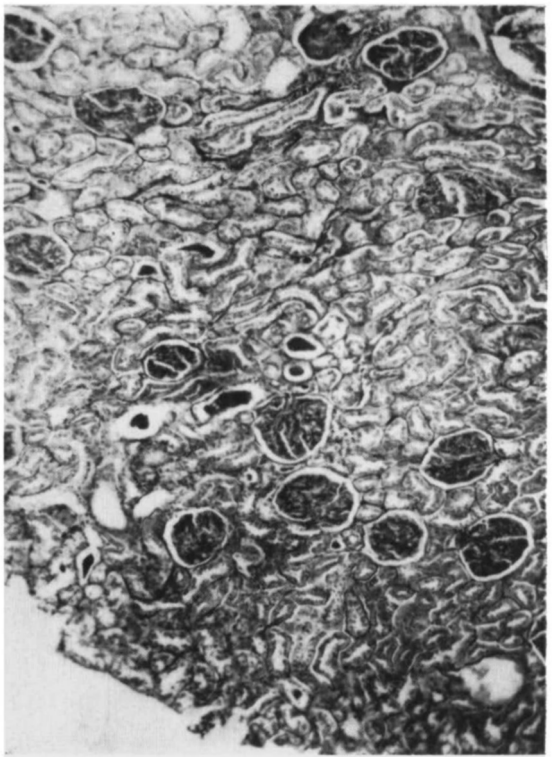

Fig. 9. Section of kidney of 24-month-old rat from Procaine β group. Discreet tubular nephritis was present in this rat. PAS stain, 160 x.

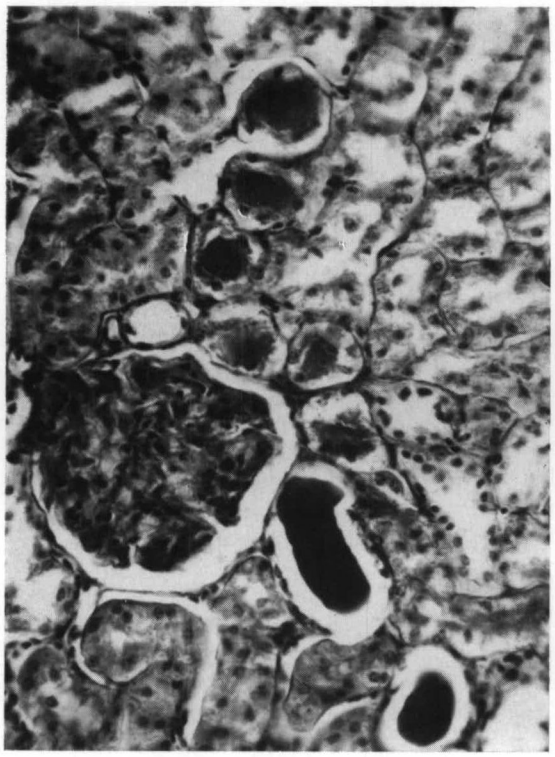

Fig. 10. Section from kidney of 24-month-old control rat which shows thickening of basal membrane from accumulation of neutral mucopolysaccharides. (PAS positive material with tubular deposits.) PAS stain, 240 x.

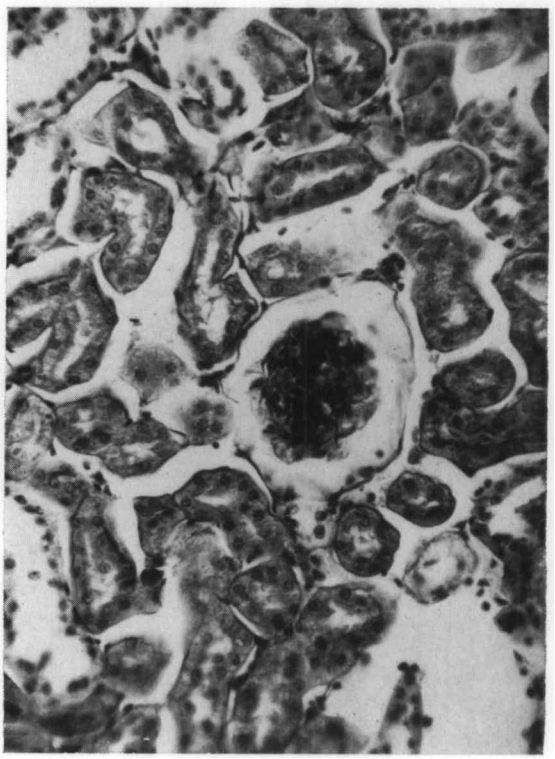

Fig. 11. Section from kidney of 24-month-old rat from Procaine β group which shows enlargement of Bowman's capsule. PAS stain, 240 x.

Aslan, A.: Eine neue Methode zur Prophylaxe und Behandlung des Alterns mit Novocain-Stoff H3—eutrophische und verjüngende Wirkung. *Therapiewoche, 7:* 14-22, 1956. (b)

Aslan, A.: Neue Erfahrungen über die verjüngende Wirkung des Novocain-Stoffs H3—nebst experimentellen, klinischen und statistischen Hinweisen. *Therapiewoche, 8:* 10-19, 1957. (a)

Aslan, A.: Recherches sur l'action de la novocaine (substance H3); action eutrophique et rajeunissante *In: 4th Congress of the International Association of Gerontology.* Tito Mattioli, Fidenza, 1957, Vol. II, pp. 468-478. (b) See also: *Gior. Geront., 6:* 246-270, 1958.

Aslan, A.: Procaine therapy in old age and other disorders. (Novocain-factor H3). *Gerontologia Clin., 2:* 148-176, 1960.

Aslan, A.: Prophylaxie du vieillissement précoce. *In: La Thérapeutique de L'asthénie et de la Sénescence Prématurée chez les Anciens Deportés et Résistants.* Ed. de la Fédération Internationale des Résistants, Vienna II, 1961.

Aslan, A.: The present stage of procain therapy in geriatrics. Summary *in:* E. Beregi (Editor), *Conference with International Character of the Hungarian Gerontologists in Budapest, Oct. 25-27, 1962.* Ianka Gyula igazgato, Budapest, 1962, No. 159.

Aslan, A., and S. Câmpeanu: Die Wirkung von Novocain und p-Amino-benzoesäure auf Sauerstoff-Verbrauch der Bierhefe. *Arzneimitt. Forsch., 8:* 116-120, 1958.

Aslan, A., C. David, and S. Câmpeanu: (Metabolic effects of procaine therapy). *Fiziol. norm. patol., 9:* 321-330, 1963.

Aslan, A., M. L. Nedler, and L. Todea: (The effects of procaine on experimental arthritis induced in white rats.) *Com. Acad. Rep. Pop. Romine, 1:* 1111-1116, 1951.

Aslan, A., A. Vrăbiescu, and L. Câmpeanu: (Morphological changes in rats in induced neurodystrophia. The influence of Gerovital H3 treatment.) *Fiziol. norm. patol., 8:* 21-27, 1962.

Berger, P.: Les effets du traitement chronique à la procaine sur la sénescence du rat blanc. *Path. biol. (Paris), 8:* 1163-1166, 1960.

Parhon, C. I., and A. Aslan: *Novocain factor eutrófico y rejuvenecedor.* Ed. N.B.P., Buenos Aires, 1957, 98 pp.

Pearse, A. G. E.: *Histochemistry; theoretical and applied.* J. E. A. Churchill, London, 1960, 998 pp.

Verzár, F.: Note on the influence of procaine (Novocaine), PABA and Diethylethanolamin on the ageing of rats. *Gerontologia, 3:* 350-358, 1959.

Verzár, F., and J. McDougall: Learning and memory tests in young and old rats. *In:* 3rd Cong. Int. Assoc. Geront., London, 1954, *Old Age in the Modern World.* E. & S. Livingstone Ltd., London, 1955, pp. 247-259.