ACCELERATION OF GROWTH OF YOUNG RATS BY TREATMENT WITH METHYLATED DIHYDROXYPURINES

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An increase in the rate of growth (by 10-24.5%) was demonstrated in rats aged from 3 to 60 days after prolonged administration of caffeine and theobromine in doses of 0.01-0.04 mg/kg. If these substances were administered simultaneously with aurantin, the action of which is similar to that of actinomycin D, the rates of growth were reduced to 12-17% below those of control rats receiving caffeine or theobromine only. Rats receiving aurantin only showed a gain in weight which was 34-44% lower than that of the control rats. The results suggest that the acceleration of growth produced by administration of methylated hydroxypurines is due to their action on nucleic acid synthesis.

Previous investigation in the writers' laboratory showed that adrenergic blocking agents (chlorpromazine and reserpine), if administered daily to rats and puppies for 1-30 days, reduce the catecholamine concentration and the acetylcholinesterase activity in the brain, blood, and adrenals, depress the basal metabolism, and lower the intensity of growth [1, 5, 10-13].

Attempts to increase the rate of growth by activating the sympathicoadrenal system by application of nociceptive stimuli or by chronic administration of iproniazid proved ineffective because this system is functioning at the limit of its powers in the young animal [5, 11] and is quickly exhausted, and also because monoamine oxidase is not present in young rats under 14 days old [18].

Bearing in mind the results of stimulation of growth, development, and regeneration by the use of methylated purines [4, 7], it was decided to investigate the effect of caffeine and theobromine on growth in rats.

EXPERIMENTAL METHOD

Rats aged 3-7 to 30-60 days were used. In series I eight rats were left in each of five litters and distributed into two groups. The four experimental rats received a daily subcutaneous injection of caffeine in a dose of 0.01 mg/kg, except in one experiment (no. 1) in which a larger dose (1 mg/kg) was given. The control rats received an injection of the same volume of physiological saline. In series II the experiment began on the 7th day; the rats of four litters received caffeine or theobromine together with aurantin (batch 52) obtained from Planel'es' laboratory [4, 8] and with an action similar to that of actinomycin D, inhibiting the synthesis of mRNA [1-3, 15, 16, 19, 20]. Litter I was the control, and in litter II half of the rats received larger doses (70-100 μg/kg) of aurantin subcutaneously, while the other half received smaller doses (35-50 μg/kg). In litter III the action of caffeine was tested in a dose of 0.01-0.04 mg/kg, while in litter IV the rats received caffeine in the same dose together with aurantin [35-50 μg/kg). Animals of litter V received theobromine in a dose of 0.01-0.04 mg/kg, and the rats in litter VI received theobromine in the same doses together with aurantin (35-50 μg/kg). The doses were increased after the 24th day. The animals were weighed on the 7th, 14th, 21st, 30th, 45th, and 60th days. The growth constant was calculated by Shaml'gauzen's (1927) formula:

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TABLE 1. Changes in Weight of Rats Receiving Caffeine (M ± m)

Litter	Age at beginning of expt. (in days)	Group of animals	Weight (in g)							
			initial (7 days)		age of rats (in days)					
					12—14		20—26		28—33	
I	4	Experimental	10,50	0,30	18,6*	0,40	50,40	0,80	68,14	0,50
		Control	10,90*	0,20	18,01	0,35	45,40	0,65	62,25	0,60
II	3	Experimental	6 42*	0,15	21,39	0,20	51,32	0,70	67,95	0,42
		Control	6,40	0,09	19,36	0,40	44,41	0,53	56,69	1,12
III	6	Experimental	8,16*	0,20	19,10	0,80	35,50	0,50	— —	
		Control	8,66	0,31	15,50	0,20	30,80	0,45	— —	
IV	7	Experimental	11,50*	0,40	—	—	31,81	0,42	57,90	0,60
		Control	11,70	0,60	—	—	25,54	0,80	52,30	0,30
V	7	Experimental	9,86*	0,08	17 90*	0,30	36,30	0,60	61,60	0,90
		Control	10,08	0,10	17,80	0,20	31,90	0,60	49,70	0,70

*Differences between experimental and control animals not significant, in other cases significant ($P < 0.001$).

$$K = \frac{\lg v_2 - \lg v_1}{\lg t_2 - \lg t_1},$$

where V_1 and V_2 are the weights of the animals and t_1 and t_2 their ages.

EXPERIMENTAL RESULTS

The experiments of series I showed that caffeine produces a significant increase in the rate of growth in all cases, to an extent which varies, however, with the dose and duration of the experiment. A large dose of caffeine (1 mg/kg), administered to litter I, led to a smaller gain in weight (9.5-11% compared with the control) than the smaller dose of 0.01 mg/kg, which produced a much greater acceleration of growth in the experimental rats of litters II-V, namely by 20-24.5% (Table 1). From information in the literature on the stimulation of bacterial reproduction and of growth and regeneration in animals by caffeine and theobromine [4, 7, 9] it was considered that acceleration of the growth of rats by caffeine may be due to its action on nucleic acid synthesis. If this hypothesis is correct, aurantin, which blocks mRNA synthesis [1-3, 15-16], ought to prevent the stimulation of growth produced by caffeine and theobromine.

The experiments of series II showed that aurantin inhibits normal growth of young rats. Rats receiving the larger doses of aurantin (70-100 μg/kg) had gained in weight by 20.5 ± 0.8 g at the age of 21 days, which was 37.2% below the control level (32.6 ± 0.4; $P < 0.001$), while at the age of 30 days their gain in weight was 43.5% less (28.1 ± 0.9 and 49.7 ± 0.7 g respectively; $P < 0.001$); the growth constants were 0.56 and 1.04 respectively. A dose of 35 μg/kg led to a smaller retardation of growth by the 21st day, by 25.2% (24.4 ± 0.7 g). An increase in dose after the 24th day to 50 μg/kg increased the retardation in growth (33.1 ± 0.8 g) by comparison with the control at that age (49.7 ± 0.7 g), corresponding to growth constants of 0.65 and 1.04. By the age of 60 days the retardation of growth was smaller; the growth constant had risen to 0.918. These experiments showed that aurantin delays normal growth, especially during the 1st month of life.

The rats of litter III (series II), which received caffeine, weighed 55.1 ± 0.5 g on the 30th day of life, while the rats of litter IV, which recieved caffeine and aurantin, weighed 47.2 ± 0.3 g ($P < 0.001$). By the 60th day the first group of animals weighed 128.1 ± 0.5 g, and the second group 98.4 ± 0.7 g ($P < 0.001$). The retardation in growth on the 30th day was 14.3% and on the 60th day 23.3%. Almost the same retardation in growth by the 60th day of life was observed in rats receiving theobromine with aurantin (103.7 ± 0.7 g), by comparison with rats receiving theobromine only (127.6 ± 0.7 g; $P < 0.001$).

It can be concluded from a comparison of the results obtained with litters III-VI in the experiments of series II with the data for the control rats that aurantin not only prevents the increase in intensity of growth linked with the effect of caffeine and theobromine, but actually reduces it by comparison with the control. However, this decrease is less marked than if aurantin alone is used.

The delay in growth of the rats receiving aurantin, as well as the blocking of the acceleration of growth induced by methylated 2,6-dihydroxypurines (litters IV and VI in the experiments of series II confirm the hypothesis that the stimulation of growth by caffeine and theobromine is due to their influence on nucleic acid synthesis. The participation of methylated purines in nucleotide metabolism has been demonstrated [17, 20]. They have a weak mutagenic action and modify the activity of ribo- and deoxyribonucleotide phosphorylase [15], and purine antagonists induce developmental anomalies [21].

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