THE INFLUENCE OF INDUCING DRASTIC CHANGES IN BODY METABOLISM UPON THE GROWTH OF SARCOMA 180 [1]

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The present report is a summary of the effect upon the growth of sarcoma 180 of three substances (dinitrophenol, thyroxin, and so-called growth hormone), which affect the general metabolism in various ways. As measured by the effect upon caloric consumption and gain or loss in body weight, these substances produced in our experiments the following changes:

Dinitrophenol:	No change in caloric consumption
	15 per cent decrease in body weight.
Thyroxin:	44 per cent increase in caloric consumption
	10 per cent increase in body weight growth.
Growth hormone:	10–20 per cent increase in caloric consumption
	10 per cent increase in body weight.

No marked changes in the behavior of sarcoma 180 were noted under these profound disturbances of the general metabolism, the greatest effect produced being a 40 per cent weight increase in tumor growth.

The technic of inoculation, the control of food consumption, and the method of recording tumor data were the same as previously described (1–3) with the exception of three experiments, two dealing with the growth hormone and one with thyroxin. In these, matched tumors arising from the same inoculation were paired and the experiment begun at the time of pairing. This procedure decreases the experimental error.

DINITROPHENOL

Toxicity: The lethal dose of dinitrophenol for mice lies between 40 and 50 mg. per kilo, when given subcutaneously as a single dose. Eight out of 8 mice survived 35 mg., 5 out of 16 mice survived 40 mg., 2 out of 9 mice survived 50 mg. When sublethal doses were given at hourly intervals a larger amount could be given during a day. Groups of mice were given hourly subcutaneous doses of 0.4 to 0.6 mg. until a maximum of 2.4 mg. was given. This represented 120 mg. per kilo, or 2.4 times the lethal dose when given in a single dose. One out of 5 mice died on this dose. Fourteen out of 15 mice survived daily subcutaneous doses of 90 mg., given at two-hour intervals.

Terada and Tainter (4) found that rats could tolerate approximately six times as much dinitrophenol administered by mouth as when given subcutaneously. To study oral administration, 0.1 per cent dinitrophenol was mixed with the calf meal which the mice received regularly. As the animals became accustomed to the taste, their food intake increased and the percentage

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of dinitrophenol was raised to 0.2. At the end of the experiment they were consuming 340 mg. dinitrophenol per kilo per day and were in good condition. Their food consumption was as great as that of the controls, but their weight remained practically stationary while the controls made their usual gain. A group of 8 mice received increasing amounts of dinitrophenol from 0.1 to 0.35 per cent of their diet until they died. Paralysis developed first in one hind leg, then in the other. Death usually came within twenty-four hours after the first symptoms of paralysis. The amount of food consumed remained high. Another group of 6 mice received 0.3 per cent dinitrophenol in calf meal. After a preliminary period, when food consumption was low,

TABLE I: *Effect of Dinitrophenol*

	Body Weight Changes in Grams				Food Intake in Grams per Mouse					Mgm. D.N.P. per Kg. per Day	Tumor Growth After Inoculation	
	1-5 days	6-10 days	11-14 days	15-19 days	1-5 days	6-10 days	11-14 days	15-18 days	Total food intake		15 days	20 days
Controls	+1.4	+1.4	+2.6		4.3	3.9	3.3		54		12.4±1.0	
Mice receiving dinitrophenol, divided dose subcutaneously	+0.7	+0.6	+0.1		4.1	3.8	3.8		55	90	10.6±1.0	
Controls	+2.4	+3.0	+3.1	+3.0	4.2	3.6	3.2	3.3	65		12.0±0.9	13.5±1.1
Mice receiving dinitrophenol with food, 0.1 per cent −0.2 per cent	+1.1	+0.5	−0.2	−0.3	3.5	3.8	3.6	3.4	65	340	11.2±0.07	14.2±0.7

they were eating more than the controls, consuming 780 mg. dinitrophenol per kg. per day. When paralysis appeared the mice were killed and sections made of heart, kidney, and liver. No pathological changes were noted.

Food Consumption, Weight Changes, and Tumor Growth: Group 1 received dinitrophenol subcutaneously. The food consumption was practically the same as that of the controls, though the body weight remained very nearly stationary. In Group 2, dinitrophenol was added to the food, in increasing amount from 0.1 to 0.2 per cent. Again the food intake was about the same as that of the controls, with the body weight remaining very nearly stationary.

Both groups of mice were inoculated with sarcoma 180 the day that the dinitrophenol was first given. The tumor was measured on the 10th, 15th, and 20th day. There was no significant change in the growth of the tumor when dinitrophenol was given, whether subcutaneously or by mouth, though a slight effect in the early stages was suggested. A larger series of animals would be necessary to establish this point and another type of tumor might well be used. The data are given in Table I.

GROWTH HORMONE

The growth hormone preparation of Squibb & Sons was used in all experiments and consisted of different lots which were of varying age. This preparation is of a higher degree of purity than the preparations originally used by us in 1932 (1) and possibly lacks physiologically active fractions present in the cruder preparations (suggested by a recent comment of Emge and Murphy, 5). The Squibb preparation is not toxic to mice as was the cruder preparation. In the four inoculation series recorded, growth hormone was

administered from the day of inoculation in two series, and from the tenth day of tumor life (matched tumors) in the other two series. Significant effects were produced in two series, a 40 per cent increase in tumor weight over the controls (Gardner reports a 30 per cent increase for carcinoma 256). In two series the 10 per cent weight increase of tumors paralleled the 10 per cent increase in body weight. A 10 per cent increase in body weight, however, is significant, while a 10 per cent increase in tumor weight is within once the standard deviation of the mean. It will be noted that old mice (30 gm.) were slightly more receptive to the action of the growth hormone than young 20 gm. mice. (Note that the standard deviation of the mean is not used in series comprising matched tumors.) The data are given in Table II.

TABLE II: *Influence of Growth Hormone (Squibb) Upon Sarcoma 180*

	Body Weight Changes in Grams					Food Intake in Grams per Mouse						Tumor Diameter		
	1–5 days	6–10 days	11–15 days	16–20 days	21–25 days	1–5 days	6–10 days	11–15 days	16–20 days	21–25 days	Total food consumed	15th day	20th day	25th day
Control	+0.7	+1.5	+1.7			4.0	4.0	3.8			55	9.4±1.1	12.6±0.9	
Mice receiving growth hormone, 0.1 c.c. per day	+1.3	+2.0	+3.2			4.5	4.4	4.0			60	11.5±0.9	15.5±0.8	
Control	+1.5	+1.5	+1.7			4.2	4.0	3.7			60	13.7±0.9		
Mice receiving growth hormone, 0.4 c.c. per day	+1.5	+2.9	+3.8			4.6	4.6	4.5			68	14.4±0.9		
Control			+0.5	+2.1	+2.4			4.1	4.1	4.0	61	6.2	9.1	12.0
Mice receiving growth hormone 0.3 c.c. per day (divided)			−0.2	+2.6	+4.1			3.6	4.8	5.0	67	7.0	11.1	14.6
Control (young)			+1.8	+1.8	+2.8			4.5	4.6	4.5	68	9.9	12.1	16.1
Control (old)			+1.3	+0.7	+0.7			4.4	4.3	4.1	64	8.2	10.9	14.4
Mice receiving growth hormone (old), 0.3 c.c. per day divided			+1.1	+1.6	+3.4			4.4	5.2	5.8	77	10.1	11.9	15.9

THYROXIN

The experiment on the effect of thyroxin differs from those previously reported by us (2) only in the diet of the mice, which instead of calf meal received a mixture of 1 part calf meal and 1 part starch. This procedure reduces the protein content of the diet by one-half. It was believed that this procedure might produce results similar to those reported by Gilroy (6) for thyroxin. No significant effect upon tumor growth was noted, in spite of the fact that caloric consumption was increased 44 per cent. See Table III.

In another experiment thyroxin treated mice were allotted food equivalent to the caloric consumption of their respective controls. In this régime the treated mice lost 7.8 gm. in body weight per mouse as compared with the controls. Three out of ten mice died. A significant retardation of tumor growth was noted the thirtieth day of tumor life.

DISCUSSION

The experiments with dinitrophenol were so planned and executed that the maximum amounts of dinitrophenol compatible with life were administered. The effect upon general metabolism may be assumed to be slight. A

TABLE III: *Influence of Thyroxin and a Low Protein Diet Upon the Growth of Sarcoma 180*

	Diet	Food Intake in Grams per Mouse per Day			Total	Body Wt. Changes			Tumor Diameter 15th day
		1–5 days	6–10 days	11–15 days		1–5 days	6–10 days	11–15 days	
Control	19% protein 54% carb.	4.2	4.2	3.5	60	+1.2	+2.4	+2.6	11.5±1.0
Control	9.5% protein 77% carb.	3.5	3.4	3.0	50	+1.3	+2.3	+2.5	12.3±1.4
Mice receiving thyroxin 0.2 mg. per day	9.5% protein 77% carb.	3.7	5.4	5.3	72	+1.1	+3.0	+4.0	11.2±1.0

similar body weight loss may be brought about by a 15 per cent decrease in caloric intake, a procedure which is without effect upon tumor growth. However interesting studies of the effect of dinitrophenol on tissue cultures or excised tissues may be, our experiment would indicate that in the mouse, at least, the transplanted neoplasm is not greatly affected by amounts which tax the organism to the limit. The results do not warrant further study.

The thyroxin experiment is interesting not only because of the effect upon body weight similar to that produced by growth hormone, but also the increase in metabolism. The body effects of the growth hormone cannot, therefore, be attributed solely to the thyrotropic factor.

A summary of all our data for the effect of the growth hormone upon normal mice bearing sarcoma 180, including both data for the cruder preparations and the later Squibb preparation, indicates that the tumor growth is not greatly accelerated over the body growth. Considering that tumor growth normally is proceeding at a much greater rate than body growth even in young mice, we must conclude that, as far as specificity is concerned, the body in the normal mouse is as sensitive as the tumor to growth hormone. It is also true that whereas the body ceases to grow in the hypophysectomized animals the neoplasm continues to grow but at a decreased rate. Ball and Samuels' (10) contention that the neoplasm is more sensitive to the pituitary secretion is not borne out by these observations.

Our results on the growth hormone are therefore in accord with those of Sugiura and Benedict (7), Gardner (8), and Reiss, Druckrey and Hochwald (9). A dissenting voice is that of Emge and Murphy (5), who worked with a growth hormone preparation which "was not standardized" but nevertheless "was found to be very active." Their data later show that the amounts used caused little or no effect upon body growth in normal animals and an effect upon body growth in only a few of the hypophysectomized animals. Only 3 to 6 animals were used in the hypophysectomized groups. We took the liberty of calculating a standard deviation for a control group in which there were 7 tumors and were amazed to find that the normal variation was so

great that 100 per cent change would be necessary to establish significance. The experimental error of Emge and Murphy was therefore greater than any effect ever reported for the growth hormone. This, in conjunction with the fact that they have little evidence of any activity in their extracts, invalidates, in our opinion, any deductions they have made. It is possible that the growth hormone does not effect the growth of the Emge and Murphy sarcoma but acceptable evidence one way or another has not been produced. McEuen and Thomson (10) also failed to note any effect of the growth hormone (upon carcinoma 256) in normal rats. These authors were, however, well aware that the amounts administered which were adequate as replacement therapy in hypophysectomized rats might not have been effective in the normal animals.

Summary

(1) The lethal dose of dinitrophenol for mice lies between 40 and 50 mg. per kilo when given subcutaneously as a single dose, over 120 mg. per kilo when given subcutaneously in divided dosage, and over 340 mg. per kilo when given orally.

In two inoculation series (mouse sarcoma 180) comprising 60 animals, dinitrophenol was given in sublethal doses, orally in one experiment and in divided subcutaneous doses in the other experiment. A 15 per cent decrease in body weight, isocaloric consumption, and no significant effect upon tumor growth, as compared with controls were noted.

(2) Daily subcutaneous doses of 0.2 mg. thyroxin in mice on a 9.5 per cent protein diet produced a 44 per cent increase in caloric consumption, a 10 per cent increase in body weight, and no significant effect upon the growth of sarcoma 180. By limiting thyroxin-treated mice to the caloric consumption of their respective controls, marked body weight loss with decrease in tumor rate growth could be produced.

(3) The effect of subcutaneous injection of Squibb's anterior lobe extract (growth hormone) was tested in four series of approximately 120 mice bearing sarcoma 180. In all series a 10 per cent increase in body weight and a 10 to 20 per cent increase in caloric consumption were noted. Significant increases in tumor growth over controls were observed in two experiments, insignificant values in two other series. The maximum effect noted was 40 per cent increase in tumor weight over the controls.

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