Reports

Effect of Reserpine and Chlorpromazine on Sarcoma 37

It has been shown (*1*) that, when reserpine is administered to mice that are carrying the lymphoid tumor L1210, the mice become deeply depressed and remain so for 6 to 8 days. During this period they neither eat nor drink. Under these conditions, the local tumor frequently regresses and survival time is significantly increased.

Because of the unusual aspects of the response of this lymphoid tumor to reserpine, it was of interest to determine how a solid tumor, such as sarcoma 37, would respond following administration of reserpine.

Twenty-five CAF_1 hybrid mice, carrying 6-day-old intramuscular implants of sarcoma 37, were given doses of reserpine subcutaneously (50 mg/kg of body weight). A similar group served as untreated controls. During the course of an hour or more, the animals that received the drug became deeply tranquilized and remained so for 5 to 6 days, by which time practically all were dead, presumably from inanition and dehydration, and perhaps from some specific effect of the drug itself.

During their depressed state, the mice kept their eyes closed and burrowed their heads in the shavings. In addition, a lowering of body temperature was observed; the mice were decidedly cool to the touch, and their urine, voided when they were handled, felt cold. Rectal temperatures were below 94°F (the lowest calibration of the thermometers used), whereas the rectal temperatures of mice bearing 6-day intramuscular implants averaged 97°F, and the temperatures of normal, nontumor-bearing mice averaged 100°F. All treated mice lost considerable weight, as was determined by making carcass measurements at death.

On the day the drug was injected, and every third day until the end of the experiment, the tumors were measured with calipers along their three diameters. The average of these measurements was used to determine the volume of the tumor, calculated as a sphere.

As can be seen from a typical experiment shown in Table 1, tumor growth in the animals that were treated with reserpine ceased at once. The tumors in untreated animals continued to grow at a normal rate, so that at the end of 6 days their average volume was more than 3 times that of the tumors in the treated mice.

To determine whether this effect was limited to some specific action of reserpine, or was merely a consequence of a prolonged state of depression regardless of how induced, other depressant drugs presumably acting by a different mechanism were tested—for example, phenobarbital, urethane, chloral hydrate, Doriden, and chlorpromazine.

Chlorpromazine placed the animals into a deeply tranquil state comparable to that following the administration of reserpine. In Table 1 are given the results of a typical experiment in which, at the end of 5 days, the tumors in the untreated animals were also about 3 times the volume of those borne by treated mice.

To ascertain whether any direct cellular effect of these two drugs contributed to the marked inhibition of tumor growth, mice tranquilized by each drug were sacrificed at the end of 6 days, and sections of the tumors were fixed in Zenker-formol and stained with hematoxylin and eosin.

Although it is difficult, because of spontaneous necrosis, to assess damage in advanced tumors (12 days old) a distinct impression was obtained that more histologic damage occurred in the treated than in the control groups. As a further check, mice with 6-day tumors were given reserpine or chlorpromazine and sacrificed 3 days later. Again the impression was that damage in the treated tumors was more extensive than in the controls.

Sarcoma 37 grown as an ascites tumor was also used. Groups of mice carrying 3-day-old ascites tumors were given doses of reserpine or chlorpromazine intraperitoneally to maintain tranquillity for several days. Other mice served as untreated controls. Every day for 4 days, samples of ascitic cells were drawn from all three groups and examined in the fresh state and also as smears which were fixed by air-drying and immersion in methanol and then staining with Giemsa.

Except for an increase in size and number of cytoplasmic vacuoles, the Giemsa preparations from both treated groups showed no cytotoxic effects. Cells in all stages of mitosis were evident.

Beginning at 48 hours after treatment, fresh smears from mice of both reserpine- and chlorpromazine-treated groups showed a progressive increase in the number and size of yellowish, refringent granules (seen as vacuoles in the alcohol-treated Giemsa smears). These stained deeply with Oil-Red-O, indicating the presence of neutral fat. Negative results with periodic acid-Schiff treatment indicated they were not polysaccharide. Cells from untreated mice did not exhibit such granules (*2*).

The induction of a deeply tranquilized state by reserpine and chlorpromazine was accompanied by an immediate ces-

Table 1. Effect of reserpine (*3*) and chlorpromazine (*4*) on growth of sarcoma 37 in CAF_1 mice. The drugs were administered in the vehicle provided with each. Neither vehicle had any effect on the growth of sarcoma 37. The figures in parentheses indicate the number of surviving mice.

Treatment	Dose (mg/kg)	Average tumor volume (mm^3)		
		Day treatment was begun	3 days	5–6 days*
Reserpine	50	1262 (25)	1129 (22)	1118 (11)
Controls (untreated)		1080 (25)	2413 (25)	3797 (25)
Chlorpromazine	50†	921 (28)	944 (26)	1040 (8)
Chlorpromazine	25†	899 (25)	876 (25)	854 (5)
Controls (untreated)		992 (25)	1899 (25)	2739 (25)

* The experiments with reserpine were terminated 6 days after the first treatment, those with chlorpromazine 5 days after treatment.
† A supplementary dose of 25 mg/kg was given 2 days after the first dose.

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sation of the growth of sarcoma 37. This phenomenon is striking; whether or not the concomitant tumor necrosis was the result of a specific action of the drugs used cannot now be answered. The effects noted may also be explained as the result of the prolonged depressed state with accompanying inanition, dehydration, changes in metabolism, lowering of body temperature, and a drop in blood pressure with consequent hypoxia. All these are factors which by themselves can produce marked tumor damage and can slow tumor growth.

The appearance of fat granules in the ascitic cells similarly may be the result of the depressed state of the host. Unlike the controls in which the ascites daily increased in volume, the ascitic fluid in treated mice was very scanty, thick and viscous, but rich in cells. As a result of the progressive dehydration and other changes, the metabolic state of the ascitic cells could well have been affected, and the fat granules (which many would call "degeneration" granules) could be the consequence of the changes.

Whether the action of these drugs is specific or is mediated through the host, it is suggested that these drugs provide an additional means for study of the host-tumor relationship, particularly in conjunction with other tumor-necrotizing drugs.

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References and Notes

1. A. Goldin *et al.*, *Science* 125, 156 (1957).
2. We wish to thank Ira Kline for preparation of the material that was treated with the special stains used in this investigation.
3. Serpasil (Ciba).
4. Thorazine (Smith, Kline and French).

26 November 1957

Effect of Gibberellic Acid on Growth of Maize Roots

Gibberellic acid has been shown to stimulate markedly stem and leaf elongation in a number of plants (*1–4*). The specific genetic constitution of a strain or variety appears to determine whether or not it will respond to applied gibberellic acid by increased shoot growth. So far the most notable responses have been observed in dwarf types. Phinney (*2*) has reported that applications of gibberellic acid to five single-gene, dwarf mutants in maize so enhanced growth that the treated plants were almost indistinguishable from plants carrying the normal alleles of the mutant genes. One other dwarf mutant made only a slight response, and another made no response. Such differential responses of shoot growth have also been reported in varieties of *Pisum, Phaseolus,* and *Vicia* containing dwarfism alleles (*1*). To date, the only experiments on root growth reported are those of Brian, Hemming, and Radley (*3*), who found that gibberellic acid had no significant effect on the growth of roots of cress seedlings.

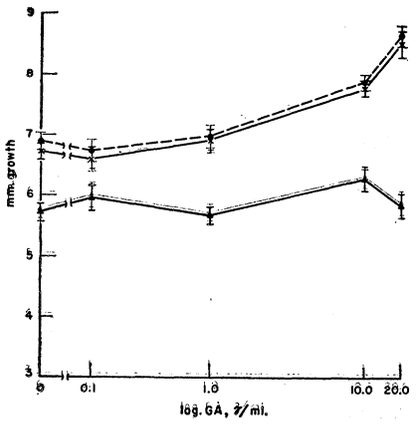

Fig. 1. Growth of excised, apical, 10-mm segments of primary roots of two maize inbreds and their hybrid as affected by gibberellic acid. The limits indicated at each graph point represent ± 2 times the standard error; ×, hybrid; ●, line 854, ▲, line 857.

In the work reported here, gibberellic acid (*5*) was added to White's supplemented solution (*6*) in which excised, apical, 10-mm segments of maize roots were grown for the 24-hour period representing the sixth to seventh day after the beginning of germination. Apical segments of both primary and adventitious seminal roots of two inbred lines of maize and their distinctly heterotic hybrid, bearing our laboratory numbers 854, 857, and 854 × 857, were used. Neither inbred line contains any dwarfism alleles, and other studies have shown that the growth rates of the inbreds beyond the very early seedling stage are comparable to the growth rate of the hybrid. The effects of gibberellic acid on growth of excised apical segments of primary roots are shown in Fig. 1. The points on the graph for 0.1, 1.0, and 10.0 μg/ml represent means of three or four replicates of at least ten roots each. The smallest number of roots represented is 35, the largest 93. The points for 20.0 μg/ml represent only single tests with 16 to 40 roots. The supply of gibberellic acid was too limited to permit repetition of the 20 μg/ml tests. The primary roots of line 857 were not affected by gibberellic acid over the range of concentrations used. Those of line 854 were significantly stimulated by concentrations of 10 μg/ml and further stimulated by 20 μg/ml. At 20 μg/ml, growth was increased on the order of 24 percent. The effect on the primary roots of the hybrid appears to be identical with that upon the roots of 854. The curves suggest that the primary roots of 854 and the hybrid might be further stimulated by higher concentrations.

The growth of the adventitious seminal roots was affected by gibberellic acid in the same manner as the growth of the primary roots, but the amount of stimulation of 854 and the hybrid was proportionately less than that in the primary roots, about 12 percent at 20 μg/ml. The adventitious seminal roots are of later origin than the primary roots, and they normally grow somewhat less than the primary roots under the conditions and during the experimental period used here.

More extensive experiments with the effects of gibberellic acid have been carried out (*7*), and it may be noted that all our results indicate that the root growth in certain genotypes of maize is significantly stimulated by gibberellic acid. The results presented in this preliminary note, showing a positive growth response of one inbred, no response by the other inbred, and a hybrid response essentially parallel to that of the first inbred, suggest direct inheritance of a growth system which can be affected by gibberellic acid.

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References and Notes

1. P. W. Brian and H. G. Hemming, *Physiol. Plantarum* 8, 669 (1955).
2. B. O. Phinney, *Proc. Natl. Acad. Sci. U.S.* 42, 185 (1956).
3. P. W. Brian, H. G. Hemming, M. Radley, *Physiol. Plantarum* 8, 899 (1955).
4. B. O. Phinney, abstr., *Plant Physiol.* 31, Suppl. 20 (1956).
5. The gibberellic acid was kindly supplied by Curt Leben, Eli Lilly and Co., through Irwin Spear.
6. P. R. White, *A Handbook of Plant Tissue Culture* (Cattell, Lancaster, Pa., 1943).
7. A paper describing our more extensive experiments is in preparation.

5 November 1956

Radiocarbon Dates from Sandia Cave, Correction

Frank C. Hibben has reported in *Science* (*1*) that in 1948 the late Kirk Bryan submitted two samples of charcoal from Sandia Cave for radiocarbon age determination to W. F. Libby, who was then at the University of Chicago. Hibben reported that these samples came from fire hearths located in the Sandia level of the cave and that "From these two samples, tentative dates of 17,000-plus years ago and 20,000-plus years ago, respectively, were derived." There is no proof that these alleged dates were ever determined by radiocarbon analysis or that Bryan ever submitted any samples from Sandia Cave to any laboratory.