period necessary for activation of vegetative growth in the Elberta peach is 950 hours of temperature below 45°F (*4*).

Two-year-old Elberta peach trees which had "normally" dropped their leaves in the fall and had been exposed to temperatures below 45°F for a maximum of 164 hours were transplanted from the orchard to large cans and placed in a storage room kept at 65°F. United States weather records, taken in the area, were used in calculating the number of hours in which the temperature was below 45°F. Lights were on in the room from 8 A.M. to 5 P.M. daily, and the trees were watered as necessary.

On 23 February 1957, 95 days after the trees had been placed in the storage room, 14 trees were placed in the greenhouse for treatment with gibberellic acid (*5*). The following concentrations of gibberellic acid were used: 0, 50, 100, 200, 500, 1000, and 4000 ppm. Solutions of each concentration were sprayed on two trees. Fourteen days later, at the time of the second application, it was noted that a large percentage of the buds on the trees that had received the 1000- and 4000-ppm applications had grown and produced small green leaves. Trees that had been sprayed with the lower concentrations (0, 50, 100, 200, and 500 ppm) did not show any growth at that time. On 29 March, after the trees had received two applications of gibberellic acid (23 February and 8 March), the trees that had been sprayed with 1000 and 40000 ppm were growing rapidly. The trees that had received 200 and 500 ppm were growing some, but trees that had been sprayed with concentrations lower than 200 ppm were still dormant (Table 1). The growth response of trees that had received the same treatment was uniform.

In another experiment, three trees that had been exposed for 433 hours to temperatures below 45°F, nearly half of the number of hours necessary to break the rest period, were sprayed with 0, 100, and 200 ppm of gibberellic acid, respectively. The trees were sprayed four times at 10-day intervals. Four days after the first application it was noted that one or two buds on the tree that had received the 200 ppm spray were opening and exhibiting small leaves. Fifty days after the first application, the tree that had been treated with 200 ppm of gibberellic acid had an average of 14 inches of new terminal growth and large, "normal" green leaves. At the same time, buds on the tree that had been sprayed with 100 ppm had 3 to 4 inches of new growth, but there were still a few completely dormant buds. Two or three buds on the untreated control were just starting to "break" dormancy. Trees that have not been exposed to the necessary chilling period commonly have a few open terminal buds after a long period of favorable growing temperatures, but the growth is usually somewhat abnormal.

Table 1. Effect of gibberellic acid on the breaking of dormancy of buds of 2-year-old Elberta peach trees. The trees had been exposed to temperatures below 45°F for 164 hours before treatment. Data were recorded 29 March 1957, 36 days after the initial application of spray.

Gibberellic acid concn. (ppm)	Leaf buds growing (No.)	Leaf buds growing (%)	Av. growth per shoot (in.)
0	0	0	0
50	0	0	0
100	0	0	0
200	17	40	0.25
500	17	50	0.5
1000	28	85	2
4000	37	98	4

Table 2. Effect of gibberellic acid on breaking the rest period in dormant peach seeds. Seeds received approximately one-half of normal stratification period before treatment. Data were recorded 16 days after the gibberellic acid treatment.

Gibberellic acid concn. (ppm)	Germination (%)
0	30
20	50
100	80
200	70
500	40
1000	30

Tests were also conducted with peach seed that had received 35 days of stratification—that is, the seed were placed in a moist medium at a temperature near freezing. The standard time for stratification of peach seed is between 60 and 100 days at 41°F, according to Kains and McQuesten (*6*). In this experiment ten seeds were soaked for 24 hours in each of the following concentrations of gibberellic acid: 0, 20, 100, 200, 500, and 1000 ppm. The seeds were then planted in a flat of sand, and after 16 days the percentage germination was recorded (Table 2).

The percentage germination of seed that had been soaked in 100- or 200-ppm concentrations of gibberellic acid was greater than that of seed that had been soaked in other concentrations. Concentrations higher than 200 ppm may have been toxic for optimum germination and growth. Twenty days after the treated seed had been planted, the plants were measured. There was little root growth and no top growth of the plants grown from seed that had been treated with concentrations in excess of 200 ppm. The root and top growth of plants grown from seed that had been soaked in 20-ppm concentration was greater than that of the untreated controls but smaller than that of plants grown from seed that had been soaked in the 100-ppm concentration. Plants grown from seed that had been soaked in the 100- and 200-ppm treatments averaged the same length and weight, although there was considerable variation within a treatment. The plants grown from seed that had been treated with 100 ppm were much larger, averaging 48 percent more top growth than the untreated control plants. Three plants that had received the same treatment and that had the same amount of top growth were selected for comparison of their root systems. The roots of plants grown from seed that had been treated with 100- and 200-ppm concentrations of gibberellic acid were much larger than the roots of the untreated controls. Seed that had been soaked in the 100 ppm solutions produced plants with root systems of 56 percent greater length and 80 percent greater weight (on a fresh-weight basis) than did the untreated seed.

Gibberellic acid apparently activates the metabolic processes or nullifies the effect of an inhibitor of growth of young Elberta peach trees. Thus gibberellic acid "replaced" the cold requirement for the breaking of the rest period in the young trees. If mature trees responded similarly, without a detrimental effect on other plant processes, southern peach growers would be able to grow varieties that required a longer chilling period. There also exists the possibility of extending the peach industry further south to new areas. Further investigations of the effect of gibberellic acid on the biochemical processes may help in understanding the rest period of plants.

CLIVE W. DONOHO, JR.
DAVID R. WALKER
Department of Horticulture, North Carolina State College, Raleigh

References and Notes

1. L. Rappaport, *California Agr.* 10, No. 12, 4 (1956).
2. A. Kahn *et al.*, *Science* 125, 601 (1957).
3. J. F. Harrington *et al.*, *Science* 125, 45 (1957).
4. J. H. Weinberger, *Proc. Am. Soc. Hort. Sci.* 67, 107 (1956).
5. Gibberellic acid was kindly supplied by Merck and Co., Inc., Rahway, N.J.
6. M. G. Kains and L. M. McQuesten, *Propagation of Plants* (Orange Judd, New York, 1950).

12 August 1957

Protection of Guinea Pigs Against Radiation Death by Cell-Free Mouse Spleen Extract

In previous studies it has been demonstrated that cell-free saline extracts of mouse spleens obtained from donor animals 6 days after their exposure to an LD 30/15 days contained a factor which protects mice against radiation-induced

Table 1. Summary of mortality data.

Time after exposure (days)	Expt. 1 (dose 550 r in air)		Expt. 2 (dose 600 r in air)		Expt. 1 + expt. 2	
	Animals alive (No.)	Mortality (%)	Animals alive (No.)	Mortality (%)	Total animals alive (No.)	Total mortality (%)
			A. Saline (0.4 ml)			
0	40	0	20	0	60	0
5	40	0	20	0	60	0
10	38	5	20	0	58	3
15	30	25	13	35	43	28
20	28	30	12	40	40	33
			B. Spleen (0.4 ml)			
0	30	0	30	0	60	0
5	30	0	30	0	60	0
10	30	0	30	0	60	0
15	26	13	28	7	54	10
20	26	13	28	7	54	10
			C. Spleen (0.2 ml)			
0	10	0	20	0	30	0
5	10	0	20	0	30	0
10	10	0	19	5	29	3
15	7	30	14	30	21	30
20	7	30	14	30	21	30

mortality (*1*). This factor was also found in the spleens obtained from unirradiated donor animals (*2*) but was missing in extracts prepared from the spleens that had been removed 6 days after exposure of the donor mice to an LD 55/15 days (*2*).

We have now been able to demonstrate that such cell-free saline extracts protect not only mice but also guinea pigs against radiation-induced mortality (*3*).

The preparation of the mouse spleen extracts was essentially the same as has been previously described (*2*). Spleens were removed as quickly as possible from unirradiated donor mice that had been killed by cervical dislocation. The organs were frozen on Dry Ice, ground in a mortar, and extracted for at least 24 hours with saline (1 ml per spleen) at refrigerator temperature. The extracts were made cell-free by centrifugation and filtration through both a Seitz and a Selas porcelain filter (*4*). All these procedures took place at low temperatures. The filtrate was lyophilized.

For injection, the lyophilized material was dissolved in sterile saline so that 1 ml corresponded to the contents of five spleens (spleen extract). Amounts of 0.2 and 0.4 ml were injected intramuscularly each day, starting on zero day shortly after irradiation of the guinea pigs, for five consecutive days.

The guinea pigs from a highly inbred Rockefeller strain, bred at the Naval Medical Research Institute, were exposed in a large animal irradiator (*5*) to dosages of 550 and 600 r in air of Co^{60} gamma radiation with a 4 π geometry in groups of ten (*6*). Mortality was observed over a period of 20 days and compared with that produced in animals that had been subjected to the same amount of irradiation and that had received 0.4 ml of saline. The results are presented in Table 1.

As may be seen in Table 1, with each of the two radiation dosages, administration of 0.4 ml of spleen extract caused a reduction in radiation-induced mortality, while the injection of 0.2 ml of extract resulted in a mortality corresponding to that of the saline-injected control animals.

A statistical analysis of these data by chi square was made (*7*); this established the fact that the difference between group *A* (saline) and group *B* [spleen (0.4 ml)] was statistically not significant in experiment 1 (χ^2, 0.8203) but was significant in experiment 2 (χ^2, 4.7482). Since the statistical analysis also proved that neither the saline controls nor the spleen groups in experiments 1 and 2 were significantly different from each other (χ^2, 0.256 and 0.186, respectively), a comparison of the combined mortality data for the two experiments for groups *A* and *B* was permissible. The combined data demonstrated a protective effect of spleen extract (0.4 ml), with a satisfactory statistical significance (χ^2, 5.3788; *P*, 0.05 to 0.02).

The favorable effect of the spleen extract on mortality was also reflected in the weight curves (*8*).

As far as we know, the foregoing data represent the first observations of protection against radiation-induced mortality with a cell-free total spleen extract obtained from heterologous material (*9*).

The importance of these observations consists in the following: (i) They give strong support to the theory that a humoral factor or factors of spleen (*10*) is the essential agent, in the protection against radiation-induced mortality, of spleen shielding, spleen transplantation, or spleen homogenates. (ii) They indicate that this agent is not a species-specific factor. (iii) They offer, therefore, a justified hope for the eventual identification and isolation of the protective factor.

Investigations along these lines are in progress.

F. ELLINGER

Pharmacology Division,
Naval Medical Research Institute,
Bethesda, Maryland

References and Notes

1. F. Ellinger, *Radiol. Clin.* 23, 299 (1954).
2. ———, *Proc. Soc. Exptl. Biol. Med.* 92, 670 (1956).
3. This is a preliminary report. The opinions or assertions contained herein are my personal ones and are not to be construed as being official or as reflecting the views of the U.S. Department of the Navy or of the Naval service at large. The technical assistance of personnel of the Radiation Technology Division, Naval Medical Research Institute, and of B. F. Lindsley, E. L. Silvers, E. L. Schloegel, and R. H. Crutcher, is acknowledged, with thanks.
4. To prove that the filtration method used to make the extracts cell-free truly removed all cellular material, the following tests were performed. A pure strain of A.T.C.C. No. 4157 of *Escherichia coli* in brain-heart infusion broth was filtered after 48 hours' incubation, first through a Seitz, and then through a Selas, microporous filter (No. 03 porosity) under pressure. The filtrate was then streaked out on a blood-agar plate, and, in addition, a brain-heart infusion broth was inoculated. Both samples were found to be sterile at the end of a 72-hour incubation period.
5. R. H. Draeger *et al., U.S. Naval Med. Research Inst. Research Rept. NM 006 012.04.64* (1953).
6. F. Ellinger, E. P. Cook, J. E. Morgan, *U.S. Naval Med. Research Inst. Rept. NM 620200, Subtask I, Rept. 1* (1957).
7. My co-workers and I are greatly indebted to J. Z. Hearon for the analysis of our data.
8. A description of the details is in preparation.
9. A. Goldfeder and G. E. Clarke [*Radiation Research* 6, 318 (1957)] described some protection of irradiated mice with protein-free extracts from calf spleen. They could not, however, fully substantiate their observations in two repeated experiments.
10. L. O. Jacobson, *Cancer Research* 12, 315 (1952).

11 September 1957

Dietary Calcium Levels and Retention of Radiostrontium in the Growing Rat

The contamination of dietary sources of calcium with radiostrontium has led to a consideration of methods by which the absorption and retention of dietary radiostrontium can be reduced. The data reported here do not imply that remedial measures are now necessary and do not evaluate the practical application of such methods. Previous work has been mainly