Table 1. Effect of age of the guinea pig on the degree of contact skin reactivity to pentadecylcatechol. The quantity of this compound in column 1 is the smallest amount that was effective. The mean end points (in micrograms) for the three age groups were: 2 mo, 1.1; 6 mo, 2.3; 2 to 3 yr, 2.7. Probabilities that mean end points are identical were: 2 mo vs. 6 mo, < 0.001; 2 mo vs. 2 to 3 yr, < 0.001; 6 mo vs. 2 to 3 yr = 0.22.

Pentadecylcatechol-reacting (μg)	No. of guinea pigs		
	2 mo	6 mo	2 to 3 yr
0.5	3	0	0
1.0	16	1	1
2.0	4	6	5
N*	0	2	4

* Negative: animals that showed no reaction at the 2-μg level. To calculate the mean end point this value was assumed to be 4 μg.

from a common population, it was found that the reactivity of the guinea pigs in the older age groups was not statistically different but that the animals of both these groups were significantly less reactive than the 2-month-old animals.

The antibody titers determined from the tanned cell agglutination test are presented in Table 2. The highest titers were found in the sera of 2- and 6-month-old animals, while the 2- to 3-year-old animals gave the lowest titers. Calculation revealed that the mean titers of the two younger groups of animals were probably not different, but that both are significantly different from those of the 2- to 3-year-old animals.

Thus, guinea pigs that are 2 to 3 years old show a lower immunological response to both delayed contact skin sensitivity and to antibody production than do 2-month-old guinea pigs. The 6-month-old animals appear to be transitional in reactivity and show a contact sensitivity similar to that of 2- to 3-year-old animals but give a serum antibody response similar to that of the 2-month-old animals.

Table 2. Effect of age of guinea pig on the titer of antibovine γ-globulin. Geometric mean titers for the three age groups were: 2 mo, 50,200; 6 mo, 54,800; 2 to 3 yr, 24,100. Probabilities that mean titers are identical (calculated from $\log_2$ of titer) were: 2 mo vs. 6 mo = 0.4; 2 mo vs. 2 to 3 yr, <0.001; 6 mo vs. 2 to 3 yr = 0.01.

Maximum serum titer $\times 10^3$*	No. of guinea pigs		
	2 mo	6 mo	2 to 3 yr
8	0	0	0
16	3	2	3
32	6	2	5
64	10	2	1
128	4	2	0
256	0	1	0

* Titers are the reciprocal of the serum dilution.

Other workers (*1–7*) have also observed that old humans and old animals respond to an immunologic stimulus, but the limited data seem to show no unanimity relative to the effect of advancing age. For example, Thomsen (*5*) observed that the injection of horse serum resulted in a reduced anaphylactic sensitivity in old guinea pigs compared with sensitivity in young adults, and Baumgartner (*6*) found that rabbits over 2 years of age produced a lower agglutinin titer to *Bacillus enteritidis* than 6- to 13-month-old animals. However, the response to typhoid vaccine in humans did not differ in individuals 15 to 78 years of age (*7*), and the antibody response in chickens (*4*) did not seem to be affected by age. These results may be due to the different species, but it has been suggested (*4*) that one of the most important variables is the genetic constitution. That the genetic make-up of animals may, in fact, markedly affect antibody response is known (*13*).

In our study, the effects of environment, sex, and genetic constitution have been largely eliminated and the age of the animals, therefore, was the main variable. Whether the results have general application cannot be unequivocally established. However, strain 13 guinea pigs are immunologically competent and have been used by others to study delayed hypersensitivity to tuberculin (*14*) and circulating antibody (*15*). P. R. B. McMaster found no difference in the antibody response of strain 13 and Hartley guinea pigs to thyroglobulin (*16*). Furthermore, the degree of sensitivity to pentadecylcatechol, as measured by the minimal quantity inducing a reaction on the skin, is approximately the same in 2- to 4-month-old Hartley strain guinea pigs (*17*) as in the strain 13 animals reported here.

Little can be said about the reason for the reduced response of old animals. However, there is some evidence that the antibody-producing tissue of old animals does not function as effectively as in younger animals (*3*). Furthermore, it was our impression that after the injection of antigen in adjuvant, the lymph nodes of the 2-month-old guinea pigs showed a much greater enlargement than those of the 2- to 3-year-old animals (*18*).

HAROLD BAER
ROBERT T. BOWSER
Division of Biologics Standards, National Institutes of Health, Bethesda 14, Maryland

References and Notes

1. E. V. Cowdry, *Problems of Ageing* (Williams and Wilkins, ed. 2, Baltimore, Md., 1942), pp. 131–134.
2. N. W. Shock, *A Classified Bibliography of Gerontology and Geriatrics*, suppl. 1, 1949–55 (Stanford Univ. Press, Stanford, Calif., 1957), p. 186.
3. T. Makinodan and W. J. Peterson, *Proc. Natl. Acad. Sci. U.S.* **48**, 234 (1962).
4. H. R. Wolfe, A. Mueller, J. Neess, C. Tempelis, *J. Immunol.* **79**, 142 (1957).
5. O. Thomsen, *Z. Immunitaetsforsch.* **26**, 213 (1917).
6. L. Baumgartner, *J. Immunol.* **27**, 407 (1934).
7. L. O. Brenner, S. O. Waife, M. G. Wohl, *J. Gerontol.* **6**, 229 (1951).
8. J. A. Bauer, *Ann. N.Y. Acad. Sci.* **73**, 663 (1958).
9. J. B. Rogers, *Anat. Record* **103**, 498 (1949).
10. J. E. Dunn, H. S. Mason, B. S. Smith, *J. Invest. Dermatol.* **6**, 323 (1945).
11. A. B. Stavitsky, *J. Immunol.* **72**, 360 (1954).
12. F. E. Croxton, *Elementary Statistics* (Dover, New York, 1953), p. 226.
13. M. A. Fink and V. A. Quinn, *J. Immunol.* **70**, 61 (1953).
14. J. A. Bauer and S. H. Stone, *ibid.* **86**, 177 (1961); S. H. Stone, *Intern. Arch. Allergy* **20**, 193 (1962).
15. P. R. B. McMaster, E. M. Lerner, E. D. Exum, *J. Exptl. Med.* **113**, 611 (1961).
16. P. R. B. McMaster, unpublished.
17. H. Baer, S. Srinivasan, R. T. Bowser, A. Karmen, *J. Allergy*, in press.
18. We thank Dr. Leon Sokoloff, National Institute of Arthritis and Metabolic Diseases, for suggesting the problem and supplying the 2- to 3-year-old animals, and Lederle Laboratories for the pentadecylcatechol. Dr. C. J. Maloney, division of Biologics Standards, National Institutes of Health, made the statistical analyses.

16 April 1963

Growth-Regulating Chemicals Persist in Plants: Qualitative Bioassay

Abstract. *Scions from untreated tomato plants were grafted on plants treated with 2,4-dichlorophenoxyacetic acid by dipping one leaf in a solution containing 1000 parts per million. Subsequent malformations of the scion shoot and leaves were used as criteria of the presence of this chemical. The chemical or a substance causing similar symptoms persisted in the plant in physiologically active amounts for approximately 60 days.*

The literature concerning the length of time for which 2,4-dichlorophenoxyacetic acid (2, 4-D) may exert morphological effects in plants is very extensive (*1*). The bulk of the evidence indicates that morphological irregularities or distortions in response to the treatment are usually all established within a few days after the application of the chemical (*2*).

On the other hand, morphological irregularities which are brought about in buds may not become evident as long as a year after the application (*3*). Morphological irregularities in the

young developing seeds likewise may not appear until the next growing season when the seeds germinate (*4*). Nevertheless, most of the evidence at hand indicates that the irregularities induced by this chemical agent have been essentially established in these tissues soon after the application.

Furthermore, 2,4-D and 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP) are effective in preventing preharvest drop of apples from 30 to 50 days (*5*). In the citrus species, they provide effective control of abscission for as long as 7 months (*6*). It is not clear whether the growth regulators themselves persist in the plants for this length of time or whether the effects which they induce carry on after the chemicals are metabolized. These results suggest that these and related chemicals may persist in some plants for several months. Dosage, method of application, and growth rate may all affect persistence in the plant. Possibly the 2,4-D is bound to a relatively insoluble compound or compounds in the plant and is not readily extractable by ordinary chemical methods.

In our qualitative bioassay technique, meristems from untreated plants are introduced into the treated plant by grafting. Tomato plants (Washington Forcing variety) were used in most of the work. The experiment was repeated on four different occasions with from 4 to 10 plants per replication. Plants, 6 to 8 weeks old, were treated by dipping one leaf (fourth from base) into a solution containing 1000 parts of 2,4-D per million. The treated leaf was excised after 8 days. Buds or short branches from untreated tomato plants were then grafted to the treated plants in the second internode above the excised leaf. Grafting was done at various intervals up to 90 days after treatment. The new growth from buds grafted up to 2 months after treatment showed definite characteristic symptoms, such as discoloration, excessive enlargement of veins, and shortening and twisting of leaves (Fig. 1*B*). At the same time intervals, buds were grafted from untreated plants to untreated plants and from treated plants to untreated plants to make certain that aerial contamination was not taking place. Those buds grafted from the untreated plants to the untreated plants produced normal growth without any characteristic malformations throughout the experiment (Fig. 1*A*). Buds from treated plants grafted to untreated plants produced shoots with typical malformations. There was an apparent transfer of 2,4-D from the scion to the stock from those grafts made within the first week from treated to untreated plants as shown by slight epinastic responses on the stock. No such stimulus was apparent from later grafts.

Fig. 1. Growth of tomato scions on untreated stocks (*A*) and treated stocks (*B*), showing symptoms typical of 2,4-D damage on the scion growing on the treated plant. The scion was grafted 40 days after one leaf of the stock was dipped in a solution containing 1000 parts of 2,4-D per million.

Inasmuch as the scions grafted on the treated plants could receive the deforming agent only from one source, the stock, it is apparent that the chemical persisted in the treated plants for at least 60 days and probably longer. From 80 to 90 days after the stock plants were treated the new growth of shoots on the stock plants was normal in appearance. This suggests that the abnormalities produced by new growth in the treated plants was a good measure of the persistence of the chemical in the plant.

The concentration used is not an abnormally high dosage since it corresponds to applications which are made for herbicide use in the field. Also, in our experiments, it was applied to only one leaf for a limited time. It is, however, a greater amount than was applied by some of the other workers, which may help to explain the apparent discrepancy of the results.

Malformations typical of those caused by the applied chemical are taken as presumptive evidence that the chemical is present in an amount sufficient to cause a growth response. On the other hand, it is possible that the 2,4-D has been changed to another compound which causes similar symptoms. Nevertheless, our evidence points to a continuing effect from a single application to one leaf.

This method is advantageous because the plant makes the "analysis." It overcomes the difficulty of determining whether the abnormalities in growth after application were established prior to the development of shoots. By introducing a meristem from untreated plants, we have established a bioassay system intimately associated with the internal metabolic processes of the plant (*7*).

T. J. MUZIK
J. W. WHITWORTH*
Department of Agronomy,
Agricultural Experiment Station,
Washington State University, Pullman

References and Notes

1. A. C. Leopold, *Auxins and Plant Growth* (Univ. of California Press, Berkeley, 1960), pp. 193–195.
2. A. J. Eames, *Am. J. Botany* **38**, 777 (1951).
3. E. C. Tullis and W. C. Davis, *Science* **111**, 90 (1950).
4. A. M. S. Pridham, *Science* **105**, 412 (1947).
5. M. B. Hoffman and L. J. Edgerton, *Proc. Am. Soc. Hort. Sci.* **59**, 225 (1952).
6. W. S. Stewart and H. Z. Hield, *ibid.* **55**, 163 (1950).
7. Scientific paper No. 2335, Washington Agricultural Experiment Station, Pullman, project No. 1374.
* Present address: Department of Agronomy, New Mexico State University, University Park.
8 April 1963