going two seizures in a 4-day period (Phase A) and rats undergoing only one seizure 4 days before gland removal (Phase B). Having a seizure 12 hr before gland removal did not contribute to gland weight. Finally, it will be seen that the only significant interaction was the triple interaction among susceptible-nonsusceptible, ACTH-water, and the Phase A-Phase B variables. This calls for an intensive experimental analysis of the relatedness of these three sets of factors.

The initial problem set for this investigation was the determination of the effect of adrenal cortical stimulation on the AGS susceptibility of laboratory rats. This problem was attacked in Phase A of the present study, and results indicate that ACTH injections sufficient to increase the weight of the adrenal gland produce no reliable change in the AGS susceptibility of either susceptible or nonsusceptible rats.

It is of interest that the adrenal glands of the AGS-susceptible groups are heavier than those of the nonsusceptible groups. It is possible that rats undergoing AGS have larger glands as a result of the seizure. The seizures induced in these animals are characterized by severe explosive bouts of running, followed by tonic-clonic convulsions, and terminating in a state of catatonia. This tremendous, though short-lived, energy release may be sufficient to produce adrenal cortical hypertrophy. It will be recalled that the adrenal glands were removed from half the rats (Phase A) 12 hr after the second of two seizure tests (the first having been 4 days before the second), and from half the rats (Phase B) 4½ days after a single seizure test. If a seizure-induced adrenal cortical hypertrophy exists, it is doubtful if 12 hr, or even 4½ days would result in a complete return to normal adrenal cortical weight. On the other hand, if seizure-induced cortical hypertrophy does occur, there might be evidence of a summation of the effects of the two separate seizures. No such trend was found in these data. Examination of Tables 1 and 2 reveals no significant or suggestive signs of a summation of the two seizures. Absence of a summation effect following two seizures does not permit the assumption that the first seizure did not produce gland hypertrophy, however. If seizure behavior produces an increase in gland weight it may well be that this increase in weight reaches a limit after one seizure. Subsequent seizures might act as a similar physiological stimulant of the gland without producing further increments in gland weight. The possibility of seizure-induced cortical hypertrophy cannot be conclusively evaluated within this study. Future studies in this laboratory will be directed toward the question of whether larger adrenal glands precede or follow audiogenic seizures. If the latter is true it will be of biological interest that such a transient, though severe, bout of activity is sufficient to produce increased adrenal cortical weight. If future evidence indicates that AGS-susceptible rats have larger adrenal cortices than nonsusceptible rats, independent of seizure occurrence, it will be an interesting and provocative correlation of a biological and behavioral characteristic. Other studies have suggested a relationship between the adrenal cortex and convulsive seizure threshold, frequency, and severity. Griffiths (*3*) observed that adrenalectomy reduced either seizure incidence or severity in rats. Woodbury and Sayers (*4*) have shown that both ACTH and cortisone lower the electroconvulsive threshold in rats pretreated with desoxycorticosterone. These studies suggest the need for exploration of the possibility that seizure-susceptible rats have larger adrenal cortices than nonsusceptibles, independent of any immediate effect of the seizure.

References

1. Hoagland, R. *J. Comp. and Physiol. Psychol.*, **40**, 107 (1947).
2. Finger, F. W. *Psychol. Bull.*, **44**, 201 (1947).
3. Griffiths, W. J., Jr. *J. Comp. and Physiol. Psychol.*, **42**, 303 (1949).
4. Woodbury, D. M. and Sayers, G. *Proc. Soc. Exptl. Biol. Med.*, **75**, 398 (1950).

Manuscript received, August 8, 1952.

Nutritive Value of Rust-infected Leaves

C. E. Yarwood, Alice P. Hall, and Marjorie M. Nelson

Department of Plant Pathology, Department of Home Economics,* and *Institute of Experimental Biology, University of California, Berkeley

The habit of certain snails, slugs, beetles, and insect larvae of eating the rusted areas of bean, broad bean, or snapdragon leaves or stems in preference to the healthy portions of these plants suggested that rusted tissues might have unique nutritive properties. The reported salutary effect of rusted tissues on farm animals and man (*1*); the greater invasiveness of a powdery mildew (*2*), of several viruses (*3*), and of Fusaria (*4*), in rusted than in normal tissues; the collection of rust spores by bees (*5*); and the high carotene content of rusted leaves (*6*), all support this idea.

Microbiological assay (*7*) of primary pinto bean (*Phaseolus vulgaris*) leaves infected with rust (*Uromyces phaseoli*) revealed a higher pantothenic acid content in rusted than in healthy leaves (Fig. 1). The pantothenic acid content of inoculated leaves increased up to at least 12 days after inoculation, at which time it was about 10 times as great as that of normal leaves. Rust uredospores alone showed 45 γ pantothenic acid/g spores, and it might therefore seem that the pantothenic acid of rusted leaves was primarily localized in the rust mycelium and spores. No clear difference between healthy and rusted leaves with respect to thiamin, riboflavin, folic acid, or niacin content was detected in a preliminary test.

Snails (*Helix aspersa*) ranging from 0.3 to 8 g in weight were confined singly in pint jars or large Petri dishes with healthy bean leaves and/or with bean leaves inoculated 6–10 days previously with

rust. The leaves and snails were weighed before and after a 2-day confinement period. Most trials were in a light laboratory at about 20° C, but two trials at 13°, 19°, and 25° C showed greater gains per unit of food consumed at 13° than at 19° and greater gains at 19° than at 25° C. Small snails made greater relative gains than large ones. Of 20 snails confined with healthy and rusted leaves in the same dish, 3 ate from healthy leaves only, 9 ate from rusted leaves only, 3 ate from both healthy and rusted leaves, and 5 selected neither. Twenty-five snails confined with healthy leaves alone gained 12% of their original weight and 0.34 g for each gram fresh weight of leaves consumed, and in the same tests 25 snails confined with rusted leaves gained 15% of their original weight and 0.45 g for each gram of leaves consumed.

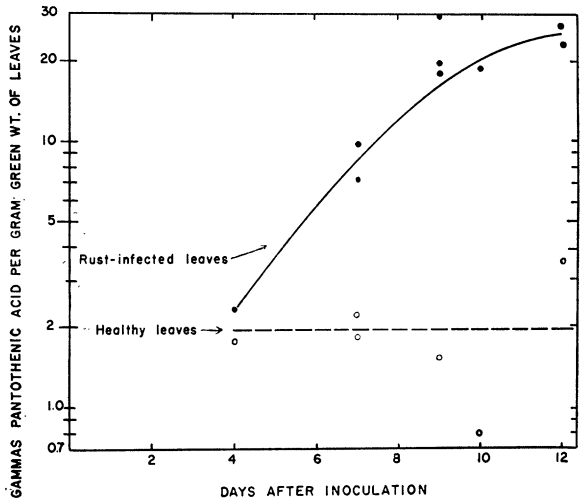

FIG. 1. The pantothenic acid content of healthy and rust-infected bean leaves at different times after inoculation.

In other trials bean leaves were sprayed with calcium pantothenate (CaP) solution to give about 1 mg CaP/g green weight of leaves. After the spray deposit had dried the CaP-sprayed and control leaves were offered separately to snails. The 13 control snails gained 7% of their original weight and 0.30 g/g of leaves consumed, whereas the 10 snails fed CaP-treated leaves gained 14% of their original weight, or 0.51 g/g leaves consumed.

Thirteen Diabrotica beetles confined for 3 days with both healthy and rusted leaves ate about 3 times as much leaf area from the rusted as from the healthy leaves, but in another test 5 Diabrotica beetles ate less from leaves bearing 1 mg CaP/g of leaves than from unsprayed leaves.

Ten rats (*Rattus norvegicus*) were maintained on a pantothenic acid–deficient diet (*8*) supplemented with biotin and folic acid for 39 days after weaning, for depletion of their vitamin stores. From these 10, 3 matched pairs of litter mates were selected. One of each pair was then given a daily supplement of 1 g dried healthy bean leaves, for a period of 18 days, and its litter mate was given a daily supplement of 1 g dried rusted bean leaves. The rats given the healthy bean leaves lost an average of 0.2 g/rat/day, whereas the rats given the rusted leaves gained an average of 5.3 g/rat/day. In contrast to this, rats on a protein-free diet, but fed dried rusted bean leaves *ad lib,* showed no significant difference in growth rate from rats given dried healthy leaves *ad lib.*

In nine trials in which CaP was added to a water suspension of crude tobacco mosaic virus (TMV) inoculum, the numbers of local virus lesions resulting on bean was consistently increased over the controls without CaP. The optimum concentration was not sharply defined but seemed to be about 1% CaP, which gave an average of 1.8 times as many lesions as the control.

When bean leaves were inoculated with TMV, detached, and incubated with the upper inoculated surfaces resting on water, very few if any virus lesions developed. This is another aspect of the inhibiting action of water in virus inoculations (*9*). When the leaves were placed with the inoculated surface on 3% CaP instead of water, large numbers of lesions developed. Apparently CaP offset the effect of water.

When young primary bean leaves from plants 7–10 days after seeding were inoculated with TMV on their upper surfaces, detached, and floated with their lower surfaces on 3% CaP in a light laboratory at about 20°C, the number of local virus lesions was commonly greater and the size of the lesions was consistently larger than for control leaves on water. In one representative test the assayed amount of virus in leaves incubated on 3% CaP was 7 times as great as for leaves incubated on water.

A similar or much greater increase in number and/or size of lesions and assayed amount of virus resulted when inoculated bean leaves were incubated on 0.001% $CuSO_4 \cdot 5H_2O$, 0.01% $ZnSO_4 \cdot 7H_2O$, or 0.00003% $AgNO_3$. This might seem to confirm with TMV on bean the apparent similarity in effect of copper and pantothenic acid as observed with rats (*10*), but evidence against this hypothesis is beyond the scope of this report. The above effects were not observed with old leaves or with leaves incubated at 31°C.

References

1. ARTHUR, J. C. *The Plant Rusts.* New York: Wiley (1929).
2. YARWOOD, C. E. *Phytopathology,* **40,** 971 (1950).
3. ———. *Science,* **114,** 127 (1951).
4. DIMOCK, A. W., and BAKER, K. F. *Phytopathology,* **41,** 536 (1951).
5. TODD, F. E., and BRETHERICK, O. *J. Econ. Entomol.,* **35,** 312 (1942).
6. SMITS, B. L., and MITCHELL, H. L. *Science,* **113,** 296 (1951).
7. SKEGG, H. R., and WRIGHT, L. D. *J. Biol. Chem.,* **156,** 21 (1944).
8. NELSON, M. M., VAN NOUHUYS, F., and EVANS, H. M. *J. Nutrition,* **34,** 189 (1947).
9. YARWOOD, C. E. *Nature,* **169,** 502 (1952).
10. SINGER, L., and DAVIS, G. K. *Science,* **111,** 472 (1950).

Manuscript received August 18, 1952.