In addition to the inhibitory effect of some plant extracts a peculiar phenomenon of disturbed growth and very often of definite stimulation of the test organisms was observed. These phenomena were similar to those described by Abraham *et al.*[10] They appeared as halos of varying sizes surrounding the zones of inhibition. The stimulation was in some cases of extraordinary strength. Boas[3] called attention to the fact that stimulative principles may be present in plant tissues together with inhibitors. A simultaneous action in this sense might explain the observation mentioned. It is also regarded possible that the inhibitor as it penetrates the agar becomes diluted to such a degree that its action reverses.

The results obtained so far indicate that a wide field is opening up for exploration. In all probability, the problems to be encountered will be of a general biological nature rather than being confined to the interrelations between bacteria and higher plants. Broader aspects are coming into the picture, heretofore merely touched but not yet developed.[1, 3, 5]

The authors envisage the applicability of some of the findings to plant pathological problems, particularly in connection with the treatment of seed-borne diseases not yet controllable. An indication of the effect of an antibiotic on plant pathogenes is given in the work of Brown and Boyle[11] in which two plant pathogenes, *Erwinia carnegieana* and *Corynebacterium sepedonicum,* are shown to be inhibited by penicillin. In the light of the work of Link and Walker[12] it is not illogical to suspect that the resistance of some plants to disease is due to the presence in the host cells of distinct chemical substances which are antibiotic to specific pathogenes. The development of antifungal principles, similar in their action to the antibacterial substances, is not outside the realm of possibility.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW PROCEDURE FOR THE BIOASSAY OF VITAMIN C[1]

THE basis of an acceptable bioassay procedure for the estimation of vitamin C in foods is a test diet which is devoid of vitamin C but otherwise adequate for the growth and development of the guinea pig. Commonly used diets amply fortified with ascorbic acid have been shown in this laboratory to be nutritionally incomplete for normal growth and reproduction of these animals.

As a result of studies over the past four years involving some 2,000 guinea pigs, a ration mixture has been devised which appears to give promise in vitamin C bioassays. It is vitamin C—free; is palatable enough to be readily eaten; supports normal reproduction of mature females and practically normal growth of the young to maturity and subsequent reproduction; and consists of ingredients of relatively low nutritional variability, thus permitting reproducible results in growth or tooth development. (Normal performance was taken as that obtained on a mixture similar to the one herein reported plus green feed fed ad libitum.)

The feed mixture consists of:

15.0	per cent.	Ground No. 1 feed oats
10.5	" "	Ground No. 5 Canadian northern wheat
25.0	" "	Dried beet pulp
10.0	" "	Linseed oilmeal
15.0	" "	Dried skimmilk
5.0	" "	Non-oily fish meal
5.0	" "	Wheat germ meal
10.0	" "	Dried brewers yeast
4.0	" "	Bone char
0.5	" "	Salt (iodized at 0.1 per cent. KI)

This mixture for feeding is pressed into pellets of about 1/16 inch diameter and 1/4 inch length. In addition to these pellets, guinea pigs are fed every second day by direct administration 0.5 cc of a feeding fish oil to supply vitamins A and D. On alternate days they are given 6 mg alpha tocopherol. These quantities are doubtless in excess of the unknown minimum requirements. Adequate ascorbic acid (not less than 2 mg/day for growing pigs) must of course also be supplied. The significance of the proportions of the diet mixture ingredients is unknown. Doubtless considerable change in the quantities of most of the foods may be made, and some may probably be omitted or replaced with others. The reduction of this formula to one consisting of purified materials is the object of studies now under way at this station.

Using this diet the eight-weeks growth response of young male guinea pigs to graded doses between 0.5 mg and 2.0 mg of ascorbic acid bears a linear relation to the log log of the dose. Female pigs have been found unsatisfactory in growth assays because of higher variability and slower growth rates. The variability in growth response in the male pigs on identical dose levels of the order of 29 per cent. and with 16 pigs per group it required 40 to 45 grams differ-

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 197.

[11] J. G. Brown and A. M. Boyle, *Phytopathology,* 43: 760, 1944.

[12] K. P. Link and J. C. Walker, *Jour. Biol. Chem.,* 100: 379, 1933.

ence in mean gain between adjacent dose levels to be statistically significant with a probability of 5 per cent.

In the course of these studies it was discovered that the maximum height (or length) to which the odontoblast cells of the incisor teeth would develop in a given length of assay was related to the dose level of vitamin C. In this procedure the size of the odontoblasts is measured in microns by microscopic examination of sections of decalcified teeth cut longitudinally through the pulp cavity in such a way as to expose the plane from the dental papilla to the crown of the tooth containing the most fully developed odontoblast cells.

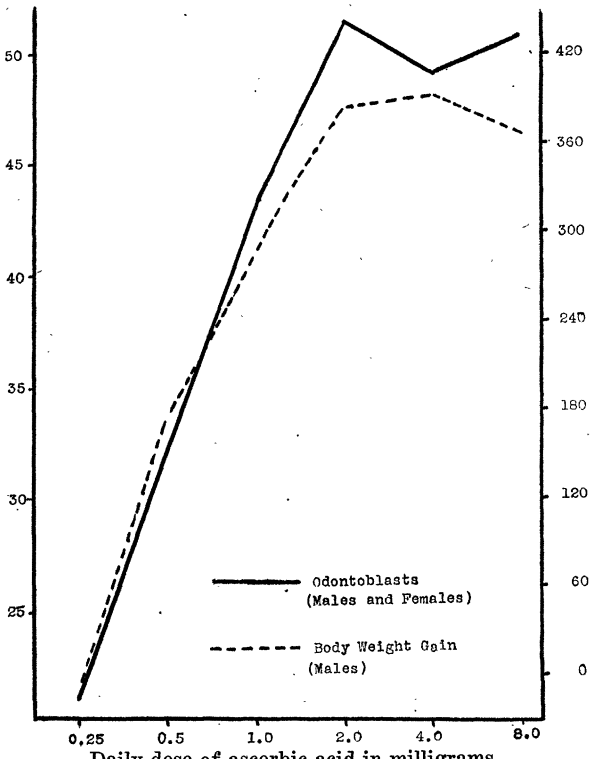

FIG. 1

When such readings were plotted against log dose of ascorbic acid, the relationship was linear between intakes of 0.5 mg and 2.0 mg ascorbic acid. No difference was found between males and females in this characteristic. Replicate tests have given the same relative results though average cell development may differ in different trials. Readings are reproducible by different technicians working independently.

Most important of all is the fact that the variability between animals treated alike is relatively low. In our eight-weeks tests the coefficient of variation in odontoblast development was 12 per cent. as compared to 29 per cent. in the case of live weight gains. The effect on numbers of animals required per treatment which this reduction in variability has is considerable. To obtain a significant mean difference of 10 per cent. between groups would require 10 animals per group in the case of the odontoblast assay as against 65 animals were growth the criterion.

It is noteworthy that the levels of vitamin C between which response by either method bears a relationship to dose are the same. The upper level (2 mg) agrees with that for maximum bone healing reported by Bourne.[2]

In our experience no macroscopic scurvy is found up to eight-weeks duration of assay in young pigs where the daily vitamin C intake has been 0.5 mgms or greater. We have taken this as the minimum level of ascorbic acid suitable for bioassay, on the premise that guinea pigs that are deficient in ascorbic acid to the extent of producing visible lesions can not be depended upon to show either predictable nor reproducible development in the characteristic being used as the criterion of vitamin C intake.

Fig. 1 shows the response by guinea pigs to ascorbic acid as measured (1) by growth and (2) by odontoblast development. For these particular tests the linear relationships were:

For growth : $y = 296.9 + 818.72\ x$, when $x = \log$ dose.
For odontoblast: $y = 8.0 + 34.09\ x$, when $x = \log$ dose.

Tests are still in progress to determine the minimum length of assay period permissible. Thus far it appears that at least a six weeks feeding period should be used. Assays on three- and four-week periods have not given linear response between dose level and cell development.

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