A REVERSIBLE GROWTH INHIBITION OF ISOLATED TOMATO ROOTS*

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Introduction.—It is known that certain bacteria are inhibited in their growth by the presence of sulfanilamide or related compounds in the nutrient medium, and it has been suggested by Fildes[1] and by Woods[2] that this inhibition is due to specific interference with the utilization of essential metabolites, in particular *p*-amino benzoic acid. In the present paper data will be presented which show that the growth of isolated tomato roots[3] is inhibited in the presence of appropriate concentrations of sulfanilamide or its derivatives, but that this growth inhibition can be in whole or in part abolished by the further presence of *p*-amino benzoic acid, a substance not otherwise essential as a supplement for the growth of isolated tomato roots.

Methods.—Isolated tomato roots were cultured aseptically in the nutrient medium described earlier.[4] One centimeter tips from vigorously growing branch roots were used as inocula for the experimental cultures. Approximately 200 roots were grown in each experiment. The two hundred initial tips were randomly divided into 10 lots of 20 tips each and each treatment carried out on one or more lots. The conclusions contained in this paper are based on a series of 42 such experiments.

All cultures were maintained in Petri dishes, four roots per dish, in the dark at 25°C. for one week. At the end of this time the growth in length of the principal axis of each root was measured. The roots were then discarded and fresh inocula from stock cultures used in further experiments.

Three clones of roots, all of the variety "San Jose Canner"[5] were used. These clones, each of which had previously been subjected to 27 weekly transfers in the standard basal medium, reacted similarly to all of the treatments discussed below.

Experimental Results.—Table 1 shows that the growth of isolated tomato roots is inhibited by sulfanilamide and by sulfapyridine. In the presence of 30 mg. per liter of either of these substances isolated tomato roots grew only 15–21% as long in one week as similar roots not receiving inhibitor. Table 1 also shows that the growth inhibition caused by sulfanilamide or sulfapyridine is in part offset by the further addition of *p*-amino benzoic acid to the culture medium in the concentration of 1 mg. per liter. Roots receiving inhibitor and *p*-amino benzoic acid grew 3.3 to 4.5 times as much in one week as roots receiving inhibitor only. This effect cannot be ascribed to a general growth-promoting effect of *p*-amino benzoic acid, since, as shown in table 2, this substance in the concentration of 1 mg. per liter is without effect on the growth of tomato roots. Increased (10 times) concentrations of nicotinic acid or pyridoxine did not affect the inhibition as did *p*-amino benzoic acid.

TABLE 1

THE EFFECT OF SULFANILAMIDE AND SULFAPYRIDINE ON THE GROWTH OF ISOLATED TOMATO ROOTS. GROWTH IN MM. PER ROOT PER WEEK

SUPPLEMENTS	SULFONILAMIDE EXPTS.	NO. OF ROOTS	MM./WEEK	SULFAPYRIDINE EXPTS.	NO. OF ROOTS	MM./WEEK
None	I-25	40	56.6 ± 2.2	I-24	38	45.0 ± 2.5
	I-31	26	49.8 ± 3.1	I-32	32	52.7 ± 1.6
30 mg. inhibitor/l.	I-25	33	12.0 ± 1.5	I-24	29	9.7 ± 1.3
	I-31	14	10.0 ± 1.3	I-32	30	7.8 ± 0.6
30 mg. inhibitor/l. 1 mg. *p*-amino benzoic acid/l.	I-25	19	39.5 ± 3.4	I-24	17	37.9 ± 2.5
	I-31	16	36.2 ± 2.3	I-32	14	35.4 ± 1.4

TABLE 2

LACK OF EFFECT OF *p*-AMINO BENZOIC ACID ON THE GROWTH OF ISOLATED TOMATO ROOTS. GROWTH IN MM. PER ROOT PER WEEK

EXPTS.	NO *p*-AMINO BENZOIC ACID: NO. OF ROOTS	LENGTH	1.0 MG./L. *p*-AMINO BENZOIC ACID: NO. OF ROOTS	LENGTH
I-27	18	52.2 ± 4.3	18	53.6 ± 3.5
I-31	26	49.8 ± 3.1	16	49.1 ± 3.5
I-37	13	42.7 ± 1.8	17	41.8 ± 1.3

Sulfathiazole also inhibits the growth of isolated tomato roots as is shown in table 3, which gives the composite results of 16 experiments. Since the growth of untreated control roots varied from one experiment to the next (as in tables 1 and 2) the growth rates in each experiment were reduced to per cent of the untreated control growth rate and it is this relative growth rate which appears in table 3. It is clear from this table that

TABLE 3

INHIBITION OF THE GROWTH OF ISOLATED TOMATO ROOTS BY SULFATHIAZOLE AND ITS REVERSAL BY *p*-AMINO BENZOIC ACID. MEAN VALUES FROM 16 EXPERIMENTS. GROWTH RATES IN PER CENT OF CONTROL ROOTS GROWN FOR 1 WEEK IN STANDARD BASAL MEDIUM. THE AVERAGE GROWTH RATE (100%) OF THE CONTROL ROOTS WAS 45.8 ± 2.33 MM. PER WEEK IN THESE EXPERIMENTS

SULFATHIAZOLE CONC.; MG./L.	*p*-AMINO BENZOIC ACID CONCENTRATION; MG./L. GROWTH PER WEEK: % OF UNTREATED CONTROLS				
	0	0.03	0.10	0.30	1.0
100	3	...	...	...	27.5
30	15.2	17.0	21.0	25.0	53.6
10	18.4	20.0	32.3	58.7	94.6
3	37.4	52.2	83.0	99.0	100.7
1	67.6	92.3	97.7	99.0	...
0	100	...	...	...	100

sulfathiazole is a relatively potent inhibitor of root growth since half inhibition is given by about 2 mg. sulfathiazole per liter. The amount of *p*-amino benzoic acid needed to abolish a fixed amount of inhibition varies with the inhibitor concentration. Thus nearly the same absolute increase in growth rate was brought about by 0.03 mg. per liter of the material in the presence of 1 mg. per liter of sulfathiazole as was brought about by 1 mg. per liter of *p*-amino benzoic acid in the presence of 100 mg. per liter of sulfathiazole. Inhibition by a given quantity of sulfathiazole was markedly decreased by the addition of a 100 times smaller quantity of *p*-amino benzoic acid in every case. Inhibition by sulfathiazole and reversal of this inhibition by *p*-amino benzoic acid would seem to depend in part on the ratio between the two substances in the nutrient medium rather than strictly on the absolute amount of inhibitor present.

Isolated tomato roots of the clones used in these experiments appear, qualitatively, to contain *p*-amino benzoic acid or related substance of similar physiological activity,[6] even when grown through 40 or more successive weekly transfers in *p*-amino benzoic acid free medium. The presence of this material in such roots suggests that isolated tomato roots of the present clones may synthesize the substance.

Discussion.—The inhibition of the growth of isolated tomato roots by sulfathiazole depends in part on the ratio between added inhibitor and added *p*-amino benzoic acid. This observation might suggest that the inhibition is in part competitive in the sense that sulfathiazole may compete with *p*-amino benzoic acid for some essential position or function in the living cell. That sulfathiazole inhibits the growth of roots not supplied with *p*-amino benzoic acid is not in disagreement with this view since isolated tomato roots appear to normally contain this substance. Other factors may also govern the inhibition.[7]

Summary.—Isolated tomato roots of 3 different clones were found to be inhibited in growth by the addition of sulfanilamide, sulfapyridine or sulfathiazole, to the nutrient medium. This inhibition was in whole or in part abolished by the further addition of *p*-amino benzoic acid to the medium. Isolated tomato roots of the clones used normally contain *p*-amino benzoic acid, or a substance having similar physiological activity.

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[1] Fildes, P., *British Jour. Expt. Path.*, **21**, 67 (1940).

[2] Woods, D. D., *Ibid.*, **21**, 74 (1940).

[3] White, P. R., *Plant Physiology*, **9**, 585 (1934). Robbins, W. J., and Bartley, Mary, *Science*, **85**, 246 (1937).

[4] Bonner, J., *Amer. Jour. Bot.*, **27**, 692 (1940). This medium contains per liter of redistilled water: 1.5 mg. ferric tartrate, 20 mg. KH_2PO_4, 65 mg. KCl, 81 mg. KNO_3, 36 mg. $MgSO_4{\cdot}7H_2O$, 236 mg. $Ca(NO_3)_2{\cdot}4H_2O$, and 20 gm. sucrose.

[5] The seed used was kindly supplied by the California Packing Corporation.

[6] The assays for p-amino benzoic acid were carried out by Prof. G. W. Beadle and Prof. E. Tatum, Stanford University, using a mutant of *Neurospora crassa* for which p-amino benzoic acid is an essential supplement.

[7] In 5 experiments sulfanilamide or its derivatives were applied to intact tomato plants grown in the greenhouse in sand or solution culture. Concentrations of up to 125 mg. per liter of nutrient were found to be without marked inhibiting effect on root or top growth.

GENERAL CONGRUENCES INVOLVING THE BERNOULLI NUMBERS

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Kummer[1] gave a result which may be expressed as follows:

$$h^n(h^{p-1} - 1)^i \equiv 0 \pmod{p^i}, \quad (n - 1 \geqq i;\ n \not\equiv 0 \pmod{\overline{p - 1}}), \qquad (1)$$

where p is an odd prime; the left-hand member is expanded in full, then b_t/t is substituted for h^t, and the b's are defined by the recursion formula

$$(b + 1)^n = b_n, \quad (n > 1),$$

in which we expand the left-hand member by the binomial theorem and substitute b_k for b^k. The latter formula gives the Bernoulli numbers. The congruences (1) have played an important part in the development of the arithmetic theory of these numbers.

In the present paper we shall employ the theorem which the writer gave in another paper,[2] the statement of which, for convenience, is repeated here:

Theorem I. *Let R be the ring of algebraic integers in an algebraic field and put*

$$f_{n_i}^{(i)}(\alpha_1, \alpha_2, \ldots, \alpha_{ki}) = \sum_{r=1}^{ki} \alpha_{ri} a_{ri}^{n_i} \qquad (2)$$

where the a's and α's are in R and the n's are rational integers $\geqq 0$. Further let there be a rational integer $d > 0$ such that for all r's in the range 1 to k_i and all i's in the range 1 to s,

$$a_{ri}^{\ d} \equiv 1 \pmod{\mathfrak{m}} \qquad (3)$$

where $\mathfrak{m}$ is a fixed ideal in R. Also, let

$$\beta_1 + \beta_2 + \ldots + \beta_s \equiv 0 \pmod{\mathfrak{m}}$$