INHIBITION OF ESCHERICHIA COLI BY *p*-AMINOBENZOIC ACID AND ITS REVERSAL BY *p*-HYDROXYBENZOIC ACID*,‡

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p-Aminobenzoic acid (PABA) not only reverses sulfonamide inhibition (2) but also, in higher concentrations, is itself a chemotherapeutically useful inhibitor of rickettsiae (3–5). The competition with sulfonamides is clearly associated with the normal metabolic function of PABA; in contrast, its mode of action as a chemotherapeutic agent is unknown.

A key to this problem was provided by the discovery, based on the nutritional requirement of certain mutants (quintuple aromatic auxotrophs) of *Escherichia coli*, that *p*-hydroxybenzoic acid (POB) is an essential metabolite in this organism (6) and is able to reverse competitively the inhibition of *E. coli* by its sulfone analogue, 4,4′-dihydroxydiphenyl sulfone (6, 7). The present communication will report that POB exerts a similar reversing effect on inhibition by high concentrations of PABA, which therefore appears to act as a competitive analogue of POB. A more limited reversal of PABA inhibition is also produced by precursors of POB. In addition, it will be shown that analogues of POB exert only a growth-retarding effect, but become completely bacteriostatic on addition of L-aspartic acid in exceedingly low concentrations.

Methods

Experiments were performed at 37°C. in minimal medium A (8) with the W strain of *E. coli* (ATCC No. 9637), as well as mutants derived from it (quintuple aromatic auxotrophs) that require tyrosine, phenylalanine, tryptophan, PABA, and POB (6, 9). Competition between inhibitor and antagonist was studied chiefly in pour plates containing 50 to 100 bacterial cells; the advantages of this method have been discussed elsewhere (10). More conventional methods, employing liquid media or streaks on solid media, were also tested for practically all the phenomena reported; they yielded similar results, except that higher concentrations were required for inhibition. In addition, resistant mutants grew out in liquid media.

Inhibition by PABA; Synergism with L-Aspartic Acid

Table I shows that growth of the W strain of *E. coli* is slowed by PABA at a concentration of 150 μg./ml. Bacteriostasis, however, is incomplete, large

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‡ A preliminary report of this work has appeared (1).

TABLE I

Inhibition of Wild Type E. coli by PABA and Aspartic Acid; Reversal by POB or Shikimic Acid

Approximately 50 cells of *E. coli* (W strain) were poured in 5 ml. of medium A agar, supplemented as indicated, in Petri dishes (50 mm. diameter). After incubation at 37° C., colony size was visually estimated, ranging from *m* (microscopic) and ½ (barely visible) to 4 (large).

PABA	Other	Colony size					
		DL-Aspartic acid 0			DL-Aspartic acid 30 μg./ml.		
		1 day	2 days	5 days	1 day	2 days	5 days
μg./ml.	μg./ml.						
—	—	3	4	4	3	4	4
100	—	2	4	4	1	4	4
150	—	0	4	4	0	0	0
200	—	0	4	4	0	0	0
400	—	0	2	4	0	0	0
800	—	0	*m*	3	0	0	0
1600	—	0	0	½	0	0	0
	POB						
150	1	3	4	4	3	4	4
200	0.2	0	4	4	0	2	4
"	0.5	1	4	4	1	4	4
"	1	2	4	4	2	4	4
"	2	2	4	4	2	4	4
400	1	0	2	4	0	0	0
"	2	½	3	4	0	1	4
"	4	1	4	4	1	3	4
800	2	0	*m*	4	0	0	±
"	4	0	1	4	0	0	2
"	8	1	3	4	½	2	4
"	16	1	3	4	1	3	4
1600	2	0	0	2	0	0	1
"	4	0	½	3	0	0	2
"	8	0	1	3	0	1	2
"	32	*m*	1	3	*m*	1	3
	Shikimic acid						
200	0.2	0	4	4	0	0	0
"	0.5	1	4	4	0	1	4
"	2	1	4	4	0	1	4
"	100	1	4	4	0	2	4
400	4	0	2	4	0	0	0
"	100	0	2	4	0	0	0

TABLE II

Synergism of Aspartic Acid and Related Compounds with PABA Inhibition

Experimental conditions as in Table I.

PABA	DL-Aspartic acid	L-Aspartic acid	Other	Colony size		
				1 day	2 days	5 days
μg./ml.	*μg./ml.*	*μg./ml.*	*μg./ml.*			
—	—	—		2	4	4
—	2000	—		1	4	4
800	—	—		0	*m*	3
"	0.5	—		0	0	2
"	1	—		0	0	1
"	2	—		0	0	0
"	—	0.25		0	0	2
"	—	0.5		0	0	0
200	—	—		0	3	4
"	0.5	—		0	2	4
"	1	—		0	0	4
"	2	—		0	0	0
"	—	0.25		0	2	4
"	—	0.5		0	0	1
"	—	1		0	0	0
			Asparagine			
"			80	0	0	4
"			40	0	2	4
			Fumaric acid			
"			40	0	0	0
"			20	0	0	3
			Malic acid			
"			200	0	3	4
			Succinic acid			
"			200	0	3	4
			Cysteic acid			
"			200	0	3	4
			D-Aspartic acid*			
"			100	0	3	4

* Kindly furnished by Dr. J. P. Greenstein.

colonies developing by 48 hours. With increasing concentrations of PABA the inhibition becomes more prolonged, but even at 800 μg./ml. small colonies appear by 48 hours, and at 1600 μg./ml. they appear by 5 days. Identical results have been obtained with the K-12 strain of *E. coli*.

Table I also shows that the addition of aspartic acid converts the retardation by PABA into essentially permanent bacteriostasis. In Table II it is seen that the synergism produced by DL-aspartic acid is complete at 2 μg./ml., while some effect is exerted by even lower concentrations.[1] In contrast, DL-aspartic acid alone produces negligible inhibition at 2000 μg./ml. L-Aspartic acid is about twice as effective as the racemic mixture, while D-aspartic acid is inactive. The concentration of aspartic acid required for synergism is relatively independent of the concentration of PABA; conversely, as is seen in Table I, the concentration of PABA required for inhibition is not altered by the addition of aspartic acid. Synergism with PABA inhibition is also shown, but only at higher concentrations, by asparagine and fumaric acid, which probably act through conversion to aspartic acid. Succinic, malic, and cysteic acids showed no synergism in concentrations up to 200 μg./ml. (Table II).

L-Aspartic acid and asparagine have previously been reported (11) to prolong the growth-retarding effect of D-serine, which interferes competitively with the conversion of β-alanine to pantothenate (10). As with PABA, synergism with D-serine is also shown by fumaric acid but not by succinic or cysteic acid (12); in addition, the same concentrations of the several compounds are required for synergism with either PABA or D-serine. The resemblance of the two systems suggested a possible connection between them, but none was found: pantothenate failed to eliminate the effect of aspartic acid on PABA inhibition, while POB failed to overcome its effect on D-serine inhibition.

The incomplete bacteriostatic effect exerted by PABA is not a peculiarity of this compound, but appears rather to be a property of competitive inhibitory systems that is revealed by the technique used here, which permits unusually prolonged observation of the interaction between individual cells and a relatively constant environment. With this technique sulfathiazole, a classical competitive inhibitor, showed a tenfold range between the concentrations that prevented appearance of visible colonies in 1 and in 5 days. Aspartic acid exerted no effect on this inhibition.

Reversal of PABA Inhibition by POB and Its Precursors

As is shown in Table I, the inhibitory effect of PABA is overcome in a competive manner by POB in a concentration about 1/100 that of PABA; delayed reversal is produced at an even lower ratio. The addition of aspartic acid,

[1] Failure to yield visible colonies by 5 days is a practical but not a rigorous index of complete bacteriostasis; after 7 days colonies did appear in the presence of PABA 200 plus DL-aspartic acid 2 μg./ml. (Table III), but not PABA 200 plus DL-aspartic acid 10 μg./ml.

despite its synergistic inhibitory effect, does not significantly alter the competitive ratio. At the lower inhibitory concentrations of PABA (200 μg./ml.) the competitive ratio is smaller (about 1/200), presumably because of the contribution of POB by the wild type cells themselves, which synthesize this substance in excess and excrete it (6). It can be noted that with increasing concentrations of PABA the growth rate is less completely restored to normal by POB, even in excess; this residual inhibition, presumably due to interference with other enzyme systems, is not affected by aspartic acid.

Shikimic acid (a 3,4,5-trihydroxycyclohexene-1-carboxylic acid), a precursor of POB as well as of several other aromatic metabolites (9, 13), also antagonizes PABA inhibition, but less effectively than POB itself. Several limitations of the reversing effect of shikimic acid are illustrated in Table I: it is ineffective against a moderate concentration of PABA (400 μg./ml.); even when added in excess it only partly restores the growth rate in the presence of a minimal inhibitory concentration (200 μg./ml.); its action is impeded by aspartic acid. Similar results were obtained with compound X,[2] a precursor of shikimic acid (9) recently isolated and identified as a dehydroshikimic acid (14). It is concluded that these precursors, even in excess, can increase to only a limited extent the intracellular concentration of POB in *E. coli*, since they can only approximately double the concentration of PABA required for inhibition.

The effect of aspartic acid on the action of shikimic acid is described in greater detail in Table III. DL-Aspartic acid at 3 μg./ml. partly prevents, and at 100 μg./ml. nearly permanently prevents, shikimic acid from reversing PABA inhibition. Furthermore, shikimic acid is no more effective at 100 μg./ml. than at 1 μg./ml. On the other hand, aspartic acid does not significantly interfere with the reversal of inhibition by POB (Table III), and could be shown to have no effect on the quantity of POB required by mutants (unpublished observations). Since aspartic acid could not be shown to interfere with the utilization of POB, it appears to interfere with its synthesis from shikimic acid, but in a manner that cannot be competitively overcome by shikimic acid. It seems unlikely, however, that this effect of aspartic acid accounts for its synergism with PABA; the reasons will be discussed in a later section.

It is of interest to note that shikimic acid also has little capacity to antagonize a competitive analogue of another of its metabolic derivatives (9, 13), PABA. Using the minimal concentration of sulfathiazole (1 μg./ml.) that produced prolonged inhibition of *E. coli* under the experimental conditions of Table I, we were unable to demonstrate significant reversal by shikimic acid, even at 100 μg./ml., though the inhibition was reversed completely by 0.3 μg./ml. PABA, and slowly by as little as 0.01 μg./ml.

[2] Generously provided by Dr. I. I. Salamon of this laboratory.

TABLE III

Interference by Aspartic Acid with the Reversal of PABA Inhibition by Shikimic Acid

Experimental conditions as in Table I.

PABA	DL-Aspartic acid	POB	Shikimic acid	Colony size			
				1 day	2 days	5 days	7 days
μg./ml.	μg./ml.	μg./ml.	μg./ml.				
—	—	—	—	1	4	4	4
200	—	—	—	0	3	4	4
"	—	0.5	—	1	4	4	4
"	—	2	—	1	4	4	4
"	—	—	1	1	4	4	4
"	—	—	100	1	4	4	4
"	3	—	—	0	0	0	3
"	"	0.5	—	1	4	4	4
"	"	2	—	1	4	4	4
"	"	—	1	0	3	4	4
"	"	—	100	*m*	3	4	4
"	30	—	—	0	0	0	0
"	"	0.5	—	½	4	4	4
"	"	2	—	1	4	4	4
"	"	—	1	0	1	4	4
"	"	—	100	0	2	4	4
"	100	—	—	0	0	0	0
"	"	0.5	—	0	2	4	4
"	"	2	—	1	4	4	4
"	"	—	1	0	0	0	4
"	"	—	100	0	0	0	1

Inhibition by Related Compounds

The inhibition of some bacteria by 4,4′-dihydroxydiphenyl sulfone (bis(4-hydroxyphenyl)sulfone) is not reversed by PABA (15) but is reversed by POB (6, 7). Both this sulfone[3] and the hydroxy analogue[3] of sulfathiazole (*N*-(2-thiazolyl)-4-hydroxybenzenesulfonamide; phenosulfazole; darvisul) are active against *E. coli* at 100 μg./ml.; they resemble PABA in requiring aspartic acid for complete bacteriostasis. The sulfonamide analogue of POB, *p*-hydroxybenzenesulfonamide,[4] is inactive in concentrations up to 1000 μg./ml., as has been reported by other investigators. *p*-Chlorobenzoic acid and *p*-fluorobenzoic acid are also inactive at 1000 μg./ml.

[3] Provided through the courtesy of the American Cyanamid Co. and E. R. Squibb and Sons.

[4] Kindly supplied by Dr. E. K. Marshall.

The competition between PABA and POB does not appear to be symmetrical; we observed no inhibition by POB in concentrations up to 2000 μg./ml. At 20 mg./ml., however, a bactericidal effect on *E. coli* has been reported (7). In contrast to the relative inactivity of POB, its esters are used as food preservatives, presumably acting as phenolic disinfectants (16, 17).

TABLE IV

Simultaneous Inhibitory Competition of p-Nitrobenzoic Acid with PABA and POB

Experimental conditions as in Table I.

p-Nitrobenzoic acid	POB	PABA	Colony size					
			DL-Aspartic acid 0			DL-Aspartic acid 30 μg./ml.		
			1 day	2 days	5 days	1 day	2 days	5 days
μg./ml.	μg./ml.	μg./ml.						
—	—	—	2	4	4	2	4	4
25	—	—	1	4	4	*m*	2	4
50	—	—	0	3	4	0	0	0
100	—	—	0	2	4	0	0	0
200	—	—	0	1	4	0	0	0
25	10	—	2	4	4	2	4	4
50	"	—	1	4	4	*m*	3	4
100	"	—	0	3	4	0	0	4
200	"	—	0	2	4	0	0	2
25	—	10	2	4	4	2	4	4
50	—	"	*m*	3	4	0	1	4
100	—	"	0	3	4	0	0	0
200	—	"	0	1	4	0	0	0
200	10	10	1	4	4	1	3	4

Simultaneous Competition of p-Nitrobenzoic Acid with PABA and POB

Table IV shows that *p*-nitrobenzoic acid (PNBA) at 50 to 100 μg./ml. produces inhibition that is reversed by POB plus PABA, but not by either vitamin alone.[5] It must be emphasized that in this experiment, in contrast to the previous ones, PABA is present in a moderate concentration and is acting as a metabolite rather than an inhibitor.

As with the other analogues of POB, the anti-POB effect (*i.e.*, the inhibition produced in the presence of PABA) is transient and requires aspartic acid for

[5] A similar double competition was observed with two samples of phenosulfazole but was traced to contamination of this material with sulfathiazole, from which it had been manufactured. PNBA was therefore recrystallized three times from different solvents; no change in activity was observed.

complete bacteriostasis. The anti-PABA effect (*i.e.*, the inhibition produced in the presence of POB) is also only transient; this property has been explained by the observed reduction of PNBA to its own reversing agent, PABA, by the bacteria (18, 19). The anti-PABA effect is markedly prolonged by DL-aspartic acid (Table IV) or L-aspartic acid; D-aspartic acid is inactive. Since L-aspartic acid does not affect inhibition by a stable analogue of PABA, such as sulfathiazole (unpublished data), its effect on the anti-PABA action of PNBA appears to imply that it interferes with the reduction of PNBA to PABA. No connection is obvious between this property of L-aspartic acid and its synergism with POB analogues or D-serine.

Inhibition of POB-Requiring Mutants

Since wild type *E. coli* synthesizes and excretes POB (6), a mutation that blocked POB synthesis would be expected to increase the sensitivity to inhibition by POB analogues. As no mutant with a single requirement for POB has been isolated, quintuple aromatic auxotrophs (83-1, 170-27) were tested in the presence of adequate quantities of their requirements (including POB 0.01 μg./ml.). For these mutants the minimal inhibitory concentration of PABA was 10 μg./ml., in contrast to 150 μg./ml. for wild type. Even greater differences between mutant and wild type (up to 25-fold) were obtained with other POB analogues; this result is not unexpected, for it is known that PABA in moderate concentrations slowly satisfies the POB requirement of the mutants (6), and hence would tend to antagonize itself.

With these mutants it was also possible to observe POB-reversible inhibition of growth by 100 to 300 μg./ml. of *p*-hydroxybenzenesulfonamide, *p*-chlorobenzoic acid, and *p*-fluorobenzoic acid, which have been noted above to be inactive against wild type at 1000 μg./ml.

The synergistic action of L-aspartic acid was observed with the mutants as with wild type.

Inhibition of Rickettsiae

The competition between POB and PABA observed with *E. coli* has led to similar experiments with rickettsiae, kindly performed by Dr. J. C. Snyder. A preliminary report of the reversal by POB of the rickettsiostatic action of PABA in chick embryos and mice has been published (20).

DISCUSSION

Following the announcement of POB as a bacterial vitamin required by a mutant (6), our attention was called to an earlier report by Levaditi *et al.* (7) that this compound reversed inhibition of *E. coli* by 4,4′-dihydroxydiphenyl sulfone. This work was unfortunately overlooked because of an abstract[6] that misquoted the reversing agent as PABA rather than POB.

[6] *Chem. Abstr.*, 1945, **39**, 3320.

The mechanism of inhibition of *E. coli* by PABA has been shown in the present paper to depend on competition with POB. PABA is known to inhibit not only this organism (21) but also other bacteria, fungi (22, 23), and rickettsiae. Of particular chemotherapeutic interest is the reversal of its rickettsiostatic action by POB *in vivo* (20).

PABA has the unusual property of acting metabolically in three different ways: as an essential metabolite, required by certain mutants in a low concentration (0.005 μg./ml.); as a replacement for, and presumably source of, POB in quintuple aromatic auxotrophs (6, 9) at a higher concentration (0.1 to 1 μg./ml.); and as a competitive inhibitor of POB utilization, in either wild type or mutants, at even higher concentrations (150 μg./ml. for wild type, 10 μg./ml. for POB-requiring mutants). This last effect implies that POB cannot be formed from high concentrations of PABA to an extent that can compensate for the inhibitory effect of PABA itself. The combined role of PABA as both competitor and source of POB finds precedent in the similar relation between *p*-nitrobenzoic acid and PABA (18, 19) and possibly between aspartic acid and β-alanine (10).

A further consequence of the close structural resemblance between PABA and POB is the simultaneous competition of *p*-nitrobenzoic acid with both these metabolites. PABA alone has been reported to reverse inhibition of *E. coli* by *p*-nitrobenzoic acid (19), but it is hardly surprising that the residual inhibition due to competition with POB should have been overlooked, since this effect is transient in the absence of aspartic acid, and is not readily demonstrated in liquid media as employed in that investigation.

The inhibition of a "sulfonamide-requiring" mutant of Neurospora by PABA (24) probably involves a mechanism quite different from the direct competition with POB reported here, since it requires only an exceedingly low concentration of PABA, and PABA can be replaced as inhibitor by methionine, a product of PABA metabolism (25).

L-Aspartic acid, and related compounds that readily give rise to it, have been shown to be synergistic with all the available competitors of POB. This relation has several remarkable features. First, aspartic acid is active in a concentration (1μg./ml.) that one might expect to be quickly metabolized,[7] yet high concentrations of this compound alone (>1000 μg./ml.) are not inhibitory. Secondly, aspartic acid substantially eliminates the whole range of partial growth inhibition by POB analogues, causing a sharp transition from lack of inhibition at one concentration (*e.g.*, 100 μg./ml. PABA) to permanent bac-

[7] It should be emphasized that the extraordinary sensitivity to aspartic acid was detected with inocula of discrete cells scattered in solid media. It could also be demonstrated in liquid media with very small inocula (10^2 cells). With ordinary inocula (10^8 cells), however, growth occurred without appreciable inhibition by PABA or synergism by aspartic acid. The cause of this growth was shown to include the outgrowth of resistant mutants, and possibly also metabolic elimination of the inhibitors and excretion of POB.

teriostasis at a slightly higher concentration (150 μg./ml. PABA). This effect parallels the fact that a large number of quintuple aromatic auxotrophs have a requirement for POB that is only partial but becomes complete on the addition of L-aspartic acid in concentrations similar to those required for synergism with PABA (unpublished observations). Thirdly, the synergistic effect of aspartic acid does not significantly change the minimal inhibitory concentration of POB analogues or their competitive ratio with POB.

The mechanism of the aspartic acid effect is not clear. Since aspartic acid does not interfere with reversal of PABA inhibition by POB, but does interfere with its reversal by shikimic acid, a precursor of POB, aspartic acid appears to impede the synthesis of POB from shikimic acid, though it is unable alone to block this reaction in wild type. It is unlikely, however, that this effect of aspartic acid entirely accounts for its synergism with POB analogues, for aspartic acid is required in very low concentration for prolongation of growth inhibition by these analogues, but in higher concentration for prolonged interference with the reversal of this inhibition by shikimic acid. Furthermore, a genetic block in the synthesis of POB causes a 15- to 25-fold increase in sensitivity to inhibition by POB analogues, yet aspartic acid does not cause even a 2-fold increase in the sensitivity of wild type. One therefore wonders whether the aspartic acid effect may not involve a more complex mechanism than the simple competitions under consideration. In particular, it seems possible that aspartic acid, though not increasing the quantitative requirement of a genetically or chemically blocked cell for POB, may directly or indirectly interfere with some POB-requiring reaction, but only when this reaction is being slowed through suboptimal supply of POB or its products. These considerations may well apply to the very similar synergism of L-aspartic acid with D-serine, which blocks the conversion of β-alanine to pantothenic acid (10); the mechanism here is equally obscure. There is no indication of any direct metabolic connection between the two systems.

Research with bacterial mutants in this laboratory has been based in part (13) on the hope of finding metabolites that are peculiar to microorganisms and absent from the animal host; such compounds would be rational models for the synthesis of analogues designed to be selectively toxic to microorganisms, and hence chemotherapeutically useful. The thought that POB might be such a useful model arose when liver extract was found to lack significant quantities of this compound (6). The relatively high concentrations of available POB analogues required for inhibition of *E. coli*, however, offer little hope that these compounds will have chemotherapeutic value against organisms whose POB metabolism quantitatively resembles that of *E. coli*. On the other hand, the proved value of PABA in rickettsial infections verifies the anticipation that POB might be a useful chemotherapeutic model, and shows that some parasites are adequately susceptible to inhibition *in vivo* by at least one of its ana-

logues. Other analogues of POB, and possibly of aspartic acid, therefore seem to merit further chemotherapeutic investigation.

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SUMMARY

p-Aminobenzoic acid (PABA) exerts three metabolic effects on *E. coli:* it acts as a normal vitamin at low concentrations, as a source of another vitamin, *p*-hydroxybenzoic acid (POB), at moderate concentrations, and as a growth inhibitor at high concentrations (150 to 1600 μg./ml.). The inhibition is competitively reversed by POB in 1/100 the concentration of PABA. The inhibition is also reversed to a limited extent by shikimic acid and compound X, precursors of POB.

p-Nitrobenzoic acid is an inhibitory competitor of both POB and PABA.

The retardation of growth produced by PABA and other competitive analogues of POB (*p*-nitrobenzoic acid; 4,4′-dihydroxydiphenyl sulfone; phenosulfazole) is converted to complete bacteriostasis by the addition of L-aspartic acid in a remarkably low concentration (1 μg./ml.), without change in the competitive ratio with POB. The mechanism underlying this synergism is not clear.

In contrast to wild type, mutants that require POB not only are inhibited by much lower concentrations of the above analogues, but also show inhibition by weaker competitors of POB such as *p*-hydroxybenzenesulfonamide, *p*-chlorobenzoic acid, and *p*-fluorobenzoic acid.

BIBLIOGRAPHY

1. Davis, B. D., *Fed. Proc.*, 1951, **10,** 406.
2. Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21,** 74.
3. Snyder, J. C., Maier, J., and Anderson, C. R., December 26, 1942, Report to Division of Medical Science, National Research Council, cited in reference 4.
4. Yeomans, A., Snyder, J. C., Murray, E. S., Zarafonetis, C. J. D., and Ecke, R. S., *J. Am. Med. Assn.*, 1944, **126,** 349.
5. Greiff, D., Pinkerton, H., and Moragues, V., *J. Exp. Med.*, 1944, **80,** 561.
6. Davis, B. D., *Nature*, 1950, **166,** 1120.
7. Levaditi, C., Mentzer, C., and Perault, R., *Compt. rend. Soc. biol.*, 1942, **136,** 769.
8. Davis, B. D., and Mingioli, E. S., *J. Bact.*, 1950, **60,** 17.
9. Davis, B. D., *J. Biol. Chem.*, 1951, **191,** 315.
10. Maas, W. K., and Davis, B. D., *J. Bact.*, 1950, **60,** 733.
11. Davis, B. D., and Maas, W. K., *J. Am. Chem. Soc.*, 1949, **71,** 1865.
12. Maas, W. K., and Davis, B. D., unpublished data.
13. Davis, B. D., *Experientia*, 1950, **6,** 41.

14. Salamon, I. I., and Davis, B. D., Abstracts 119th meeting Am. Chem. Soc., 1951, 21C.
15. Jensen, K. A., and Schmith, K., *Z. Immunitätsforsch.*, 1942, **102,** 261.
16. Suter, C. M., *Chem. Rev.*, 1941, **28,** 209.
17. Cavill, G. W. K., and Vincent, J. M., *J. Soc. Chem. Ind.*, 1947, **66,** 175.
18. Miller, J. K., *J. Pharm. and Exp. Therap.*, 1941, **71,** 14.
19. Johnson, O. H., Green, D. E., and Pauli, R., *J. Biol. Chem.*, 1944, **153,** 37.
20. Snyder, J. C., and Davis, B. D., *Fed. Proc.*, 1951, **10,** 419.
21. Lecoq, R., and Solomides, J., *Compt. rend. Acad. sc.*, 1947, **225,** 1392.
22. Cavill, G. W. K., and Vincent, J. M., *Nature*, 1945, **155,** 301.
23. Cavill, G. W. K., and Vincent, J. M., *J. Soc. Chem. Ind.*, 1948, **67,** 25.
24. Zalokar, M., *Proc. Nat. Acad. Sc.*, 1948, **34,** 32.
25. Zalokar, M., *J. Bact.*, 1950, **60,** 191.