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Rapid Methods for Determining Decarboxylase Activity: Arginine Decarboxylase

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A rapid biochemical method for the determination of arginine decarboxylase (EC 4.1.1.19) activity has been developed for use in the routine clinical microbiology laboratory and correlated with similar procedures for ornithine and lysine decarboxylase (EC 4.1.1.18) systems. It is based on the detection of agmatine, the amine end product formed during growth on a synthetic medium containing arginine as the key amino acid. A modified diacetyl reagent is used to detect this amine after a differential butanol extraction of the cultures. This procedure can be used to detect this amine after a 1- to 4-hr incubation period (with the use of an initial concentrated inoculum) or with an overnight culture. Thus, both an indirect measurement based on the alkalinization of the medium and a lengthy incubation period were avoided. Parameters for optimal enzyme activity and the pertinent enzyme systems involved in arginine and agmatine catabolism are discussed in detail.

The decarboxylation of ornithine, lysine, and arginine has been routinely employed in the identification and characterization of the *Enterobacteriaceae* and other gram-negative organisms such as the pseudomonads (4–7, 12, 16, 18, 21). The present standard procedure, first devised by Møller (12), is based on the development of an alkaline reaction after several days of incubation in a complete medium containing the desired amino acid and a *p*H-sensitive indicator.

Several authors (1, 3, 9) have developed rapid biochemical methods for detecting cadaverine and putrescine, the respective end products of lysine and ornithine decarboxylase, but no one has reported a similar procedure for arginine decarboxylase. This paper presents a rapid method for the specific determination of this enzyme. It is based on a differential butanol extraction of agmatine, the amine end product, and its subsequent reaction with a modified diacetyl reagent specific for the guanidino portion of the molecule. The relevant enzyme systems involved in arginine and agmatine catabolism are discussed. Several parameters for optimal decarboxylase activity are reported.

MATERIALS AND METHODS

Media. Synthetic, Møller's, and maintenance media were prepared as described in the preceding paper (9). The synthetic medium contained 0.5 g of L-tyrosine and 0.25 g of L-methionine per liter. The final *p*H and arginine concentration are discussed later.

Organisms, inoculum preparation, and incubation. The organisms used in this study were clinical isolates and stock cultures, including several from the American Type Culture Collection (ATCC). Procedures involving maintenance, preparation of inocula, and conditions of incubation have been described previously (9).

Extraction and detection of agmatine. Agmatine is not soluble in chloroform. The procedure of Goldschmidt and Lockhart (Anal. Chem., *in press*) was used: 2 ml of a NaCl-saturated 10% KOH solution were added to the culture tubes, and the tubes were agitated on a Vortex mixer; 2 ml of *n*-butanol was then added, and the tubes were agitated for 45 to 60 sec. From the top butanol layer, 0.5 ml was removed for reaction with the diacetyl reagent. A dark red-orange color developed within 3 min if agmatine was present. The diacetyl reagent (Goldschmidt and Lockhart, Anal. Chem., *in press*) was prepared as follows. Solution A: 60 μliters of diacetyl (2,3-butanedione) was diluted to 100 ml with distilled water and refrigerated in a dark glass bottle storing for no longer than 2 weeks. Solution B: 1 g of 1-naphthol, 6 g of NaOH, and 20 g of NaCl were dissolved in distilled water, diluted to 100 ml, and stored in a dark glass bottle at 5 C for no longer than 3 weeks. This solution remains a liquid in the freezer. To each 0.5-ml sample of the butanol extract, 0.3 ml of solution A and 0.15 ml of solution B were added. The two solutions could also be first combined in these proportions each day before use.

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Extraction and detection of putrescine. Putrescine was differentially extracted from the cultures as described in the preceding paper (9). The ninhydrin reagent (9) was also prepared and used as described.

Chromatography. Ascending one-dimensional silica-gel thin-layer chromatograms were prepared as described previously (9). The solvent system consisted of phenol-acetic acid-water (6:1:6). Ninhydrin, diacetyl, and Sakaguchi spray reagents were prepared by the method of Goldschmidt and Lockhart (Anal. Chem., *in press*). Samples of cell extracts and known compounds were spotted on the plates in amounts of 100 to 200 μliters of each.

Spectrophotometric determinations. Cultures and samples of agmatine and putrescine were differentially extracted in butanol and reacted with the diacetyl reagent. After color development, the samples were pipetted into cuvettes and placed in a Beckman DK-2A ratio recording spectrophotometer to obtain absorption curves in the visible spectrum.

RESULTS AND DISCUSSION

> "When I use a word, said Humpty Dumpty . . . it means just what I choose it to mean—neither more nor less. The question is, said Alice, whether you can make words mean so many different things."
>
> —Lewis Carroll

Møller's method (12) for detecting amino acid decarboxylation reactions in the *Enterobacteriaceae* has been adopted as the standard reference method for most clinical microbiologists (4–7, 15, 18, 21). The designations of ornithine and lysine decarboxylase are very straightforward. However, much confusion has resulted from the terminology employed to describe the reactions occurring with arginine. Arginine decarboxylase (2, 6, 8, 12, 13), arginine dihydrolase (5, 7, 17, 20), arginine desimidase (EC 3.5.3.6; 12, 14), and arginase (EC 3.5.3.1; 10, 14, 16, 18) have been used interchangeably in the literature. Ewing used the term dihydrolase in his publications (5–7). However, he did not detect either urea or citrulline in his cultures and therefore commented that the term arginine decarboxylase should probably be used (E. H. Ewing, *personal communication*).

The test itself is responsible for this confusion, because it depends only on the change in a *p*H-sensitive indicator and does not specifically identify or differentiate the possible end products. Table 1 lists some of the enzymes which can attack arginine or agmatine to produce an alkaline reaction in the medium. The pathways shown here for the enzymes attacking arginine are considerably more complex than those reported for ornithine or lysine decarboxylase. The amines produced by the latter two enzymes are stable, whereas agmatine and the other intermediates of arginine catabolism can be further converted to other amines or alkaline end products.

TABLE 1. *Some enzymes involved in the catabolism of arginine and agmatine*

Enzyme	Substrate	End products
Arginine decarboxylase	Arginine	Agmatine + CO_2
Arginase	Arginine	Ornithine + urea
Arginine dihydrolase[a]	Arginine	Ornithine + CO_2 + NH_3
Agmatinase[b]	Agmatine	Putrescine + urea
Agmatine dihydrolase	Agmatine	Putrescine + CO_2 + NH_3

[a] This is actually two enzymes: arginine desimidase (converting arginine to citrulline) and citrulline ureidase (converting the citrulline to ornithine).

[b] This enzyme has also been termed agmatine ureohydrolase.

Slade and Slamp (17) reported an aerobic dihydrolase system in the streptococci and isolated ornithine, NH_3, and CO_2 from their cultures. Møller (12) initially limited the use of the term arginine dihydrolase to describe "early" alkalinization of the medium (within 24 to 48 hr), whereas arginine decarboxylase referred to the late or delayed reactions (3 to 10 days). However, he also stated that both enzymes could act simultaneously in some organisms. Unfortunately, his end-product analyses were done with 10-day cultures, and similar data for the 24- to 48-hr cultures (on which he based his differential terminology) were never published. We have been able to detect agmatine within 4 hr by use of a large initial inoculum. The additional formation of putrescine in these cultures depended in most cases on the initial *p*H of the medium. The significance of this finding will be discussed in the section dealing with *p*H.

Media. Arginine decarboxylase is inducible in microorganisms (8). Thus, no enzymatic activity is found in the absence of arginine. When we began these studies using a large inoculum in a complex medium containing yeast extract and peptone (such as Møller's medium), we observed several false-positive reactions (9). Møller (12) also found them and added paraffin oil to his tubes to slow down the alkalinization of the controls (which could be caused by amine formation or NH_3 production). He also reported various *p*H shifts during the incubation period. Because of this, we derived a synthetic medium which more accurately related amine production to the single "parent" amino acid present in the medium, thus eliminating false reactions in the

controls. The same basal medium can be used for all three decarboxylase systems, thus standardizing methods and results.

Melnykovych and Snell (11) studied the nutritional requirements for the formation of arginine decarboxylase in *Escherichia coli* and reported a requirement for several additional compounds for optimal enzyme activity. These included asparagine, tyrosine, methionine, and iron. We found that tyrosine and methionine stimulated this enzyme under our conditions, whereas iron and asparagine had little or no activity. Growth in the presence of these amino acids did not induce agmatine formation in the absence of arginine.

The best glucose concentration was 1.5%. Arginine concentrations of 0.05 to 1% were tested in the short-term (1 to 16 hr) experiments, and 0.15 to 0.5% was found to give earlier and more strongly positive results than 0.5 to 1%. A concentration of 0.3% was chosen for the rest of these experiments.

Oxygen tension. Preliminary experiments indicated that a paraffin or mineral oil overlay was not necessary, even though Møller (12) recommended anaerobic conditions. Several authors (11, 13, 19, 20) have also reported excellent decarboxylase activity under aerobic conditions with organisms growing in stationary cultures, shake flasks, or chemostats.

Temperature. Previous investigators have differed in their reports on the optimal temperature for arginine decarboxylase activity (2, 8, 16). Temperatures ranging from 22 to 37 C have been cited. Table 2 presents data collected at 27 and 37 C. The effect of temperature on arginine decarboxylase activity was similar to that earlier observed with the procedures developed for ornithine and lysine. Positive results were obtained 1 to 2 hr earlier at 37 C in the organisms containing the enzyme. Temperature and *p*H studies were run simultaneously to find the most favorable combination. When a large initial inoculum was used, all argining decarboxylase-positive organisms produced agmatine within 3 hr at 37 C and a *p*H of 5.3. Overnight cultures were positive regardless of temperature or initial *p*H. A temperature of 37 C, therefore, was used in the remainder of these experiments.

pH. A *p*H range from 3.5 to 9.5 was used to determine the optimal *p*H for rapid agmatine production. Several authors (2, 8, 11, 13) reported *p*H optima ranging from 4.25 to 5.5, whereas others (11–13, 15, 16, 19) used a neutral or basic *p*H. In fact, Morris and Pardee (13) reported that *E. coli* contained two different arginine decarboxylase enzymes, one having a *p*H optimum at *p*H 5 and the other one around *p*H 8. This latter decarboxylase enzyme was reportedly involved in the formation of putrescine from arginine through a pathway in which agmatine, rather than ornithine, was an intermediate. They used the term ureohydrolase for the enzyme that converted agmatine to putrescine, although the term agmatinase is probably more common. See Table 1 for these pathways.

We therefore decided to observe both agmatine and putrescine production at different *p*H levels. Since preliminary studies indicated that the very acid or alkaline conditions were deleterious to the growth of many organisms (*p*H 3 to 5 and 8.5 to 9.5), *p*H 5.3 and 7.8 were chosen. The effect of *p*H on the time of formation of agmatine is shown in Table 2. As can be seen, agmatine appeared 1 to 2 hr earlier in cultures grown at the

TABLE 2. *Effect of pH and temperature on agmatine formation*

Organism	Time (hr) for positive reaction[a]			
	27 C		37 C	
	*p*H 5.3	*p*H 7.8	*p*H 5.3	*p*H 7.8
Escherichia coli K-12	3W	4W	2W	3W
E. coli 11246 (ATCC)	3W	4	2W	3W
E. coli B	1W	1W	1W	1W
Proteus vulgaris	N	N	N	N
P. morganii	N	N	N	N
P. mirabilis	N	N	N	N
Salmonella typhi	N	N	N	N
S. paratyphi	2	3W	1	3W
S. typhimurium	2	3W	1	3
S. newport	3W	4W	2W	4
Klebsiella pneumoniae	N	N	N	N
Providencia alcalifaciens	N	N	N	N
Enterobacter cloacae	1	3W	2	3
E. hafniae	N	N	N	N
Arizona	1W	2W	1	1W
Citrobacter 8090 (ATCC)	2W	—	1W	3W
Serratia marcescens HY	3	4	2	4
S. marcescens	N	N	N	N
Shigella flexneri	1	4	1	2
S. dysenteriae	1W	2W	1	2W
S. sonnei 9290 (ATCC)	1W	4W	1W	2W
Pseudomonas aeruginosa	N	N	N	N
P. stutzeri	N	N	N	N
P. maltophilia	3	4	3	3

[a] W = weak reaction followed by a strong reaction within the next hour; — = negative within 4 hr but positive after overnight incubation. N = not positive after overnight incubation (organism lacking arginine decarboxylase). Overnight cultures grown in the basal synthetic medium were washed and resuspended in the synthetic medium plus 0.3% arginine.

TABLE 3. *Effect of pH on the formation of agmatine and putrescine*[a]

Organism	Agmatine		Putrescine	
	pH 5.3	*pH* 7.8	*pH* 5.3	*pH* 7.8
Klebsiella pneumoniae	−	−	−	−
Escherichia coli K-12	+	+	−	+
E. coli 11246 (ATCC)	++	++	−	++
E. coli B	++	++	−	++
Citrobacter 8090 (ATCC)	++	++	+	++
Arizona	++	+	−	++
Proteus morganii	−	−	−	−
P. mirabilis	−	−	−	−
P. vulgaris	−	−	−	−
Salmonella paratyphi	++	++	++	++
S. anatum	+	++	−	++
S. typhimurium	++	++	+	++
S. newport	+	+	−	+
S. typhi	−	−	−	−
Shigella sonnei 9290 (ATCC)	++	+	+	++
Enterobacter cloacae	+	+	+	++
E. hafniae	−	−	−	−
Enterobacter sp. (group A)	+	+	−	++
Pseudomonas stutzeri	−	−	−	−
P. aeruginosa	−	−	−	−
P. maltophilia	+	+	−	++
Serratia marcescens HY	+	+	+	+
S. marcescens	−	−	−	−

[a] Symbols: + = positive; ++ = strongly positive; − = compound absent. Overnight cultures were grown in the basal synthetic medium, washed, and resuspended in the synthetic medium plus 0.3% arginine. Cultures were harvested after 16 hr and tested for the presence of agmatine and putrescine. The temperature of incubation was 37 C.

initial *p*H of 5.3. All organisms which contained the enzyme produced agmatine at this *p*H within 4 hr when a large initial inoculum was used. Agmatine formation was delayed at the higher *p*H. For example, agmatine was observed within 2 hr in cultures of *Citrobacter* at *p*H 5.3 but not until 6 to 16 hr when the initial *p*H was 7.8. With overnight or 24-hr cultures, the initial *p*H made little difference in agmatine formation.

As can be seen in Table 3, the initial *p*H of the medium had a definite effect on putrescine production. Since agmatine production was delayed in some organisms grown at *p*H 7.8, 16-hr cultures were used for this study. In all of the organisms observed so far, those producing agmatine also formed putrescine when grown at this *p*H, regardless of the ornithine decarboxylase potential of the cells. Interestingly enough, several of the organisms that were arginine decarboxylase- and ornithine decarboxylase-positive (A+O+), such as *Arizona* and several of the *Salmonella* species, did not produce putrescine when grown at the acid *p*H. Those organisms (A+O+) which did, *Shigella sonnei* and *Enterobacter cloacae*, usually produced very small amounts. These data tended to substantiate the conclusions of Morris and Pardee (13) that the enzymes involved in the conversion of arginine to putrescine via agmatine were operative at *p*H 7.5 to 8.0 rather than at the lower *p*H. In addition, our earlier studies with ornithine decarboxylase (9) indicated that a *p*H of 7.5 was more favorable for rapid putrescine formation than was the acid *p*H. Thus, *p*H 5.3 did not provide favorable conditions for rapid production of putrescine, and very little was formed through this pathway as a result.

E. coli 11246 (ATCC) was A+O−, so putrescine must have arisen from agmatine rather than ornithine. These pathways are presented for comparison in Table 1.

When *Citrobacter* (A+O−), *E. coli* 11246 (A+O−), and *Salmonella typhimurium* (A+O+) were grown on the synthetic medium containing agmatine instead of arginine, putrescine was produced by all three. When citrulline, an intermediate in the dihydrolase system was used as the substrate here, only *S. typhimurium* (A+O+) converted it into putrescine. When another A+O+ organism, *Arizona*, was grown on citrulline, no putrescine was detected. If arginine dihydrolase was functioning in all four organisms and citrulline was converted to ornithine by citrulline ureidase, only *S. typhimurium* and *Arizona* would be able to form putrescine via the ornithine decarboxylase pathway. Since we were primarily interested in a rapid method for arginine decarboxylase, we did not examine cultures for ornithine or urea at this time. We can only deduce from the data with citrulline as a substrate that *Arizona* did not contain the dihydrolase system under our conditions, whereas *S. typhimurium* did (although both would be called dihydrolase positive with Møller's procedure). *E. coli* and *Citrobacter* might contain this enzyme system but were unable to form putrescine from ornithine since both were O−. Since putrescine was formed when agmatine was used as the substrate, we can safely assume that all of these organisms contained agmatine ureohydrolase, agmatinase, or the other enzymes postulated to convert agmatine to putrescine. One indisputable fact emerged very clearly, however: *all* of the organisms discussed above converted arginine to agmatine via arginine decarboxylase (Tables 2 and 3).

Comparison of methods. To continue this detective game of postulating which enzymes might be operative, we grew various organisms on both the synthetic medium (detecting agmatine) and Møller's medium (detecting an alkaline reaction

TABLE 4. *Comparison of methods used to determine arginine decarboxylase activity*[a]

Organism	Synthetic	Møller	Literature[b]
Klebsiella pneumoniae	−	−	−
Escherichia coli 11246 (ATCC)	++	+	d
Citrobacter 8090 (ATCC)	++	++	d
Arizona	++	+	+
Proteus morganii	−	−	−
P. vulgaris	−	−	−
P. mirabilis	−	−	−
Salmonella paratyphi	++	+	+
S. anatum	++	+	+
S. typhimurium	++	+	+
S. typhi	−	−	−
Enterobacter cloacae	+	++	+
E. hafniae	−	−	−
Pseudomonas maltophilia	+	+	+
P. aeruginosa	−	+	+
Serratia marcescens HY	+	−	d
S. marcescens	−	−	d
Providencia alcalfaciens	−	−	−
Shigella flexneri	+	+	d
S. dysenteriae	+	+	d

[a] Symbols: + = positive reaction; ++ = strongly positive; − = negative reaction; d = variable reaction. Cultures were observed after 4 hr of growth in the synthetic medium and after 4 days on Møller's medium. A 24-hr culture, grown in the basal synthetic medium, was used as an inoculum.

[b] Most of the literature refers to dihydrolase rather than decarboxylase production (4–7, 12, 16, 18, 21). The data above with the synthetic medium refer to agmatine production and those with Møller's to the formation of an alkaline reaction in the medium.

in the medium). These data are presented in Table 4. Ornithine decarboxylase activity had been previously determined with these organisms (9).

All of the *Shigella* and *Salmonella* strains tested were A+O+ on both media, except for *S. typhi* (A−O−). To indicate the confusion in interpreting accurately the biochemical reactions which *could* have been operating individually or simultaneously in these A+O+ organisms, we have listed the pertinent enzyme systems below, formulated on the basis of data reported in the literature (5–7, 12, 13, 16, 18, 20) and obtained in this study: (i) arginine decarboxylase (all produced agmatine); (ii) arginine dihydrolase (all produced putrescine from arginine); (iii) agmatine ureohydrolase, agmatinase, or agmatine dihydrolase (putrescine formed from agmatine); (iv) ornithine decarboxylase [putrescine formed from ornithine by arginine dihydrolase (2) or arginase]. These would all result in an alkaline reaction in both media.

The strains of *E. coli*, *Citrobacter*, and *Pseudomonas maltophilia* used in these experiments were A+O−. Since agmatine was produced from arginine, these organisms all contained arginine decarboxylase. The formation of putrescine from either agmatine or arginine indicated that agmatine ureohydrolase, agmatinase, or agmatine dihydrolase were present. Since no putrescine was produced from citrulline, the arginine dihydrolase system was either inactive or the reaction stopped at ornithine. Again, these reactions would result in an alkaline *p*H in the media.

Several strains of *Pseudomonas* (mostly clinical isolates) were tested in addition to those mentioned in Tables 2–4. They all gave results similar to those listed for *P. aeruginosa*. None produced agmatine from arginine or putrescine from ornithine, arginine, or agmatine (all were A−O−). The pseudomonads were usually designated in the literature as dihydrolase-positive. Thornley (20) reported citrulline and NH_3 formation in these organisms. An alkaline reaction was produced in Møller's medium. Probably the arginine dihydrolase system is truly functioning here, forming ornithine (or citrulline), CO_2, and NH_3. Our method would report these as A− or arginine decarboxylase-negative since no agmatine was produced. This is one of the differences between the two methods. Our procedure does not detect dihydrolase activity.

Klebsiella pneumoniae (A−O−), the three species of *Proteus* (A−O±), *Providencia alcalfaciens* (A−O−), and *Enterobacter hafniae* (A−O+) were negative on both media. No putrescine was formed by these organisms, regardless of their ability to decarboxylate ornithine. Since they were negative on Møller's medium, they were probably arginine dihydrolase-negative as well.

When biochemical reactions are used to characterize and identify microorganisms, the accuracy of the interpretation depends upon the specificity of the test. The detection of agmatine, the primary end product of arginine decarboxylase activity, provided a more sensitive and definitive reflection of arginine catabolism than the indirect measurement of an alkaline reaction in the medium.

Extraction and detection methods. When the butanol extraction procedure was used for differentially extracting agmatine from the medium containing arginine, putrescine was also found in the butanol layer. However, the diacetyl reagent was specific for the guanidino group of agmatine and did not react with putrescine. Sakaguchi's reagent, which also reacted with guanidino groups,

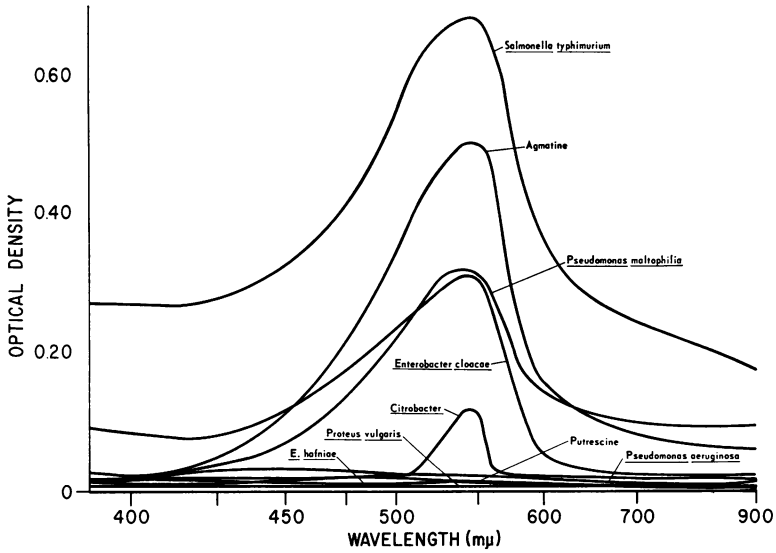

FIG. 1. *Absorption spectra obtained when the diacetyl reagent was added to the butanol extracts of organisms grown for 4 hr on the synthetic medium containing arginine. Controls of agmatine and putrescine were similarly treated.*

was initially investigated for this procedure. However, the control tubes were tinted, and the color complex was unstable. The diacetyl reagent was finally selected because it had none of these faults.

Several organisms were grown in the synthetic medium containing arginine and extracted with the *n*-butanol. The extracts were reacted with the diacetyl reagent and placed in a Beckman ratio-recording spectrophotometer. Samples of agmatine and putrescine were treated similarly. Figure 1 shows the curves for several organisms. *S. typhimurium*, *P. maltophilia*, *E. cloacae*, and *Citrobacter* exhibited peaks in the same portion of the spectrum as agmatine. Although putrescine was present in these extracts, no interfering peak was recorded. The extracts from *Proteus vulgaris*, *Pseudomonas aeruginosa*, *E. hafniae*, and the control sample of putrescine were negative across the whole spectrum and did not interfere in the color reaction with the guanidino groups.

As indicated in the preceding paper (9), we have tried to adapt these procedures for the routine laboratory by utilizing pipettors, similar techniques, and a minimal amount of glassware and handling. Organisms which were strongly positive could be determined by adding the diacetyl reagent directly to the culture tube after butanol extraction. There was some color formed at the interface and in the aqueous layer where the diacetyl reacted with the residual arginine or any agmatine not completely extracted into the top butanol layer. The addition of a few drops of dimethylsulfoxide to the diacetyl reagent helped somewhat. However, the most accurate results were obtained by placing 0.5 ml of the butanol layer in either a spot plate or a small test tube before adding the diacetyl reagent.

Diacetyl and 1-naphthol were soluble in butanol. However, no colored complex formed with agmatine when these compounds were prepared in butanol and the NaOH and NaCl were eliminated. Similarly, the ninhydrin reagent would not react with the amines in the absence of hydroxyl groups.

Thin-layer chromatography. Duplicate ascending one-dimensional thin-layer chromatography plates were run on the butanol extracts from several positive and negative organisms. One set was sprayed with diacetyl or Sakaguchi's reagent to detect specifically the guanidino portion of agmatine (Fig. 2). The other was sprayed with a ninhydrin reagent to detect compounds containing NH_2 groups such as putrescine and agmatine (Fig. 3). Samples of agmatine and putrescine were included as controls. A comparison of these chromatograms showed that both agmatine and putrescine were present in the butanol extracts from cultures of *S. typhimurium* (A+O+) and *E. coli* (A+O−) but were absent in the extract from *P. vulgaris* (A−O−). In addition, the Sakaguchi or diacetyl reagent was specific for agmatine, because the area where putrescine was located remained colorless. The plate in Fig. 2 was sprayed with Sakaguchi's reagent. Diacetyl also gave the same results,

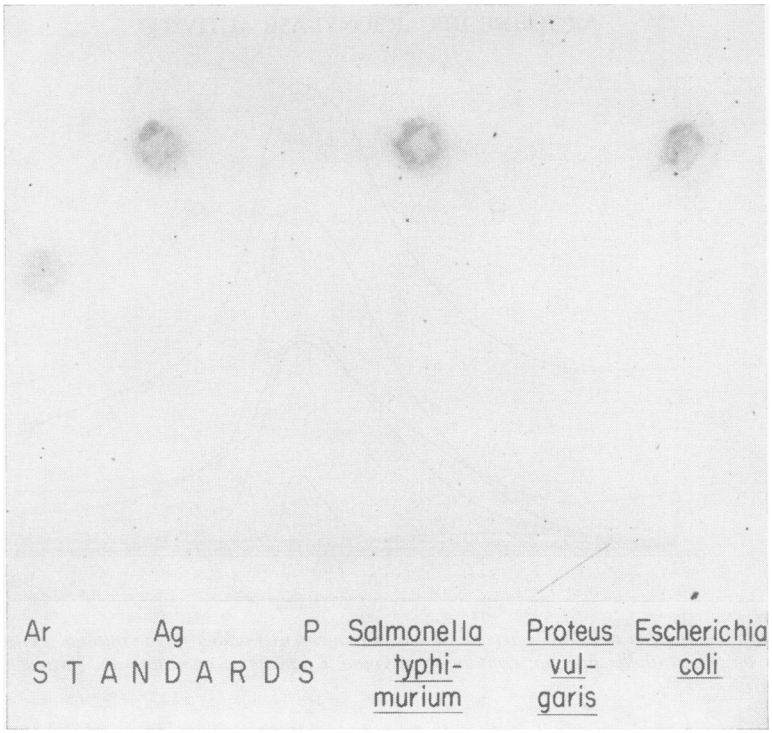

FIG. 2. *Thin-layer one-dimensional chromatogram of the butanol extracts from several organisms grown for 4 hr in the synthetic medium containing arginine. The chromatogram was developed by spraying with Sakaguchi's reagent to detect compounds containing a guanidino group. This chromatogram was a duplicate of that shown in Fig. 3. Ar = arginine; Ag = agmatine; P = putrescine. These were used as standards.*

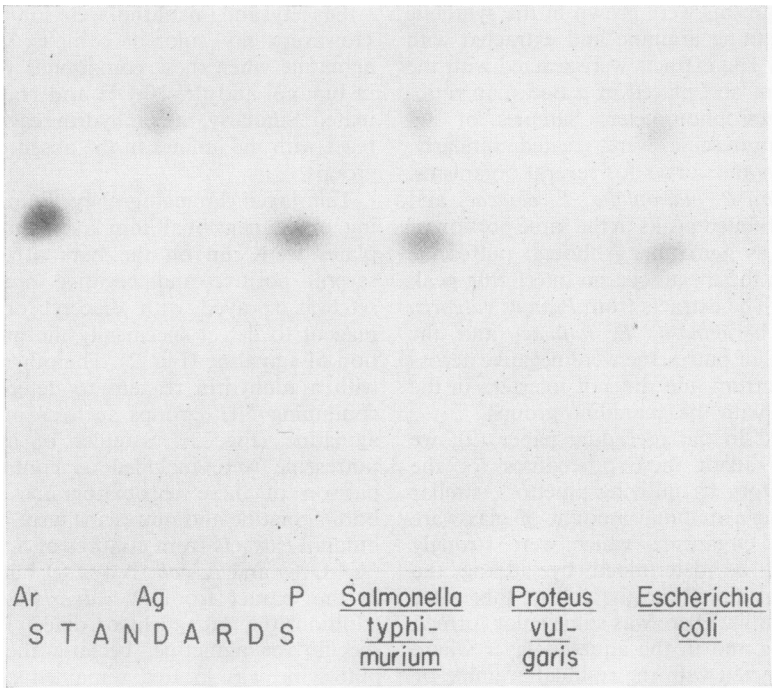

FIG. 3. *Thin-layer one-dimensional chromatogram of the butanol extracts from several organisms grown for 4 hr in the synthetic medium containing arginine. The chromatogram was developed by spraying with a modified ninhydrin reagent. This chromatogram was a duplicate of the one shown in Fig. 2. Ar = arginine; Ag = agmatine; P = putrescine. These were used as standards.*

but the background became discolored and the contrast was not distinctive enough for photographic purposes.

These chromatograms also indicated that no other amines or diacetyl-reacting compounds were present in these extracts. Although not shown here, chromatograms of cultures grown in the basal synthetic medium did not contain agmatine, putrescine, or other amines. Since citrulline and ornithine were not extracted by butanol, they would not appear in these chromatograms. Cadaverine, the amine end product of lysine decarboxylase, was extractable by butanol. However, this amine was never found in any of the chromatograms made from cultures which were grown in the synthetic medium containing arginine, regardless of the genetic potential of the organisms. This was even true when the initial inoculum for these short experiments was prepared from washed cultures grown in a complex medium, because no false-positive results were observed in the controls. This would be of value in the clinical microbiology laboratory where cultures were usually isolated on complex media.

Thus, in summary, a rapid and specific biochemical method for the determination of arginine decarboxylase activity has been developed. It was based on the differential extraction of agmatine, the primary end product, from cultures grown on a synthetic medium containing arginine as the key amino acid. Since the enzyme was inducible, the use of a large inoculum allowed early expression of decarboxylase activity without necessarily requiring concurrent growth. This method correlated with procedures developed for rapid determination of ornithine and lysine decarboxylase activity.

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