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Biosynthesis of Putrescine in the Prostate Gland of the Rat

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In the rat ventral prostate gland the biosynthesis of putrescine, a precursor of spermidine and spermine, is shown to occur by the direct decarboxylation of L-ornithine. Some properties of a soluble pyridoxal phosphate-dependent L-ornithine decarboxylase are described. The findings are discussed in relation to other enzymic reactions involved in the biosynthesis of polyamines by the prostate gland.

The prostate gland of sexually mature males of many mammalian species contains large amounts of the polyamines spermine and spermidine (Raina, 1963; Mann, 1964; Tabor & Tabor, 1964). In some mammals, spermine or spermidine or both may appear in the secretions elaborated by prostatic epithelial cells (Mann, 1964; Tabor & Tabor, 1964). It is well established that polyamines can interact with, and stabilize, ribosomes, mitochondria, cell membranes and various polynucleotides from many different types of cells (Tabor, Tabor & Rosenthal, 1961; Tabor & Tabor, 1964). Such actions of spermine and spermidine may be of physiological significance in living cells, especially with regard to the formation and turnover of ribopolynucleotides and proteins. However, the functions of the polyamines in the prostate gland and its fluids remain obscure. An understanding of the enzymic basis of polyamine biosynthesis in the prostate gland would throw considerable light on this problem, as it might enable the concentrations of prostatic polyamines to be controlled by application of specific inhibitors of the enzymes involved in this process.

There is clear evidence that putrescine can act as a precursor for the synthesis of polyamines by bacterial (Tabor, Rosenthal & Tabor, 1958; Tabor & Tabor, 1964) and mammalian (Raina, 1963, 1964; Tabor & Tabor, 1964; Jänne & Raina, 1966; Siimes & Jänne, 1967; Jänne, 1967) cells *in vivo*. Pegg & Williams-Ashman (1968) demonstrated the enzymic synthesis of spermidine by soluble cell-free extracts of the rat ventral prostate gland, an organ that is exceptionally rich in polyamines (Rhodes & Williams-Ashman, 1964). The decarboxylation of (−)-*S*-adenosyl-L-methionine by such prostatic preparations is markedly and specifically enhanced by the addition of putrescine. In the presence of putrescine there is a close correspondence between the liberation of carbon dioxide from (−)-*S*-adenosyl-L-methionine and the incorporation of radioisotope from labelled putrescine into spermidine. Many of the properties of the prostatic enzyme system catalysing this putrescine-dependent decarboxylation of (−)-*S*-adenosyl-L-methionine (Pegg & Williams-Ashman, 1968) are different from those described by Tabor (1962*a*) for a (−)-*S*-adenosyl-L-methionine decarboxylase from *Escherichia coli*. Further insight into the enzymic pathways responsible for polyamine biosynthesis in the prostate gland requires delineation of the enzymic reaction(s) catalysing the formation of putrescine in this organ.

The enzymic synthesis of putrescine by certain bacteria and higher plants has been the subject of a number of reports. Gale (1940) discovered an inducible enzyme catalysing the conversion of L-ornithine into putrescine in *E. coli*. Morris & Pardee (1965, 1966) found that *E. coli* contains separate 'inducible' and 'biosynthetic' L-ornithine decarboxylases, which differ from each other in many properties, and which require different conditions for their formation. In addition, *E. coli* contains two separate L-arginine decarboxylases of the 'inducible' and 'biosynthetic' type, which catalyse the degradation of L-arginine to agmatine. *E. coli* (Morris & Pardee, 1966) and mycobacteria (Zeller, Van Orden & Vögtli, 1954) contain enzymes that hydrolyse agmatine to form putrescine and urea. Certain higher plants convert agmatine into putrescine via the intermediate formation of *N*-carbamoylputrescine (Smith & Garroway, 1964).

Decarboxylation of ornithine has also been reported to occur in etiolated pea seedlings (Hasse, Ratych & Salnikow, 1967).

The enzymic synthesis of putrescine by cell-free preparations of mammalian tissues does not appear to have been described previously, although experiments involving administration of ^{14}C-labelled precursors have suggested that avian and mammalian tissues degrade arginine and ornithine to putrescine *in vivo* (Raina, 1963, 1964; Jänne & Raina, 1966; Jänne, 1967). It is shown in the present paper that the rat ventral prostate gland contains a soluble L-ornithine decarboxylase. Some properties of this enzyme, which requires pyridoxal phosphate, are described.

MATERIALS AND METHODS

Animals. Adult male rats of the Sprague–Dawley strain weighing 275g. or more were fed on a diet of rat cake and water *ad libitum*.

Materials. Putrescine, spermidine, spermine and pyridoxal phosphate (crystalline, approx. 98% pure) were purchased from the Sigma Chemical Co., St Louis, Mo., U.S.A. Paper-chromatographic (Herbst, Keister & Weaver, 1958) and paper-electrophoretic (Raina, 1964) analyses indicated that the specimens of putrescine, spermidine and spermine were uncontaminated by each other. L-Ornithine, DL-ornithine and L-arginine were obtained from Mann Research Laboratories Inc., New York, N.Y., U.S.A.

DL-[1-^{14}C]Ornithine (2·3mc/m-mole), L-[U-^{14}C]ornithine (172mc/m-mole) and [1,4-$^{14}C_2$]putrescine dihydrochloride (5·22mc/m-mole) were provided by the New England Nuclear Corp., Boston, Mass., U.S.A. L-[U-^{14}C]Arginine (172mc/m-mole) and $NaH^{14}CO_3$ (22·8mc/m-mole) were obtained from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A. Solutions of amines that were supplied as hydrochloride salts were adjusted to pH7·2 with tris base.

Preparation of tissue extracts. Rats were killed by cervical dislocation, and the ventral prostate glands were freed of fat and connective tissue *in situ*. The ventral prostate tissue was placed in ice-cold solutions containing 0·25M-sucrose, 10mM-tris–HCl buffer, pH7·7, 1mM-2-mercaptoethanol and 0·1mM-EDTA (disodium salt). After being minced with scissors at 0°, the tissue was homogenized at 0–2° in a glass apparatus with a rotating Teflon pestle. The homogenate was centrifuged at 10000*g* for 10min. and the pellet discarded. The supernatant fluid was centrifuged at 45000rev./min. for 90min. in a no. 50 rotor of a Spinco model L-2 ultracentrifuge. All centrifugations were carried out at 2°. The supernatant fluid obtained after ultracentrifugation was passed through a glass funnel containing a plug of glass wool.

Crude nuclear, mitochondrial and microsomal fractions were obtained by centrifugation at 2° of the homogenate at 600*g* for 10min., 10000*g* for 10min. and 100000*g* for 90min. respectively. The nuclear and mitochondrial pellets were washed twice by resuspension in the homogenizing medium, followed by the appropriate centrifugations. Nuclei were also isolated from ventral prostate homogenates prepared in 2·2M-sucrose by the method of Chauveau, Moulé & Rouiller (1956).

Ultracentrifuged prostatic extracts were fractionated with $(NH_4)_2SO_4$ as follows. To each 10ml. of solution was added 15ml. of saturated $(NH_4)_2SO_4$ soln. with stirring at 0°. [The $(NH_4)_2SO_4$ soln. was saturated at 0° and adjusted to pH6·9 with aq. NH_3 soln.; the $(NH_4)_2SO_4$ was of low heavy-metal content, obtained from Mann Research Laboratories Inc.] After all the $(NH_4)_2SO_4$ had been added, the solution was stirred gently for a further 30min. and then centrifuged at 12000*g* for 15min. To each 25ml. of the supernatant fluid was added 25ml. of saturated $(NH_4)_2SO_4$ soln. The precipitate that formed after stirring for 15min. was collected by centrifugation and suspended in a medium containing 20mM-tris–HCl buffer, pH7·7, 5mM-2-mercaptoethanol and 0·05mM-pyridoxal phosphate. The extracts were dialysed at 2° overnight against 3l. of the same medium.

Assay of ornithine decarboxylase activity. The reactions were followed by measurement of release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine. The composition of the reaction mixtures is shown in the legends to the Tables and Figures. The reactions were carried out in conical glass flasks equipped with rubber stoppers; each flask carried a polypropylene well, in which was placed 0·2ml. of ethanolamine–ethylene glycol monomethyl ether (2:1, v/v). (These assemblies were supplied by the Kontes Glass Co., Vineland, N.J., U.S.A.) The reactions were initiated by addition of the enzyme solution. After incubation for various time-intervals at 37°, 0·5ml. of 5N-H_2SO_4 was injected through the rubber cap to halt the reaction and to release bound CO_2 from the reaction mixture. A further incubation for 15min. at 37° was allowed to ensure complete absorption of the CO_2 into the solution contained in the polypropylene well. The wells and their contents were then removed, and placed in glass counting vials containing 15ml. of a scintillation-counting fluid of the following composition: xylene–dioxan–ethanol (1·0:1·0:1·1, by vol.) containing 7·5% (w/v) naphthalene, 0·45% (w/v) 2,5-diphenyloxazole and 0·0045% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene. When this procedure was checked by using various accurately determined concentrations of $NaH^{14}CO_3$ in the flasks, it was found that the recovery of labelled CO_2 was more than 99% over a range of concentrations similar to those obtained in the enzymic decarboxylation experiments.

Measurement of the stoicheiometry between the release of carbon dioxide and the formation of putrescine. In these experiments, L-[U-^{14}C]ornithine was used as the substrate, and the release of CO_2 was estimated as described above, except that the reactions were halted by the addition of 0·5ml. of 20% (w/v) trichloroacetic acid instead of 5N-H_2SO_4. After the polypropylene wells had been removed for measurement of the CO_2 formed, the acidified reaction mixtures were quantitatively transferred to tubes and centrifuged. The precipitate of protein was washed twice by resuspension in 1ml. of 5% (w/v) trichloroacetic acid followed by centrifugation. The combined acid-soluble material and washes were extracted twice at room temperature with diethyl ether to remove trichloroacetic acid. The solution was then extracted with butan-1-ol, as described by Raina (1964). The butan-1-ol extract was evaporated to dryness in a rotary evaporator at 50°, and the residue was dissolved in 0·1ml. of 0·1N-HCl. Portions (5μl.) of this solution were applied to strips of Whatman no. 1 paper (31cm.×4cm.) and subjected to electrophoresis at 300v for 100min. with 0·05M-sodium citrate buffer, pH3·4.

After the completion of the electrophoresis, the papers were dried and cut into strips 1 cm. in width. The strips were placed in counting vials, covered with 15 ml. of scintillation-counting fluid, and the radioactivity was determined. This procedure gave excellent separation of putrescine from ornithine. A sample of ^{14}C-labelled putrescine was added to similar reaction mixtures and processed in the same fashion concurrently with the other samples to correct for losses during the extraction. It was found that 92% of the added putrescine was recovered in the butan-1-ol fraction, and the results were corrected accordingly. The putrescine formed by the enzymic decarboxylation of ornithine migrated to the same position as authentic samples of putrescine both on paper electrophoresis and in the paper-chromatographic system of Herbst *et al.* (1958). The position of putrescine and ornithine on the papers was determined by spraying them with a solution of ninhydrin.

Determination of radioactivity. Radioactivity was assayed with a Nuclear–Chicago liquid-scintillation system no. 722. The counting efficiency for ^{14}C was in the range 52–55%. Suitable corrections were applied for background counts, and for radioactivity of zero-time control samples in all enzyme experiments. Sufficient counts were recorded to obtain S.D. of the net count rate less than $\pm 5\%$.

Other methods. All pH measurements were made with a glass electrode, and referred strictly to the stated temperatures. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Homogenates of rat ventral prostate were found to be able to catalyse the release of labelled carbon dioxide from DL-[1-^{14}C]ornithine (Table 1). The results shown in Table 1 for the activity of undialysed fractions may be an underestimate, since any endogenous ornithine present would decrease the specific radioactivity of the labelled ornithine used as substrate. However, measurements performed at two separate substrate concentrations showed a similar distribution of decarboxylase activity between the various subcellular fractions. Most of the detectable activity was present in the fraction of the homogenate that was not sedimentable by ultracentrifugation at 100000*g* for 90 min. The interrelationship between polyamines and nucleic acids in the cell (Caldarera, Barbiroli & Moruzzi, 1965; Dykstra & Herbst, 1965; Kostyo, 1966; Cohen & Raina, 1967; Dion & Herbst, 1967; Jänne, 1967) raises the possibility that some or all of the enzymes involved in polyamine synthesis are of nuclear origin. Since it is known that certain nuclear enzymes readily leak out of nuclei prepared by homogenization in such dilute sucrose media, nuclei were prepared by the method of Chauveau *et al.* (1956) and tested for ornithine decarboxylase activity. However, as shown in Table 1, very little activity was found in nuclei prepared in this manner. Thus it appeared that the ornithine decarboxylase of rat ventral prostate gland is present almost exclusively in the soluble portion of the cytoplasm.

Table 1. *Decarboxylation of ornithine by prostate homogenates and subcellular fractions*

The incubation medium for Expt. *A* contained 50 μmoles of tris–HCl buffer, pH 7·1, 0·05 μmole of pyridoxal phosphate, 2·5 μmoles of 2-mercaptoethanol, 0·21 μmole of DL-[1-^{14}C]ornithine (2·3 mc/m-mole) and approx. 10 mg. of protein of each fraction in a total volume of 1 ml. In Expt. *B*, 2·1 μmoles of DL-[1-^{14}C]ornithine (0·23 mc/m-mole) were added; the other components were the same as for Expt. *A*. Incubation was for 1 hr. at 37°. The tissue fractions were prepared as described in the Materials and Methods section. Nuclei (*a*) were prepared by homogenization in 0·25 M-sucrose and nuclei (*b*) by homogenization in 2·2 M-sucrose, as described in detail in the text.

Fraction added	CO_2 formed ($\mu\mu$moles/hr./mg. of protein) Expt. *A*	Expt. *B*
Nuclei (*a*)	24	95
Nuclei (*b*)	5	32
Mitochondria	14	—
Microsomes	3	—
Soluble fraction	95	484
Dialysed soluble fraction	280	568
Ammonium sulphate fraction after dialysis	817	1450

The protein precipitated by ammonium sulphate between 60% and 80% saturation contained most of the ornithine decarboxylase activity, and exhibited about three times the specific activity of the initial 100000*g* supernatant extracts. This fraction was used as an enzyme source for most of the experiments described in this paper. Even with this preparation, the activity was very small compared with that of bacterial amino acid decarboxylases (Gale, 1946) and necessitated the use of an isotopic assay method. For economic reasons the commercially available DL-[1-^{14}C]-ornithine was used as a substrate in most of the experiments. As shown in Table 2, the release of labelled carbon dioxide from this substrate was decreased by the addition of unlabelled L-ornithine or DL-ornithine. The production of labelled carbon dioxide was decreased by the addition of DL-ornithine to a value similar to that obtained when half the concentration of the L isomer was used. This finding indicated that only the L isomer is decarboxylated, and that the presence of equal amounts of the D isomer does not affect the reaction.

Even with crude undialysed extracts the ornithine decarboxylase activity was appreciably increased by the addition of pyridoxal phosphate. After fractionation with ammonium sulphate and dialysis, the requirement for pyridoxal phosphate became absolute. Table 3 shows these results and

Table 2. *Effect of unlabelled* DL-*ornithine and* L-*ornithine on decarboxylation of labelled* L-*ornithine*

The basal medium and incubation conditions were similar to those of Table 1, Expt. *A*. Approx. 2mg. of protein (dialysed ammonium sulphate fraction) was added. The results shown have been corrected for the radioactivity of zero-time control samples. The CO_2 released was calculated by assuming that only L-ornithine takes part in the reaction.

Addition to medium	Radioactivity released (counts/min. released/hr.)	CO_2 released ($\mu\mu$moles/hr.)
None	3284	1148
5 μmoles of DL-ornithine	375	3410
2·5 μmoles of L-ornithine	387	3520
2·5 μmoles of DL-ornithine	685	3090
1·25 μmoles of L-ornithine	677	3040
0·50 μmole of DL-ornithine	1828	2165
0·25 μmole of L-ornithine	1898	2250

Table 3. *Effects of inhibitors and pyridoxal phosphate*

The complete medium contained 50 μmoles of tris–HCl buffer, pH 7·1, 0·05 μmole of pyridoxal phosphate, 2·5 μmoles of 2-mercaptoethanol and 0·21 μmole of DL-[1-^{14}C]ornithine (2·3mc/m-mole) in a total volume of 1·0ml. In Expt. *A* about 5mg. of protein (dialysed ammonium sulphate fraction) was added, and in Expt. *B* about 10mg. of protein (100000*g* supernatant fraction) was added.

System	CO_2 released ($\mu\mu$moles/hr./mg. of protein) Expt. *A*	Expt. *B*
Complete	320	111
Pyridoxal phosphate omitted	12	38
KCN (0·1mM) added	190	—
Isonicotinic acid hydrazide (0·5mM) added	15	3
4-Bromo-3-hydroxybenzyl-oxyamine (0·1mM) added	7	4

also that the reaction was inhibited by the well-known inhibitors of pyridoxal phosphate-requiring enzymes, isonicotinic acid hydrazide and 4-bromo-3-hydroxybenzyloxyamine (Levine, Sato & Sjoerdsma, 1965). The concentration, 0·05mM, of pyridoxal phosphate always used gave a maximal rate of decarboxylation.

Enzymic activity was rapidly lost, particularly after preparation of the ammonium sulphate fraction followed by dialysis. This loss of activity could be retarded, but not completely prevented, by the addition of pyridoxal phosphate to the solution of enzyme and dialysing medium (Table 4). Similar stabilization of pyridoxal phosphate-requiring enzymes by the cofactor has been described previously (Shukuya & Schwert, 1960; Thompson & Richardson, 1967). The considerable decline of activity on storage (even in the presence of pyridoxal phosphate) could not be prevented by freezing, or by the addition of glycerol or other reagents known to stabilize certain enzymes. This problem, coupled with the difficulties of obtaining large amounts of starting material, thwarted the further purification of this mammalian ornithine decarboxylase. A similar enzymic activity could be detected in rat liver, but this tissue was at least 10 times less active than the prostate gland when results were expressed in terms of carbon dioxide liberated/g. wet wt. of tissue.

No requirement of the prostatic enzyme for any metal activator could be demonstrated: Mg^{2+}, Mn^{2+}, Ca^{2+}, Co^{2+}, Zn^{2+} and Fe^{3+} were tested at a range of concentrations between 1mM and 20mM. The optimum pH for the reaction with either tris–hydrochloric acid or sodium phosphate buffer was about 7·0 (Fig. 1).

The stoicheiometry of the reaction was investigated with L-[U-^{14}C]ornithine as a substrate. The labelled carbon dioxide released was counted as described above, and the putrescine formed was estimated by extraction into butan-1-ol from alkaline solution followed by paper electrophoresis. No radioactive product other than putrescine was found, and there was a close correspondence between the amount of putrescine formed and the amount of carbon dioxide released (Table 5).

In initial experiments, the concentration of labelled DL-ornithine used was 0·21mM, which, although giving a linear rate of incorporation for at least 60min. incubation (Fig. 2), was not a saturating concentration. With this ornithine concentration there was a three- to four-fold increase in activity after dialysis of the prostate supernatant fraction (Table 1). This increase in activity on dialysis was markedly diminished when 2·1mM-DL-ornithine was used as substrate, and therefore was more likely to be due to removal of competitive inhibitors of the reaction than to dilution of the labelled ornithine with endogenous ornithine.

Despite the inherent difficulties of interpreting

Table 4. *Stabilization by pyridoxal phosphate*

The 100000*g* supernatant fraction was dialysed for 12hr. as described in the Materials and Methods section, or with the omission of pyridoxal phosphate from the dialysis medium. After dialysis the fractions were stored at 4° until assayed. The assay medium was as described in Table 3.

	Ornithine decarboxylase activity ($\mu\mu$moles/hr./mg of protein.)		
Time after homogenization (hr.) ...	18	42	66
Treatment of fraction			
Dialysed without pyridoxal phosphate	190	145	98
Dialysed with 0·1 mM-pyridoxal phosphate	244	212	194

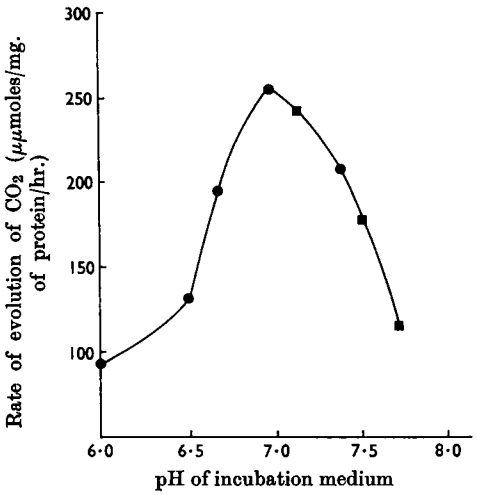

Fig. 1. Effect of pH on decarboxylation of ornithine. The medium contained 50μmoles of tris–HCl buffer (■), or sodium phosphate buffer (●), of the pH shown, 0·05μmole of pyridoxal phosphate, 2·5μmoles of 2-mercaptoethanol, 0·21μmole of DL-[1-^{14}C]ornithine (2·3mc/m-mole) and about 5mg. of protein (dialysed supernatant fraction), in a total volume of 1·0ml.

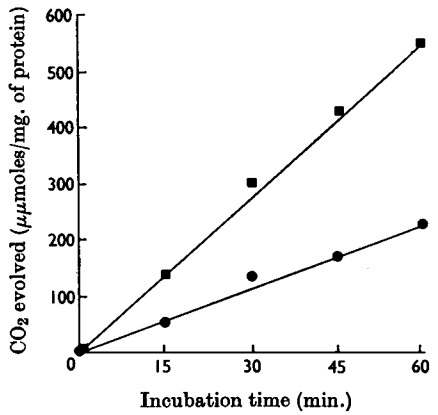

Fig. 2. Time-course of decarboxylation of ornithine by supernatant fraction. The medium contained 50μmoles of tris–HCl buffer, pH 7·1, 0·05μmole of pyridoxal phosphate and 2·5μmoles of 2-mercaptoethanol in a total volume of 1·0ml. The concentration of DL-[1-^{14}C]ornithine was 0·21mM (●) or 1·21mM (■). About 5mg. of protein from supernatant fraction was added.

kinetic data obtained with such a crude enzyme preparation, an approximate K_m for L-ornithine of 0·19mM was obtained from the Lineweaver–Burk plot shown in Fig. 3. The maximal velocity obtained at saturating ornithine concentrations corresponded to a decarboxylation of about 30mμmoles of substrate/hr./g. wet wt. of tissue.

The reaction product, putrescine, was a competitive inhibitor of the reaction, having a K_i of approx. 1·2mM. The related amines, spermidine and spermine, were also competitive inhibitors, with K_i values of approx. 2·7mM and 9·0mM respectively (Fig. 3). The inhibition by spermine, although manifest only at relatively high concentrations, was not due to contamination of the spermine samples by spermidine or putrescine, since less than 1% of these compounds was present in the spermine used.

Table 5. *Stoicheiometry of reaction*

The incubation medium contained 50μmoles of tris–HCl buffer, pH 7·1, 0·01μmole of pyridoxal phosphate, 2·5μmoles of 2-mercaptoethanol, about 5mg. of protein (dialysed supernatant fraction) and the L-[U-^{14}C]ornithine (172mc/m-mole) concentration given below, in a total volume of 1ml. The CO_2 released and the putrescine formed were determined as described in the Materials and Methods section. Incubation was for 60min. at 37°.

Concn. of ornithine in medium (mM)	CO_2 formed ($\mu\mu$moles)	Putrescine formed ($\mu\mu$moles)
0·029	840	720
0·229	1560	1680

Since considerable amounts of spermine and spermidine are present in the rat ventral prostate gland (Rhodes & Williams-Ashman, 1964; Tabor &

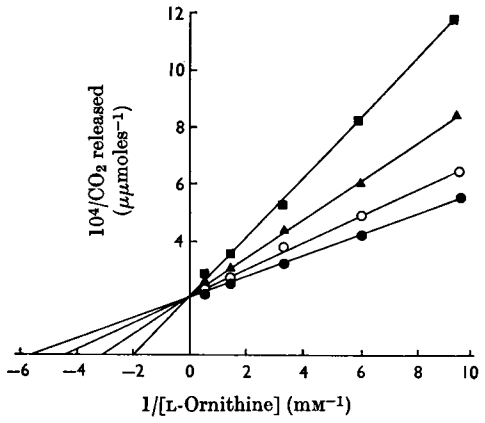

Fig. 3. Effect of putrescine, spermidine and spermine at various ornithine concentrations. The basic incubation medium contained 50μmoles of tris–HCl buffer, pH 7·1, 0·05μmole of pyridoxal phosphate, 2·5μmoles of 2-mercaptoethanol, the L-[U-^{14}C]ornithine (0·2mc/m-mole) concentration shown and about 4mg. of protein (dialysed ammonium sulphate fraction). Incubation was for 60min. at 37°. Results are shown for incubations with L-ornithine alone (●) and in the presence of 2μmoles of spermine (○), 2μmoles of spermidine (▲) and 2μmoles of putrescine (■).

Tabor, 1964), and hence in the supernatant fraction of the homogenates of this tissue, it is possible that the increase in ornithine decarboxylase activity of this fraction on dialysis described above is due to the removal of these compounds.

DISCUSSION

These experiments demonstrate the existence in rat ventral prostate gland of an enzyme forming putrescine by decarboxylation of L-ornithine. We have been unable to find any evidence of the existence of an L-arginine decarboxylase in prostate gland, even when homogenates were tested in the presence of pyridoxal phosphate and Mg^{2+} ions, which are needed for the *E. coli* enzyme (Morris & Pardee, 1966). Hence it appears that the putrescine required for the biosynthesis of spermidine and spermine in this mammalian tissue is produced by the direct conversion of ornithine. It is possible that the provision of ornithine for this reaction is the reason for the existence of arginase in tissues in which the other enzymes of the urea cycle are not detectable (Greenberg, 1960; Knox & Greengard, 1965). Low but definite activities of arginase have been reported to occur in the rat prostate gland (Kochakian, 1944). Whether the decarboxylation of ornithine is responsible for the biosynthesis of putrescine in all mammalian tissues remains to be determined. Incorporation of labelled ornithine into spermidine and spermine *in vivo* has been reported in the chick embryo (Raina, 1963), and labelled ornithine or arginine is incorporated into polyamines in regenerating liver (Jänne & Raina, 1966; Jänne, 1967). However, arginine could be converted readily into ornithine in the liver, and we have been able to detect a similar ornithine decarboxylase in rat liver.

In *E. coli* it has been shown that spermidine synthesis proceeds by the addition to putrescine of the propylamine moiety from 5′-deoxy-5′-*S*-(3-methylthiopropylamine)sulphonium adenosine. The latter compound is produced by the decarboxylation of (−)-*S*-adenosyl-L-methionine (Tabor & Tabor, 1964). Two distinct enzymes catalysing these separate reactions have been obtained (Tabor, 1962*a*,*b*). *E. coli* contains appreciable amounts of both putrescine and spermidine and, in fact, the concentration of putrescine and its acetylated derivatives considerably exceeds that of spermidine (Tabor & Tabor, 1964; Raina & Cohen, 1966). Mammalian tissues contain only trace amounts of putrescine in relation to the concentration of spermine and spermidine (Tabor & Tabor, 1964; Jänne, 1967). In the rat ventral prostate gland we have shown that the decarboxylation of (−)-*S*-adenosyl-L-methionine is markedly stimulated by putrescine with the stoicheiometric formation of spermidine (Pegg & Williams-Ashman, 1968). It is noteworthy that the activity of ornithine decarboxylase that we have measured is considerably less than that of (−)-*S*-adenosyl-L-methionine decarboxylase in the rat ventral prostate when this is measured under optimum conditions. Ornithine decarboxylase may therefore be an important site for the control of polyamine biosynthesis.

The physiological significance of the competitive inhibition of prostatic ornithine decarboxylase activity by putrescine (the product of the reaction) and by the related amines spermidine and spermine is not clear, for, although the combined amounts of these amines reported to be present in the rat ventral prostate gland (Rhodes & Williams-Ashman, 1964) is likely to be sufficient to exert some effect on the ornithine decarboxylase activity, it is not known how much of the spermine and spermidine of the gland is present extracellularly in prostatic secretion. It must be emphasized, however, that in measurements of the ornithine decarboxylase activity of crude tissue homogenates care must be taken to ensure that the system is saturated with the substrate.

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