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ORNITHINE DECARBOXYLASE AND HISTAMINASE (DIAMINE OXIDASE) ACTIVITIES IN RAT THYMUS AND THEIR RELATIONSHIP TO THE THYMUS LYMPHOCYTE

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Abstract— Two enzymes associated with the synthesis and degradation of polyamines, ornithine decarboxylase and diamine oxidase (histaminase), were found in high levels in rat thymus. The ornithine decarboxylase and, to a smaller extent, the histaminase activity declined with age or upon depletion of thymus of its lymphocytes by treatment with dexamethasone. The ornithine decarboxylase activity was located exclusively in the lymphocytes and the histaminase activity was located outside the lymphocytes in the surrounding thymus stroma. Studies with labeled precursor, DL-[2-^{14}C]ornithine, *in vitro* showed that the amino acid was taken up by the isolated thymus lymphocytes and was converted to [^{14}C]putrescine, the first of the amines in the polyamine metabolic pathway. The labeled putrescine accumulated in the cell and did not diffuse readily in or out of the cell. It is suggested that the ability of the thymus lymphocytes to synthesize polyamines may be related to their capacity to proliferate at a rapid rate in thymus. These cells may serve, therefore, as a useful model to study the role of polyamines in cell and tissue growth.

The polyamines, putrescine, spermidine and spermine, are thought to play an important role in the regulation of rapid cell and tissue growth [1]. A correlation between polyamine accumulation and RNA synthesis has been noted under conditions of rapid growth as, for example, in regenerating rat liver after partial hepatectomy [1, 2] or in stress-induced cardiac hypertrophy in rabbits [3]. A key enzyme in the polyamine biosynthetic pathway is ornithine decarboxylase (L-ornithine carboxy-lyase, EC 1.4.3.6) which catalyzes the synthesis of putrescine from ornithine [1, 4], the first and rate-limiting step in this pathway [4]. Spermidine and spermine are derived from putrescine by the addition of aminopropyl groups [5]. Putrescine is oxidatively deaminated in tissues by the enzyme, diamine oxidase (diamine: O_2 oxidoreductase [deaminating], EC 1.4.3.6). This enzyme deaminates histamine as well as diamines such as putrescine, and it has been identified as the enzyme responsible for the histaminase activity in a number of body tissues [6].

Our interest in the role of polyamines in thymus was generated by the observations that the thymus in young animals has one of the most rapid rates of turnover of small lymphocytes of all lymphoid organs [7], high levels of spermidine and spermine [8], and, as seen in our studies, high levels of diamine oxidase as measured by histaminase activity. Our preliminary studies also indicated that thymus possessed high levels of ornithine decarboxylase activity in addition to diamine oxidase [9].

The present study is an attempt to characterize further these two enzyme activities in thymus and to identify their location. The study includes the investigation of the influence of strain and age of the animal on the enzyme levels, the effect of depletion of thymus lymphocytes by dexamethasone treatment, and measurement of enzyme activities in isolated thymus lymphocytes and stroma. Lastly, the possibility of using isolated thymus lymphocytes to investigate polyamine synthesis in intact cells *in vitro* was explored.

METHODS

Preparation of animals and tissues. Pathogen-free spontaneously hypertensive (SH/NIH) or normotensive Wistar (Wistar/NIH) rats were obtained at 4 weeks of age from the Division of Research Services, NIH. Sprague–Dawley and Osborn–Mendel rats were obtained from Taconic Farms, Inc., Germantown, N.Y. Animals were housed at 22–24° and were fed *ad lib.* a diet of distilled water and Purina laboratory chow. Dexamethasone (500 μg/kg) was administered subcutaneously as the sodium phosphate salt in 0·2 ml of isotonic saline at 24 hr or, in some experiments, 48 hr before removal of the thymus. The animals were killed by cervical dislocation. The thymus was removed, rinsed in water, blotted, weighed and homogenized in 9 vol of 0·1 M, pH 6·8, sodium phosphate buffer. All homogenizations were carried out at a slow speed in a loose-fitting glass Tenbroeck homogenizer.

Thymus lymphocytes were isolated by placing the thymus in 0·1 M phosphate buffer, pH 6·8, tearing open the thymus capsule with sharp forceps and gently teasing out the lymphocytes into the buffer. The whole mixture was strained through gauze. The tissue retained on the gauze was washed with buffer and squeezed gently to liberate the remaining lymphocytes. Thymus tissue retained by the gauze (thymus residue) was homogenized in phosphate buffer. The filtrate and washing were pooled and centrifuged at 4000 *g* for 20 min at 4° to deposit lymphocytes. The supernatant fluid (first wash) was removed and the pellet was resuspended in phosphate buffer and recentrifuged. The supernatant fluid was again collected (second wash) and the second pellet was resuspended in phosphate

buffer (lymphocyte suspension). The number of lymphocytes was determined by counting in a blood-cell counting chamber. The volume of phosphate buffer used for the above steps was 9 times the volume of the original thymus.

In one experiment, lymphocyte suspensions (on ice) were sonicated for two 15-sec periods, 1 min apart, in a Bronwill Biosonik sonicator at a setting of 40.

*Assay of histaminase activity.** Histaminase activity was determined by the procedure of Beaven and Jacobsen [10]. The tissue or cell homogenate, 20 μl, was incubated for 60 min at 37° with [β-^{3}H]histamine, 0·1 μCi (15 nmoles), and pH 6·8 phosphate buffer, 0·1 M, to a volume of 0·2 ml. Tritiated water, which is formed upon demamination of [β-^{3}H]histamine by histaminase, was assayed by sublimation of water from the incubation mixture using Thunberg tubes.

Assay of ornithine decarboxylase activity and measurement of labeled polyamines by isotope dilution. Ornithine decarboxylase activity was assayed in two ways: by measurement of the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine and by measurement of the formation of [^{14}C]putrescine from DL-[2-^{14}C]ornithine. The DL-[2-^{14}C]ornithine from commercial sources was purified to remove the 0·5 to 0·9% [^{14}C]putrescine found in these materials by extracting of the amine (to less than 0·04%) into butanol according to the procedure described for the purification of labeled histidine [11]. The [1-^{14}C]ornithine was not repurified.

Each incubation contained 0·5 ml of homogenate or cell suspension; DL-[1-^{14}C]ornithine or DL-[2-^{14}C]ornithine, 0·1 μCi, and unlabeled L-ornithine, 250 nmoles; pyridoxal phosphate, 10 nmoles; aminoguanidine, 50 nmoles, and sodium phosphate buffer 0·1 M, pH 6·8, to a total volume of 1·0 ml. Assay blanks were prepared with 0·5 ml of phosphate buffer in place of the tissue homogenate. Incubations were for 90 min at 37°.

The labeled CO_2 was trapped in Hyamine as described elsewhere [12]. The [^{14}C]putrescine was assayed by extraction and derivatization with benzene sulfonyl chloride by the following procedure. Unlabeled putrescine, 40 mg (455 μmoles), was added as a solution (200 μl in water) to the incubation mixture. Two ml of 0·8 M perchloric acid was then added and the mixture was centrifuged to remove the precipitated protein. The labeled and carrier amine were extracted into *n*-butanol and back into dilute HCl according to the procedure used by Shore *et al.* [13] for the extraction of histamine from tissues. The same quantities of reagents and solvents were used for the extraction and washing procedures, except that 2 ml of 0·6 M HCl was used for the final extraction of putrescine from the organic phase. Powdered sodium bicarbonate, 500–550 mg, and 180 mg of benzene sulfonyl chloride in 3 ml of *p*-dioxane were added to the HCl extract. The reaction of putrescine with the benzene sulfonyl chloride was allowed to proceed for 30 min at room temperature with occasional shaking of the tubes. After 30 min, water was added in 1-ml increments until crystallization of the derivative was complete. The crystals were washed twice with 10 ml of 25% ethanol in water, dried overnight, dissolved in 2 ml acetone and recrystallized. The recrystallization procedure was repeated twice more. The crystalline derivative (generally 35–40 mg) was weighed and dissolved directly in 10 ml scintillation fluid for assay of ^{14}C.

The amount of [^{14}C]putrescine originally present in the incubation mixture was determined for the assay blanks and samples from the formula:

$$^{14}\text{C (dis/min)} \times \frac{169^{\dagger}}{\text{mg of derivative}} = \text{dis/min } ^{14}\text{C-putrescine in incubation mixture.}$$

The final results were expressed as nmoles $^{14}CO_2$ or [^{14}C]putrescine formed/hr/g of tissue or per whole thymus. The rate of decarboxylation was linear up to 90 min and was proportional to the number of lymphocytes, up to 2×10^8 cells/ml.

[^{14}C]Spermidine and [^{14}C]spermine were measured in separate incubations. After the incubation, 150 mg spermidine trihydrochloride or spermine tetrahydrochloride was added to the reaction mixture and the labeled and unlabeled amine were extracted by the butanol extraction procedure described above. The final 0·6 M HCl extract was freeze-dried. The extracted amine hydrochloride was crystallized from 90% (v/v) ethanol in water (about 2·0 ml), from 93% (v/v) and finally from 95% (v/v) ethanol. Crystals were washed twice with 0·5 ml absolute ethanol between each crystallization. The final crystalline product (yield 15–35 mg) was dissolved in 0·3 ml Hyamine hydroxide and 0·1 ml water and then mixed with a liquid scintillation mixture [11] for assay of radioactivity. Less than 0·03 per cent [2-^{14}C]ornithine and 1 per cent [2,3-^{3}H]putrescine was carried over by these procedures.

Studies with isolated lymphocytes. The lymphocytes were isolated from several rat thymuses, pooled and incubated with DL-[2-^{14}C]ornithine, 2·0 μCi; unlabeled L-ornithine, 50 nmoles; pyridoxal phosphate, 10 nmoles; aminoguanidine, 50 nmoles; and phosphate buffer, pH 6·8, to a volume of 1·0 ml. After incubation (90 min), the mixture was centrifuged at 4000 *g* for 20 min. The supernatant fraction was collected. The lymphocyte pellet was washed in 0·5 ml phosphate buffer and then resuspended and homogenized in 0·5 ml buffer. In one experiment, the labeled putrescine was assayed by the extraction and derivatization procedure described above. In another experiment, the labeled amines were separated and assayed by paper electrophoresis using the system described by Pegg and Williams-Ashman [4].

Materials. Dexamethasone sodium phosphate and NSD-1055 (4-bromo-3-hydroxybenzyloxyamine) were gifts from Merck Sharp & Dohme (Rahway, N.J.) and Smith & Nephew, Ltd. DL-[1-^{14}C]ornithine, DL-[2-^{14}C]ornithine, [2,3-^{3}H] putrescine and [^{14}C]putrescine were purchased from New England Nuclear Corp., Boston, Mass., and the unlabeled compounds from Mann Research Laboratories, Orangeburg, N.Y. [β-^{3}H]histamine, 4(5)-(2-[2-^{3}H]aminoethyl) imidazole, was prepared from [β-(side chain)-^{3}H]-L-histidine as described previously [10]. Pyridoxal phosphate was obtained from Calbiochem, Los Angeles,

* The histaminase activity in thymus appears to be due to the enzyme, diamine oxidase, as indicated in studies of inhibitors and substrate competition. A close correlation between histamine and putrescine deaminating activity has also been observed for thymus and other rat tissues (M. A. Beaven and R. E. Shaff, *Biochem. Pharmac.*, in press).

† The theoretical yield of dibenzene sulfonyl derivative derived from 40 mg putrescine is 169.

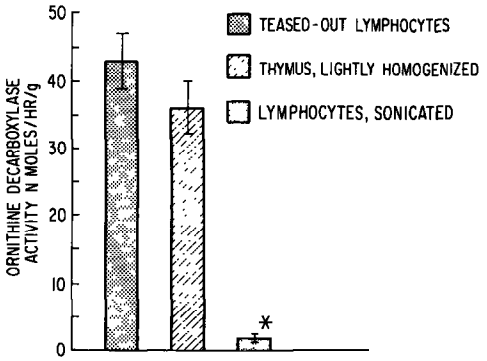

Fig. 1. Ornithine decarboxylase activity in whole thymus homogenate, isolated thymus lymphocytes and sonicated lymphocytes. Bars and brackets indicate mean ± S.E.M. of five experiments and the asterisk depicts significant decrease ($P < 0.01$) in enzyme activity. Enzyme activity was measured by $^{14}CO_2$ release from DL-[1-^{14}C]ornithine. A similar loss (36 to 2 units/g) of activity was observed after sonication of lymphocytes when enzyme activity was assayed by measurement of [^{14}C]putrescine formation from DL-[2-^{14}C]ornithine.

Calif., and Hyamine hydroxide from Packard Instrument Corp., Downers Grove, Ill. α-Methyldopa and α-hydrazinohistidine were obtained from Merck Sharp & Dohme Laboratories.

RESULTS

Assay of ornithine decarboxylase activity in thymus —evaluation of assay procedures. The initial studies with the $^{14}CO_2$-release assay indicated that ornithine decarboxylase activity was present in both thymus homogenates and isolated thymus lymphocytes (Fig. 1). The enzyme activity was not reduced by homogenizing lymphocyte suspensions at a slow speed in a loose-fitting glass homogenizer. This procedure did not disrupt the lymphocytes and was adopted for all assays. More extensive homogenization led to appreciable and variable (40–98 per cent) losses of activity and sonication resulted in almost complete loss of enzyme activity (Fig. 1).* Similar results were obtained with either Tris or phosphate buffer and with or without the addition of mercaptoethanol or Cleland's reagent (5 mM).

Of the two assay procedures used, the $^{14}CO_2$-release assay permitted the simultaneous assay of a large number of samples and was the only convenient method in experiments with large numbers of animals. The assay was reproducible and gave low values for blanks (<0·03 per cent of label). Breakdown of [1-^{14}C]ornithine by other metabolic pathways could yield $^{14}CO_2$, however, and it was also necessary to assay ornithine decarboxylase activity by measurement of [^{14}C]putrescine formation from [2-^{14}C]ornithine.

Major difficulties were encountered when extraction and electrophoretic separation procedures were used for the measurement of labeled putrescine and the polyamines. Salts and other tissue constituents interfered in the separation. If the labeled amines constituted less than 0·5 per cent of the total radioactivity, they did not give discrete peaks of activity, and generally concentrations of 1–2 per cent were required for quantitative measurements.

The isotope dilution procedures, on the other hand, gave reproducible and quantitative results. Recovery of exogenously added labeled amines was better than 98 per cent for [^{14}C]putrescine and 96 per cent for spermidine and spermine. Little (<0·5 per cent) of the labeled spermidine and spermine was carried over with the putrescine sulfonyl derivative or labeled putrescine (<0·5 per cent) with the spermidine or spermine hydrochloride salts. Elemental analysis of the crystalline derivative of putrescine indicated that a disulfonyl amide derivative was formed. When purified [2-^{14}C]ornithine was taken through the procedure (i.e., as assay blanks), 0·05 to 0·07 per cent of the label was recovered as [^{14}C]putrescine.† The agreement between duplicate determinations was within 0·03 per cent. A limitation, however, was the time required for completion of the assay (4 days or more).

When the two assays were compared on the same preparation of thymus homogenates or isolated lymphocytes ($n = 10$), the amount of [^{14}C]putrescine recovered from incubations of DL-[2-^{14}C]ornithine was 40–56 per cent (mean 50 per cent), [^{14}C]spermidine, 11–15 per cent, and [^{14}C]spermine, <1 per cent, of the amount of $^{14}CO_2$ released from DL-[1-^{14}C]ornithine. The values were similar whether thymus homogenate, supernatant fraction or isolated lymphocytes were used. These data indicated that a major part (~65 per cent), but not all, of the $^{14}CO_2$ released was attributable to the formation of putrescine.

* The isolation and properties of thymus ornithine decarboxylase activity will be the subject of a separate report.

† Assay blanks prepared with [1-^{14}C]ornithine, which does not form labeled putrescine, gave values of 0·02 to 0·03 per cent. The difference in these values from those obtained with [2-^{14}C]ornithine was attributed to residual [^{14}C]putrescine in the purified preparation of [2-^{14}C]ornithine.

Table 1. Thymus ornithine decarboxylase and histaminase activities in various rat strains*

Rats	n	Age (days)	Ornithine decarboxylase activity (nmoles/hr/g)	Histaminase activity (pmoles/hr/g)
SH/NIH	5	40	43 ± 5	1381 ± 78
Sprague–Dawley	5	52	36 ± 4	632 ± 164
Osborne-Mendel	6	47	41 ± 5	1093 ± 156
Wistar/NIH	5	50	39 ± 6	631 ± 70

* Values are mean ± S.E.M.

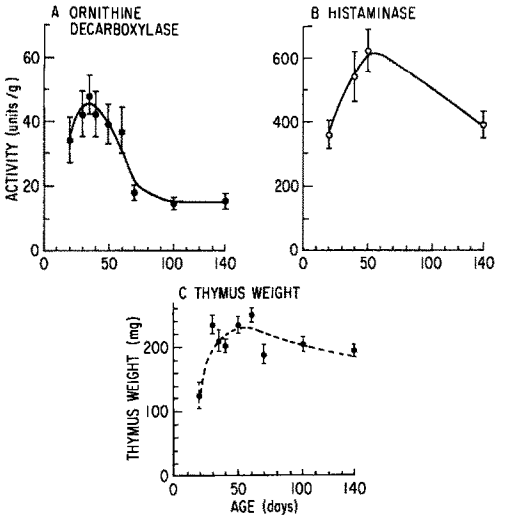

Fig. 2. Changes in ornithine decarboxylase activity (A), histaminase activity (B), and thymus weight (C) with age. Thymuses from 5–12 female Wistar/NIH rats were assayed in each group. The points depict mean value and brackets S.E.M. Units of activity are defined in Materials and Methods.

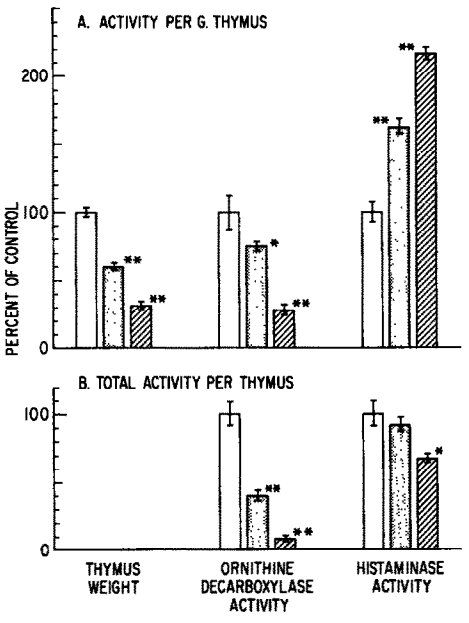

Fig. 3. Effect of dexamethasone treatment on thymus ornithine decarboxylase and histaminase activities. Female Wistar/NIH rats, 35 days of age, received saline (□), or dexamethasone, 500 μg/kg, one dose (◫) or two doses one day apart (■), and the animals were killed 24 hr thereafter. The bars and brackets show mean ± S.E.M. of values from 12 rats. Enzyme activities were calculated as activity per g of thymus (A) and per thymus (B) and are expressed as per cent of control values. Asterisks denote significant difference from control rats * $P > 0{\cdot}01$; ** $P > 0{\cdot}001$. The data from two experiments (six animals per group per experiment) were pooled for presentation in this figure. The same significant differences were observed in both experiments.

Variation in ornithine decarboxylase and histaminase activities with strain and age. Ornithine decarboxylase activity was similar in thymus of all strains tested, unlike histaminase activity, which varied from strain to strain (Table 1). In the Wistar/NIH strain, the amount of ornithine decarboxylase activity in thymus increased with age, reached a maximum at about 30 days of age and then declined rapidly after 60 days of age (Fig. 2). Thymus histaminase activity also declined in older rats, but to a lesser extent than did ornithine decarboxylase activity (Fig. 2).

Changes in enzyme activities after depletion of thymus lymphocytes by dexamethasone treatment. Dexamethasone, in a single dose of 500 μg/kg, produced by 24 hr a significant decrease in thymic weight and ornithine decarboxylase activity (Fig. 3). In contrast, histaminase activity was increased per g of thymus, but histaminase activity per thymus remained unchanged (Fig. 3). The data indicated that ornithine decarboxylase activity, but not histaminase activity, was depleted by dexamethasone treatment.

When dexamethasone was administered on 2 successive days, the difference in enzyme activities was more evident (Fig. 3). Thymic weight decreased approximately 70 per cent, ornithine decarboxylase activity per g of thymus decreased by 75 per cent and histaminase activity increased by 100 per cent. In terms of enzyme activity per thymus, ornithine decarboxylase decreased by 90 per cent and histaminase decreased by 34 per cent. The number of thymic lymphocytes decreased by 80 per cent after two doses of dexamethasone.

Loss of ornithine decarboxylase activity was also evident by measurement of [^{14}C]putrescine, After two doses of dexamethasone, the activity declined from 26–29 (mean, 27) to 2–5 (mean, 3) nmoles/hr/g (88 per cent decrease) compared with a decline from 45–57 (mean, 51) to 7–10 (mean, 8) nmoles/hr/g (85 per cent decrease) when activity was measured by the $^{14}CO_2$-release assay.

Location of ornithine decarboxylase and histaminase activities in thymus. Most of the thymus lymphocytes

Table 2. Distribution of ornithine decarboxylase and histaminase activities in thymus fractions

Thymus fraction	Ornithine decarboxylase activity* (nmoles/hr/g)	Histaminase activity* (pmoles/hr/g)
Lymphocytes	38 (50,31,37)	18 (10,22,22)
First washing	4 (6,4,3)	485 (366,519,542)
Second washing		39 (25,44,47)
Residual stroma	5 (1,10,4)	929 (577, 988, 925)

* Values are calculated per g of original thymus. Figures are the mean and, in parentheses, individual values.

could be separated from the thymus stroma by repeated washing and squeezing of the residual thymus tissue. Under these conditions, 80 per cent of the ornithine decarboxylase activity was recovered in the isolated thymus lymphocytes and 98 per cent of the histaminase activity in the washings and residual stroma (Table 2).

Formation and accumulation of putrescine in isolated thymus lymphocytes. When intact isolated lymphocytes were incubated with DL-[2-^{14}C]ornithine and [^{3}H]putrescine and the labeled products separated by paper electrophoresis, two peaks of ^{14}C-labeled material, [^{14}C]ornithine and [^{14}C]putrescine, were identified in the lymphocytes and one peak, [^{14}C]ornithine, was identified in the incubation medium (Fig. 4). The ^{14}C-ornithine in the lymphocytes accounted for 34–45 per cent of the L-[^{14}C]ornithine in the incubation. The [^{3}H]putrescine was unchanged and remained largely (~ 90%) in the incubation medium.

In a second experiment, the labeled putrescine was measured by isotope dilution; [^{14}C]putrescine was again found in the lymphocytes, but not in the medium. Table 3 compares the data obtained in the two experiments.

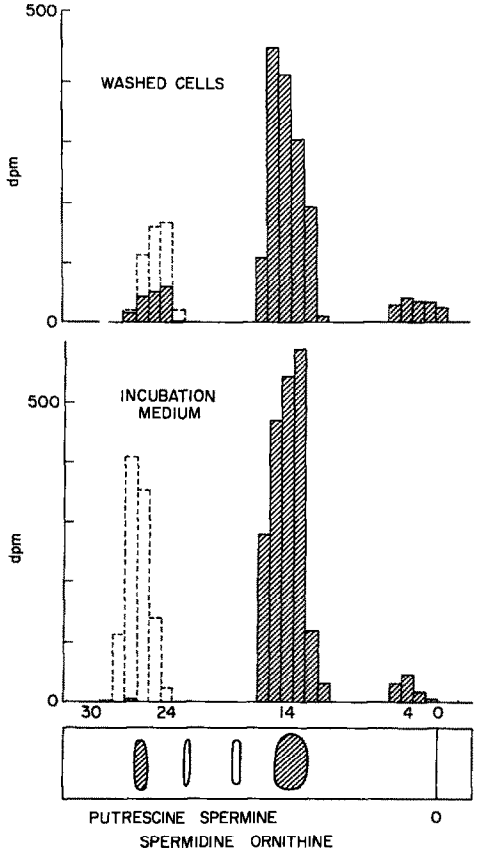

Fig. 4. Separation of labeled products by paper electrophoresis in washed thymus lymphocytes (top panel) and incubation medium (lower panel) after incubation of thymus lymphocytes with DL-[2-^{14}C]ornithine and [^{3}H]putrescine in a volume of 1 ml. After the incubation, the lymphocytes were separated, washed and extracted into 0·5 ml as described in Materials and Methods; 20 µl of the washed cell extract and 10 µl of the incubation medium were applied to the paper for electrophoresis. ^{14}C-label is shown by striped bars; ^{3}H-label is shown by dashed line. Numbers indicate distance (from origin) in cm.

Table 3. Formation and accumulation of [^{14}C]putrescine in thymus lymphocytes *in vitro**

	[^{14}C]putrescine content (nmoles)	
	Isotope dilution Expt. 1	Paper electrophoresis Expt. 2
Whole incubation	1·8	4·2
Medium	0·2	<0·2
Lymphocytes	1·6	4·0

* Each incubation contained: 250 nmoles L-ornithine and (200 nCi), DL-[2-^{14}C]ornithine and (Expt. 1) 90 × 10^{6} and (Expt. 2) 1·25 × 10^{9} cells. Values show [^{14}C]putrescine content (1-hr incubation) in nmoles. The assumption was made that [^{14}C]putrescine was derived exclusively from the L-isomer.

Inhibition of ornithine decarboxylase activity in lymphocytes. Various inhibitors of pyridoxal-dependent histidine and the aromatic amino acid decarboxylases [14, 15] were tested for their effects on the lymphocyte ornithine decarboxylase activity. The lymphocyte enzyme activity was not inhibited by 10^{-3} α-methyldopa [15], a specific inhibitor of aromatic L-amino acid decarboxylase, but was inhibited by 10^{-3} M NSD 1055 (98 per cent) and 10^{-3}M α-hydrazinohistidine (78 per cent), both of which inhibit histidine and the aromatic L-amino acid decarboxylase.

DISCUSSION

The present results confirm our earlier finding of high ornithine decarboxylase and histaminase activities in rat thymus and that these activities decline with age [9]. The results further show that the ornithine decarboxylase activity is located within the thymus lymphocytes, whereas the histaminase activity lies outside the lymphocytes in the surrounding stroma. Depletion of thymic lymphocytes by administration of dexamethasone leads to almost complete loss of ornithine decarboxylase activity but produces no loss of histaminase activity. If dexamethasone treatment is continued, however, there is some loss of histaminase activity.

The ornithine decarboxylase activity was destroyed by sonication or by freezing and thawing of the lymphocytes, as has been observed for this enzyme in cultures of Don-C cells [16]. The properties of ornithine decarboxylase in the intact cell may differ from those of the isolated enzyme. Unlike the soluble preparations of ornithine decarboxylase from prostate [17] or regenerating liver [18], enzyme activity in the intact lymphocyte was not enhanced by addition of mercaptoethanol or dithiothreitol or by the addition of pyridoxal phosphate.

Several conclusions can be drawn from the studies with intact lymphocytes. The similar activities in thymus homogenates and intact lymphocyte suspension indicate that transport of ornithine into the cell is not a limiting factor in putrescine synthesis. The accumulation of ornithine suggests that ornithine may be actively transported across the cell membrane before conversion to putrescine in the cell. If so, the transport system provides an additional site for inhibiting putrescine synthesis. The accumulation of [^{14}C]putrescine within the cell and the exclusion of [^{3}H]putrescine from the cell indicate that the cell membrane is relatively impermeable to the amine. It would

appear, therefore, that putrescine is an intracellular constituent and that the action of exogenous putrescine may not parallel the intracellular actions of this compound.

Although a substantial part of the $^{14}CO_2$ release was accounted for by conversion of ornithine to putrescine, some of the $^{14}CO_2$ release was not accounted for, and may have been due to other metabolic reactions or metabolism of the polyamines.

The role that polyamines play in biological tissues has been a subject of much speculation. One line of evidence has been presented to show that in bacterial and mammalian systems polyamines stabilize ribosomal particles [5], reduce swelling of mitochondria in hypotonic media [5], inhibit the degradation of RNA [5, 19], and have a stabilizing effect on cell walls [5]. Another line of evidence indicates that the polyamines may be involved in tissue or cell growth. It has been proposed that putrescine may act as a growth factor in human fibroblast cultures [20] and that the polyamines are specific regulators of rRNA synthesis [21]. It is not entirely clear, however, whether the increase in polyamine levels accompanies or precedes the RNA accumulation that occurs during growth. Large increases have been observed, for example, in the levels of putrescine, spermidine and spermine, as well as in RNA and protein during concanavalin A-induced blast transformation of small lymphocytes isolated from lymph nodes [22]. These authors noted that the increase in amine levels occurred after the increase in cellular RNA, and it was considered unlikely that the polyamines regulated RNA synthesis during this process.

As far as the present study is concerned, one might speculate that the large production of putrescine in the thymus lymphocytes is associated with the rapid proliferation of the thymus lymphocyte *in vivo*. Whether putrescine has a regulatory role or its production is merely associated with some event in cell growth or division remains to be determined. Whatever its role, it would appear that an efficient mechanism exists for the destruction of putrescine outside the thymus lymphocyte, since diamine oxidase is present in high levels in the surrounding stroma. Presumably, the diamine oxidase serves to destroy putrescine which may leak out of the thymus lymphocyte. Because the ornithine decarboxylase and diamine oxidase activities are clearly separated in thymus, this organ may provide a useful model to investigate the synthesis, metabolism and role of polyamines in the intact cell. The possibility that ornithine decarboxylase is a marker for thymus-derived lymphocytes also warrants further investigation.

Recently Janne and Holta [23] confirmed our earlier finding that rat thymus contains high levels of diamine oxidase and ornithine decarboxylase activities. In addition, these authors found that two other enzymes associated with polyamine synthesis, spermidine synthetase and adenosylmethionine decarboxylase, were present in high levels in this organ. The levels of these enzyme activities declined with age as observed for diamine oxidase and ornithine decarboxylase.

REFERENCES

1. A. Raina and J. Janne, *Fedn. Proc.* **29,** 1568 (1970).
2. D. H. Russell and S. H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* **60,** 1420 (1968).
3. M. J. Feldman and D. H. Russell, *Am. J. Physiol.* **222,** 1199 (1972).
4. A. E. Pegg and H. G. Williams-Ashman, *Biochem. J.* **108,** 533. (1968).
5. H. Tabor and C. W. Tabor, *Pharmac. Rev.* **16,** 245 (1964).
6. E. A. Zeller, *Fedn. Proc.* **24,** 766 (1965).
7. J. F. A. P. Miller and D. Osoba, *Physiol. Rev.* **47,** 437 (1967).
8. J. Janne, A. Raina and M. Siimes, *Acta physiol. scand.* **62,** 352 (1964).
9. M. A. Beaven and W. deJong, *Biochem. Pharmac.* **22,** 257 (1973).
10. M. A. Beaven and S. Jacobsen, *J. Pharmac. exp. Ther.* **176,** 52 (1971).
11. M. A. Beaven, Z. Horakova, W. B. Severs and B. B. Brodie, *J. Pharmec. exp. Ther.* **161,** 320 (1968).
12. M. A. Beaven, Z. Horakova and W. B. Severs, *Eur. J. Pharmac.* **11,** 233 (1970).
13. P. A. Shore, A. Burkhalter and V. H. Cohn, *J. Pharmac. exp. Ther.* **127,** 182 (1959).
14. R. J. Levine, T. L. Sato and A. Sjoerdsma, *Biochem. Pharmac.* **14,** 139 (1965).
15. W. Lovenberg, in *Methods in Enzymology* (Ed. H. Tabor and C. W. Tabor), Vol. XVII, p. 652. Academic Press, New York (1971).
16. S. J. Friedman, R. A. Bellantone and E. S. Canellakis, *Biochim. biophys. Acta* **261,** 188 (1972).
17. J. Janne and H. G. Williams-Ashman, *J. biol. Chem.* **246,** 1725 (1971).
18. A. Raina and J. Janne, *Acta chem. scand.* **22,** 21 (1968).
19. L. Stevens, *Biol. Rev.* **45,** 1 (1970).
20. P. Pohjanpelto and A. Raina, *Nature New Biol.* **235,** 247 (1972).
21. D. H. Russell and T. A. McVicker, *Biochem. J.* **130,** 71 (1973).
22. R. H. Fillingame and D. R. Morris, *Biochemistry, N.Y.* **12,** 4479 (1973).
23. J. Janne and E. Holta, *Acta chem. scand.* **27,** 2399 (1973).