An inhibitor of ornithine decarboxylase in the thymus and spleen of dexamethasone-treated rats

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A marked decrease in activity of ornithine decarboxylase in thymus and spleen occurs soon after treatment of rats with a glucocorticoid. In the present study, evidence was obtained that extracts of these tissues prepared 5h after administration of dexamethasone, when the enzyme activity is very low, contain an inhibitor of ornithine decarboxylase. The inhibitor is also present at 12h after treatment and, in lesser amount, at 2.5h, but was not evident at 24h. The inhibitory activity was destroyed by treatment with heat or with trypsin, and was not lost on dialysis of the extract. Preliminary experiments indicate that the M_r of the inhibitor is greater than 50 000, which differentiates it from antizyme, an inhibitor of ornithine decarboxylase found in several other cell types. The inhibitor seems to act by a non-catalytic and non-competitive mechanism. The inhibition is dependent on the amount of inhibitor and does not change with time. Since inhibition is not changed by dialysis of the inhibitory extract, its activity apparently does not require small- M_r substances. This differentiates it from inhibitors which inactivate ornithine decarboxylase by covalent modification, such as the polyamine-dependent protein kinase or transglutaminase. The formation of this inhibitor is an early event in lymphoid tissues in response to dexamethasone and may be important in causing the inhibition of cell division which precedes the destruction of lymphocytes.

The activity of ODC, the first and rate-limiting enzyme in polyamine biosynthesis in mammalian cells, is closely linked to the proliferative state of a cell population. ODC activity is very low in quiescent cells, but increases substantially (10–500-fold) in response to a variety of trophic stimuli, including hormones, carcinogens, tumour promoters, nutrient manipulation and organ regeneration (Morris & Fillingame, 1974; Tabor & Tabor, 1976; Jänne *et al.*, 1978; Russell, 1980; Bachrach, 1984). In contrast with this marked increase in ODC activity in tissues stimulated to divide or to respond anabolically, a large decrease in ODC activity has been observed in hormone-treated tissues in which the hormone has a catabolic effect. It was found that ODC activity in rat thymus was very low 16h after treatment with glucocorticoids (Atkins & Beaven, 1975), an effect attributed to involution of the thymus as a result of lymphocytolysis (Beaven & de Jong, 1973). A decrease in ODC would be expected in a tissue where cells are being

actively destroyed. However, more recent studies have demonstrated that a decrease in ODC activity occurred in thymus and spleen as soon as 2–5h after treatment with dexamethasone (Richards, 1978; Scalabrino *et al.*, 1979; Richards *et al.*, 1981), much earlier than previously reported, and preceding by several hours the dissolution of lymphocytes. This suggested that the change in ODC activity might be an important early event in the response of the tissues to glucocorticoids and was not necessarily the result of cell destruction.

The aim of our present studies was to determine the reason for the very large difference in ODC activity in thymus and spleen from untreated and dexamethasone-treated rats. Possible reasons for the low enzyme activity in tissue preparations from hormone-treated animals include (i) a change in turnover rates of the enzyme resulting from a change in rate of synthesis or degradation (Prouty, 1976; Erwin *et al.*, 1983; Seely & Pegg, 1983), (ii) inactivation owing to covalent modification of the ODC molecule (Kuehn & Atmar, 1982; Russell, 1981), (iii) the presence of a specific enzyme inhibitor (Canellakis *et al.*, 1979). Results of experiments reported here show that an inhibitor

Abbreviation used: ODC, ornithine decarboxylase (EC 4.1.1.17).

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of ODC occurs in thymus and spleen of dexamethasone-treated rats and that it differs in certain fundamental properties from other documented inhibitors of ODC.

Materials and methods

Treatment of animals

Immature female Wistar rats (UBC strain), age 4–8 weeks, were used in these experiments. After delivery from the animal supply unit, rats were kept in a room with 12h-dark/12h-light cycle for at least 48h before use, since stress from the transfer had resulted in the ODC activity of thymus being very low. Dexamethasone, prepared in ethanol and diluted in 0.9% (w/v) NaCl to a final ethanol concentration of 0.5%, was given by intraperitoneal injection. Each hormone-treated animal received 200 µg of hormone. Control animals received an equal volume of vehicle, or were not treated. The specific activity of ODC was the same in extracts of thymus from saline-treated and non-injected control animals. Animals were killed by cervical dislocation after brief exposure to CO₂.

Preparation of tissue extracts

Thymus glands and spleens were removed and homogenized in a Potter–Elvehjem homogenizer in 4 ml of buffer A/g of tissue. Buffer A contained 50 mM-Hepes, 10 mM-dithiothreitol and 100 µM-EDTA at pH 7.4. The homogenates were centrifuged at 20000g for 20 min at 4°C and the supernatant fraction was filtered through gauze. This extract contained all of the ODC activity extractable from thymus and spleen. Complete extraction of the ODC inhibitor from tissues of hormone-treated animals required that the material pelleted by centrifugation (see above) was re-homogenized in the original volume of buffer B [buffer A containing 1% (w/v) Lubrol], and centrifuged as above. The supernatant fractions from the centrifugations were pooled.

Assay of ODC activity

Samples of tissue extract were incubated in a total volume of 400 µl with L-ornithine (137 µM; 0.125 µCi of L-[1-¹⁴C]ornithine hydrochloride) and 200 µM-pyridoxal phosphate. The reaction was allowed to proceed for 30 min and then was terminated by acidification with 200 µl of 2 M-citric acid. Radioactive ¹⁴CO₂ was collected in Hyamine hydroxide (1 M in methanol) contained in a centre well suspended from the stopper of the reaction tube during a further 30 min incubation. In some experiments, the concentration of ornithine was varied as described in the Results section.

Under these conditions, ODC activity was linear with time over 60 min, and was dependent

on protein content from 0.2 to 5.0 mg per assay. Where indicated, ODC was partially purified from thymus by chromatography on DEAE-Sepharose CL-6B (Richards *et al.*, 1981) and on a pyridoxamine phosphate affinity column (Boucek & Lembach, 1977).

Assay of S-adenosylmethionine decarboxylase activity (EC 4.1.1.50)

Activity of this enzyme was measured by a modification (Richards *et al.*, 1982) of a procedure described by Nawata *et al.* (1980). The assay mixture contained 0.6 mM-S-adenosylmethionine (0.1 µCi of S-adenosyl-L-[carboxy-¹⁴C]methionine), 2.5 mM-putrescine and 0.1 M-sodium phosphate buffer, pH 7.1, and tissue extract in a final volume of 250 µl.

Detection and assay of ODC-inhibitory activity

ODC activity was measured in extracts of thymus from control rats and of thymus or spleen from dexamethasone-treated animals, and in mixtures, in various proportions, of the control extract with tissue preparations from treated rats. The presence of an inhibitor of ODC was indicated when the ODC activity observed in the mixing assay was less than the sum of the activities in the extracts measured separately. In some experiments, ODC partially purified (50-fold; P. Bishop, unpublished work) from rat thymus was used as a source of enzyme in the mixing assay used to detect the inhibitor. Results are expressed as % inhibition (= 100 – % predicted activity).

Trypsin digestion of tissue extracts

Extracts of thymus from dexamethasone-treated rats were incubated at 37°C with 1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK')-treated trypsin (1000 units/ml of thymus extract) at pH 7.6 for periods of 0–60 min. At the selected times, samples were removed and incubated with trypsin inhibitor, (2000 units/ml of thymus extract) for 5 min at 0°C before assay for residual ODC-inhibitory activity.

Ultrafiltration of thymus extracts

In estimating the size of the ODC inhibitor, extracts of thymus from dexamethasone-treated animals were fractionated by ultrafiltration, by using Amicon Diaflo membranes with solute retentions of *M_r* 10000, 30000 and 50000. In each case, the retained material and the filtrate were assayed for the presence of ODC inhibitor.

Sephacryl S-200 gel filtration

The resin was packed in a Pharmacia column (1.5 cm × 90 cm) to a total bed volume of 100 ml. The column was calibrated with Blue Dextran (*M_r*,

2.0×10^6), aldolase ($M_r 1.58 \times 10^5$), bovine serum albumin ($M_r 6.7 \times 10^4$), ovalbumin ($M_r 4.3 \times 10^4$) and chymotrypsinogen A ($M_r 2.5 \times 10^4$).

A partially purified thymus ODC inhibitor preparation (eluted from a DEAE-Sephacrose CL-6B column) was applied to the Sephacryl column and eluted with standard buffer. Each 1.0 ml fraction was assayed for inhibitory activity by using the mixing assay.

Miscellaneous procedures

In the experiments indicated, the thymus extracts were dialysed before assay for ODC and ODC-inhibitory activity. A 3-ml volume of each extract was dialysed separately for 21 h at 0–4°C against 3×800 ml of buffer A. The dialysis tubing retained materials of M_r greater than 12000. The sensitivity of the ODC inhibitor to heat was determined by incubating samples of thymus extract, prepared from rats treated with dexamethasone, in a water bath at 55°C or 100°C for 2 min. The samples were then cooled on ice for 5 min before being assayed for the presence of the ODC inhibitor. Protein concentration of the tissue preparations was determined by the method of Bradford (1976). A Philips PW 4700 liquid-scintillation counter was used for measuring radioactivity.

Materials

L-[1- ^{14} C]Ornithine hydrochloride (58 Ci/mol) and Hyamine hydroxide were obtained from Amersham Corp. (Oakville, Ont., Canada). S-Adenosyl-L-[carboxy- ^{14} C]methionine (60 Ci/mol) was purchased from New England Nuclear (Lachine, Que., Canada). Dithiothreitol, pyridoxal phosphate, L-ornithine, putrescine, TPCCK-treated trypsin, and soyabean trypsin inhibitor were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). S-Adenosylmethionine was purchased from Calbiochem-Behring (San Diego, CA, U.S.A.). Dialysis tubing was obtained from Arthur H. Thomas Co. (Philadelphia, PA, U.S.A.).

Results

ODC activity was measured in samples of the aqueous extract of control thymus and the combined aqueous/detergent extracts of thymus and spleen from hormone-treated animals, and in mixtures of samples of the two types of extracts. The results in Table 1(a) confirm our previous finding that the ODC activity in thymus and spleen from dexamethasone-treated animals was much less than that found in tissues of control animals (Richards, 1978). When the extracts were

Table 1. An inhibitor of ODC is induced in thymus and spleen of dexamethasone-treated rats

Rats received either saline/ethanol (control group) or dexamethasone (200 µg), 5 h before being killed. In (a) tissues were pooled from three or four rats per group, and prepared as in the Materials and methods section. In (b), tissues were pooled as in (a), but the thymus glands from both control and hormone-treated rats were extracted with buffer, and buffer containing Lubrol.

(a) Occurrence of inhibitor in tissues of treated rats

Source of tissue in assay		ODC activity (pmol of CO ₂ /30 min)		Inhibition (%, ±S.E.M.)
Treatment of animals	Tissue	Observed	Predicted	
1. Control	Thymus	242	—	—
2. Hormone-treated	Thymus	12	—	—
3. Hormone-treated	Spleen	11	—	—
4.	1 + 2	87	254	66 ± 5.9
5.	1 + 3	47	253	81 ± 5.7

(b) Absence of inhibitor from thymus extracts from control rats

Tissue preparation in assay	ODC activity (pmol of CO ₂ /30 min)	Inhibition (%, ±S.E.M.)
1. Partially purified rat thymus ODC (25 µl)	120	—
2. Thymus extracts, control animals (100 µl)	114	—
3. Thymus extracts, hormone-treated animals (100 µl)	5	—
4. 1 + 2	253	Nil
5. 1 + 3	53	58 ± 4.9
6. 2 + 3	59	50 ± 4.3

assayed together, the enzyme activity observed was considerably less than the sum of the activities in the extracts assayed separately. Since the difference between the predicted sum of activities and the observed activity was greater than the activity in the extract from treated animals, destruction or inhibition of ODC in the extract of control thymus must have occurred. The total amount of protein used in the mixing assay did not exceed the range where enzyme activity was linear with the amount of extract assayed (see the Materials and methods section). Since enzyme activity was linear with time for 60 min, the observed inhibition is unlikely to be due to limitation of substrate or cofactor. In a series of some 20 experiments similar to that in Table 1(a), inhibition by thymus or spleen from hormone-treated rats ranged from 30 to 80%.

In control experiments, it has been shown that Lubrol does not affect the activity of ODC in extracts of thymus when added directly to the assay mixture at a final concentration of 2%, which is much higher than the maximum 0.25% reached in the mixing assays. However, ODC activity in extracts of spleen is inhibited by Lubrol (30% inhibition at 0.5% Lubrol), and so we have not been able to use spleen as a source of ODC in mixing assays to detect inhibitor in buffer/detergent extracts of tissues from treated animals. It has, however, been possible to show that the ODC of spleen is also inhibited by the substance in thymus and spleen of dexamethasone-treated animals. Part of the inhibitor, but not all, can be extracted from the tissues of the treated animals with only buffer A, which does not contain Lubrol. When these aqueous extracts were assayed for the presence of inhibitor with extracts of spleen from control animals, inhibition of control spleen ODC was observed (results not shown).

Several experiments were designed to test for the presence of the inhibitor in thymus from untreated or saline-injected rats. The results in Table 1(b) show that ODC activity of a partially purified enzyme preparation was additive when assayed with a buffer/detergent extract of thymus from control rats. Inhibition of ODC was observed when the enzyme preparation was assayed with a buffer/detergent extract of thymus from dexamethasone-treated animals.

Results in Table 2 show that the extent of inhibition of thymus ODC was dependent on the proportion of the extracts from control and hormone-treated animals in the mixing assay. The observed inhibition of ODC in a constant amount of a control thymus extract increased as the amount of extract from thymus of treated animals was increased. In the reverse experiment, the inhibitory effect of a sample of thymus extract

Table 2. *Effects of ratio of inhibitor to enzyme on ODC activity*

Extracts of thymus were prepared from control rats (as source of enzyme) and from dexamethasone-treated rats (source of inhibitor). Tissues were pooled from four to six animals per group. ODC inhibition was measured as described in the text, by using different amounts of the two extracts. Assays were done in duplicate or triplicate.

Amount of enzyme (μ l)	Amount of inhibitor (μ l)	Inhibition of ODC (% $\pm$ S.E.M.)
100	0	0
100	50	37 $\pm$ 2.4
100	100	56 $\pm$ 5.1
100	250	72 $\pm$ 6.9
50	100	73 $\pm$ 6.1
100	100	56 $\pm$ 4.9
250	100	34 $\pm$ 2.2

from dexamethasone-treated rats decreased when assayed with increasing amounts of thymus extract from control animals.

The results illustrated in Fig. 1 show that ODC activity in thymus and spleen extracts from control rats, from dexamethasone-treated rats and in 1:1 mixtures of extracts from control and hormone-treated rats is linear with time of incubation of the assay. The extent of inhibition of thymus ODC by extracts of thymus and spleen from treated rats did not change with time of incubation. These experimental results show that inhibition of ODC by spleen extracts ranged from 52 to 57%, and from 27 to 32% for thymus, measured at 15–60 min of incubation. Similar results were obtained in other experiments where the actual extent of inhibition was altered by changing the proportions of tissue extracts from control and treated animals (results not shown).

When the inhibitory activity of thymus extracts was measured with different amounts of substrate, it was found that the inhibition remained at 68–73% over a range of ornithine concentrations of 10–500 μ M. This showed that the activity of the inhibitor was not related to the amount of available substrate. When the data were plotted (Fig. 2), the apparent K_m for ornithine was 80 μ M in extracts of control thymus and in the mixing assays, indicating that the inhibitor acts non-competitively.

In studying some of the properties of the inhibitor, it was observed that it shows some specificity for ODC. When ODC and S-adenosyl-methionine decarboxylase activities were determined in extracts of thymus from control and hormone-treated animals, and in mixtures of the two types of extracts, the observed S-adenosyl-

Fig. 1. Effect of the ODC inhibitor at different times of incubation

The effects of (a) thymus extract and (b) spleen extract from dexamethasone-treated rats on thymus ODC activity were measured after different periods of incubation. Control thymus ODC activity (100 μ l/assay) is shown as (●) in (a) and (b). Activity of tissue extracts from hormone-treated animals (100 μ l/assay), are shown as (□) in (a) and (Δ) in (b). ODC activity in the mixtures of the two types of extract is shown as (■) in (a) and as (▲) in (b). Percentage inhibition of ODC is shown as ○--○ in each case.

Fig. 2. Measurement of K_m for ornithine of thymus ODC in a mixing assay

Thymus extracts were prepared from tissues of rats treated with saline or dexamethasone (200 μ g/animal) 5h before being killed. ODC activity (v , pmol of CO₂/30min) was measured in 100 μ l samples of thymus extract from control animals alone (●) and in the presence of 100 μ l samples of thymus from hormone-treated animals (○) at concentrations of substrate of 5–500 μM .

methionine decarboxylase activity in the mixing assay was very close (85–90%) to the predicted sum of the activities in the extracts assayed separately. ODC activity in the mixing assay was only 30% of the predicted sum of activities in the individual extracts. These results suggest that the inhibitory effect in the thymus extract from treated rats is not the result of a general proteolysis. The ODC-inhibitory activity in the thymus extract from dexamethasone-treated rats is heat-sensitive and was totally inactivated by heating to 100°C for 2min (Table 3a). Treatment of the extract from treated rats with trypsin before assay also inactivated the inhibition. Results in Table 3(b) show that inhibition of ODC by a sample of thymus extract from treated animals decreased from 62% to 22% after 60min of treatment with trypsin. These results suggest that the ODC inhibitor is a polypeptide. Other experiments indicate that the inhibitory activity is not associated with a small molecule, nor does it require small- M_r substances for its action. The inhibitor was not removed by dialysis, and dialysis of thymus extracts from control and treated animals (i.e. source of enzyme and source of the inhibitor) before assay did not change significantly the extent of inhibition of ODC. When the thymus extract from dexamethasone-treated rats was fractionated by ultrafiltration using Amicon Diaflo membranes with solute retentions of M_r 30000 or 50000, all of the ODC-inhibitory activity was retained by filters. The filtrates had no effect on ODC activity in a mixing assay (results not shown). The M_r of the thymus ODC inhibitor was estimated by gel filtration on

Table 3. *Properties of ODC inhibitor in thymus of dexamethasone-treated rats*

An extract of thymus was prepared from tissues of four to five animals, each treated with 200 µg of dexamethasone 5 h before being killed. The procedure used for each of these experiments is described in the Materials and methods section. ODC activities of control thymus extracts used in assays were 237 pmol of CO₂/30 min in *a*, and 120 pmol of CO₂/30 min in *b*. In *a*, observed activity refers to ODC activity in the inhibitor preparation.

(a) Effect of heat

Treatment of extract in assay	ODC activity (pmol of CO ₂ /30 min per 100 µl)	
	Observed	Inhibition (%, ±S.E.M.)
—	15	68 ± 5.9
55°C for 2 min	Nil	11 ± 0.9
100°C for 2 min	Nil	2 ± 0.05

(b) Effect of trypsin

Time of trypsin treatment (min)	Inhibition of thymus ODC (%, ±S.E.M.)
0	62 ± 5.5
30	44 ± 4.1
60	22 ± 1.9

(c) Effect of dialysis

Source of tissue	Dialysis	ODC activity (pmol of CO ₂ /30 min per 100 µl)	
		Observed	Inhibition (%, ±S.E.M.)
1. Control	—	258	—
2. Hormone-treated	—	11	—
3. 1 + 2	—	102	62 ± 5.5
4. Control	+	270	—
5. Hormone-treated	+	11	—
6. 4 + 5	+	99	65 ± 5.5

Sephacryl S-200 standardized as described in the Materials and methods section. Fig. 3 shows the apparent M_r to be 50000–54000.

Previous studies in this laboratory demonstrated that ODC activity was decreased in thymus and spleen of rats at 2–12 h after injection of dexamethasone (Richards, 1978; Richards *et al.*, 1981). In the current work, the presence of the ODC inhibitor was investigated at different times after treatment with the hormone. Experimental results in Table 4 show that the inhibitor was present in largest amounts at 5 and 12 h after dexamethasone treatment. The inhibitory effect of the thymus extract from animals killed 2.5 h after administration of hormone was much less, and no inhibition of ODC was observed in a mixing assay with thymus extract prepared from rats 24 h after treatment, even though the ODC activity in the tissue was very low. Since the thymus glands from

Table 4. *Activity of ODC and ODC inhibitor in thymus extracts prepared from rats at different times after dexamethasone treatment*

Thymus extracts were prepared from rats (three or four per group) at times indicated after treatment with dexamethasone. ODC activity was measured in duplicate on 100 µl of each extract, and ODC-inhibitory activity was determined in a mixing assay as described in the Materials and methods section. ODC activity of control thymus used in the mixing assays was 226 pmol of CO₂/30 min.

Time after treatment (h)	Specific activity of ODC (% of control)	Inhibition of ODC in mixing assay (%, ±S.E.M.)
2.5	32	12.3 ± 1.1
5.0	5	58.1 ± 4.2
12.0	5	47.5 ± 3.1
24.0	15	0

Fig. 3. Determination of M_r of thymus ODC inhibitor

A thymus extract was prepared from tissues of 16 rats which had been treated with dexamethasone 5 h before being killed. The inhibitor was partially purified as described in the Materials and methods section and applied to the Sephacryl S-200 column. V_0 (void volume) of the column was 52.6 ml. Standards used were aldolase, bovine serum albumin, ovalbumin and chymotrypsinogen A. ODC activity of the thymus extract used in the mixing assays was 267 pmol of $\text{CO}_2/30$ min.

the latter group were much smaller than those from control animals, the low ODC activity may be due to depletion of lymphocytes.

Discussion

Several previous studies have demonstrated that a decrease in ODC activity in lymphoid cells may be an important early event in inhibiting the division of those cells and is not simply a result of cell destruction or loss of viability. We have found that ODC activity of Nb2 rat lymphoma cells decreased when exponentially growing cells ceased to divide and became stationary after transfer to a medium lacking an essential mitogenic lactogen (Richards *et al.*, 1982). The stationary-phase cells remained viable, however, and responded to the addition of prolactin by going through DNA synthesis and cell division, events preceded by large increases in ODC activity. Thus the low

ODC activity observed in the stationary Nb2 cells was apparently associated with the arrest of cell division and accumulation of the cells in G_1 phase. Similar results have been reported in S49 mouse lymphoma cells which, on exposure to cyclic AMP or dexamethasone, become arrested in G_1 phase. A large decrease in ODC occurred shortly after addition of the nucleotide or hormone (3–4 h), but cell viability and cell numbers were not affected until 18–20 h after treatment (Insel & Fenno, 1978; Insel & Honeysett, 1981; Russell *et al.*, 1981). Studies with intact animals have shown also that a decrease in ODC activity in thymus and spleen of dexamethasone-treated rats occurred much earlier than previously reported. The change was observed as early as 2–5 h after treatment, long before involution owing to lymphocytolysis was present (Richards, 1978; Scalabrino *et al.*, 1979; Richards *et al.*, 1981). Since the same enzymic response to dexamethasone was observed in adrenalectomized or hypophysectomized animals, it probably resulted from a direct effect of the hormone on the lymphoid tissues and was not an indirect result of the interaction of dexamethasone with another endocrine tissue (Scalabrino *et al.*, 1979). This rapid decrease in ODC seemed potentially important as part of a mechanism for inhibiting multiplication of the lymphoid cells, and a knowledge of its mechanism would add to our understanding of the role of polyamines in cellular control.

The results reported here suggest that the early decrease in ODC activity in thymus and spleen of dexamethasone-treated rats is related to the presence of a polypeptide inhibitor of the enzyme which appears in response to the hormone treatment. In many ways, the inhibitor resembles ODC antizyme, a specific inhibitor of ODC found in a variety of tissues (Canellakis *et al.*, 1979) and which may be induced by polyamines or other physiological stimuli (Fong *et al.*, 1976; Friedman *et al.*, 1977; McCann *et al.*, 1977; Kallio *et al.*, 1979; Brosnan *et al.*, 1983; Lesiewicz & Goldsmith, 1983). Like antizyme, the dexamethasone-induced inhibitor in thymus is a protein which inhibits ODC non-competitively. The extent of inhibition does not change with time, indicating that the inactivation of ODC is not an enzymic process. If the inhibitor acted by a catalytic mechanism, inhibition of ODC would increase during prolonged incubation. The inhibitor of ODC found in thymus differs from antizyme in its size. The hormone-induced inhibitor has an apparent M_r of 54000, much larger than the 26000 reported for antizyme in several biological systems. A more definitive determination of M_r by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis will require extensive purification of the thymus inhibitor.

Since the thymus inhibitor apparently does not act catalytically, and does not require small M_r materials for activity, it is differentiated from protein kinase and transglutaminase, enzymes which inactivate ODC by phosphorylation (Kuehn & Atmar, 1982) or covalent addition of putrescine (Russell *et al.*, 1981). The ATP, putrescine or polyamines required for these reactions would have been removed from the thymus extracts by dialysis, and could not have contributed to the inhibition of ODC in the experiments reported here.

The production of the inhibitor of ODC may be an important early event in the response of rat lymphoid cells to glucocorticoids, since it appears several hours before cell destruction and involution of the tissues (Dougherty, 1952; Claman, 1972). Protein synthesis is partially inhibited and protein degradation is stimulated in lymphocytes from glucocorticoid-treated animals and in lymphoid cells exposed to the hormone *in vitro*. However, the effects on individual proteins may differ. The activity of thymidine kinase in rat thymus did not decrease until 20 h after treatment with cortisone, whereas other enzyme activities showed little change after treatment (Greengard & Machovich, 1972; Greengard & Lempert, 1973). Therefore the effect on ODC must be regarded as being a selective and specific response to hormone treatment. It has been demonstrated that protein synthesis is actually required for dexamethasone to exert its long-range toxic effects on lymphoid cells. Changes in nuclear morphology and cell destruction in lymphoid cells exposed to the hormone can be prevented by treatment with cycloheximide (Nicholson & Young, 1978; Thomas & Bell, 1981; Thomas *et al.*, 1983). Production of an inhibitor of ODC may be part of the required synthetic process.

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