

Markedly elevated angiotensin converting enzyme in lymph nodes containing non-necrotizing granulomas in sarcoidosis*

(angiotensin I/hippuryl-L-histidyl-L-leucine/dipeptidyl carboxypeptidase/epithelioid cell/macrophage)

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ABSTRACT Sarcoidosis is a disease of unknown etiology that is characterized by the generalized formation of granulomas and is accompanied by elevation in the serum in less than half the patients of angiotensin converting enzyme, a dipeptidyl carboxypeptidase that catalyzes the conversion of the decapeptide, angiotensin I, to the pressor octapeptide, angiotensin II, and L-histidyl-L-leucine. Mean activity of angiotensin converting enzyme was elevated generally more than 10-fold in granuloma-containing lymph nodes, but not in lung in which normally it is abundant, in 19 of 20 patients with sarcoidosis. Angiotensin converting enzyme in lymph nodes from subjects with sarcoidosis was similar to the enzyme from normal lung and lymph node with respect to activity as a function of pH, inhibition of activity by EDTA and *o*-phenanthroline, gel filtration on Sephadex G-200, and requirement for chloride for activity, but appeared to be more heat labile. The data suggest that the granulomas in sarcoidosis may be the source of the elevated serum enzyme and that cells of the granulomas, particularly the epithelioid cells which appear by electron microscopy to have active protein biosynthesis, may be actively synthesizing the enzyme.

Sarcoidosis is a disease of unknown etiology and world-wide distribution characterized by the generalized formation of non-necrotizing granulomas, composed characteristically of epithelioid cells and occasional giant cells, as well as lymphocytes. The stimulus for the formation of the granulomas in sarcoidosis is unknown. Certain unelucidated cellular and humoral immunological, as well as metabolic, aberrations are frequently associated with the disease (1-7).

Epithelioid cells have been observed by electron microscopy to contain mucoglycoprotein, and it has been suggested that these cells are predominantly biosynthetic rather than phagocytic (8). Recently an elevated level of angiotensin converting enzyme (ACE) activity has been demonstrated in the serum of many patients with sarcoidosis, but not with tuberculosis or other chronic lung diseases[¶] (9, 10). This enzyme is a dipeptidyl carboxypeptidase which catalyzes the conversion of the decapeptide, angiotensin I, to the pressor octapeptide, angiotensin II, and L-histidyl-L-leucine. The present work was undertaken to elucidate the source of the increased circulating ACE by enzymatic study of lymph node and lung biopsies which were made for morphologic diagnosis in patients with clinical sarcoidosis. The results suggest that granulomas in sarcoidosis contain higher concentrations of ACE than does normal lung, the richest major organ source, and that the granulomas may be actively synthesizing the enzyme. A brief account of this work has been given (11).

Abbreviation: ACE, angiotensin converting enzyme.

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MATERIALS AND METHODS

Chemicals. Hippuryl-L-histidyl-L-leucine was purchased from Bachem Fine Chemicals; ethyl acetate (analytical reagent grade, redistilled prior to use) from the J. T. Baker Chemical Co., and 2 N phenol reagent from the Fisher Scientific Co. All inorganic reagents were analytical reagent grade.

Tissues. Lymph node and lung specimens were obtained surgically from patients with clinical sarcoidosis, tuberculosis, and other conditions. The specimens were mainly diagnostic biopsies; some were portions of specimens removed therapeutically. Normal lung was obtained from surgical resections for carcinoma. Specimens were frozen on solid CO₂ after surgical removal. Histologic sections stained with hematoxylin and eosin were obtained on an unfrozen portion of the specimen as well as on portions of frozen specimens after fixation in 10% formalin. Sarcoidosis specimens were also stained and cultured for acid fast bacilli and fungi. No organisms were observed or cultured except when the diagnosis of tuberculosis is noted, in which case *Mycobacterium tuberculosis* was cultured. The diagnosis of sarcoidosis was based on the presence of non-necrotizing granulomas, the absence of etiologic agents morphologically or by culture, and compatible clinical features. Blood was obtained on most patients from the antecubital vein and the serum collected with a pasteur pipette after clotting at room temperature for 1-2 hr. All tissues and sera were stored at -75° to -85°.

Tissue Extracts. A 2 or 5% tissue homogenate in 50 mM potassium phosphate, pH 8.3, was made by grinding tissue in a Teflon pestle type tissue grinder immersed in an ice-water bath at 10-sec intervals interspersed with 30-sec rest periods until only a small amount of whitish connective tissue remained. The homogenates were then centrifuged at 254 × *g* at about 5° for 10 min. The supernatants were assayed immediately and frozen at -85° for possible further use.

Angiotensin Converting Enzyme activity was assayed spectrophotometrically (12) in duplicate, using 25 or 50 μl of low-speed supernatant, unless a spectrofluorimetric assay (13) with the same substrate, hippuryl-L-histidyl-L-leucine, is noted.

Protein was assayed by the method of Lowry *et al.* (14).

RESULTS

The mean specific activity of ACE in 18 sarcoidosis lymph nodes containing non-necrotizing granulomas and varying degrees of nonextreme hyalinization and fibrosis was about 15-fold higher than in 10 nonsarcoid lymph nodes (Table 1). There was no overlap between the two groups, and the difference was statistically highly significant (*P* < 0.001). Extensively fibrotic or hyalinized sarcoidosis lymph nodes were either within the normal range or only moderately elevated,

Table 1. Angiotensin converting enzyme in lymph node, lung, and serum of patients with and without sarcoidosis

Condition	No.	ACE activity
<i>Lymph node</i> (nmol/min·mg protein)		
Sarcoidosis ^a	18	37.4 ± 3.52, ^b (20.6–69.3) ^c
Sarcoidosis ^d	2	6.90 (3.7, 10.1)
Tuberculosis	2	5.97, 3.44
Nonsarcoidosis ^e	10	2.56 ± 0.68 (0–6.8)
<i>Lung</i> (nmol/min·mg protein)		
Sarcoidosis	10	17.5 ± 2.23 (8.27–31.7)
Normal	3	17.7 ± 1.56 (15.0–20.4)
Tuberculosis	1	14.5
<i>Serum (nmol/min·ml)^f</i>		
Sarcoidosis ^g	29	59.0 ± 17.2 (20.7–182)
Nonsarcoidosis	9	20.1 ± 2.01 (13.4–32.0)

^a The difference between sarcoidosis and nonsarcoidosis values was statistically highly significant ($P < 0.001$). All specimens were mediastinal except for one cervical (69.3 nmol/min·mg of protein).

^b Standard error of the mean.

^c Range.

^d Extensive fibrosis or hyalinization.

^e Six histologically normal (one pericarotid, one inguinal, one mediastinal and paratracheal, and three abdominal); one chronic reactive accumulation of histiocytes and fibrosis; two mediastinal carcinoma.

^f Fluorimetric assay (13).

^g The difference between sarcoidosis and nonsarcoidosis values was highly significant ($P < 0.005$).

suggesting a fall in ACE with obliteration of granuloma mass. In this regard it was of interest that the mean duration of disease for the two fibrotic lymph nodes with low ACE levels (52.5 ± 31.5 months) was much longer than for 18 nodes with high ACE levels (4.41 ± 0.877). The correlation between ACE and duration of disease for the 20 sarcoidosis lymph nodes ($r = -0.463$) was not statistically significant ($P > 0.05$). The negative correlation may be related to the finding that serum ACE levels generally appear to fall with increasing duration of sarcoidosis.^{||}

It was therefore of considerable interest to determine whether elevated ACE is specific to the granulomatous tissue of sarcoidosis by assessing ACE in granuloma-containing tissues in other granulomatous disease. Thus far, the ACE activity has been determined in two patients with tuberculosis; it was within the range of other nonsarcoidosis lymph nodes and below the levels of 18 sarcoidosis lymph nodes that were not extensively fibrotic or hyalinized. The specific activity of ACE in lung, which is normally highest of the major organs (15), was similar in 10 sarcoidosis patients with multiple non-necrotizing granulomas in the lung, three patients with lung tumor in which normal segments of resected lung were assayed, and one patient with pulmonary tuberculosis (Table 1). In general, the amount of granuloma per unit mass of tissue appeared to be higher in lymph nodes than in lung in histologic sections, perhaps relating to the higher mean ACE in sarcoidosis lymph node as compared to lung (Table 1).

Whereas ACE was elevated in granulomatous lymph nodes of 19 of 20 patients with sarcoidosis whose lymph nodes were assayed, serum ACE was elevated (>52 nmol/min·ml) (13) in 7 of 19 and also in 13 of 29 sarcoidosis patients who underwent lymph node or lung biopsy. The correlation between lymph node and serum ACE in sarcoidosis ($r = 0.277$) was not signif-

Table 2. Effect of sodium chloride on ACE in normal lung and sarcoid lymph node

Enzyme source	ACE activity, nmol/min·ml	
	With 0.3 M NaCl	Without NaCl
Normal lung	2.41	0.06
Sarcoid lymph node	2.46	0.00

Assay conditions are noted in *Materials and Methods*. 15 μ l of low-speed supernatant was used.

icant ($P \gg 0.05$). The difference in serum ACE between the sarcoidosis and nonsarcoidosis groups was statistically significant ($P < 0.005$; Table 1).

Work was also directed toward determining whether the elevated lymph node ACE in sarcoidosis had characteristics corresponding to those of authentic ACE and whether it was identical to the lung ACE. ACE from both normal lung and sarcoid lymph node was inactive in the absence of added NaCl (Table 2), as expected for ACE (12) and unlike many proteolytic enzymes. The pattern of inhibition by EDTA and *o*-phenanthroline was similar for normal lung and lymph node as compared to sarcoid lymph node and corresponded to results in other species (Table 3) (12). ACE samples from normal lung and sarcoid lymph node were indistinguishable with respect to activity as a function of pH. There was a broad maximum in activity in the region of pH 8.3 (Fig. 1). They both were also similarly predominantly particulate; about 90% of activity sedimented at $26,800 \times g$ in 40 min (Table 4) (16). Solubilized ACE samples from both normal lung and sarcoid lymph node appeared in the void volume peak of a Sephadex G-200 column coincident with blue dextran, indicating that the molecular weight of both exceeds 200,000 (Fig. 2).

ACE samples from lung and sarcoid lymph node were distinguishable, however, with respect to heat stability at 50° and especially at 53°. At these temperatures lymph node ACE lost more activity than lung ACE, whereas both were essentially inactivated at 60° in 5 min (Fig. 3). At 54°, sarcoid lymph node

Table 3. Inhibition of ACE of normal lung and lymph node and sarcoid lymph node^a

Compound	Inhibition, %		
	Lung	Sarcoid lymph node	Normal lymph node
EDTA (1 mM)	81	87	
EDTA (0.2 mM)	79	85	
EDTA (0.05 mM)		76.8	71.3
<i>o</i> -Phenanthroline (1 mM)	99.3	99.3	
<i>o</i> -Phenanthroline (0.1 mM)		27	12

^a A particulate fraction containing ACE was prepared by centrifuging 0.5 ml of 5% homogenate (prepared as described in *Materials and Methods*) at 18,000 rpm for 60 min in a Sorvall SS-34 rotor ($25,300 \times g$). The supernatant was discarded and the precipitate was suspended in 1 ml of 0.05 M potassium phosphate, pH 8.3, again recovered by centrifugation at 18,000 rpm for 60 min, and resuspended uniformly in 0.5 ml of fresh buffer by brief homogenization in a tissue grinder with a Teflon pestle. Activity was measured fluorimetrically with hippuryl-L-histidyl-L-leucine substrate as described elsewhere (13). Activity was linear with time, indicating removal of L-histidyl-L-leucine peptidase activity.

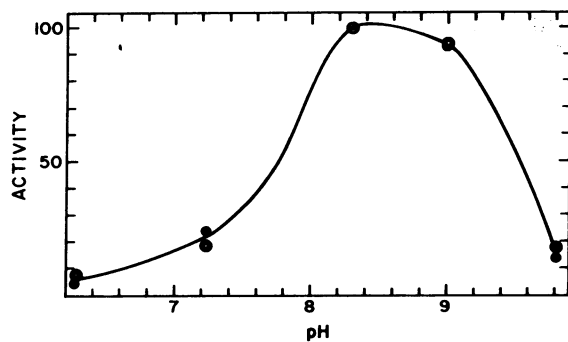


FIG. 1. pH-activity curve of ACE from normal lung (●) and non-necrotizing granulomatous lymph node (○). The buffer at pH 6.3, 7.2, and 8.3 was 0.1 M potassium phosphate and at pH 9.0 and 9.8 was 98 mM borate (24.5 mM sodium tetraborate). The 40-min reaction was initiated by addition of 25 μ l of tissue extract (see *Materials and Methods*) that had been dialyzed overnight at 2–4° against 5 mM potassium phosphate at pH 7.6. Activities were normalized to 100% at pH 8.3.

ACE was similarly more rapidly inactivated than normal lymph node ACE (Fig. 4).

Since atypical lymphocytes are present in sarcoidosis (17) and monocytes are thought to be precursor to epithelioid cells (18), it was of interest to determine whether ACE is elevated in white blood cells in sarcoidosis and whether the elevation would be observed in nearly every patient, as in sarcoid lymph nodes, instead of in less than half the patients, as observed with serum ACE (10). However, no activity was found in sonicates of white blood cells** (prepared from fresh citrated blood by several centrifugations and removal of accompanying erythrocytes by lysis in 0.2% NaCl) of six patients with sarcoidosis and two without sarcoidosis under conditions in which specific activities of 0.1 nmol/min-mg of protein would have been detectable (13). The same white blood cell sonicates contained readily assayable lysozyme activity. The activity of human or rat ACE from various tissues was not altered by sonication.

In order to determine whether the elevated ACE activity seen in sarcoidosis is due to the presence of an activator rather than to a greater number of enzyme molecules, mixing and gel filtration experiments were done. Activities of serum ACE from sarcoidosis patients and subjects without sarcoidosis were additive, and 86% of an elevated serum ACE from a patient with sarcoidosis was recovered from a Sephadex G 200 column, suggesting that the elevated ACE activity in sarcoidosis is not due to a soluble, dissociable activator.

DISCUSSION

This work demonstrates that ACE is markedly increased in non-necrotizing granulomatous lymph nodes in sarcoidosis, suggesting that the granulomas, the hallmark of sarcoidosis, may be the source of elevated serum ACE observed in somewhat less than half of a large series of patients with sarcoidosis (10)^{ll}. Elevated lymph node ACE in the absence of massive fibrosis may be of ancillary diagnostic aid, along with the morphologic criterion of non-necrotizing granuloma.

Despite the presence of varying amounts of fibrosis and nongranulomatous tissue in sarcoidosis lymph nodes, the specific

** The whole white blood cell fraction was used since the main interest was to develop a clinically applicable diagnostic assay for sarcoidosis, and in order not to miss ACE activity in an unsuspected cell type. The lack of activity obviated the need to determine which cells contained ACE activity.

Table 4. Localization of ACE activity in the particulate fraction in normal lung and sarcoid lymph node^a

Tissue	ACE activity, nmol/min-ml	
	Supernatant	Precipitate
Normal lung	19.9	150.2 (88.3%)
Sarcoid lymph node	8.49	95.8 (91.9%)

^a Low-speed supernatants from 5% homogenates (0.5 ml; see *Materials and Methods*) were centrifuged at 18,500 rpm (26,800 \times g) in a Sorvall SS-34 rotor at 5° for 40 min. Precipitates were re-suspended in the original volume of 50 mM potassium phosphate at pH 8.3.

activity of ACE was generally higher than that in normal lung, which has the highest level of the enzyme of the major organs (15), suggesting the possibility that ACE may be synthesized at a high, perhaps maximal, rate by cellular components which may be epithelioid cells of the granulomas. If this is the case, the absence of ACE in circulating white blood cells in sarcoidosis suggests either that synthesis of ACE is turned on at a later stage in the transformation of monocytes to epithelioid cells (18) in sarcoidosis or that circulating monocytes (or other leukocytes) may not be the precursor. The possibility of active synthesis of ACE by sarcoidosis granulomas receives support from recent electron microscopic studies of granulomas (8), which indicate that epithelioid cells may be primarily engaged in biosynthesis rather than phagocytosis and catabolism. Also supportive of the possibility of active ACE synthesis in sarcoidosis granulomas is the lack of elevation of lung ACE, normally a large source of the enzyme, and the presence of increased circulating ACE in a significant number of sarcoidosis patients. Alternative explanations for the increased ACE in sarcoidosis lymph nodes,

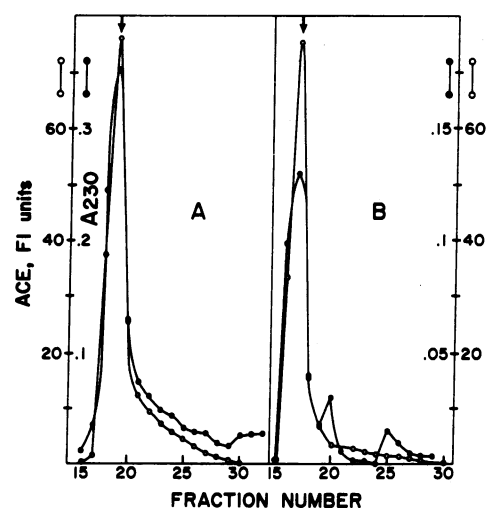


FIG. 2. Similarity of gel filtration of ACE from sarcoid lymph node (A) and normal lung (B) on a Sephadex G-200 column (1 \times 105 cm, 1.95-ml fractions). A particulate fraction (prepared as described in the legend of Fig. 4) was homogenized (30 sec at 10-sec intervals, 0°) in 0.5–0.7 ml of deoxycholate (1.5 mg/ml of 50 mM phosphate, pH 8.3), frozen at –85° overnight for 2 days, thawed at 0°, and centrifuged at 18,000 rpm. The supernatant, containing more than 90% of ACE activity [measured fluorimetrically (13); 0.1 ml of filtrate was assayed in duplicate], was used for the gel filtration. Onto the column was placed ACE activity of 31 nmol/min-ml in 0.55 ml (A) or 46.5 nmol/min-ml in 0.25 ml (B). An FI (fluorescence intensity) of 100 is equivalent to 8.20 (A) or 8.84 (B) nmol/min-ml of ACE. ○, FI; ●, A₂₃₀. Arrows indicate blue dextran peak. Note ACE in A and B filters with the void volume.

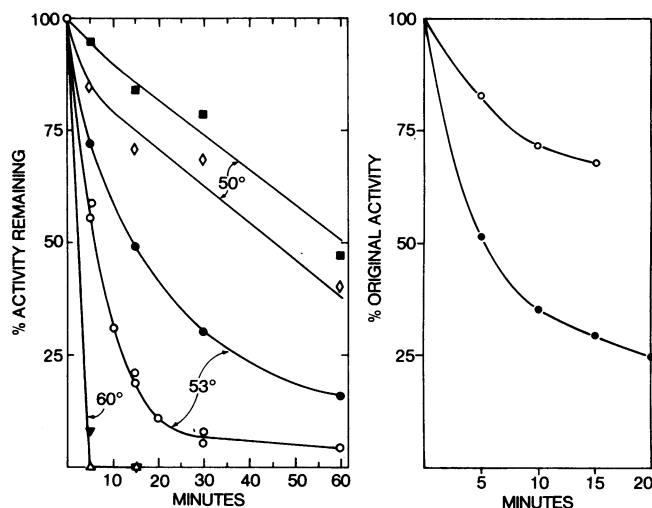


FIG. 3. (left). Effect of temperature on the activity of ACE from normal lung and sarcoid lymph node. Low-speed supernatants (see *Materials and Methods*) were diluted in 50 mM potassium phosphate, pH 8.3, to an activity of about 60 nmol/min-ml. Enzyme in tightly sealed test tubes was heated at the temperatures and for the times indicated in the figure, and held at 0° until assay. Normal lung: ■, 50°; ●, 53°; ▼, 60°. Sarcoid lymph node: ◇, 50°; ○, 53°; △, 60°.

FIG. 4. (right). Increased heat instability of ACE from sarcoidosis as compared to normal lymph node. Low-speed supernatants of 5% homogenates (see *Materials and Methods*) were centrifuged for 40 min at 18,000 rpm at 5° in a Sorvall SS-34 rotor, washed with 1 ml of buffer and again centrifuged, and uniformly resuspended in 50 mM potassium phosphate, pH 8.24, at the volume of the initial low-speed supernatant by homogenization in a tissue grinder. Fifty microliters of the enzyme preparation were heated at 54° at each point noted for the indicated time and duplicate fluorimetric assays were performed with 10 μ l of enzyme (13). ○, Normal lymph node; ●, sarcoid lymph node. Note that dilution of ACE increases its sensitivity to heat inactivation (compare Figs. 3 and 4).

e.g., specific concentration and storage of circulating ACE in the lymph nodes or specifically decreased degradation, appear to be less likely due to the required specificity, although these mechanisms cannot be rigorously excluded by the present data. There is no generalized increase in enzymes in granulomatous lymph nodes in sarcoidosis (19).

It is of great interest to consider how ACE biosynthesis may be turned on in sarcoidosis and what relationship this process may have to the pathogenesis of the disease (20). The etiology of sarcoidosis is unknown, but it is generally assumed that it is a response to an unknown antigenic stimulus that eludes detection. An alternative possibility is that precursor cells form sarcoidosis granulomas through an intrinsic biochemical alteration which converts them to granuloma cells that actively synthesize ACE and perhaps other clinically significant molecules. This alteration might be integration of viral DNA into the host chromosome, persistence of a viral plasmid, or perhaps a chromosomal mutation. It is of interest that elevated serum and spleen ACE has recently been observed in the majority of subjects with genetically determined Gaucher's disease (21). Transmission of the granulomatous lesion in animals in a manner suggestive of the possibility of a biologic agent has recently been demonstrated (22). A nonviral agent that is difficult to detect can also not be excluded.

An important aspect in this regard is the nature of the ACE found in granulomatous tissues in sarcoidosis. We have found ACE from normal lung and lymph node and sarcoidosis lymph node to be catalytically similar with regard to activity as a function of pH and modulators. Similarly, no difference in K_m ,

effect of modulators, filtration on Sephadex G-200, and electrophoresis on polyacrylamide gel was observed between serum ACE of sarcoidosis patients and control subjects (23). ACE of both normal lung and sarcoid lymph node appeared in the void volume on filtration through a Sephadex G-200 column, unlike the smaller serum enzymes which filtered similarly in the included volume prior to lactate dehydrogenase. Serum ACE appears to correspond to a purified particulate glycoprotein ACE from rabbit lung (24) and may be derived from the larger tissue form, perhaps biologically, since this transformation could not be achieved by purification *in vitro* of the rabbit enzyme (24). However, ACE in sarcoid lymph node appeared to be more readily inactivated by heat than ACE from normal lung or lymph node, indicating that it may differ from the normal enzyme.

Serum ACE levels were measured on the same patients in whom lymph node biopsies were taken in order to ascertain the relationship between the two. Although as a group there was a statistically significant difference in serum ACE level between these biopsied sarcoidosis patients and controls (Table 1), as has been found previously in a large series (10), in more than half the individual patients the serum level was not above the normal range although the lymph node ACE was markedly elevated in almost all patients. Perhaps the level of serum ACE may reflect the total mass of nonfibrotic granulomas present in the patient and the leakiness of their constituent cells for the enzyme, as well as the rates of enzyme synthesis, degradation, perhaps urinary excretion, and transport to the serum from the cells in which they are synthesized.

An important question under investigation is whether a higher level of ACE is characteristic of the granulomatous process in general regardless of etiology, or whether it is specific for the granuloma of sarcoidosis. Thus far it appears that elevated ACE is not characteristic of the granulomatous process generally, although sarcoidosis epithelioid cells are morphologically similar to those of other granulomas (8). ACE was not elevated in tuberculous lymph nodes, and there was a statistically significant difference in serum ACE in patients with tuberculosis and sarcoidosis in a large series of patients with the two granulomatous diseases, but not between patients with tuberculosis and normal controls (10)¹¹. Further, no increased ACE has been observed in subcutaneous Freund's adjuvant granulomas in the rat, and ACE was either undetectable or low in rat, rabbit, or mouse peritoneal or alveolar macrophages that were not activated or activated despite their apparent relationship to the epithelioid and giant cell constituents of granulomas (18, 23, 25).

It was originally anticipated that there might be difficulty in distinguishing an elevation of ACE in lung, which normally has a large amount of the enzyme. No difference between normal and sarcoidosis lung was actually obtained. Perhaps the fibrosis and inactivation of ACE production in areas of the lung that normally produce significant amounts of the enzyme offsets any increased synthesis of the enzyme by granulomas that may be occurring. In addition, the generally smaller density of granulomas in lung as compared to lymph node may also be a factor. This result also suggests that the increased serum ACE in sarcoidosis does not result from a general increase of lung enzyme as a result of some stimulus to increased synthesis, although an increased turnover rate cannot be excluded by these experiments.

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1. Mitchell, D. N. & Scadding, J. G. (1974) "Sarcoidosis," *Am. Rev. Respir. Dis.* **110**, 774-802.
2. James, D. G. (1975) "Sarcoidosis," in *Textbook of Medicine*, eds. Beeson, P. B. & McDermott, W. (W. B. Saunders Co., Philadelphia, Pa.), pp. 164-175.
3. James, D. G. (1973) "Modern concepts of sarcoidosis," *Chest* **64**, 675-677.
4. Topilsky, M., Siltzbach, L. E., Williams, M. & Glade, P. R. (1972) "Lymphocyte response in sarcoidosis," *Lancet* **1**, 117-120.
5. Naito, M., Akiyama, Y., Kato, S., Tachibana, T. & Sakai, T. (1973) "Studies on antibody surveillance to EB virus-induced antigen in patients with sarcoidosis and nasopharyngeal carcinoma by indirect immunofluorescence," *Biken J.* **16**, 141-148.
6. Hirshaut, Y., Glade, P., Vieira, L. O. B. D., Ainbender, E., Dvorak, B. & Siltzbach, L. E. (1970) "Sarcoidosis, another disease associated with serologic evidence for herpes-like virus infection," *N. Engl. J. Med.* **283**, 502-506.
7. Wahren, B., Carlens, E., Espmark, Å., Lundbeck, H., Lofgen, S., Madar, E., Henle, G. & Henle, W. (1971) "Antibodies to various herpes viruses in sera from patients with sarcoidosis," *J. Natl. Cancer Inst.* **47**, 747-756.
8. James, E. M. V. & Jones Williams, W. (1974) "Fine structure and histochemistry of epithelioid cells in sarcoidosis," *Thorax* **29**, 115-120.
9. Lieberman, J. (1975) "Elevation of serum angiotensin-converting-enzyme (ACE) level in sarcoidosis," *Am. J. Med.* **59**, 365-372.
10. Silverstein, E., Friedland, J., Lyons, H. & Kitt, M. (1975) "Serum angiotensin converting enzyme in sarcoidosis," *Clin. Res.* **23**, 352A.
11. Silverstein, E., Friedland, J., Lyons, H. & Gourin, A. (1975) "Elevated angiotensin converting enzyme activity in non-necrotizing granulomatous lymph nodes in sarcoidosis," *Clin. Res.* **23**, 352A.
12. Cushman, D. W. & Cheung, H. S. (1971) "Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung," *Biochem. Pharmacol.* **20**, 1637-1648.
13. Friedland, J. & Silverstein, E. (1976) "Sensitive fluorimetric assay for angiotensin converting enzyme," *Am. J. Clin. Pathol.*, in press.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) "Protein measurement with the folin phenol reagent," *J. Biol. Chem.* **193**, 265-275.
15. Cushman, D. W. & Cheung, H. S. (1971) "Concentrations of angiotensin-converting enzyme in tissues of the rat," *Biochim. Biophys. Acta* **250**, 261-265.
16. Lanzillo, J. J. & Fanburg, B. L. (1974) "Membrane-bound angiotensin-converting enzyme from rat lung," *J. Biol. Chem.* **249**, 2312-2318.
17. Biberfeld, P. & Hedfors, E. (1974) "Atypical blood lymphocytes in sarcoidosis: morphology, cytochemistry and membrane properties," *Scand. J. Immunol.* **3**, 615-625.
18. Weiss, L. P. & Fawcett, D. W. (1953) "Cytochemical observations on chicken monocytes, macrophages and giant cells in tissue culture," *J. Histochem. Cytochem.* **1**, 47-65.
19. Palva, T., Dammert, K. & Palva, A. (1973) "On enzyme histochemistry of sarcoidosis," *Acta Pathol. Microbiol. Scand. Sect. A.* **81**, 189-194.
20. Silverstein, E. (1976) "Pathogenesis of sarcoidosis: an hypothetical model," *Med. Hypotheses*, in press.
21. Silverstein, E. & Friedland, J. (1976) "Elevated serum and spleen angiotensin converting enzyme (ACE) and serum lysozyme (L) in chronic Gaucher's disease (GD)," *Clin. Res.*, **24**, 478A.
22. Mitchell, D. N. & Rees, R. J. W. (1969) "A transmissible agent from sarcoid tissue," *Lancet* **2**, 81-84.
23. Friedland, J. & Silverstein, E. (1976) "Similarity in some properties of serum angiotensin converting enzyme from sarcoidosis patients and normal subjects," *Biochem. Med.*, in press.
24. Das, M. & Soffer, R. L. (1975) "Pulmonary angiotensin-converting enzyme structural and catalytic properties," *J. Biol. Chem.* **250**, 6762-6768.
25. Silverstein, E. & Friedland, J. (1976) "Lack of elevation of angiotensin converting enzyme in Freund's adjuvant granuloma in the rat," *Fed. Proc.* **35**, 717 (no. 2820).