Serum lactate dehydrogenase isoenzyme activities in patients with asthma

D. J. USHER¹, R. J. SHEPHERD², and T. DEEGAN

Liverpool Regional Cardiac Centre, Sefton General Hospital, Liverpool L15 2HE

Usher, D. J., Shepherd, R. J., and Deegan, T. (1974). *Thorax*, 29, 685–689. Serum lactate dehydrogenase isoenzyme activities in patients with asthma. Increases in the serum activities of several enzymes have been reported in patients with asthma. Liver damage, resulting from altered tensions of oxygen and carbon dioxide in the circulation, has been held to be responsible for the majority of this increase, although it has also been suggested that allergic reactions in the lungs might make some contribution.

This communication describes the application of a more specific enzyme assay, the serum lactic dehydrogenase (LDH) isoenzyme pattern, to asthma patients in an attempt to elucidate the source of the increase in enzyme activity. Raised activities of two isoenzymes, LDH-3 and LDH-5, comprised the bulk of the increase in total LDH activity; in contrast, the activities of LDH-1 and LDH-2 were virtually unaltered. Analysis of the distribution of isoenzyme activity in lung tissue homogenate, coupled with knowledge of that in liver, suggested that both tissues contributed towards the effects observed. It appeared probable that the increment in LDH-3 activity arose from lung involvement, whereas the major portion of the increment in LDH-5 activity was derived from the liver.

Colldahl (1960) demonstrated increased serum levels of aspartate aminotransferase (glutamic oxaloacetic transaminase, GOT), alanine aminotransferase (glutamic pyruvic transaminase, GPT), and of the liver-specific enzyme, ornithine carbamyl transferase (OCT), in patients with asthma. It was concluded that the increases in serum enzyme activity resulted from liver damage caused by the altered tensions of oxygen and carbon dioxide in the circulation. Subsequently, El-Shaboury, Thomas, and Williams (1964) reported a rise in serum GOT activity in 40% of patients in status asthmaticus, and suggested, although with no supporting evidence, that a possible contribution to the increase in enzyme activity arose from allergic reactions in the lung.

Separation and quantitation of the five isoenzymes of lactate dehydrogenase (LDH) by serum electrophoresis provides a more informative diagnostic tool than single enzyme assays, due to the variation in the isoenzyme composition of different tissues. This has been exemplified particularly by the increase in concentration of

the more anodic isoenzymes (LDH-1 and LDH-2) following myocardial infarction (Latner and Skillen, 1961), and by the increase in the cathodic iso-enzyme (LDH-5) in diseases of the liver (Wilkinson, 1971).

The present study details investigations into the changes in LDH isoenzyme patterns of asthma patients and attempts to establish the tissues responsible for the increased enzyme activity.

SUBJECTS AND METHODS

SUBJECTS Thirteen patients, seven men and six women, were investigated. All were suffering asthma attacks of sufficient severity to warrant referral to hospital. Clinical examination showed that all patients had marked expiratory wheezing and 11 had a history of atopy. Chest radiograph and ECG findings were normal, as were blood urea concentrations. Except for one patient with diabetes mellitus, controlled by diet alone, no other conditions were apparent. The severity of the attacks varied from moderately severe to severe status asthmaticus, but no cases required intermittent positive pressure ventilation. This was considered carefully in one patient, who had a P_{CO_2} of 76 mm on admission, but was avoided on improvement of both the clinical state and the blood gas analysis following treatment.

¹Present address: Department of Chemical Pathology, For esterhill Aberdeen AB9 2ZD

²Present address: The Royal Southern Hospital, Liverpool L8 5SH

Normal values for both total LDH activity and the separate isoenzyme activities were established in a group of 17 staff members, none of whom had evidence of conditions known to influence these measurements.

METHODS Blood specimens were withdrawn from the antecubital fossa within 12 hours of admission in all cases, and in some, further specimens were taken at intervals over a period of three days. No criteria were imposed regarding the specimens from the control subjects. After collection, all specimens were allowed to clot at room temperature, and serum was collected by centrifugation at 2000 g for 10 minutes.

Total LDH activity was estimated at 30° by the spectrophotometric method of Wroblewski and LaDue (1955). Separation of LDH isoenzymes was achieved by electrophoresis on 0.5% agarose gel in 0.05 M barbital buffer, pH 8.6, at 5 V/cm for 75 minutes. The resultant zones were visualized with a tetrazolium salt incubation mixture (van der Helm, 1961) and were quantitated by measurement of the intensity of the developed stain with a Chromoscan reflectance densitometer (Joyce Loebel and Co. Ltd., Gateshead). The activities of the individual isoenzymes were derived in each case from the total activity and the percentage distribution of the staining intensities in the separated zones; the latter were proportional to the integrated areas under the individual peaks of the densitometric scans (Figure).

LUNG TISSUE HOMOGENATES Small samples (ca 1g) of normally functioning lung, removed during lobectomy, were homogenized within 1 hour in 0.1 M phosphate buffer, pH 7.4, at 4° with a Potter-Elvehjem apparatus. Total enzyme activity and the isoenzyme

pattern were determined on the supernatant obtained after centrifugation at 16,000 g for 10 minutes. In all, nine samples were analysed to provide mean values for the percentage isoenzyme distribution in lung tissue.

All activities were expressed as international units per litre (U/l). Group levels were expressed as mean±standard deviation. When more than one sample was taken from a patient, the peak activity was used in the derivation of the mean levels for the asthma group. Mean levels were compared by the appropriate Student's *t* test, with a 5% level of significance.

RESULTS

GROUP FINDINGS The mean values for the total LDH activity and the separate isoenzyme activities of the normal subjects and of the asthma patients are presented in Table I. The total LDH activity

TABLE I
COMPARISON OF TOTAL AND ISOENZYME ACTIVITIES IN NORMAL SUBJECTS AND IN PATIENTS WITH ASTHMA

Subjects	Total LDH (U/l)	Isoenzyme Activity (U/l)				
		1	2	3	4	5
Normal (n = 17)	236 ±36	53 ±12	86 ±12	64 ±12	18 ±5	14 ±7
Asthma patients All (n = 13)	325 ±85 0.001	52 ±16 0.9	89 ±23 0.7	95 ±28 0.001	35 ±12 0.001	54 ±25 0.001
Group I (n = 6)	402 ±59 0.001	60 ±20 0.4	107 ±20 0.01	119 ±20 0.001	44 ±9 0.001	72 ±15 0.001
Group II (n = 7)	259 ±27 0.2	44 ±7 0.1	74 ±15 0.1	75 ±11 0.1	26 ±7 0.01	39 ±23 0.001

¹Comparison of normals with asthma patients by Student's *t* test.

FIGURE Densitometric scans of the LDH isoenzyme patterns obtained from A, a normal subject; B, an asthma patient; and C, lung tissue extract.

was increased significantly in the patients and this increase was distributed in the main between LDH-3 and LDH-5, with a somewhat smaller increase in LDH-4. The activities of the LDH-1 and LDH-2 fractions, however, were identical in the two groups. Typical densitometric scans of a normal subject and an asthma patient illustrate these differences (Figure).

The total LDH activity was not increased above the upper limit of normalcy (i.e., >mean+2SD) in every patient. This allowed division of the patients into two subgroups: I, six patients with significantly raised activities, and II, seven patients with activities within the normal range.

Comparison of group I with the normal group (Table I) again showed significant increases in the

activities of LDH-3 and LDH-5, with lesser increases in those of LDH-2 and LDH-4. LDH-1 values were identical in both groups.

Patients in Group II also had some alteration in the isoenzyme pattern, even in the situation of normal total enzyme activity. The activities of LDH-1 and LDH-2 tended to be reduced ($0.05 < P < 0.1$) and that of LDH-3 increased ($0.05 < P < 0.1$). At the same time small but significant increases in the mean activities of both LDH-4 and LDH-5 ($P < 0.01$) were detected. The most interesting alteration from the normal distribution was the equal activities of LDH-2 and LDH-3.

DIFFERENTIAL CHANGES IN ACTIVITY Two patients, J. T. and S. O. (Table II), showed increases in

fell by 67%, 46%, and 28% respectively during a period of two days, whereas LDH-2 and LDH-1 remained unchanged. It was considered that such effects were minimal in terms of the isoenzyme distribution at the time of peak LDH activity.

RELATIONSHIPS WITH CLINICAL FACTORS Attempts to relate the changes in isoenzyme activities to clinical factors were abortive. The extent of the increase in total LDH activity was independent of either the severity of the asthmatic episode, as assessed clinically on admission, or of its duration. The paradox of waxing or waning of the total LDH activity in the presence of clinical improvement has already been noted.

Treatment with drugs either before or after hospital admission also did not influence the changes in activity. In particular, steroid therapy had been given to two patients for two days and to one patient for three weeks before admission. No progressive effect due to the length of treatment was detected, nor were the isoenzyme results in these patients different from those in the patients who did not receive steroids. The same conclusion was reached for the patients who received bronchodilator therapy, six in all, one of whom was the patient who had received steroids for three weeks. Antibiotic therapy was equally unrelated to the enzyme responses.

SOURCE OF ISOENZYME ACTIVITY The increases in isoenzyme activities described above require the organ or organs of origin to contain sizeable amounts of LDH-3 and LDH-5. The composition of lung tissue extract (Figure), although showing all five isoenzymes, contained predominantly LDH-3 and LDH-4 and suggested that this tissue was the probable source of the increased activity of these moieties. This was supported by comparison of the mean increments in activity of the five isoenzyme species in the group I patients, which together contributed to the increase in mean total LDH activity of 166 U/l above normal, with the species distribution in lung tissue of an equivalent amount of total LDH activity (Table III). Reasonable concordance between the values was apparent for LDH-1, LDH-2, and LDH-3, but LDH-4 was overestimated and LDH-5 underestimated. The discrepancy in the case of LDH-5 was probably more than that estimated, due to the short biological half-life of this entity, and indicated that its source was an alternative tissue. In view of the specific increase in serum OCT activity reported previously (Colldahl, 1960), and the predominance of LDH-5 in liver—greater than

TABLE II
INDIVIDUAL CHANGES IN LDH ACTIVITY IN ASTHMA PATIENTS

Patient	Total LDH (U/l)	LDH Isoenzyme Activity (U/l)				
		1	2	3	4	5
J.T.						
Admission	260	73	78	73	10	26
+ 2 days	460	64	115	143	41	97
Change in activity	+200	-9	+30	+70	+31	+71
S.O.						
Admission	295	67	86	88	27	27
+ 1 day	410	57	102	135	53	62
Change in activity	+115	-10	+16	+47	+26	+35
G.M.						
Admission	360	40	83	104	54	79
+ 2 days	250	42	82	75	25	26
Change in activity	-110	-2	-1	-29	-29	-53

total LDH activity during the two days following admission to hospital. In terms of the changes in the individual isoenzyme activities, the increases were mainly in the LDH-3 and LDH-5 fractions and to a lesser extent in LDH-4, in agreement with the overall picture observed on comparison of the patients with the normal subjects. These increments in activity took place in an improving clinical situation in both patients.

Any assessment of the extent of day-to-day increases in isoenzyme activities is complicated by the different biological half-lives of the separate moieties, which vary from 8 hours for LDH-5 to 40 hours for LDH-1 (Boyd, 1967). This is exemplified by the results from patient G. M. (Table II), in whom LDH-5, LDH-4, and LDH-3

TABLE III
POSSIBLE CONTRIBUTION OF LUNG TISSUE ISOENZYMES
TO THE INCREASE IN ENZYME ACTIVITY IN ASTHMA
PATIENTS

	Total LDH (U/l)	Isoenzyme Activity (U/l)				
		1	2	3	4	5
Main increase in Grade I patients	+166	+ 7	+21	+55	+26	+58
Lung extract ¹	+166	+14	+31	+47	+42	+32

¹Calculation based upon mean percentage isoenzyme distribution from analyses of nine extracts of lung tissue.

80% of the total LDH content (Cohen, Djordjevich, and Ormiste, 1964)—it appeared that this organ was the source of the increase in this fraction.

DISCUSSION

The technique used in this investigation for the separation and quantitation of the individual serum LDH isoenzymes has provided values in normal subjects comparable to those obtained previously by agarose electrophoresis (Elevitch, Aronson, Feichtmeir, and Enterline, 1966), and, when applied to patients suffering asthma attacks, has shown increased activities due mainly to LDH-3 and LDH-5.

Earlier work suggested that the raised levels of serum enzymes found in patients with asthma originated from the liver (Colldahl, 1960). Support for this hypothesis was obtained from the nature of the enzymes released, in particular the increase of the activity of OCT, a liver specific enzyme (Reichard, 1960), and from experimental studies on the effects of hypoxaemia and hypercapnia, both of which states are known to occur in asthma attacks (Lukas, 1951; Schiller and Lowell, 1954; Williams and Zohman, 1960). Subjection of rats to either severe experimental asthma or to low oxygen and high carbon dioxide tensions resulted in increased serum GOT activity (Colldahl, 1943). In addition, centrilobular necrosis has been detected at necropsy in 5% and steatosis in 23% of patients dying from asthma (Colldahl, 1971).

El-Shaboury *et al.* (1964), however, found no correlation between serum GOT activity and liver function tests in patients suffering asthma attacks, and suggested that the increase in circulating enzyme activity may have arisen from lung tissue damaged by allergic reaction.

The present finding of an increased quotient of LDH-5 activity in asthma patients supports the premise that liver damage accompanies this condition. The associated increase in LDH-3 activity,

however, cannot arise from this source, since liver is not abundant in this isoenzyme species.

LDH-3 activity is present in many tissues; those with the highest contents are pancreas, spleen, thyroid gland, adrenal gland, and lymph node, in all of which greater than 40% of the total LDH complement is present in this fraction (Wilkinson, 1971). Lung represents the major tissue of second order abundance—the present investigation has shown 28% to be in the LDH-3 form, a value in close agreement with a previous estimate (Wroblewski and Gregory, 1961).

Changes in isoenzyme distribution have been reported in cases of carcinoma of the bronchus (Wieme, van Hove, and van der Straeten, 1968) and in experimental pulmonary embolus (Bloor, Sobel, and Henry, 1970). In the former study, 76 patients were investigated; marked increases in LDH-3 activity were recorded in 38 patients and mild increases in 22 patients, in some of whom the total LDH activity was normal. In addition, transient increases in both LDH-3 and LDH-5 isoenzymes were noted in some patients soon after commencement of cytostatic therapy. The occurrence of LDH-3 increments in experimental pulmonary embolus agrees with the frequent but by no means universal finding of similar changes in clinical cases (van der Helm, Zondag, Hartog, and van der Kooi, 1962; Amelung, 1963; Mager, Blatt, and Abelmann, 1966; Papadopoulos and Kintzios, 1967).

These observations in conditions of pulmonary involvement suggest that lung tissue may be the source of the increase in LDH-3 activity in asthma. Support for this contention is provided by the close correlation between the absolute increase in activity of the isoenzyme in the circulation and the concentration of the same fraction in an aliquot of lung tissue of equivalent total LDH activity. It is possible that hypoxaemia may also play a part in such enzyme release from lung tissue by producing a transient but reversible alteration in membrane permeability. A similar change is seen in artificially induced stasis in muscle and results in increased levels of both LDH-3 and LDH-5 species. Restoration of the circulation promotes a return to normal levels, more rapidly in the former than in the latter fraction (Starkweather, Green, Spencer, and Schoch, 1966). However, in view of the frequency of detection of increments in LDH-3 activity in a variety of clinical conditions (Wilkinson, 1971), absolute definition of the source of this activity must await immunospecific identification of the tissue involved.

Past attempts to relate increases in serum GOT levels with prognosis in asthma patients have been unsuccessful (El-Shaboury *et al.*, 1964). The present finding of a lack of correlation between the clinical condition of the patient and the various isoenzyme levels, coupled with the further increases noted in certain clinically improved patients, indicates that the more specific assay is equally inapplicable for this purpose.

We wish to express our thanks to Dr. P. I. Adnitt, Dr. N. Coulshed, Dr. A. C. C. Hughes, and Dr. M. W. W. Wood for permission to study their patients, and to Mr. B. J. Bickford and Mr. H. R. Matthews for providing the samples of fresh lung tissue.

REFERENCES

- Amelung, D. (1963). Klinische Erfahrungen mit dem Zellulose-adsorptionstest zur Differenzierung der Lactat-Dehydrogenase des Serums. *Deutsche medizinische Wochenschrift*, **88**, 1940.
- Bloor, C. M., Sobel, B. E., and Henry, P. D. (1970). Autologous pulmonary embolism in the intact unanesthetized dog. *Journal of Applied Physiology*, **29**, 670.
- Boyd, J. W. (1967). Rates of disappearance of L-lactate dehydrogenase isoenzymes from plasma. *Biochimica et Biophysica Acta*, **132**, 221.
- Cohen, L., Djordjevic, J., and Ormiste, V. (1964). Serum lactic dehydrogenase isozyme patterns in cardiovascular and other diseases, with particular reference to myocardial infarction. *Journal of Laboratory and Clinical Medicine*, **64**, 355.
- Colldahl, H. (1943). Gaswechsel und Gewebsatmung beim experimentellen Asthma des Meer-schweinchens. *Acta Physiologica Scandinavica*, Supplement No. 18.
- (1960). A study of serum enzymes in patients suffering from periods of respiratory insufficiency, especially asthma and emphysema. *Acta Medica Scandinavica*, **166**, 399.
- (1971). Serum enzymes in patients with asthma and emphysema, II. *Acta Allergologica*, **26**, 291.
- Elevitch, F. R., Aronson, S. B., Feichtmeir, T. V., and Enterline, M. L. (1966). Thin gel electrophoresis in agarose. *American Journal of Clinical Pathology*, **46**, 692.
- El-Shaboury, A. H., Thomas, A. J., and Williams, D. A. (1964). Serum transaminase activity in status asthmaticus. *British Medical Journal*, **1**, 1220.
- Latner, A. L. and Skillen, A. W. (1961). Clinical applications of dehydrogenase isoenzymes. *Lancet*, **2**, 1286.
- Lukas, D. S. (1951). Pulmonary function in a group of young patients with bronchial asthma. *Journal of Allergy*, **22**, 411.
- Mager, M., Blatt, W. F., and Abelman, W. H. (1966). The use of cellulose acetate for the electrophoretic separation and quantitation of serum lactic dehydrogenase isozymes in normal and pathological states. *Clinica Chimica Acta*, **14**, 689.
- Papadopoulos, N. M. and Kintzios, J. A. (1967). Quantitative electrophoretic determination of lactate dehydrogenase isoenzymes. *American Journal of Clinical Pathology*, **47**, 96.
- Reichard, H. (1960). Ornithine carbamyl transferase activity in human tissue homogenates. *Journal of Laboratory and Clinical Medicine*, **56**, 218.
- Schiller, I. W. and Lowell, F. C. (1954). Pulmonary function in bronchial asthma. *Journal of Allergy*, **25**, 364.
- Starkweather, W. H., Green, R. A., Spencer, H. H., and Schoch, H. K. (1966). Alteration of serum lactate dehydrogenase isoenzymes during therapy directed at lung cancer. *Journal of Laboratory and Clinical Medicine*, **68**, 314.
- van der Helm, H. J. (1961). Simple method of demonstrating lactic acid dehydrogenase isoenzymes. *Lancet*, **2**, 108.
- , Zondag, H. A., Hartog, H. A. P., and van der Kooi, M. W. (1962). Lactic dehydrogenase isoenzymes in myocardial infarction. *Clinica Chimica Acta*, **7**, 540.
- Wieme, R. J., van Hove, W. Z., and van der Straeten, M. E. (1968). The influence of cytostatic treatment on serum lactate dehydrogenase patterns of patients with bronchial carcinoma and its relation to tumor regression. *Annals of the New York Academy of Science*, **151**, 213.
- Wilkinson, J. H. (1971). *Isoenzymes*, 2nd Edition, pp. 134–203. Chapman and Hall, London.
- Williams, M. H. and Zohman, L. R. (1960). Cardiopulmonary function in bronchial asthma. *American Review of Respiratory Disease*, **81**, 173.
- Wroblewski, F. and Gregory K. F. (1961). Lactate dehydrogenase isozymes and their distribution in normal tissues and plasma and in disease states. *Annals of the New York Academy of Science*, **94**, 912.
- and LaDue, J. S. (1955). Lactic dehydrogenase activity in blood. *Proceedings of the Society for Experimental Biology and Medicine*, **90**, 210.

Request for reprints to: Dr. T. Deegan, Liverpool Regional Cardiac Centre, Sefton General Hospital, Liverpool L15 2HE.