A Study on the Isozymic Alterations of Lactic Dehydrogenase in the Tissues of Rats Following Sulfur Dioxide Exposure

Sook Pyo Kwon

Department of Preventive Medicine, Yonsei University College of Medicine
Seoul, Korea

(Received for Publication: 31-Dec.1968)

ABSTRACT

Lactic dehydrogenase (LDH) isozyme patterns were examined in rats after exposing the animal to 250 ppm of sulfur dioxide gas. The isozymes of the respective tissues were separated on cellulose-acetate strips from the brain, lung, heart, liver, kidneys, and muscle, and visualized as the isozyme bands by the formazan reaction and analyzed by densitometry.

As well as the above experiment, room-air and room-air+SO₂ were aerated through tissue homogenates in-vitro, accompanied by pure oxygen aeration in order to see the in vitro effect of the gases on the LDH activity in the tissues mentioned with the following conclusions.

1. The H-type of LDH activity dominated in the normal heart tissue of rats, M-type in the normal lung, liver, and muscle tissues of the animal.

2. The kidney tissue of normal rats exhibited preponderance of LDH-1 and-5 isozymes, brain tissue in LDH-1 and 4 isozymes.

3. When rats inhaled sulfur dioxide gas in the concentration of 250 ppm, it appeared that the M-type tended to predominate in the anaerobic tissues and the H-type in the aerobic tissue.

4. The degree of oxygen tension seemed to be correlated with the low level of LDH activity in the anaerobic tissues such as liver and muscle and with the increased activity in the aerobic tissues, such as heart and lung.

5. The low oxygen tension seems to favor synthesis of M-type LDH and high oxygen tension H-type LDH in the tissues of rats.

INTRODUCTION

The influence of gases on tissue enzymes has been investigated as shown by the studies of alterations in lung enzyme levels as affected by inhalation of irritant agents, such as nitrogen dioxide (Buckley 1967) and ozone (Stokinger 1965). This report stated that inhalation of nitrogen dioxide resulted in increased QO₂ values not only in the lung but also in kidney tissue for each study period, with accompanying significant elevation of aldolase activities in the lung and liver of guinea pigs. But the inhalation of nitrogen dioxide resulted in a decrease in the relative amounts of the fast moving (aerobic) isozyme and an increase in the slow moving (anaerobic) isozyme in the lungs of the animal. The observation of the relation of gas concentration to pulmonary enzymes is of particular significance, since the presence of a specific gas in cigarette smoke and in air pollution of urban and industrial atmosphere endanger the vitality of men living in the contaminated area. It may be expected, therefore, that a certain correlation exists between enzyme activity levels and exposure of animals to such an irritant gas for instance, with particular reference to its sensitivity to oxygen tension, manifested by its isozymic patterns specific to organs.

In Buckley's review of 1967, alterations in enzyme activities in the tissues of animals exposed to irritant agents were noted to occur rather early and sometimes preceded clinical signs of tissue damage. For example
succinic dehydrogenase and succinic oxidase activities were elevated and cytochrome c oxidase activity was decreased in guinea pig lung exposed to silica dust before the usual increase in collagen formation occurred (Kilroe-Smith 1963). Even a single exposure of rats and mice to ozone, as reported by Mountain (1961), resulted in a lowered level of alkaline phosphatase and succinic dehydrogenase activity in the lung and elevated levels in the serum of the animals.

These reports favor the idea that any enzyme activity alterations in serum and tissue, as influenced by inhalation of an air pollutant, are a sensitive indicator of toxicity or of possible tissue damage. In view of these recent studies, correlating enzymes to irritant agents, the author was tempted to examine organ-specific LDH isozyme variations when an animal is exposed to sulfur dioxide gas, the study of which has been scanty or non-existent. The gas has as harmful an effect as nitrogen dioxide in the industrial and urban areas.

Over the past several years, the probable significance of the lactic dehydrogenase (LDH) isozyme system has come to light, with contributions by Kaplan and his co-workers (Silsen 1964, Kaplan 1960, 1961, Salthe 1965). The remarkable reviews of this have already been presented to the Symposium on Multiple Forms of Enzymes and Control Mechanisms by a few outstanding pioneers (Kaplan 1963, Stadtmann 1963, Gorini 1963), which stimulated a large number of reports. These were concerned with normal distribution, tissue and species specificity, genetic and developmental characteristics, and clinical involvement, as well as varieties of methodological investigations.

LDH isozymes were first separated by electrophoresis (Markert 1959). Almost every tissue examined, in general, contains five types (LDH-1 through LDH-5), but the relative proportions of every type vary from organ to organ. These five isozymes are produced at random, tetrameric association of two different polypeptide subunits which are under separate genetic control (Appella 1961, Cahn 1962; Markert 1963; Shaw 1963). Kaplan (1963) has designated the two polypeptides as the M and H subunits. M stands for skeletal muscle and H for heart muscle respectively and the composition of the five isozymes are denoted as either M4H0, M3H1, M2H2, M1H3, and M0H4 for LDH-5 through LDH-1.

It is therefore, apparent now that the first and fifth isozymes are homogenous tetramers, while the remaining three isozymes are heterogenous hybrids of the two.

These subunits aggregate both in-vivo and in-vitro in all possible combinations, thus yielding the five isozymes of specific compositions not only under genetic control but also physiological and pathological influences. According to Kaplan and his coworkers (Cahn 1963, Peace 1963), the two enzymes also differ with respect to their amino acid composition. This is so particularly with respect to their difference in histidine content, stability and catalytic characteristics, as indicated by their affinities for pyruvate as well as their reaction with the coenzyme analogues. They also display different finger printing patterns after tryptic digestion.

The author was particularly interested in the fact that two types of LDH, M and H as described above, predominate in tissues whose metabolic requirement favor its existence, and display their activity in such a way that tissues which require constantly high energy supplies demonstrate relatively greater LDH activity of an isozyme inhibited by high concentrations of pyruvic acid. This was clearly indicated by a report of Dawson et al (1964). In other words, when a higher concentration of pyruvate is needed to inhibit the enzyme, the more energy supplies are needed by the tissue, which reflects the organ-specific LDH isozyme patterns indicating the degree of selective tissue damage.

Hoping to understand the variations in the isozymes of LDH in tissues of rats when exposed to sulfur dioxide, the author assayed the enzyme in tissue homogenates.

**MATERIALS AND METHODS**

(1) **In vivo experiment**

*Experimental animals and inhalation of sulfur dioxide gas:*

Male albino rats of body weights ranging from 120 to 160 gm were divided into two groups—20 rats.
serving as the control and 20 rats as the experimental group. The latter group inhaled about 250 ppm (125–314 ppm) of sulfur dioxide for 6 hours using an apparatus as depicted in Fig. 1.

![Apparatus for Inhalation of Sulfur Dioxide by Rats](image)

**Fig. 1.** Apparatus for Inhalation of Sulfur Dioxide by Rats
1: Air inlet
2: Dual infusion/Withdrawal pump
3: Exposing chamber
4: Gas monitor
5: Gas flow meter
6: Rotary pump

The animals were placed in the tightly closed exposure chamber, of which the gas content approximated 210 l., and 250 ppm of sulfur dioxide appropriately diluted gas was introduced into the air stream entering from the inlet to the chamber. A fan was operated within the chamber during the exposure to obtain a homogenous atmosphere by diffusing the gas thus introduced. Since the minute respiratory volume per rat is reported to be about 100cc., a sufficient volume amounting to as much as 2,000 cc/min. of air as measured by the gas flow meter was supplied and controlled by the rotary pump. By introducing about 20% sulfur dioxide at a rate of 2cc./min. through a Dual Infusion/Withdrawal pump (Model 600–910/920, Harvard Apparatus Co.) the concentration of the gas at the level of 250 ppm was kept constant.

The concentration of the gas in the chamber was monitored by the Midget Impinger gas analyzer at 15 min. intervals for 6 hours, and when depleted additional gas was introduced through the auxiliary gas supplier.

The sulfur dioxide used in the present investigation was evolved from heating metallic copper with conc. sulfuric acid. It was collected in a polyvinyl bag through tightly fitted glass tubing in order for the gas not to leak or be absorbed.

Following exposure of the animals to the gas, they were sacrificed by a throat-cut with large scissors and exanguinated, accompanied by immediate removal of heart, lung, liver, kidney and gluteal muscle.

**Preparation of tissue homogenate.**

All the tissues were immediately homogenized in an ice-cold 0.25 M sucrose solution with an all-glass homogenizer in an ice-bath for a few min., and appropriately diluted with the sucrose solution to obtain 20% (W/V) homogenate. The homogenate was centrifuged at 6000 x g for 15 min. to be free from the nuclear fraction. The supernatant was again centrifuged at 12, 500 x g for 30 min. to remove the mitochondrial fraction, the resultant supernatant being used for enzyme assay and separation of isozymes by electrophoresis as follows. All centrifugations were done with the refrigerated international centrifuge, Model PR-2 (International Equipment Co.)

**Electrophoretic separation of LDH isozymes.**

Horizontal gel electrophoresis was performed according to the principles proposed by the method of Preston et al. (1965), using a cellulose-acetate strips (Sepaphore III, Gelman) on microscope slides in an Spinco electrophoretic chamber (Beckman).

With the aid of the Spinco applicator, 20 μl. of the homogenate was applied to the strip about 1.5 cm. toward the cathodal side. The samples were run on the strip at a constant current of 6 mA/6 slide for approximately 90 min. in order to have the isozymes completely separated. The samples were run in five separate strips to obtain the mean value of analyses.

**Visualization of the LDH isozymes on the strip**

LDH activity was identified directly on the strip utilizing nitro blue tetrazolium (Nutritional Biochemicals) according to the method developed by Preston et al (1965). Phenazine methosulfate (Sigma,
Chemical Co.) served as the electron transporter. A brilliant purple color produced by the reduction of the tetrazolium salt to the formazan appeared at the site of LDH activity on the cellulose-acetate strip. The incubating medium for the acetate strip consisted of Preston (1965):

- Sodium lactate, 1.0 M 1.0 ml.
- Nitroblue tetrazolium, 1 mg/ml. 3.0
- Phenazine methosulfate, 1 mg/ml. 0.3
- β-nicotinamide adenine dinucleotide (NAD), 1 mg/10 ml. 1.0
- 5.3 ml.

At the end of the incubation time the strips were taken out and placed in 5% acetic acid for about 5 min. The strips were then rinsed in distilled water, blotted and air-dried, followed by scanning with the Analytrol, and the percent of total for each fraction was calculated with the use of a planimeter.

(II) *In vitro* experiment

*Aeration of tissue homogenate*

Six male albino rats of about 120 g body weight were sacrificed and 20% (W/V) tissue homogenate in ice-cold 0.25 M sucrose solution was also prepared in the same manner as described in the experiment *in vivo*.

The homogenate was divided into four portions for different aeration conditions. Each 20 ml-portion was put into a separate tube. The portions consisted of:

1. Control portion without aeration.
2. Control portion aerated with room air for 20 min.
3. Experimental portion aerated with SO₂ (5 ppm) in the room air for 20 min.
4. (3)-group followed by 10 min. aeration with pure oxygen.

In other words, (1) and (2) served as controls to (3), while (4) were to see the effect of pure oxygen after exposure to sulfur dioxide.

The aeration to bring the gas into close contact with the homogenated tissue *in vitro* was performed as illustrated in Fig. 2, introducing the sulfur dioxide in the concentration of 5 ppm per tube through the flow regulator in order to maintain a constant rate of gas flow as well as concentration.

*Assay of LDH activity:*

LDH activity of each homogenate was measured, as follows (Neilands 1955).

Exactly 1.8 ml of 0.1 M glycine buffer, pH 10.0, 0.1 ml of 0.5 M sodium lactate solution, and 0.1 ml of 2 x 10⁻² M NAD solution was placed in a 1 cm. silica cell in the holder of the Beckman DU spectrophotometer. The enzyme solution, 0.2 ml, was pipetted into the cell and rapidly stirred into the reaction mixture. The shutter was opened and the optical density change at 340 mμ, E₁₀₀₀, was recorded as a function of time; and the turnover number (enzyme activity) was defined as the μmoles of NADH formed per min. per ml., calculating on the basis of the extinction coefficient of 6.22 x 10⁴.

*Determination of protein content.*

The protein content was monitored by the method of Lowry et al. (1951) Using bovine serum albumin (Nutritional Biochemicals) as a standard, the nitrogen content of which was previously analyzed by the micro-Kjeldahl technique, the Folin-Ciocalteau's phenol reagent was added to an appropriate amount of the sample to develop color and its optical densities at 750 mμ read with the Spectronic-20.

Thus the specific activities of the enzyme were expressed as the turnover number per min. per ml. per mg. protein content of the sample.
RESULTS

(1) Experiment in-vivo:

Mean values of more than fifteen samples determined for percentage activity of total LDH following electrophoretic separation of tissues of normal and sulfur dioxide-exposed albino rats are tabulated in Table I and Fig. 3. Standard deviations and t-values are included in the tables.

Table I. Distribution of LDH Isozyme in Each Organ of Rats Exposed to SO₂ Gas and Control Group

<table>
<thead>
<tr>
<th>Organ (No of sample)</th>
<th>LDH Isozyme Band</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain (n=20)</td>
<td>Control</td>
<td>39.3±5.8</td>
<td>10.8±3.0</td>
<td>8.0±2.0</td>
<td>29.8±2.5</td>
<td>12.1±4.8</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>21.9±2.71</td>
<td>20.6±1.2</td>
<td>20.4±0.8</td>
<td>28.3±0.9</td>
<td>8.8±2.2</td>
</tr>
<tr>
<td>Lung (n=15)</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27.0±3.5</td>
<td>73.0±3.0</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25.3±4.8</td>
<td>74.7±2.9</td>
</tr>
<tr>
<td>Heart (n=20)</td>
<td>Control</td>
<td>26.5±5.9</td>
<td>25.4±4.9</td>
<td>20.1±3.9</td>
<td>18.1±4.6</td>
<td>9.9±2.8</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>29.8±3.2</td>
<td>26.7±5.4</td>
<td>18.7±2.8</td>
<td>17.8±4.9</td>
<td>7.0±4.5</td>
</tr>
<tr>
<td>Liver (n=20)</td>
<td>Control</td>
<td>0.6±1.1</td>
<td>1.8±1.94</td>
<td>5.2±3.3</td>
<td>20.4±6.7</td>
<td>72.0±11.2</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14.5±2.0</td>
<td>85.5±3.2</td>
</tr>
<tr>
<td>Kidney (n=20)</td>
<td>Control</td>
<td>35.2±7.61</td>
<td>8.3±2.6</td>
<td>7.1±1.8</td>
<td>19.9±5.6</td>
<td>29.6±4.8</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>20.1±2.4</td>
<td>13.4±1.4</td>
<td>8.5±2.0</td>
<td>25.9±2.1</td>
<td>32.1±2.7</td>
</tr>
<tr>
<td>Muscle (n=20)</td>
<td>Control</td>
<td>3.6±1.4</td>
<td>4.7±1.9</td>
<td>11.0±2.7</td>
<td>24.8±6.1</td>
<td>55.9±4.2</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>1.2±0.3</td>
<td>3.6±0.9</td>
<td>4.7±1.5</td>
<td>12.5±2.5</td>
<td>78.0±10.9</td>
</tr>
</tbody>
</table>

Lung tissue disclosed almost un-detectable amount of LDH-1, -2, and -3 in both groups, showing only LDH-4 and -5 in relative proportions of about 27.0 and 73.0% in normal lung and 25.3 and 74.7% in the exposed group (Table I). There was, therefore, no recognizable discrepancy observed between the two groups, the ratio of LDH-5 to -4 being 2.70 in the normal and 2.95 in the exposed group.

In the case of heart tissue, the normal values for LDH-1 through -5 were respectively 26.5, 25.4, 20.1, 18.1, and 9.9% in decreasing order, while the exposed values were 29.8, 26.7, 18.7, 17.8 and 7.0 in the same gradually decreasing order (Table I). This shows that 1 and 2 isozymes tended to be elevated after exposure, 3,4 and 5 isozymes tended to appear to be reduced due to exposure to sulfur dioxide.

Brain LDH isozymes distributed 1 through 5 as 39.3, 10.8, 8.0, 29.8 and 12.1% in normal and 21.9, 20.6, 20.4, 28.3 and 8.8% in exposed animals respectively (Table I), among which the 1 and 4 isozymes in both groups were the largest. It was noted that reduction in 1 isozyme and the elevation in 2,3 isozyme were significant respectively as is apparent from the Table I.

Liver tissue was particularly interesting, revealing that M-type predominated in both groups, in contrast to the general tendency of distribution observed in heart tissue.

Liver tissue displayed an opposite phenomenon
Fig. 3. Alteration of LDH Isozyme in Each Organs by SO2-Gas Aeration to Rats.
as compared to heart tissue when exposed to the sulfur dioxide, showing a specific increase in M-type LDH activity, with almost unrecognizable H-type LDH activity in faster moving isozyme bands. The normal values for 1 through 5 isozymes were respectively 0.6, 1.8, 5.2, 20.4, and 72.0% while no activity was detected in 1, 2, and 3 isozymes in the exposed group; however, 4 and 5 isozymes in the exposed group showed 14.5% and 85.5% of total activity (Table I), which is illustrated in Fig. 3. This suggests that all the bands wherever H-type prevailed have disappeared due to the exposure, leaving only those prevailing in the M-type of the enzyme, that is in 4 and 5 isozymes.

The relative distribution pattern among the five isozymes in the kidney tissue, the normal and exposed, were 35.2, 8.3, 7.1, 19.9, and 29.6% of the total in the former and 20.1, 13.4, 8.5, 25.9, and 32.1% of the total in the latter (Table I), showing uniformly increased proportions throughout the isozymes except the 1 isozyme. The decreased 1 isozyme in the exposed group was about 60% of the comparable isozyme in the normal kidney tissue.

The results obtained with muscle tissue resembled those of liver tissue; 1 through 5 isozymes in normal muscle tissue were 3.6, 4.7, 11.0, 24.8, and 55.9% of the total, whereas those of the exposed animals were 1.2, 3.6, 4.7, 12.5, and 78.0% of the total respectively (Table I).

Exposure of the animals to sulfur dioxide resulted in marked prevalence in the M-type LDH activity, particularly in the homogenous M tetramer, 5 isozyme, and in depressed activities throughout 1 to 4 isozymes.

The increase in the slowest-moving activity in the exposed group was almost one and half times as much as the activity in the normal group. It was interesting to note that 1 isozyme was decreased with the elevations in 2 through 5 isozymes in the kidney tissue. On the other hand, 1 through 4 isozymes were decreased with the elevation in 5 isozyme in the muscle tissue when exposed to the sulfur dioxide gas.

(II) Experiment in vitro:

Mean values obtained from the triple determina-

Table II. Protein Alteration in Tissues Examined (Protein, mg/ml homog.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Organ</th>
<th>Aerated with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Air</td>
</tr>
<tr>
<td>Control</td>
<td>Brain</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>7.38</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>7.63</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>16.00</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>8.95</td>
</tr>
</tbody>
</table>

Table III. LDH Activities in Each Organ of Rats (LDH, 10⁻⁵ mole NADH/ml homog.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Organ</th>
<th>Aerated with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Air</td>
</tr>
<tr>
<td>Control</td>
<td>Brain</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>106.0</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>49.8</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>67.6</td>
</tr>
</tbody>
</table>

Table IV. Specific activity of the LDH Enzyme in Each Tissue (LDH Activity/mg protein)

<table>
<thead>
<tr>
<th>Group</th>
<th>Organ</th>
<th>Aerated with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Air</td>
</tr>
<tr>
<td>Control</td>
<td>Brain</td>
<td>6.48</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>14.36</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>2.32</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>3.11</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>7.55</td>
</tr>
</tbody>
</table>

Protein alteration in the tissues examined (Table III) showed a general and unique tendency to have reduced values when sulfur dioxide gas was aerated through the tissue homogenate,—the reduction in the content of protein in every tissue tested occurred even with air-aeration only and was further decreased by sulfur dioxide aeration in the presence of air. Brain protein was reduced by the gas treatment from
4.2 mg/ml homogenate (20%, W/V) to 3.79 and 3.02 mg/ml respectively by air and air+SO$_2$ aeration heart protein from 7.38 to 6.4 and 5.36 mg/ml, kidney from 10.0 to 9.6 and 8.02 mg/ml, lung from 7.63 to 6.02 and 4.42 mg/ml, liver from 16.0 to 10.3 and 9.50 mg/ml, and muscle from 8.95 to 8.85 and 6.90 mg/ml respectively. These values increased somewhat again when the tissue homogenate was aerated with pure oxygen; brain tissue, for example, from 3.02 to 3.16, heart from 5.36 to 5.72, kidney from 8.02 to 8.13, lung from 4.42 to 4.77, liver from 9.50 to 11.61, and muscle from 6.90 to 9.00 mg/ml, which appeared to be due to oxygen stimulation of an activity of protein biosynthetic machinery.

The LDH activity affected by the same treatment as above did not follow the results obtained in the protein determinations, but rather fluctuated widely in different organs (Table III). Brain LDH activity, 27.2 unit/ml homogenate, fell abruptly to 20.9 unit/ml when aerated with room air, the level of which was maintained even when aerated with the sulfur dioxide in air. Activity (22.1 unit/ml) was slightly increased following aeration with pure oxygen. Liver and muscle were also characterized by a similar fall after aeration with room air, from 49.8 to 41.8 and 67.6 to 55.5 unit/ml respectively; unlike the case of the brain tissue, with aeration of air+SO$_2$, both tissues showed somewhat increased activity, 49.8 unit/ml in the former and 58.0 unit/ml in the latter. This was accompanied by falls in their activity to 45.0 and 54.7 unit/ml respectively with the aeration of pure oxygen. The heart, kidney, and lung tissues, on the contrary, increased their enzyme activity after aeration with room air, 106.0 to 109.4 in the heart, 33.0 to 35.2 in the kidney, and 17.7 to 18.5 unit/ml in the lung tissue. This was followed by greater increases in activity both in the heart and lung tissues thereafter by air+SO$_2$ and even oxygen aeration, amounting to 112.6 and 122.6 unit/ml in the heart and 20.1 and 21.5 unit/ml in the lung, with the exception that kidney tissue showed fluctuation by a fall with air+SO$_2$, 27.4 unit/ml and an increase with pure oxygen aeration, 37.0 unit/ml.

In general, SO$_2$ gas has resulted in either no or slightly increased activity among the tissues examined, with the exception of kidney tissue, but the aeration with pure oxygen brought either decreased or increased activity among the tissues examined. It was generally seen that those anaerobic tissues, such as brain, liver, and muscle, followed the same tendency, while aerobic tissues, such as the heart and lung, disclosed other effects. Kidney tissue showed only a little deviation from all of the above patterns.

With regard to specific activity of the enzyme/mg protein in the tissue, as tabulated in Table V, the tissues exhibited generally increased values presumably due to greatly decreased protein contents of the tissues when exposed to the room air or air+SO$_2$, regardless of the fluctuations in activity. It was interesting to note that the specific activity in liver and muscle tissues decreased significantly with oxygen aeration, falling from 5.24 to 3.88 in liver and from 8.41 to 6.08 unit/mg protein in muscle respectively. Other tissues however either an increase, as in the kidney or no change as in brain, heart and lung.

As observed from the values in the specific activity, aerobic tissues behaved in a common fashion differing from the anaerobic tissues which, in turn, behaved in a common category, while, the kidney tissue followed rather a different pattern of its own when subjected to gas aeration.

**DISCUSSION**

The atmospheric air of most urban areas is polluted with sulfur dioxide gas from smoke from industrial plants, electric power stations, and boiler houses. As neatly summarized by Stokinger (1965), the gas makes animals susceptible to infection, irritating the respiratory as well as the digestive tract, and is readily absorbed from the trachea and lungs into all tissues, including the brain, even when inhaled in concentrations as low as 1 ppm.

As is apparent from the present data, every tissue examined showed fluctuations in LDH isozyme pattern (characteristically); the liver and muscle for instance showed increased percentages in their LDH-5, while tendencies of decreased percentages occurred either in LDH-1 (kindey) or in LDH-1 through -
The Effects of Sulfur dioxide on Tissue LDH isozyme — 45 —

(liver and muscle). Those tissues obviously are richer in M-type than H-type LDH, since the tissues mentioned should be resistant to pyruvate inhibition (Cahn 1962; Dawson 1964) and through glycolysis energy must be supplied because the tissues are relatively anaerobic and therefore less efficient in the tricarboxylic acid cycle turnover.

According to Wroblewski and Gregory's statement (1961), the $K_m$ value is lower for fast-moving isozymes, although all LDH isozymes are inhibited by excess pyruvate. Therefore, it is logical and biological that in tissues whose energy turnover is rapid and sustained the fast-moving isozyme predominates. The need of muscle and liver tissues for constant and sustained energy is lower than for the heart, and a slow-moving isozyme and higher $K_m$ value for pyruvate is thus found in these tissues. Since skeletal muscle has high energy requirements for relatively short periods of time, an isozyme which functions at higher pyruvate concentrations would allow muscle to obtain additional energy through the production of NAD⁺ during the formation of lactate from pyruvate. Lactate then enters the aerobic metabolism during a rest or recovery period. As Dawson et al (1964) clearly pointed out, low oxygen tensions favor the synthesis of the anaerobic, or M-type, while high oxygen tensions favor the synthesis of H-type.

When exposed to SO₂ gas, the efficiency of the tricarboxylic acid cycle is probably reduced because of the presence of gas other than oxygen in the tissues, not to mention the direct effect of the gas by coming in close contact with the tissues. This inevitably causes the preference to M-type LDH than the H-type LDH in the liver, muscle and possibly kidney, as was illustrated in the present work, showing depressed percentage of H-LDH activity (Fig. 3).

It is, therefore, apparent that SO₂ gas interferes with aerobic glycolysis and stimulates anaerobic glycolysis, resulting in the piling up of pyruvate contents, which in turn may stimulate the anaerobic isozyme, M-type, and suppress the aerobic isozyme, H-type in the liver and the like.

On the contrary, the heart tissue, where the H-type predominates, (displays quite otherwise) shows the tendency of increased percentage of LDH-1 activity, indicative of the opposite mechanism triggered by the SO₂ gas in the heart tissue. The observed changes in lung tissue of animals exposed to SO₂ suggests that the irritant gas had no influence over in bringing any change in the isozyme pattern. This is presumably because the gas rapidly diffuses into other tissues leaving no opportunity to affect the enzyme in the lung itself. In brain tissue, however, both of the isozyme species were repressed, with the relative increase in the 'hybrid' enzymes when exposed to SO₂ gas, which is quite different in response to the gas from the other organs and awaits further elucidation.

The protein contents in tissue homogenate in experiment in vitro showing progressive decreases in all tissues with aeration of air or air+SO₂. But it seems probable from the increased values, though slight, that pure oxygen can enable the tissue homogenates to increase or at least sustain their LDH, particularly M-type, activity, which may be due to oxygen tension as mentioned (Kaplan 1963).

It is clear from the present work (Table IV) that there was observed in the experiment in vitro three major categories; the first, as in the brain tissue (possibly including the kidney tissue), slight increase or no change of enzyme activity brought by air and SO₂ accompanied by equally slight increase with the addition of oxygen; next, as in the liver and muscle tissues (anaerobic), an increase with the air and SO₂ followed by decrease in activity with oxygen; and last, as in the heart and lung tissue, (aerobic) progressively increasing activity with air, SO₂ and almost no changed activity with pure oxygen.

Although there was little or no effect of SO₂ gas on the brain LDH activity, lung-heart tissue and liver-muscle tissue showed the same tendencies towards the SO₂ gas, that is, increased LDH activities; however, the lung, heart and liver muscle tissues showed opposite results when exposed to pure oxygen, the former having no change in activity and the latter a decreased, even though the heart tissue dominated in the H-type and the liver and muscle in the M-type when aerated with SO₂ gas.

It is probable, therefore, that either inhalation or
aeration with irritating gas induces elevated \( \text{QO}_2 \) and increased glycolytic efficiency in order to overcome the irritation to the body by producing more energy in the form of ATP. This was previously proved by Buckley and Balchum (1967) with the use of \( \text{NO}_2 \) gas, reporting that there were significant elevations in the activity of aldolase, and LDH as well as \( \text{QO}_2 \) in the tissues including the lung, liver, kidney, and spleen of the guinea pigs. They suggested possible involvement of the gas in a general physiological 'stress' reaction and/or interaction of the gas with lung and blood tissue as a possible explanation for their results. This may also be attributable to the present results with one questionable result obtained with the brain tissue.

However, when pure oxygen was aerated through the tissue after gas treatment to see if the tissue could recover, from the gas effect the liver-muscle group showed a decreased level, while the aerobic tissues showed increased levels of enzyme activity. As Kaplan and his coworkers (1960, 1962) observed using muscle or uterus explants in the culture medium, elevation of oxygen tension of the culture suppressed the overproduction of M-type LDH (by uterine muscle explants) and rather enhanced the production of H-type LDH by heart explants. Cahn (1963) also observed enhancement of the synthesis of H-type LDH in chick heart explants incubated in oxygen.

According to Dawson et al. (1964), when monkey heart cells (Salk) were incubated in an atmosphere of 95% nitrogen and 5% \( \text{CO}_2 \), they produced more M-type than control cells incubated in 20% oxygen and 5% \( \text{CO}_2 \). These changes in the synthesis of subunits with changes in oxygen tension are consistent with the proposed physiological role of M and H enzyme. The low oxygen tension favors synthesis of M-type, the form best suited for anaerobic metabolism. High oxygen tension favors synthesis of the H enzyme, the form which predominates in aerobic tissues. In this connection fluctuating results due to the distinct countercurrent pattern of circulation in the kidney (Leonhardt, 1963), showing the preponderance of H-type in the cortex, the M-type in the medulla.

The foregoing discussion illustrates the specific activities in respective tissues. With only the exception of kidney, all the tissues showed increased specific activity, because of elevated activities plus decreased protein contents. It is evident from the decreases in specific activity of the liver and muscle tissues that there was suppressed biosynthesis of LDH particularly the M-type in these tissues due to the lowered oxygen tension.

REFERENCES


The Effects of Sulfur dioxide on Tissue LDH isozyme — 47 —

Bergmeyer, H., Bernt, E. and Hess, B.: The Method of
Silson, A. C., Kaplan, N. O., Levine, L., Peace, A.,
211, 1963.
Stokinger, H. E.: Respiration, Vo. II. Fenn, W. O. and
Rahn, H., American Physiological Society, Washington,