Variations in the Beta-Glucuronidase Activity of Tuberculous Pleural Effusions*

S. H. LAWRENCE, M.D.†
San Fernando, California

Since the first description of a glucuronoside-splitting enzyme, there has been growing interest in its variation in disease conditions. The glucuronidase activity has been suggested as a test for certain types of cancer; it has been shown to be very much altered in pregnancy, and associated with toxemia; and it has been proved to be related to the elimination of many toxic agents by the body. Glucuronidase of bacterial origin has also been described. Glucose is normally oxidized during metabolic processes on the number 1 or aldehyde carbon to form gluconic acid. Under certain circumstances oxidation occurs on the number 6 carbon forming a 6 carbon chain with a carboxyl group on one end and an aldehyde group on the other. This is known as glucuronic acid. Details of its function are not entirely clear, but it is known that it combines with several organic poisons, permitting them to be excreted in detoxified form. Inasmuch as the enzyme Beta-glucuronidase is found in abnormal concentration in such intoxications, it is thought to play a role in the conjugation as well as the hydrolysis of the substrate. Fishman's studies on the Beta-glucuronidase activity of metastatic pleural effusions suggested the determination in connection with our own experiments on pleural effusions of inflammatory origin.

Methods

Beta-glucuronidase determinations were carried out according to the method described by Fishman, Springer, and Brunetti. Some of the substrate, phenolphthalein glucuronoside, was obtained from a commercial source and some was prepared in our laboratory. Determinations of glucuronidase activity were made on fluid from all our routine thoracic aspirations.

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\begin{align*}
\text{Glucuronic Acid} & = \text{O} \quad \text{O} \\
& \quad \text{OH} \quad \text{OH} \\
& \quad \text{H} \quad \text{H} \quad \text{H} \\
& \quad \text{HO} \quad \text{O} \quad \text{O} \\
\end{align*}
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*From the San Fernando Veterans Administration Hospital.
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†George Kidd, Technical Assistant.
Results

1) Purulence of the fluid: All fluids were classified visually according to their turbidity as 1, 2, 3, 4, 5 plus or clear. It was found that the Beta-glucuronidase activity was roughly proportional to the turbidity (Figure 1). Inasmuch as white blood cells have been found to contain the enzyme in considerable concentration, this result might have been expected, since the turbidity is largely due to white blood cells. That the enzyme is liberated freely into its environmental fluid was demonstrated by the fact that centrifuged specimens of turbid fluid also showed elevated activities, although not to the extent of the mixed fluid (Table I). As will be noted in Figure 1, although the enzyme concentration in the greatest number of the clear fluids is below 500 units per 100 cc., there are quite a number of specimens scattered between 1,000 units and 1,500 units per 100 cc. When compared with the levels in the blood serum, it would seem that some are in osmotic equilibrium with it, while another distinct group rises high above it (Table II). This leads to the question, where does the extra activity come from? We have already shown the white blood cells to be an added

| TABLE I: Showing Elevation of Glucuronidase Activity in the Clear Supernatant After Removal of Pus Cells by Centrifugation. |
|---|---|---|
| Patient Number | MIXED | CENTRIFUGED |
| | Turbidity | Activity* | Turbidity | Activity* |
| 1 | +1 | 444 | 0 | 360 |
| 2 | +5 | 5244 | 0 | 3374 |
| 3 | +5 | 4574 | 0 | 3374 |
| 4 | +4 | 2500 | 0 | 1050 |
| 5 | +4 | 2100 | 0 | 1050 |
| 6 | +5 | 3912 | +2 | 3150 |

*B-glucuronidase activity expressed in units per 100 cc.

| TABLE II: Glucuronidase Activity of Clear Chest Fluids Compared With the Level in the Blood Serum. |
|---|---|---|
| Patient | Serum | GLUCURONIDASE ACTIVITY (Units/100 cc.) |
| | | Chest Fluid |
| 1 | 413 | 4800 |
| 2 | 166 | 666 |
| 3 | 333 | 1866 |
| 4 | 200 | 1428 |
| 5 | 180 | 150* |
| 6 | 181 | 204* |
| 7 | 333 | 277* |
| 8 | 200 | 184* |

*Fluids considered to be in equilibrium with the blood serum.
source of the enzyme, but this cannot explain this situation because the fluid is clear and the number of white blood cells present is small. Still there is a marked increase in concentration above the blood serum.

There could be two explanations for this increase in concentration. Either tubercle bacilli growing in the fluid produce the enzyme, or the pleural walls act as a differential secretory membrane when inflamed.

To further clarify this, tubercle bacilli were grown to optimal growth in Dubos' medium. This was centrifuged and the cell-free fluid showed no Beta-glucuronidase activity. Inasmuch as certain bacteria produce the enzyme only in the presence of a substrate, 0.35 mgm. of phenolphthalein glucuronoside were added to 1 cc. of the medium. The organism failed to

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**FIGURE 1:** Comparing the B-glucuronidase activity in chest fluid with the turbidity of the fluid.

**FIGURE 2:** Showing marked rise and fall of glucuronidase activity of chest fluid of patient developing fibrin.
hydrolyse any of this substrate, and the fluid which remained after cen-
trifugation showed no glucuronidase activity.

An attempt was also made to compare the glucuronidase activity of
fluids with the presence of M. tuberculosis in the fluid, but no correlation
could be made. Certain pleural irritants, e.g., Streptokinase-Streptodornase
and Trypsin have caused a marked elevation in the Beta-glucuronidase
activity. This will be reported in greater detail later. All these data are
interpreted as evidence that the pleural membrane when irritated secretes
the enzyme into the pleural fluid.

2) Studies with relation to the duration of effusion: In a few instances
it was possible to follow an effusion in one patient over a prolonged period
of time. One such case, J. P., showed an interesting pattern. A brief history
follows:

The patient was a 41 year old white male who was hospitalized because of a
small tuberculous cavity in the right upper lobe. Pneumothorax was begun shortly
after hospitalization and was followed in one month by a pneumonolysis. The
adhesion was a rather broad one and after it was severed, bleeding was observed
through the thoracoscope. It was felt that this bleeding was controlled by cauter-
ization after a few minutes. On the day following the operation, the right chest
cavity was found to be about one-half filled with fluid which on aspiration was
found to be nearly pure blood. Aspirations were performed at daily intervals for
one week, and then at longer intervals. The fluid gradually assumed a clear straw-
color. During the next month, however, the fluid became pocketed by fibrinous
deposits and the pneumothorax was abandoned. Aspirations were continued with
addition of Streptokinase-Streptodornase until a solid fibrinous deposit made
further aspirations impossible. Figure 2 shows the gradual rise of enzyme activity
until the formation of fibrin and its rapid fall after this.
It was also observed that those fluids which were of short duration contained in general, much higher enzyme levels than those of long duration (Figure 3).

3) Beta-glucuronidase activity compared with the extent of effusion: Figure 4 shows the variation in activity in three classes of effusions following pneumothorax. The first column, minimal, refers to those fluids which on x-ray film inspection are below the dome of the diaphragm. Moderate implies that the fluid is above the dome of the diaphragm. In the last column, no pneumothorax means that this collapse procedure has been present but was discontinued and all air absorbed.

Discussion

While there seems to be some correlation between the Beta-glucuronidase activity of pleural effusions and their clinical course (Figures 3 and 4), the exact meaning of an elevation of activity is not clear. It would have been interesting to have compared these values with those on cases of idiopathic pleural effusions, but 10 of these are available at this institution.

We can, however, compare our results with those reported by Fishman, et al., in which the activities of 10 effusions, which were not associated with infection or cancer, were analyzed. It is seen that they compare favorably with many of our clear effusions, but again the few tuberculous effusions with high glucuronidase activity are prominent (Table 3).

We cannot speculate on the relationship of the elevated Beta-glucuronidase levels reported in these studies and those reported by Fishman,
et al., in carcinomatous conditions. It would seem to indicate, however, that the enzyme activity may be an index of any non-specific inflammation of the pleura, in which case it would be interesting to know what factors cause the pleural membrane to become a differential secretory organ for the enzyme.

At the present it is impossible to state whether high levels of Beta-glucuronidase activity of pleural fluid in a pneumothorax space presage future difficulties with the pneumothorax. Additional studies are being continued along these lines.

Cell counts, specific gravities and protein determinations were done on the fluids but it was not felt that their results contributed anything to this study.

**SUMMARY**

1) Determinations of Beta-glucuronidase activity were done on specimens of fluid from all chest aspirations of tuberculous patients at this hospital during the period of study.

2) Many of these effusions contained pus cells in sufficient quantity to appear turbid. The glucuronidase activity appeared roughly proportional to the turbidity.

3) In certain clear fluids the glucuronidase activity seemed to be in osmotic equilibrium with the blood serum. The activity of others, however, rose high above that of the blood serum, and some speculations were presented regarding the source of this extra glucuronidase activity.

4) As long as fluid remained clear, the glucuronidase activity seemed to be higher in effusions of short duration than in those of long standing.

5) Certain clear fluids had a very high activity out of proportion to others of their age.

6) No correlation could be seen between the glucuronidase activity and the presence of the tubercle bacillus in the fluid.

**RESUMEN**

1) Se hicieron las determinaciones de la actividad de la Beta-glucuronidasa en muestras de líquido de todas las aspiraciones del torax de pacientes tuberculosos, realizadas en este hospital durante el periodo de estudio.

2) Muchos de esos derrames contenían plóctitos en suficiente cantidad para aparecer turbios. La actividad de la glucuronidasa pareció ser proporcional a la turbidez.
3) En ciertos líquidos claros, la actividad de la glucuronidasa parece estar en equilibrio osmótico con el suero sanguíneo. La actividad de otros líquidos sin embargo, ascendía muy por encima de la del suero sanguíneo, y se hacen algunas suposiciones en relación al origen de esta extra actividad de la glucuronidasa.

4) Según el tiempo que el líquido permanece claro, la actividad de glucuronidasa parece ser más alta en los derrames recientes que en los antiguos.

5) Ciertos líquidos claros tienen una actividad sumamente alta, sin relación con otros de su misma fecha.

6) No pudo verse ninguna correlación entre la actividad de la glucuronidasa y la presencia de bacilos tuberculosos en el líquido.

RESUME

1) L'activité Beta-glucuronidique a été déterminée sur tous les épanchements pleuraux qui furent constatés chez les malades pendant la période de cette étude.

2) Beaucoup de ces épanchements renfermaient des cellules de pus en quantité suffisante pour qu'ils apparaissent louches. L'activité glucuronidique a semblé grossièrement proportionnelle à l'opacité du liquide.

3) Dans certains épanchements clairs, l'activité glucuronidique semble être en équilibre osmotique avec le sérum sanguin. Pour d'autres cependant, cette activité s'est élevée fortement au-dessus de celle du sérum sanguin. L'auteur fait quelques considérations sur les origine de cette activité extra-glucuronidique.

4) Tant que le liquide reste clair, l'activité glucuronidique apparaît plus élevée dans les épanchements qui ont peu duré que sur ceux qui existent depuis longtemps.

5) Certains épanchements clairs ont une activité très élevée, sans rapport avec les autres épanchements du même âge.

6) L'auteur n'a remarqué aucune corrélation entre l'activité glucuronique et la présence de bacilles tuberculeux dans l'épanchement.

REFERENCES