

## $\beta$ -GLUCURONIDASE OF HUMAN WHITE BLOOD CELLS

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MUCH can be learned of the function of white blood cells by studying the constituent enzymes of each cell type. Our present knowledge of white-cell enzymes has been reviewed in recent papers by Barnes<sup>1</sup> and Rebuck.<sup>21</sup> Such an obvious experimental approach to the physiology of the white cell has been neglected for so long because cell suspensions containing one cell type only are difficult to prepare. Haight and Rossiter<sup>12</sup> have recently shown that it is possible, by statistical means, to determine the distribution of either acid or alkaline phosphatase in mixed white-cell suspensions containing polymorphonuclear leukocytes and lymphocytes. For both man and the rabbit it was found that the acid phosphatase was largely confined to the lymphocyte, while the alkaline phosphatase was almost entirely in the polymorphonuclear leukocyte. In this paper the distribution of  $\beta$ -glucuronidase in cell suspensions containing polymorphonuclear leukocytes and lymphocytes from human blood has been investigated by similar methods.

In 1948, Fishman, Springer and Brunetti<sup>8</sup> reported that a high percentage of the  $\beta$ -glucuronidase activity of human blood was in the buffy-coat layer. They also presented evidence to support the view that most of the glucuronidase of the buffy coat was in the white cells rather than in the platelets. Rossiter and Wong<sup>23</sup> studied the  $\beta$ -glucuronidase of the polymorphonuclear leukocyte of the rabbit. The properties of this enzyme are similar to those of the enzyme described by Fishman and his co-workers.<sup>20</sup> A review of the literature on  $\beta$ -glucuronidase and a discussion of the function of the enzyme in white cells can be found in the paper by Rossiter and Wong.<sup>23</sup>

### METHODS

*Cell Preparations.* Mixed white-cell suspensions were obtained from freshly-drawn human blood either by centrifuging in a constricted centrifuge tube, such as was used by Butler and Cushman<sup>2</sup> for the determination of ascorbic acid in white cells, or by flotation on a solution of gum acacia as described by Spear.<sup>25</sup> Although heparin was used as an anticoagulant, the concentration in the final cell suspension was negligible.

Total and differential cell counts were made by the usual methods. Five individual dilutions were made on each suspension and one total white-cell count done on each dilution. The figure recorded was the mean of these five counts. For the differential counts, 500 cells were examined on each film. The cells were divided into polymorphonuclear leukocytes (which included basophils and eosinophils) and lymphocytes (which included monocytes). In general, the percentage of polymorphonuclear cells was greater in suspensions obtained by the centrifuging method<sup>2</sup> and less in those obtained by the flotation method.<sup>25</sup> With the latter method the polymorphonuclear cells tended to clump together. These clumped cells were removed from the suspensions by filtration through fine gauze.

*$\beta$ -Glucuronidase Determination.* The glucuronidase activity of the suspensions was determined by the method of Fishman et al.,<sup>8</sup> using as substrate phenolphthalein mono- $\beta$ -glucuronide, prepared according to the directions given by these workers. The details of the test have already been described.<sup>23</sup> The enzyme was extracted from the cells by a solution of saponin. Rossiter<sup>22</sup> has shown that certain surface-

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active substances, such as saponin, alkyl sulfate and bile salts, liberate the enzymes phosphomonoesterase and ali-esterase from white cells into the surrounding fluid. Rossiter and Wong<sup>23</sup> showed that the same was true for  $\beta$ -glucuronidase.

*Recording of Results.* The results were recorded as the number of units of glucuronidase per 100 cc. cell suspension where, following Fishman et al.,<sup>8, 26</sup> one glucuronidase unit is defined as the amount of

TABLE I.— $\beta$ -Glucuronidase Activity of Suspensions of White Blood Cells

	No. Observations	Glucuronidase (Units/10 <sup>10</sup> Cells.)	Standard Devia- tion
Males .....	20	15,000	$\pm 4,800$
Females .....	5	14,900	$\pm 3,800$
Rabbit .....	15	9,700	$\pm 3,800$

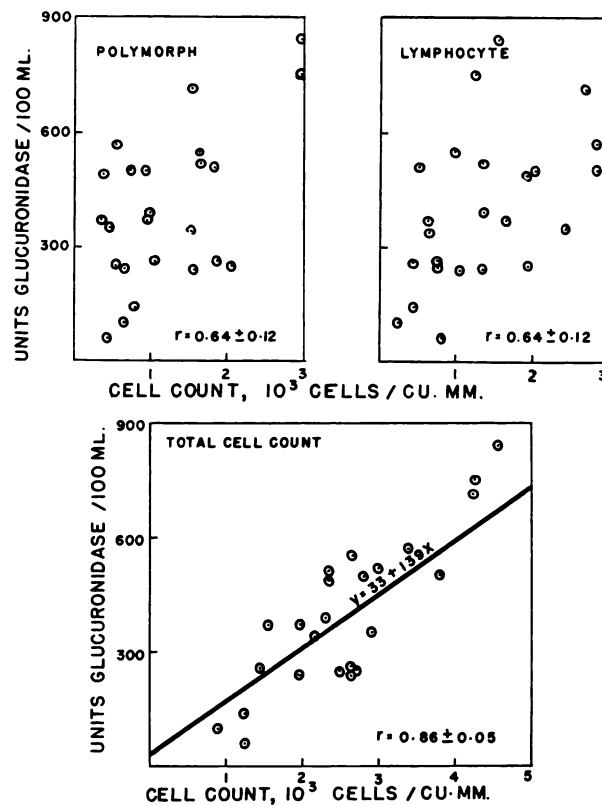


FIG. 1.—Glucuronidase activity of suspensions of human white cells plotted against polymorphonuclear leukocyte cell count, lymphocyte cell count, or total white cell count.

enzyme that would liberate one  $\mu$ g. phenolphthalein in one hour under the standard conditions of the test. In order that the results might be compared with the findings for other species and for other white-cell enzymes, they have also been expressed as the number of glucuronidase units per  $10^{10}$  cells.

#### RESULTS

Table I gives a summary of the results obtained for 25 mixed white-cell suspensions. Since it has been suggested that glucuronidase may be concerned with the

conjugation of estrogens,<sup>6</sup> the mean  $\beta$ -glucuronidase activity of cells from females was compared with that of cells from males. There was no significant difference, so in the subsequent statistical analysis the results for both sexes were pooled. Table 1 also shows that the mean level for the cells from man was somewhat greater than that for cells from the rabbit. The findings for rabbit cells have previously been reported.<sup>23</sup>

Figure 1 shows the concentration of glucuronidase in human cells, expressed in units per 100 cc., plotted against the total cell count, the polymorphonuclear leukocyte cell count, or the lymphocyte cell count. There was a high correlation between glucuronidase activity and total cell count ( $r = 0.86 \pm 0.05$ ), but a less significant correlation between glucuronidase activity and either polymorphonu-

TABLE 2.—Correlation between  $\beta$ -glucuronidase Activity and Cell Count of Suspensions of White Blood Cells

	Type of Correlation	Coefficient of Correlation (r)	S. E. of r.
$r_1$	Coefficient of correlation between $\beta$ -glucuronidase activity and total white cell count.	0.86	$\pm 0.05$
$r_{12}$	Coefficient of correlation between $\beta$ -glucuronidase activity and polymorphonuclear leukocyte count.	0.64	$\pm 0.12$
$r_{13}$	Coefficient of correlation between $\beta$ -glucuronidase activity and lymphocyte count.	0.64	$\pm 0.12$
$r_{23}$	Coefficient of correlation between polymorphonuclear leukocyte count and lymphocyte count.	0.10	$\pm 0.20$
$r_{12.3}$	Coefficient of partial correlation between $\beta$ -glucuronidase activity and polymorphonuclear leukocyte count (lymphocyte count excluded).	0.76	$\pm 0.09$
$r_{13.2}$	Coefficient of partial correlation between $\beta$ -glucuronidase activity and lymphocyte count (polymorphonuclear leukocyte count excluded).	0.76	$\pm 0.09$
$R_{1.23}$	Coefficient of multiple correlation between $\beta$ -glucuronidase activity and both polymorphonuclear leukocyte count and lymphocyte count.	0.87	$\pm 0.05$

clear leukocyte count ( $r_{12} = 0.64 \pm 0.12$ ) or lymphocyte count ( $r_{13} = 0.64 \pm 0.12$ ). This suggests that, unlike the acid or the alkaline phosphatase activity,<sup>12</sup> the glucuronidase activity is divided fairly evenly between the polymorphonuclear leukocytes and the lymphocytes.

Table 2 shows that for the suspensions used there was no significant correlation ( $r_{23} = 0.10 \pm 0.20$ ) between the polymorphonuclear leukocyte cell count and the lymphocyte cell count. This indicates that the significant correlation between the glucuronidase activity and the cell count for one of the major cell types could not be merely the result of a significant correlation between the enzyme activity and the cell count for the other cell type together with a highly significant correlation between the counts for each of the two cell types. In fact the coefficient of partial correlation between the enzyme activity and the polymorphonuclear leukocyte cell

count with the lymphocyte count excluded and that between the enzyme activity and the lymphocyte cell count with the polymorphonuclear leukocyte count excluded ( $r_{12,3}$  and  $r_{13,2} = 0.76 \pm 0.09$ ) was, in each instance, greater than the coefficient of correlation between the enzyme activity and each of the individual cell counts ( $r_{12}$  and  $r_{13} = 0.64 \pm 0.12$ ).

The multiple regression equation expressing the enzyme activity in terms of the cell count for each type of cell was found to be:

$$Y = 27 + 159 X_{\text{poly}} + 128 X_{\text{lymph}}$$

where  $Y$  is the glucuronidase activity in units per 100 cc. suspension,  $X_{\text{poly}}$  is the polymorphonuclear leukocyte count in  $10^3$  cells per cu. mm. and  $X_{\text{lymph}}$  is the lymphocyte count in  $10^3$  cells per cu. mm. From this it is seen that, on the average,

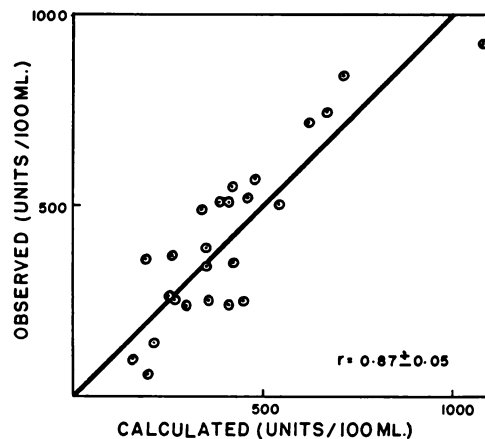


FIG. 2.—Relation between observed glucuronidase activity of suspensions of human white cells and glucuronidase activity calculated from the polymorphonuclear leukocyte and lymphocyte cell count, using the multiple regression equation presented in the text.

the ratio of the enzyme activity in the polymorphonuclear leukocyte to that in the lymphocyte is 159:128, or approximately 5:4. The observed glucuronidase activity is plotted against the value calculated from the multiple regression equation in figure 2. The coefficient of multiple correlation between the glucuronidase activity and the cell count for each of the two cell types ( $R_{1,23}$ ) was  $0.87 \pm 0.05$  (table 2).

#### DISCUSSION

The finding that the glucuronidase activity is divided fairly evenly between the polymorphonuclear leukocytes and the lymphocytes would support the conclusion of Fishman et al.<sup>8</sup> that "there does not seem to be any correlation of the buffy coat glucuronidase with the percentage of polymorphonuclear leucocytes and lymphocytes in the blood." The technic employed in our study was, however, different from that used by Fishman and his associates. We made no attempt to recover all the white cells from a given volume of blood. Counts were done on homogeneous

suspensions of white cells after the cells had been separated from the blood and after any clumped cells had been removed. If the figures of Fishman et al.<sup>8</sup> are converted into glucuronidase units per  $10^{10}$  cells, it will be seen that the figures of table 1 are considerably greater. This may in part be due to the difference in technique and in part due to the fact that, in our experiments, the enzyme was extracted from the cells with saponin, a procedure that in our hands is more efficient than the freezing-thawing method used by Fishman. This may also be the reason why we found a closer correlation between the  $\beta$ -glucuronidase activity of a cell suspension and the white cell count than the data of Fishman et al.<sup>8</sup> would suggest. These workers concluded that "although greater glucuronidase activities are frequently found in the presence of high white cell counts, a strict dependence of the glucuronidase level upon the number of white cells is not evident."

That there is a high concentration of  $\beta$ -glucuronidase in the white cells is, perhaps, to be expected, since Sera<sup>24</sup> demonstrated the presence of the enzyme in the spleen. The distribution of  $\beta$ -glucuronidase in the animal body has been undertaken by Oshima<sup>20</sup> and Talalay et al.<sup>26</sup> Both groups of workers found a high enzyme activity in the spleen, and this organ has been the source of many of the partially purified  $\beta$ -glucuronidase preparations that have been described.<sup>4, 9, 11, 18</sup> Although Oshima<sup>20</sup> showed that the thymus was rich in the enzyme, Fishman and Anlyan<sup>7</sup> reported that the concentration of lymph nodes was low. One might, therefore, have anticipated that the ratio of the concentration of the enzyme and the polymorphonuclear leukocyte to that in the lymphocyte would have been greater than 5:4.

The finding of a high concentration of  $\beta$ -glucuronidase in the white cells of man, and also in those of the rabbit,<sup>23</sup> raises the question of whether the white cells might not be a possible source of the enzyme in the plasma. Also, as was pointed out for alkaline phosphatase,<sup>3</sup> there is the possibility that the white cell may have obtained its  $\beta$ -glucuronidase by adsorption from the plasma. If this were so, the cells would have to have the property of concentrating the enzyme many times, for the mean concentration of  $\beta$ -glucuronidase in white cells is some 380,000 units per 100 Gm. packed cells, which is of a different order from that found in plasma.<sup>8, 23</sup> Additional evidence that the  $\beta$ -glucuronidase is within the cell rather than adsorbed to the cell surface is provided by the histochemical studies by Friedenwald and Becker,<sup>10</sup> who demonstrated the presence of  $\beta$ -glucuronidase in the cytoplasm of cells of the spleen, lymphatic nodules and bone marrow.

It is interesting to speculate upon the function of  $\beta$ -glucuronidase in white cells. If, as has been suggested,<sup>5, 6, 20</sup>  $\beta$ -glucuronidase has to do with metabolic conjugation and, possibly, detoxication, it would be attractive to assume that white cells aggregate at sites of infection to inactivate toxic substances. As pointed out previously,<sup>23</sup> however, the theory that  $\beta$ -glucuronidase is concerned with metabolic conjugation and detoxication has been severely criticized.<sup>13-16</sup> The function of the enzyme appears to be concerned with hydrolysis of glucuronides rather than with their synthesis.<sup>17, 19</sup> The role of the enzyme in the white cell may thus be concerned with the hydrolysis of some glucuronic acid derivative normally present in

the tissues. It is possible that  $\beta$ -glucuronidase may play some part in the complex tissue reaction of inflammation.

## SUMMARY

1. The  $\beta$ -glucuronidase activity of suspensions of mixed white cells, obtained from human blood by the centrifuging technic or by flotation on gum acacia, has been studied.
2. The correlation between  $\beta$ -glucuronidase activity and total white-cell count was greater than that between the activity of the enzyme and either the polymorphonuclear leukocyte count or the lymphocyte count.
3. On the average, the ratio of the enzyme activity in the polymorphonuclear leukocyte to that of the lymphocyte was 5:4.

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