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PRUVATOKINASE AND PHOSPHOGLYCOMUTASE ACTIVITY
OF GRANULOCYTES AND LYMPHOCYTES

Following is the translation of an article by F. Belfiore and J. Meldolesi in the Italian-language periodical Bollettino della Societa Italiana di Biologia Sperimentale (Bulletin of the Italian Experimental Biology Society), Naples, Vol 39, No 23, 1963, pages 1568-1571.]

From the Pathological Medicine Institute of Catania University
Catania Section -- 26 July 1963 session.

The study of enzymes in leucocytes, though limited to the activities revealed by these elements when considered indiscriminately, provides us with information which is not at all convincing; this is probably due to the fact that this is an expression of the metabolism of a heterogeneous cellular population basically made up of a mixture of granulocytes and lymphocytes in ratios varying from one case to the next. This brings us to the problem of defining the activity of the granulocytes and the lymphocytes not only for the purpose of establishing the activity of these two types of leucocytes but also in order to be able to compare the activities revealed under various morbid conditions to the activities of the corresponding normal cells.

In view of the difficulty encountered in obtaining preparations of granulocytes and lymphocytes in a state of sufficient integrity and purity, some authors (1) have adopted a mathematical method which enables them to calculate the activity of the individual categories of leucocytes starting from the activities of the leucocytary preparation considered as a whole and using the correlation between the behavior of the enzyme and the leucocytary formula in the case histories studied. A few critical considerations on this method, which were expressed earlier (2), have caused one of us (2, 3) to propose a procedure through which it is possible, by means of relatively simple calculations, to determine the activity of the granulocyte and the lymphocyte. Using this procedure, we were able to show in earlier research that the glucose-6-phosphate dehydrogenase (4) and arginase (2) reveal a considerably higher activity in the granulocytes than in the lymphocytes; we were also able

to show that glutamic-oxalacetic transaminase (5), isocitricodehydrogenase, and glutamate dehydrogenase (6) reveal the opposite behavior, with higher values in the lymphocytes; finally, we were able to show that aldolase and lacticodehydrogenase (7), malicodehydrogenase (8), succinodehydrogenase (9), phosphohexoseisomerase and acid phosphatase (10) do not reveal noticeable differences between the two types of leucocytes.

In this particular report we considered the behavior of the phosphoglycomutase and pyruvatokinase activities in the leucocytes; the next step, after this, was the determination of the behavior in the granulocyte and lymphocyte. On this particular topic, as far as we know, literature offers us only information pertaining to the observation by Loehr (11) according to whom pyruvatokinase activity is considerably higher in the granulocytes than in the lymphocytes.

Materials and methods. In this investigation, we studied the phosphoglycomutase and pyruvatokinase activities of leucocytes in a group of normal subjects between the ages of 20 and 65 years.

Phosphoglycomutase activity was studied in 26 subjects, 15 of whom were male and 11 of whom were female; we used the method described by Bon-signore and his associates (12), based on the spectrophotometric measurement-- in the amount of 366 m μ --of the reduction in TPN in a combined reaction, catalyzed by glucose-6-phosphate dehydrogenase; the results are expressed in units and related to 1 ml of leucolysate diluted 1/10. Pyruvatokinase activity was studied in 31 subjects, 18 of whom were male and 13 of whom were female; we determined these figures according to the technique of Bock and his associates (13); the results are expressed in units and are related to 1 ml of leucolysate diluted 1/20.

The leucolysate was prepared by adding -- to a specific volume of leucocytes, isolated by means of the previously described method (2)--distilled water in the previously indicated ratio. The results this time were related -- instead of to a specific number of leucocytes -- to a specific volume of leucocytes, hence, to an always constant cellular mass which would be independent of the prevalence of the cells in the larger volume (granulocytes) or of those in the smaller volume (lymphocytes) (2, 3).

After obtaining the values of the enzyme activities in the leucocytes as a whole, we calculated the activity of the two principal categories of leucocytes, that is, of the granulocytes and the lymphocytes, using a mathematical method described elsewhere (2, 3), always relating the data to a certain volume of these elements.

Results. The results which we have obtained are summarized in the attached table and show that the phosphoglycomutase activity of the leucocytes as a whole gives us an average value of 228 U/ml of leucolysate diluted 1/10. Using the method pointed out above (2, 4) we were able to establish a difference in activity between granulocytes and lymphocytes; we have an average

value of 303 U, in the granulocytes and an average value of 126 U in the lymphocytes.

The difference in activity between these two types of leucocytes was statistically significant ($P < 0.01$).

The pyruvate kinase activity in the leucocytes showed an average value of 4,290 U/ml of leucolysate diluted at 1/20; in the granulocytes we had an average value of 4,843 U, in the lymphocytes we had an average value of 2,133 U. The difference between the value of the granulocytes and that of the lymphocytes was statistically significant ($P < 0.02$).

The data given above agree with the data given by Loehr (11) on the finding that pyruvate kinase activity prevails in the granulocytes, although the difference in activity reported by this author as far as the two types of leucocytes are concerned was even more accentuated than the difference we observed. This slight difference may be explained if we realize that Loehr related enzyme activity to a specific number of leucocytes whereas we, as we emphasized earlier, preferred to relate the results to a specific volume of cells and hence to an always constant cell mass. If we remember that the volume of lymphocytes is smaller than the volume of granulocytes, then we will understand why numerically equivalent leucocytary preparations contain a larger cell mass if they are made up only of granulocytes and a smaller mass if they are made up only of lymphocytes; this might explain the lower activity observed by Loehr in the lymphocytes as compared to our data.

On the basis of the information given above we can say that we were able to confirm the results of our earlier research (2, 10), in other words, that granulocytes and lymphocytes, along with a difference of a morphological nature, also reveal a definite difference as far as their enzyme picture is concerned.

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PHOSPHOGLYCOMUTASE AND PYRUVATOKINASE ACTIVITIES OF
LEUCOCYTES IN NORMAL SUBJECTS

(1)	(2)	(3)	(4)	(5)	(6)	(7)
(8)						
1	68	32	83.38	16.62	—	5600
2	68	32	83.38	16.62	—	3000
3	75	25	87.63	12.37	—	3600
4	65	35	81.10	18.60	—	2000
5	70	30	81.61	15.36	252	2200
6	70	30	81.61	15.36	180	1600
7	72	28	85.85	14.15	176	2100
8	74	26	87	13	560	3200
9	75	25	87.70	12.30	240	4000
10	68	32	83.38	16.62	261	8800
11	68	32	83.38	16.62	56	9200
12	89	11	95.01	4.96	72	3000
13	64	36	80.76	19.24	200	4600
14	68	32	83.38	16.62	160	8200
15	68	32	83.38	16.62	112	3600
16	90	10	95.51	4.49	375	4600
17	62	38	79.38	20.62	372	38400
18	70	30	84.64	15.36	212	3600
(9)	71.34	28.66	85.27	14.73	231	4733
(10)	(11)	(12)			133	2692
					35.56	635
					61-301	3488-5978
(13)						
1	60	40	78	22	—	2800
2	43	57	64.03	35.97	184	2800
3	60	40	78	22	256	3800
4	38	62	59.01	40.98	216	2800
5	60	40	78	22	148	2400
6	55	45	74.20	25.80	160	4200
7	57	43	75.79	24.21	500	6800
8	48	52	68.56	23.44	112	3000
9	47	53	67.67	32.33	416	4600
10	56	44	74.91	25.06	216	3800
11	57	43	75.79	24.21	308	4600
12	50	50	70.24	29.76	220	4600
13	60	40	78	22	80	3200
(9)	53.15	46.85	72.48	27.52	217	3800
(10)	(11)	(12)			225	1191
					65	330
					90-344	3154-4446
(14)						
(9)	(10)	(11)	(12)		303	4843
					25	289
					252-354	4266-5421
					125	1580
(15)						
(9)	(10)	(11)	(12)		126	2133
					62	1065
					0-252	0-4266
					310	5825

Legend: (1) Case number; (2) number of granulocytes; (3) number of lymphocytes; (4) volume of granulocytes; (5) volume of lymphocytes; (6) phosphoglycomutase activity; (7) pyruvatokinase activity; (8) cases where granulocytes prevailed; (9) mean; (10) standard deviation; (11) standard error; (12) confidence interval; (13) cases where lymphocytes prevailed; (14) granulocyte activity; (15) lymphocyte activity.