CLVIII. ENZYMES IN CANCER.

II. THE GLYCEROPHOSPHATASES OF HUMAN ERYTHROCYTES.

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THE β -glycerophosphatase activity of erythrocytes from human cancer patients has been found to be frequently higher than that of erythrocytes from normal subjects, whilst the absolute increase in activity when magnesium ions were added appeared to be the same in both instances [Schoonover and Ely, 1935]. Therefore, the portion of the cancer erythrocyte β -glycerophosphatase which furnished the increase over the normal amount seemed to be incapable of magnesium activation.

There exists in animal organs [Davies, 1934; Bamann and Riedel, 1934] an enzyme which could be responsible for this phenomenon, and it may conceivably occur in the red blood cells. On the other hand, it is also possible that peculiar conditions of natural activation could be the cause.

A comparison of the erythrocyte type of phosphoesterase and the organ type of phosphoesterase shows that their most outstanding similarity lies in their acid p_H optima. The erythrocyte phosphatase [Davies, 1934] as well as the phosphatases of bottom yeast [Schäffner and Bauer, 1935] and of *Propionibacterium jensenii* [Pett and Wynne, 1933] hydrolyse α -glycerophosphate more rapidly than the β -isomerase and are readily activated by magnesium ions. The organ phosphatase [Davies, 1934; Bamann and Riedel, 1934], like the acid phosphatases of urine [Kutscher, 1935], of top yeast [Albers and Albers, 1934, 2] and of *Clostridium acetobutylicum* [Pett and Wynne, 1933], prefers the β -ester and is seldom activated by magnesium at or below its optimum p_H . With lower hydrogen ion concentrations, the organ enzyme shows activation; the others are inhibited. It has been suggested by Albers and Albers [1934, 1] that this activation is due to imperfect removal of erythrocytes from the enzyme preparations.

Phosphatase activity may be augmented by materials other than magnesium ions. Jenner and Kay [1931] found that phosphoesterases from various sources, especially from the red blood cells, retain a large part of their activity when freed from all but traces of magnesium. Moreover, erythrocyte phosphatase was sometimes found to be activated by magnesium ions. Bamann, Riedel and Diederichs [1934] have shown that the capacity for magnesium activation of the alkaline phosphatase is a function of the age and purity of the enzyme preparation and also of the solubility of the enzyme complex.

In an attempt to define the origin of the behaviour of cancer erythrocytes towards β -glycerophosphatase, some experiments were made with the α - and β -glycerophosphatase activities of the haemolysates of normal and of cancerous human erythrocytes. In accord with the theory that the organ enzyme, which

prefers the β -ester, is present in large amounts in cancer erythrocytes, no difference was found between the average α -glycerophosphatase activities of normal and of cancer erythrocytes. The weight of the evidence, however, makes it unlikely that this theory is correct. Both α - and β -glycerophosphatase showed quite similar behaviour with regard to magnesium activation. Artificial conditions produced a destruction of the capacity of both glycerophosphatases for magnesium activation, although considerable dephosphorylating power towards both substrates persisted. α -Glycerophosphatase activity exhibits considerable variation from one subject to another; it is the β -, and not the α -glycerophosphatase activity, which appears to be the more constant in the normal individual. The peculiarities observed in the β -glycerophosphatase activity of cancerous erythrocytes, then, are a distinct departure from the normal state, and make it possible that other phosphoric acid esters also may be hydrolysed in some unusual manner.

EXPERIMENTAL PROCEDURE.

The erythrocyte samples used in this investigation were obtained from a new series of cancer patients and normal individuals; only two of the latter have furnished data previously. It was not always possible to secure patients whose malignant condition had advanced as far as it had in the series reported previously [Schoonover and Ely, 1935].

The buffered substrates were made up daily until it became apparent that new lots of chemicals affected the p_H of these solutions to an undesirable extent. Later, they were made up in quantity and kept under the conditions recommended by Bodansky [1933] for the preservation of the more alkaline substrates. Quinhydrone p_H determinations at room temperature were conducted on each buffered substrate. There was no appreciable change in p_H , nor was any formation of inorganic phosphate found, over an interval of 3 weeks. The phosphatase units (mg. of inorganic phosphorus liberated by 10 ml. of erythrocytes in 24 hours at 37°) observed were plotted against the p_H values of the buffered substrates; from these p_H -activity curves the value for any desired p_H within the range could be read off.

Buffered substrates containing β -glycerophosphate were made up in the proportions described before and covered the approximate p_H range 5.0–6.0 at intervals of about 0.2 p_H . Buffered substrates containing α -glycerophosphate were prepared from a fresh stock solution of sodium α -glycerophosphate and sodium acetate, which was obtained in the following fashion: to 100 ml. of 0.6 *M* calcium α -glycerophosphate (neutral, DAB 6, Fraenkel and Landau) in *N* acetic acid there were added 4.5 ml. of water, and then, with shaking, 95.5 ml.¹ of 0.6 *M* oxalic acid and 240 ml. of *N* NaOH. After 10–15 min., the mixture was filtered, and 300 ml. of the filtrate, together with 35.4 g. of anhydrous sodium acetate, were made up to 500 ml. with water. Two ml. of this solution, diluted to 10 ml., as in the reaction mixture, contain an amount of phosphorus corresponding closely to that of the β -glycerophosphate substrates; moreover, the 10 ml. are 0.2 *M* in acetate ion, also as in the β -glycerophosphate substrates. The individual substrate solutions, then, were prepared by diluting 2 vols. of the sodium α -glycerophosphate-sodium acetate solution to 5 vols. with *M* magnesium chloride (when magnesium was to be added to the reaction mixture),

¹ 95.5 ml. was the quantity selected because the precipitate of calcium oxalate, after the addition of NaOH, corresponded to 95.4 ml. of oxalic acid. When 100 ml., the theoretical quantity, was employed, the calcium oxalate precipitate corresponded to only 95.7 ml. of oxalic acid.

0.5*N* HCl in sufficient quantity to produce the p_H required and water. The p_H range 5.0–6.0, at intervals of about 0.2 p_H , was covered in this way.

To 5 ml. of each β -glycerophosphate substrate, after 5 min. previous heating at 37°, were added 5 ml. of a 1 : 50 haemolysate of unwashed erythrocytes. The remaining haemolysate was diluted with an equal volume of water, and after mixing, 5 ml. of this 1 : 100 dilution were added to each substrate containing α -glycerophosphate. This procedure was rendered necessary by the extremely rapid cleavage of α -glycerophosphate by erythrocytes. The rest of the experimental procedure was exactly the same as that described in the previous paper. The use of the Bodansky [1932] table and factors to allow for the effect of Beer's law and for the influence of β -glycerophosphate and trichloroacetic acid on the colorimetric readings was found to be advantageous when the α -ester had replaced the β -ester.

p_H optimum for erythrocyte α -glycerophosphatase activity in the range 5.0–6.0.

Fig. 1 shows that the optimum substrate p_H for the hydrolysis of α -glycerophosphate by normal and by cancerous human erythrocytes is found to be close

Fig. 1. p_H -activity curves for the α -glycerophosphatase activity of human erythrocytes. — normal subjects; - - - - untreated cancer patients.

to 5.6 under the experimental conditions described. This is the same optimum as that for the hydrolysis of β -glycerophosphate.

Relation of α - to β -glycerophosphatase activity.

In Table I may be seen examples of the rates of hydrolysis of α - and β -glycerophosphates by red blood cell haemolysates from normal and cancerous individuals. α -Glycerophosphate is split by both haemolysates at rates which vary considerably from one subject to another. The cancer α -glycerophosphatase figures fall within the normal limits. Half of the cancer β -glycerophosphatase values, on the other hand, lie above the highest normal figure. The low values observed in the other half of the cancer cases are possibly due to the slighter degree of advancement in the malignant process. An examination of the ratios of the rates of hydrolysis of the two isomerides shows that there is no constant ratio in either the normal or the cancerous state; in fact, there appears to be an independence of submission to enzymic attack.

Table I. *Comparison of the rates of hydrolysis of α - and β -glycerophosphates by erythrocytes from the same individuals.*

No.	Type	p_H 5.0			p_H 5.6			p_H 6.0		
		α -Gly- cero- phos- phate	β -Gly- cero- phos- phate	α/β	α -Gly- cero- phos- phate	β -Gly- cero- phos- phate	α/β	α -Gly- cero- phos- phate	β -Gly- cero- phos- phate	α/β
		Units	Units		Units	Units		Units	Units	
1	Normal	16	4.1	3.9	38	8.4	4.5	31	7.7	4.0
2	"	20	4.0	5.0	52	8.7	6.0	39	7.5	5.2
3	"	33	5.7	5.8	54	11.2	4.8	37	11.2	3.3
4	"	26	5.2	5.0	56	9.2	6.1	44	7.2	6.1
5	"	39	6.4	6.1	62	8.9	7.0	38	—	—
6	"	25	5.1	4.9	64	9.9	6.5	57	8.8	6.5
7	"	27	5.4	5.0	65	10.4	6.3	51	10.3	5.0
8	"	27	6.4	4.2	73	11.9	6.1	50	9.8	5.1
9	"	45	7.1	6.3	84	11.9	7.1	70	12.9	5.4
1	Cancer	22	6.2	3.6	44	10.9	4.0	—	—	—
2	"	19	5.2	3.7	57	9.5	6.0	45	8.5	5.3
3	"	39	19.5	2.0	57	20.2	2.8	36	19.0	1.9
4	"	18	4.7	3.8	58	9.7	6.0	47	8.8	5.3
5	"	38	10.1	3.8	61	15.5	3.9	47	14.2	3.3
6	"	28	5.3	5.3	62	10.3	6.0	49	9.7	5.1
7	"	26	6.2	4.2	66	11.4	5.8	47	10.2	4.6
8	"	46	10.2	4.5	69	19.6	3.5	55	15.2	3.6
9	"	30	7.2	4.2	78	13.0	6.0	55	10.8	5.1
10	"	33	7.7	4.3	81	13.4	6.0	63	12.0	5.3

Activation of α - and β -glycerophosphatases by magnesium.

Erythrocyte α -glycerophosphatase activity, like erythrocyte β -glycerophosphatase activity, is augmented by the presence of magnesium chloride. According to the preliminary data recorded in Table II, 0.02 *M* magnesium

Table II. *Effect of magnesium chloride in large concentrations on the α -glycerophosphatase activity of normal and cancer erythrocytes.*

p_H	Normal				Cancer			
	<i>M</i> MgCl ₂ added to reaction mixture				<i>M</i> MgCl ₂ added to reaction mixture			
	0	0.02	0.05	0.10	0	0.02	0.05	0.10
	Units	Units	Units	Units	Units	Units	Units	Units
5.0	27	46	47	43	31	48	51	43
5.6	65	95	84	61	60	97	88	62
6.0	51	82	67	37	50	—	—	—
6.2	31	—	—	—	37	75	56	27

chloride appears to be the most nearly favourable concentration of the three tried for effecting complete activation.

In subsequent studies, when sufficient material was available, an extensive survey was made of the effects of magnesium chloride upon the rate of cleavage of both phosphoric acid esters. In others, where experiments could be carried out only under selected conditions, the results are reported by average. The increases produced in the actions of normal and cancer erythrocyte α - and β -glycerophosphatase are presented in Table III.

The qualitative effect of magnesium chloride upon the hydrolysis of the two substrates is much the same. That is, the increase with low concentrations of magnesium chloride tends to become greater as the p_H approaches that of neutrality, actually resulting in a displacement of the p_H optimum. The best examples for comparison are found in the cases of α -glycerophosphate in the presence of 0.01 M magnesium chloride and of β -glycerophosphate in the presence of 0.02 M magnesium chloride. This relation is to be expected, since the amount of enzyme source added to the α -glycerophosphate solution is one-half that added to the β -glycerophosphate solution.

Cancerous erythrocytes appear to respond less readily, in general, to magnesium stimulation than do normal erythrocytes. In the former communication [Schoonover and Ely, 1935] it was stated that the increases in β -glycerophosphatase activity produced by magnesium in cancer red blood cells were usually equal to those found in normal red blood cells. However, the cases which are reported in full in Table III represent those which displayed β -glycerophosphatase activities within or only slightly above the normal range (see Table I). Therefore, the previous observation that cancer erythrocyte β -glycerophosphatase is capable of less proportionate, or percentage, activation by magnesium still retains its validity. This finding is borne out by the averages of scattered values, where the cancer average now includes cases with higher β -glycerophosphatase activities as well. I have not been able, unfortunately, to observe the tremendous activation observed formerly [Schoonover and Ely, 1935] when 0.1 M magnesium

Table III. *Unit increases produced in α - and β -glycerophosphatase activities of normal and cancerous human erythrocytes by the addition of various concentrations of magnesium chloride to the reaction mixture.*

No. from Table I	Type	p_H 5.0					p_H 5.6					p_H 6.0				
		α		β			α		β			α		β		
		M $MgCl_2$		M $MgCl_2$			M $MgCl_2$		M $MgCl_2$			M $MgCl_2$		M $MgCl_2$		
		0.01	0.02	0.02	0.05	0.10	0.01	0.02	0.02	0.05	0.10	0.01	0.02	0.02	0.05	0.10
1	Normal	3	8	6.4	9.3	8.2	28	24	17.1	18.4	14.6	31	23	21.5	19.1	16.6
2	"	3	1	6.9	8.1	6.8	20	24	14.0	17.1	11.1	35	29	19.0	19.2	13.1
4	"	-1	10	4.9	7.4	6.8	21	22	14.8	15.8	10.4	24	23	21.3	18.9	8.8
6	"	12	16	5.9	7.9	7.9	29	24	13.8	15.1	10.6	23	10	18.3	16.2	7.0
8	"	10	14	5.9	9.0	8.8	22	26	17.6	18.0	10.0	29	20	22.1	17.2	8.0
2	Cancer	12	7	4.9	7.8	5.6	17	14	13.6	14.7	5.7	15	9	17.1	13.3	3.8
4	"	13	8	6.0	7.6	7.7	17	3	14.0	14.8	9.0	17	6	18.9	15.4	6.7
6	"	-7	5	4.3	5.9	5.7	11	14	11.9	12.3	6.2	16	9	15.5	12.4	4.5
7	"	5	7	5.9	8.2	6.9	19	22	13.8	15.4	10.2	—	24	18.4	14.6	6.5
9	"	5	7	3.0	5.1	3.7	12	10	10.1	10.9	3.6	18	13	15.2	12.5	3.4
Normal average		5	10	6.0	8.3	7.7	24	24	15.5	16.9	11.3	28	21	20.4	18.1	10.7
Cancer average		6	7	4.8	6.9	5.9	15	13	12.7	13.6	6.9	17	12	17.0	13.6	5.0
Average for all subjects from Table I including above:																
Normal average		—	12	—	—	7.6	—	28	—	16.6	—	—	27	19.1	—	—
Cancer average		—	10	—	—	6.7	—	25	—	14.8	—	—	24	18.6	—	—

chloride was added to the β -glycerophosphate reaction mixtures of p_H 5.0 which contained erythrocytes from normal male subjects. This may be due to slight variations in the hydrogen ion concentrations of the earlier buffered substrates, for the p_H -activity curve rises sharply in the region of p_H 5.0. The results, consequently, show no sex difference and hence are no longer segregated according to sex.

Further examination of the relative effects of the most nearly optimum magnesium chloride concentrations, *i.e.* the lower ones, upon the hydrolysis of the two esters reveals a noteworthy result. The increase in α -glycerophosphatase activity compared with that in β -glycerophosphatase activity is somewhat greater in terms of mg. of inorganic phosphorus released. The increase is, however, not nearly as great as might be expected from the ratios of the rates of hydrolysis of the two glycerophosphates when no magnesium has been added to the reaction mixture.

Constancy of α - and β -glycerophosphatase activities in vivo and in vitro.

The β -glycerophosphatase activity of erythrocytes from the same normal subjects has been studied at different times. Table IV illustrates the constancy of this enzymic ability in the normal individual, both in its state of natural activation and in its response to magnesium stimulation. There appears to be no seasonal variation in β -glycerophosphatase activity. Several normal subjects studied during the summer yielded results within the limits imposed by data obtained from other normal subjects during the winter. Nos. 6 and 7 in Table IV furnish examples in which there are available for comparison figures obtained from the same subjects at other seasons.

Table IV. *Constancy of β -glycerophosphatase activity in the normal individual.*

No.	Date	p_H 5.0				p_H 5.6				p_H 6.0			
		Units with 0.10 M $MgCl_2$		Unit in-crease	% in-crease	Units with 0.05 M $MgCl_2$		Unit in-crease	% in-crease	Units with 0.02 M $MgCl_2$		Unit in-crease	% in-crease
		Units	$MgCl_2$	crease	crease	Units	$MgCl_2$	crease	crease	Units	$MgCl_2$	crease	crease
1	Jan. 11	3.1	12.7	9.6	301	6.4	24.9	18.5	289	—	—	—	—
	Feb. 21	2.3	11.9	9.6	417	5.9	25.7	19.8	334	—	—	—	—
2	Jan. 15	5.7	20.2	14.5	254	13.4	30.8	17.4	130	12.2	—	—	—
	May 16	5.7	12.6	6.9	121	11.2	27.2	16.0	143	11.2	—	—	—
3	Feb. 14	5.2	12.4	7.2	138	10.0	25.5	15.5	155	10.0	25.7	15.7	157
	Apr. 23	5.4	11.5	6.1	115	10.4	26.2	15.8	152	10.3	27.9	17.6	171
4	Feb. 20	4.7	—	—	—	9.3	—	—	—	—	—	—	—
	Oct. 8	4.3	—	—	—	8.9	—	—	—	—	—	—	—
5	Mar. 7	4.4	13.1	8.7	198	8.5	25.1	16.6	195	8.3	28.8	20.5	247
	Apr. 30	4.4	12.4	8.0	182	8.8	24.3	15.6	177	8.1	27.3	19.2	237
6	May 13	7.1	15.1	8.0	113	11.9	28.8	16.9	142	12.9	29.8	16.9	131
	July 15	6.0	12.9	6.9	115	11.3	29.7	18.4	163	10.6	28.9	18.3	173
7	June 26	6.9	14.7	7.8	113	13.1	31.5	18.4	140	11.3	34.5	23.2	205
	Oct. 31	6.4	15.2	8.8	138	11.9	29.9	18.0	151	9.8	31.9	22.1	226

The α -glycerophosphatase activity of both normal and cancer erythrocytes is not so constant. It decreased remarkably in summer, although magnesium chloride was still able to exert its usual influence and sometimes an even greater influence. This statement is derived from the averages contained in Table V, which may be compared with those in Tables I and III. The summer alteration in the α -glycerophosphatase activity forms a close parallel with the apparent alteration in α -glycerophosphatase when only one half the usual amount of

enzyme source is present in the reaction mixture. Thus, it may be attributed to the dilution of a natural activator. (Whilst identical figures, calculated as mg. of inorganic phosphorus liberated by 10 ml. of erythrocytes, were obtained by the use of either 0.10 or 0.05 ml. of erythrocytes, the use of 0.025 ml. resulted in the data to be seen in Table V.)

Table V. *α -Glycerophosphatase activities of normal and cancer erythrocytes in summer and in reaction mixtures containing one half the usual amount of enzyme source.*

	p_H 5.0				p_H 5.6				p_H 6.0			
	Units with 0.02 M $MgCl_2$		Unit in-crease	% in-crease	Units with 0.02 M $MgCl_2$		Unit in-crease	% in-crease	Units with 0.02 M $MgCl_2$		Unit in-crease	% in-crease
	Units				Units				Units			
Summer average:												
Normal (5)	19	39	20	146	33	79	46	237	19	65	46	352
Cancer (4)	33	48	15	47	40	75	35	91	13	60	47	409
Normal No. 6 from Table IV:												
May 13	45	60	15	33	84	113	29	35	70	99	29	41
July 15	33	56	23	70	69	97	28	41	21	59	38	181
Normal No. 7 from Table IV:												
Oct. 31	27	41	14	52	73	99	26	36	50	70	20	40
June 26	33	57	24	73	49	93	44	90	30	68	38	127
One half the usual amount of enzyme source (average):												
Normal (2)	11	31	20	210	17	55	38	225	10	51	41	491
Cancer (2)	25	45	20	82	44	79	35	93	24	63	39	182

Table VI. *Effect of preservation on the β - and α -glycerophosphatase activities of haemolysed and unhaemolysed erythrocytes from normal males.*

	Date	p_H	β -Glycerophosphatase M $MgCl_2$ added				α -Glycerophosphatase M $MgCl_2$ added		
			0	0.02	0.05	0.10	0	0.01	0.02
			Units	Units	Units	Units	Units	Units	Units
Haemolysed red blood cells from a normal male subject	Oct. 14	5.2	—	—	—	—	31	51	55
	" 24	—	—	—	—	—	32	28	33
	" 14	5.6	8.7	23.8	25.3	18.8	—	—	—
	" 24	—	8.5	18.3	22.5	18.0	—	—	—
	" 14	5.8	—	—	—	—	47	77	76
	" 24	—	—	—	—	—	37	48	48
Unhaemolysed red blood cells from a normal male subject	Oct. 17	5.0	4.2	8.9	12.0	11.8	16	21	25
	" 24	—	2.8	5.7	7.4	9.1	9	15	22
	" 17	5.2	6.3	13.7	16.8	16.6	—	—	—
	" 24	—	5.2	10.7	13.3	14.6	—	—	—
	" 17	5.4	7.3	18.8	21.1	19.2	—	53	52
	" 24	—	7.7	16.4	20.4	18.5	—	58	62
	" 17	5.6	—	—	—	—	—	62	—
	" 24	—	—	—	—	—	—	73	—
	" 17	5.8	8.2	27.5	25.6	24.5	—	—	—
	" 24	—	10.1	27.0	25.6	19.5	—	—	—
	" 17	6.0	—	—	—	—	33	63	54
	" 24	—	—	—	—	—	48	72	65
	" 17	6.2	8.0	28.9	27.0	13.1	—	—	—
	" 24	—	9.9	28.7	28.1	12.2	—	—	—

Table V contains a slight inaccuracy. At one time during the summer the hydrogen ion concentrations of the buffered substrates were not checked with sufficient frequency. Within this period, all the α -glycerophosphate reaction mixtures were slightly less acid than they were supposed to be. Therefore, the optimum p_H appeared to shift to the acid side of p_H 5.6. Subsequent experiments, however, still showed the decrease in α -glycerophosphatase activity, together with the practically unchanged response to magnesium chloride, which apparently accompany warm weather.

Fig. 2 A.

Fig. 2 B.

Fig. 2 A. p_H -activity curves for the β -glycerophosphatase activity of fresh and 19-day old erythrocytes. The four upper curves are from fresh erythrocytes; the four lower curves from 19-day old erythrocytes. — no $MgCl_2$ added to the reaction mixture; - - - - 0.02 M $MgCl_2$ added; — — — 0.05 M $MgCl_2$ added; - · - · 0.10 M $MgCl_2$ added.

Fig. 2 B. p_H -activity curves for the α -glycerophosphatase activity of fresh and 19-day old erythrocytes. The three upper curves are from fresh erythrocytes; the three lower curves from 19-day old erythrocytes. — no $MgCl_2$ added to the reaction mixture; - - - 0.01 M $MgCl_2$ added; — — — 0.02 M $MgCl_2$ added.

This behaviour is not due merely to phosphate inhibition, as might be supposed from the statement of Havard and Reay [1925] that blood phosphorus is increased during the summer months. No especially high inorganic phosphorus content was found in these red blood cell samples. Inhibition of neither

α - nor β -glycerophosphatase activity could in addition be observed when phosphoric acid-sodium hydroxide mixtures of suitable p_H were added to the substrates in amounts representing $1\frac{1}{2}$ -30 times the amount of inorganic phosphorus present in the erythrocyte haemolysates.

The enzyme activity with and without magnesium chloride addition persists for some time in both laked and unlaked red blood cells from normal male subjects, when the samples are kept under toluene in the ice-box. The β -glycerophosphatase system is, however, the more stable. The data are found in Table VI. The studies were made at scattered p_H values, but the substrates were from the same lot as that employed for the fresh samples.

After 19 days in the ice-box under toluene, the intact red cells of a normal male subject lost much of their glycerophosphatase activity. They also lost nearly all of their ability to be activated by magnesium chloride, inhibition being noted under most conditions. The data obtained before and after the 19-day interval may be found in Figs. 2 A and 2 B.

DISCUSSION.

In both normal and cancerous erythrocytes, the α - and β -glycerophosphatase activities show similarity in their p_H optima and in their capacity for magnesium activation over the p_H range 5.0-6.0. The hydrolysis of both glycerophosphates seems to involve two enzymic factors, one of which is activated by magnesium, while the other is not. The first enzymic factor appears to act upon both α - and β -glycerophosphates and is present, on an average, in equal amounts in both normal and cancerous erythrocytes. The existence of the second enzymic factor is made probable by the work of Jenner and Kay [1931], who found activity of erythrocyte phosphatase in the presence of minimum amounts of magnesium, whilst a form of alkaline phosphoesterase incapable of magnesium activation was reported by Bamann *et al.* [1934]. This possibility is supported by the observation, in this investigation, that only a part of the α - and β -glycerophosphatase activities is lost, whilst nearly all of the capacity for magnesium activation is lost, if unhaemolysed erythrocytes are allowed to stand for some time.

It is unlikely that this second enzymic factor is the acid organ phosphatase, unless it can be shown that the presence of erythrocytes, in the organ preparations they used, caused some of the phenomena observed by Davies [1934] and by Bamann and Riedel [1934]. Thus, at p_H 6.0 with 0.02 *M* $MgCl_2$, both the organ and the erythrocyte enzymes should be activated, and the sum of the increases should produce an apparent activation of the erythrocyte phosphatase greater than that observed in normal erythrocytes under these conditions. Such was not found. Furthermore, if the yeast enzyme which prefers the β -ester is analogous to the organ enzyme, one would expect to find that the progressive inhibition which Albers and Albers [1934, 2] found in this acid phosphoesterase with increasing magnesium chloride concentrations would be reflected in cancer erythrocytes. That is, the apparent activity should be decreased, because this inhibition should detract from the effect of magnesium on the true erythrocyte phosphatase. This decrease with high concentrations of magnesium chloride was found, but with the α - as well as the β -glycerophosphatase, and the organ enzyme is not supposed to be responsible for much of the attack on the α -isomeride. Another argument against the presence of the organ enzyme may be offered. A normal ratio of rate of hydrolysis of α -glycerophosphate to that of β -glycerophosphate, when a β -ester-preferring enzyme would not be expected

to be present in increased amount, is accompanied by a decreased capacity for magnesium activation in cancer erythrocytes.

If this enzymic factor is a natural activator or a phosphatase accompanied by a natural activator, there are no certain indications as to whether it is the same for α - as for β -glycerophosphatase. There is, however, a distinct possibility that it is not the same in view of the stability of the β -glycerophosphatase in the normal individual, whilst the α -glycerophosphatase shows not only considerable variation from individual to individual but also susceptibility to climatic conditions.

It is not possible on the basis of my experimental evidence to identify the source of the abnormal behaviour of cancer erythrocytes with regard to their glycerophosphatase, particularly with regard to their β -glycerophosphatase, activities. Nevertheless, it seems more likely that this source is to be found in abnormal conditions of activation, rather than in the admixture of the organ enzyme. Of interest should be the fact that whilst α -glycerophosphatase activity seems to be a rather unstable manifestation, the β -glycerophosphatase is constant and characteristic of the normal individual over a long period. Alterations in the β -glycerophosphatase activity, then, ought to be significant, especially since other substrates of physiological importance may also be attacked in an abnormal manner.

SUMMARY.

Normal and cancerous human erythrocytes hydrolyse α -glycerophosphate optimally at substrate p_H 5.6, usually at a rate which is about six times as great as that for the β -ester. The increase in α -glycerophosphatase activity with magnesium chloride is similar to, but not so great in proportion as, that in β -glycerophosphatase activity. The relative effect of magnesium chloride on both glycerophosphatases appears to be less in cancerous erythrocyte haemolysates than in normal erythrocyte haemolysates. Indications have been found that a part of the erythrocyte glycerophosphatase activity is independent of the presence of magnesium ions. The erythrocyte β -glycerophosphatase system seems to be much more stable than the α -glycerophosphatase system in the normal individual. It appears that it is the β -glycerophosphatase, and not the α -glycerophosphatase, system which has a tendency to become altered in cancer erythrocytes. A possible explanation for these phenomena is suggested.

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REFERENCES.

- Albers and Albers (1934, 1). *Hoppe-Seyl. Z.* **232**, 165.
- — (1934, 2). *Hoppe-Seyl. Z.* **235**, 47.
- Bamann and Riedel (1934). *Hoppe-Seyl. Z.* **229**, 125.
- — and Diederichs (1934). *Hoppe-Seyl. Z.* **230**, 175.
- Bodansky (1932). *J. biol. Chem.* **99**, 197.
- (1933). *J. biol. Chem.* **101**, 93.
- Davies (1934). *Biochem. J.* **28**, 529.
- Havard and Reay (1925). *Biochem. J.* **19**, 882.
- Jenner and Kay (1931). *J. biol. Chem.* **93**, 733.
- Kutscher (1935). *Hoppe-Seyl. Z.* **235**, 62.
- Pett and Wynne (1933). *Biochem. J.* **27**, 1660.
- Schäffner and Bauer (1935). *Hoppe-Seyl. Z.* **232**, 66.
- Schoonover and Ely (1935). *Biochem. J.* **29**, 1809.