The effect of Digitalis on the activity of glucose-6-phosphate dehydrogenase (a)

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Marks[1], Mc Kearns and Kaleita[2] described the inhibitory effect of certain steroids on the activity of glucose-6-phosphate dehydrogenase of animal origin. They also verified that even the most potent inhibitory steroids, namely dehydroisoandrosterone were without effect on the enzyme from yeast. In a previous paper we confirmed and extended this finding[3].

In this paper we have investigated the effect of other substances chemically related to steroids, and among these we studied digitalics drugs.

Unexpectedly, we found that these drugs enhance the activity of both yeast and erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD).

Material and methods

The sources of G-6-PD were the purified enzyme from yeast, supplied by Böhringer, and hemolyzates of human erythrocytes, prepared as described by Zynkham and coauthors[4]. No further purification of the erythrocyte enzyme was attempted.

The assay of G-6-PD was made by a spectrophotometric method adapted from Horecker[5]. The incubation mixture contained: 1.6 ml Tris, 0.2M, pH 7.5, 0.5 ml 0.14M Sodium Chloride, 0.2ml, 0.15M Magnesium Sulphate, 0,2ml 0.002M TPN, 0.1ml of enzyme source and 0.3ml ethyl alcohol or alcoholic solution of the digitalic drug. The reaction was started by the addition of glucose-6-phosphate (0.02M, 0,1ml).

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It was found necessary to add ethyl alcohol to the controls, since previous experiments showed a statistically significant inhibition of G-6-PD by ethyl alcohol at this concentration.

The digitalic drugs used were Digitaline "Nativelle" (digitoxin solution in alcohol 1/1000) and Cedilanid "Sandoz". Both were obtained commercially.

Results

1 - Effect of digitalis on yeast G-6-PD

a) Table NΩ.1 describes the first experiment. We prepared two systems in quadruplicate. The first group of assay systems contained 0.3ml absolute alcohol and the second group contained 0.3ml of alcoholic solution of digitoxin, 1/1000. Yeast G-6-PD diluted in water 1/1000 was used as source of enzyme. In each activity determination, we recorded five consecutive readings at 340 mμ and took the average change in optical density.

The first column presents four experiments with ethyl alcohol and the second column presents four experiments with digitalis.

The difference is statistically significant at the level $p < 0.01$.

TABLE NΩ. 1

assay systems	with 0.3ml alcohol	with 0.3ml digitalis
Average OD/min	8.6	17.2
	10.4	16.8
	11.8	16.0
	9.8	14.0
Average	10.2	16.0

b) In a second experiment, we prepared fifteen assay systems: five controls, five with 0.3ml "Digitaline Nativelle" and five with 0.3 "Cedilanid Sandoz".

The enzyme solution had been prepared 28 hours before and kept in the refrigerator.

The results present in table Nº.2 show that both drugs reactivate the enzyme.

Statistically both drugs differ from the control at the level of $p < 0.01$ and Cedilanid differs from Digitalis at the level of $p < 0.05$

TABLE Nº. 2

assay systems	with 0.3ml alcohol	with 0.3ml digitalis	with 0.3ml cedilanid
Average OD/min	6.0	18.0	17.0
	6.0	21.0	16.0
	8.0	17.0	16.0
	7.0	20.0	18.0
	7.0	18.0	15.0
Average	6.8	18.8	16.6

2 - Effect of digitalis on erythrocyte G-6-PD

It was impossible to add 0.3ml of digitalis to the assay mixture since occasionally a small amount of flocculation was detected, which interferes with the determination of activity. But with 0.2ml no flocculation was detected. In order to have 3.0 ml of final solution, 0.1ml tris was added.

In order to see whether precipitation was interfering with the determinations of optical density, we did two sorts of experiments. First, we followed the variation of optical density at 340 mμ of the assay system without TPN. No change was observed. Second, we followed the variation of the complete assay system at 400 mμ . Also no change in optical density was found.

Table Nº.3 shows an experiment, similar to the ones previously described in which hemolysed erythrocytes are the enzyme source. The activation by digitalis is statistically significant at the level $p < 0,01$.

TABLE NO. 3

tube	with 0.2 alcohol	with 0.2 digitalis
Average OD/min	16.2	19.0
	15.7	20.3
	15.2	19.6
	16.0	20.0
Average	15.8	19.7

Discussion

The results obtained were unexpected to us. In fact, we expected to find some degree of inhibition, or, more probably, no effect at all, but not an activation of G-6-PD by substances of the digitalis group of drugs. Since G-6-PD is a very labile enzyme[6] and can be reactivated following the addition of its own substrates, there is no reason to believe that the same activation could not occur in the presence of digitalis.

A second point that we would like to mention is related to the description of an unspecific activator of G-6-PD in red blood cells by different authors[7-9]. Although this activator has never been isolated, we could very well think that a substance chemically related to digitalis could exist naturally in the red blood cell and act as an activator, by protecting the enzyme from denaturation.

Summary

The authors demonstrate that both digitoxin an lanatoside C activate glucose-6-phosphate dehydrogenase when added to an incubation mixture prepared for determination of the activity of this enzyme.

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