CLII. FACTORS INFLUENCING THE FORMATION OF ROBISON ESTER

By HERMANN LEHMANN¹

From the Biochemical Laboratory, Cambridge

(Received 1 July 1939)

COMPOUNDS which influence Robison ester formation from glycogen and inorganic phosphate in muscle extract may act either on the first step: glycogen + inorganic phosphate $\rightleftharpoons$ glucose-1-phosphate (Cori ester)—or on the second step: Cori ester $\rightarrow$ hexose-6-phosphate (Robison ester), or on both these steps. Several factors which increase or inhibit Robison ester formation could be shown to act on one or the other of the two processes. Glucose inhibits the initial phosphorylation [Lehmann, 1938, 1; Cori *et al.* 1939]. Insulin influences the second step [Lehmann, 1938, 2, 3]. Hexosediphosphate activates the formation of Cori ester [Gill & Lehmann, 1939]. KCN and reduced glutathione increase Robison ester formation [Gill & Lehmann, 1939]. Cori *et al.* [1939] report that they could find no influence of KCN and reduced glutathione on Cori ester formation, which indicates that these factors act upon the second step, the conversion of Cori ester into Robison ester.

In this paper experiments are reported which were performed to elucidate the exact point of attack of various factors. The methods used are identical with those given in a previous communication in greater detail [Gill & Lehmann, 1939]. The experiments were performed at 37°. When adenylic acid and Mg were added this is specially mentioned. The first was used as the Na salt and the second as the chloride. Old extracts were used and usually no coenzyme was added. It was found that under these conditions an appreciable amount of Cori ester was formed, the accumulation or disappearance of which it was possible to study. All values are expressed as mg. per ml. reaction mixture.

Glutathione

More Robison ester is formed in the presence of SH glutathione than in its absence. In cases where the total hexosemonophosphate production remains

Table I. *The influence of glutathione on Cori ester and Robison ester formation*

Time min.	Addition	Cori ester formed mg.	Robison ester formed mg.
5 days old undialysed rabbit muscle extract. Extract : total volume = 1 : 2. Initial glycogen 6 mg. Initial P 1.3 mg.			
60	—	0	1.1
120	—	0.1	2.1
120	At 60 min. + M/20 GSSG	0.7	1.3
15 days old undialysed rabbit muscle extract. Extract : total volume = 1 : 4. Initial glycogen 10 mg. Initial P 1.1 mg.			
45	—	0.5	2.0
	M/20 GSH	0.2	2.2
	M/100 GSH	0.3	2.3
	M/40 GSSG	0.9	0.6
	M/200 GSSG	0.8	1.2

¹ Beit Memorial Fellow.

unaltered, the increase in Robison ester is due to a loss in Cori ester (Table I, Exp. 2). This supports the view that reduced glutathione acts only on the step Cori ester $\rightarrow$ Robison ester. Strongly oxidizing compounds like quinone and oxidized catechol effect a complete inhibition of phosphorylation. When the mildly oxidizing agent SS glutathione is used a difference can be observed in the two steps of Robison ester formation regarding their sensitivity towards oxidation. The initial phosphorylation takes place whereas the conversion of the 1-ester into the hexose-6-phosphate is inhibited (Table I).

Alloxan and caffeine

Alloxan is an oxidizing agent [Strecker, 1862; Wieland & Bergel, 1924]. Its effect on thiol groups of proteins [see Labes & Freisburger, 1930] has been employed for inactivation of enzymes by oxidizing their SH groups [Purr, 1935, 1, 2; Hopkins *et al.* 1938; Rapkine, 1939]. Alloxan inhibits Robison ester formation (Table III). Whilst SS glutathione even at very high concentrations only inhibits the step Cori ester $\rightarrow$ Robison ester, alloxan at concentrations above $M/100$ inhibits both Cori and Robison ester formation (Table III). Experiments were made to compare its action with that of caffeine, which at a concentration of $M/100$ was found to have an inhibitory influence on both Robison ester and Cori ester formation (Table II). At lower concentrations of the purine derivative only Robison ester formation is inhibited (see also Tables III and IV). Yet whereas SS glutathione allows accumulation of Cori ester to go on whilst Robison ester formation is inhibited, the lower concentrations of alloxan and caffeine are still able to prevent this increase (Tables I and III).

Table II. *Influence of caffeine on Cori ester and Robison ester formation*

10 days old undialysed rabbit muscle extract. Extract : total volume = 1 : 4. Initial glycogen 6 mg. Initial P 1.6 mg. Time of exp. 30 min.

Addition	—		+0.15 mg. Mg		+1.5 mg. adenylic acid	
	Cori ester formed	Robison ester formed	Cori ester formed	Robison ester formed	Cori ester formed	Robison ester formed
—	0.9	2.3	0.8	2.9	1.2	2.7
$M/100$ Caffeine	0.3	1.1	0.5	0.9	0.6	2.0

Table III. *Influence of alloxan and caffeine on Cori ester and Robison ester formation*

15 days old undialysed rabbit muscle extract. Extract : total volume = 1 : 4. Initial glycogen 10 mg. Initial P 1.1 mg. Time of exp. 45 min.

Additions	Cori ester formed	Robison ester formed
—	0.5	2.0
$M/20$ Alloxan	0.3	0.2
$M/100$ Alloxan	0.5	0.9
$M/500$ Alloxan	0.5	1.3
$M/100$ Caffeine	0.2	0.9
$M/500$ Caffeine	0.5	1.4

The action of alloxan also differs from that of SS glutathione in that it is not completely reversed by SH glutathione. In Table IV is shown the annihilation of the effect of SS glutathione by an equivalent amount of the reduced tripeptide; $M/25$ reduced glutathione only partially reverses the action of $M/125$ alloxan

and has no influence on the effect of *M*/150 caffeine (Table IV). The action of caffeine on protein in general has usually been described as denaturation. There are indications that it exercises a dehydrating influence [Handovsky, 1910; Pauli & Falek, 1912; see also Szelöczy, 1929 and Brühl, 1929]. The last-named author finds evidence for a complex formation between amino-acids and caffeine. Alloxan may act in a similar way besides oxidizing the thiol groups.

Table IV. *Different reversibility by SH glutathione of the action of SS glutathione, alloxan and caffeine*

16 days old undialysed rabbit muscle extract. Extract: total volume = 1 : 5. Initial glycogen 8 mg. Initial P 0.9 mg. Time of exp. 60 min.

Additions	Cori ester formed	Robison ester formed
—	0.6	1.9
<i>M</i> /25 GSH	0.3	2.4
<i>M</i> /50 GSSG	1.0	0.5
<i>M</i> /50 GSSG + <i>M</i> /25 GSH	0.6	2.0
<i>M</i> /125 Alloxan	0.6	0.8
<i>M</i> /125 Alloxan + <i>M</i> /25 GSH	0.7	1.4
<i>M</i> /150 Caffeine	0.7	0.7
<i>M</i> /150 Caffeine + <i>M</i> /25 GSH	0.7	0.7

Zinc and cobalt

Zn has an inhibiting, Co an increasing effect on Robison ester formation. Zn in small concentrations only inhibits the Robison ester formation and not the Cori ester formation. Co increases Robison ester formation but has no influence on the production of Cori ester (Table V).

Table V. *Influence of Zn and Co on Cori ester and Robison ester formation*

26 days old undialysed rabbit muscle extract. Extract: total volume = 1 : 3.4. Initial glycogen 5.9 mg. Initial P 1.4 mg. Time of exp. 80 min.

Additions	Cori ester formed	Robison ester formed
—	0.1	2.6
<i>M</i> /850 ZnSO ₄	1.0	1.6
<i>M</i> /850 Co(NO ₃) ₂	0.1	3.6

It has to be emphasized that the effect of Co cannot be exactly the same as that of the reducing compounds. It acts at a much lower molarity and actually removes free SH groups. This was demonstrated in a model experiment with thiolacetic acid [Gill & Lehmann, 1939], and can also be seen by incubating a washed muscle brei in a *M*/200–*M*/500 Co solution for a few minutes. The absence of free SH groups can be demonstrated by the negative nitroprusside test after washing. Zn does not have this effect on muscle protein and had no effect on thiolacetic acid.

Experiments on Cori ester

The increasing or decreasing influences of several compounds on Robison ester formation can be seen also by studying the conversion of Cori ester into Robison ester. I am grateful to Dr C. S. Hanes for a generous supply of the ester which he is able to prepare on a large scale by means of a new method which is not yet published.

Conversion of Cori ester into Robison ester

10 days old undialysed rabbit muscle extract + 1.9 mg. Cori ester. Extract : total volume = 1 : 4.
Time of exp. 8 min.

Additions	Robison ester formed	± % of Robison ester formed without addition of agents
—	0.3	—
M/20 GSSG	0.1	— 70 %
M/10 GSH	0.6	+ 100 %
M/5 KCN	1.2	+ 300 %
M/100 Caffeine	0.1	— 70 %
M/1500 Zn	0.1	— 70 %
M/1500 Co	0.8	+ 170 %
1 % Glucose	0.3	± 0

It may be noted that glucose as already reported [Gill & Lehmann, 1939] does not influence the step Cori ester → Robison ester.

SUMMARY

SH glutathione increases Robison ester formation but has no influence on Cori ester formation, SS glutathione inhibits Robison ester formation but has no influence on Cori ester formation.

Alloxan and caffeine both inhibit Robison ester formation, they also inhibit Cori ester formation at concentrations higher than about M/100.

The inhibition by SS glutathione can be completely reversed by SH glutathione. SH glutathione partially reverses the alloxan inhibition, it has no influence on the caffeine inhibition.

Cobalt acts on Robison ester formation like reduced glutathione, yet it is effective at much lower concentrations and it removes SH groups from washed muscle brei.

Zinc in small concentrations inhibits the formation of Robison ester but not the formation of Cori ester. Although it acts in this respect like oxidized glutathione, it is effective at much lower concentrations and it does not remove SH groups from washed muscle brei.

REFERENCES

- Brühl (1929). *Biochem. Z.* **212**, 291.
 Cori, Colowick & Cori (1939). *J. biol. Chem.* **127**, 771.
 Gill & Lehmann (1939). *Biochem. J.* **33**, 1151.
 Handovsky (1910). *Biochem. Z.* **25**, 511.
 Hopkins, Morgan & Lutwak-Mann (1938). *Biochem. J.* **32**, 1829.
 Labes & Freisburger (1930). *Arch. exp. Path. Pharmac.* **156**, 226.
 Lehmann (1938, 1). *Nature, Lond.*, **141**, 470.
 — (1938, 2). *Nature, Lond.*, **141**, 690.
 — (1938, 3). *Chem. Ind. Rev.* **57**, 976.
 Pauli & Falek (1912). *Biochem. Z.* **47**, 269.
 Purr (1935, 1). *Biochem. J.* **29**, 5.
 — (1935, 2). *Biochem. J.* **29**, 13.
 Rapkine (1939). Private communication.
 Strecker (1862). *Ann. Chem.* **123**, 363.
 Szelöczy (1929). *Biochem. Z.* **206**, 290.
 Wieland & Bergel (1924). *Ann. Chem.* **439**, 196.