CII. THE RELATION OF INTERMEDIARY METABOLIC PRODUCTS TO ARGINASE ACTIVATION.

By ARNULF PURR and LEOPOLD WEIL.

Cancer Research Laboratories, University of Pennsylvania Graduate School of Medicine, Philadelphia, Pa.

(*Received February 16th, 1934.*)

The catheptic enzymes, which are concerned with proteolysis in animal tissue, undergo activation when treated with certain substances, such as HCN or H_2S. These characteristic activation phenomena became of increased physiological interest with the discovery of the occurrence in tissues of natural activators, as for example, zookinase [Waldschmidt-Leitz *et al.*, 1930], which has been shown by Waldschmidt-Leitz and Purr [1931] to be identical with reduced glutathione. The same activator has been described by Grassmann *et al.* [1931] for the plant proteases (papain, bromelin and yeast-proteinase). In later experiments it was established [Waldschmidt-Leitz, Scharikova and Schäffner, 1933] that arginase, another enzyme concerned with intermediary protein metabolism, is also activated directly by metal complexes of SH compounds, for example, by reduced glutathione and ferrous or cuprous ions.

The interpretation of these activations as a specific function of the sulphydryl group [Waldschmidt-Leitz, Weil and Purr, 1933] cannot be considered as valid, for we have found that other types of substances, as for example, ascorbic acid-$Fe^{=}$, methylglyoxal-$Fe^{=}$, and alloxan-$Fe^{=}$ exercise the same activating effect on arginase as is obtained with the sulphydryl-$Fe^{=}$ system (see Tables V, II and I). These newly demonstrated activators however possess in common the property of forming reversible redox systems. The hypothesis of a connection between arginase activation and the oxidation-reduction potential, for the establishment of which oxygen [Waldschmidt-Leitz, McDonald *et al.*, 1933] or hydrogen [Edlbacher *et al.*, 1933] is necessary, is in harmony with the experimental findings and accounts for the activations observed with the above-mentioned compounds.

Investigations reported by various writers during the past year have shown that in general these oxidations and reductions are regulated by reversible redox systems, which in the presence of available oxygen or hydrogen show variable potentials. Of significance to the investigation is the fact that the potential is determined not by the total amount of the system present but only by the ratio of the reduced to the oxidised phase. The total amount acts to a certain extent however as a buffer. With a constant ratio of reduced form to oxidised form, it is apparent that the redox potentials will vary also with the p_H. With increasing alkalinity they become more negative (more intense), and with increasing acidity, less negative (less intense). The particular physiological significance of such biological oxidations and reductions is dependent on the one hand on the assumption of a capacity to absorb such reduction potential (specificity) and on the other hand on the intensity of such potential. It will

thus be necessary, in enzyme activations, to distinguish between a specificity factor, and an intensity (activation) factor, which latter is known to be influenced by p_H and is responsible for the attainment of the full activity of enzymes.

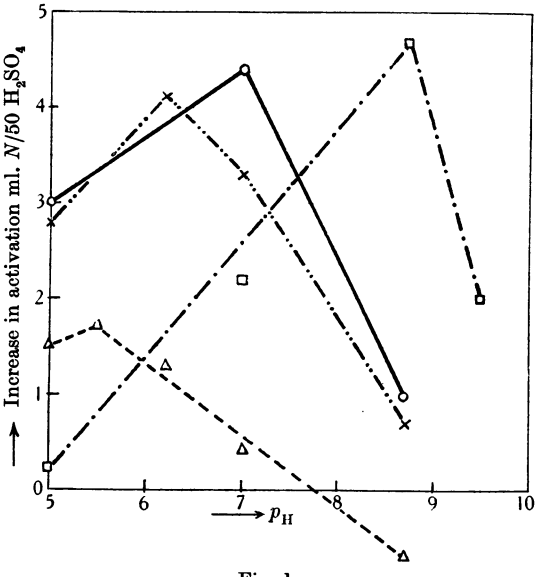

Fig. 1.

Cysteine-iron ($Fe^{=}$). — · — Cysteine.
- - - - Bivalent iron. —··— Ascorbic acid-iron ($Fe^{=}$).

From the curves (Fig. 1) which represent the experiments with $Fe^{=}$, cysteine, cysteine-$Fe^{=}$ and ascorbic acid-$Fe^{=}$, it is clear that the optimum development of the intensity factors of arginase, which is the development of the full activity, is dependent, as previously mentioned, on the p_H. Thus one obtains, by shifting the p_H to the alkaline side, the same activation intensity optimum as is obtained at neutral reaction by the addition of bivalent iron. While cysteine in acid medium is indifferent or even inhibiting, bivalent iron shows, to a less extent, the opposite picture. Bivalent iron therefore acts in a certain sense as a p_H buffer with respect to the development of the specific intensity potential which is required by the arginase, and it may also act in the same way in the organism. With ascorbic acid-$Fe^{=}$, the activation intensity optimum is shifted still more to the acid side than is the case with cysteine-$Fe^{=}$. In agreement with these results, Hill and Michaelis [1933] have found that the potential developed in the system alloxantin-$Fe^{=}$ is also dependent on the p_H, the bivalent iron apparently being a determining factor.

In a previous communication [Purr, 1933] an activation of arginase by ascorbic acid-$Fe^{=}$ was reported. In connection with this finding, the question was raised as to what extent other intermediary metabolic products exert a regulating effect on intracellular enzymic syntheses or hydrolyses. The experiments here reported, which have been carried through from this point of view, have shown that the activation mechanism of this enzyme is probably connected with a specific oxidation-reduction potential, and is not due simply to a specific effect of the SH group.

Experimental.

The activation of arginase.

The organs studied were rat liver and transplantable rat sarcoma, Philadelphia # 1[1]. Immediately after extirpation these tissues were cut up with scissors and frozen by dipping into liquid nitrogen, then pulverised by crushing with a hammer. This finely crushed material was suspended as follows: cancer (Philadelphia # 1 rat sarcoma) in 90 % glycerol (1: 10); rat liver in 90 % glycerol (1: 10); glycerol extract from liver acetone-ether dry preparation

Table I. *The effect of alloxan, allantoin, alloxantin and uric acid.*

The enzyme was activated by 0·5 ml. 0·1 *N* $FeSO_4$ or 20·0 mg. alloxan, 20·0 mg. allantoin, 20·0 mg. alloxantin, 20·0 mg. uric acid, respectively. p_H 7.

	Enzyme		
Activator system	Liver acetone-ether dry preparation (ml. 0·02 *N* H_2SO_4)	Rat liver suspension (ml. 0·02 *N* H_2SO_4)	Cancer suspension (Philadelphia # 1 rat sarcoma) (ml. 0·02 *N* H_2SO_4)
Initial activity	6·8	7·0	2·3
$Fe^{=}$	14·6	6·4	3·3
Cysteine	7·1	8·1	5·8
Cysteine-$Fe^{=}$	20·4	15·2	7·9
Alloxan	8·4	—	3·2
Alloxan-$Fe^{=}$	19·3	—	8·0
Allantoin	8·6	—	—
Allantoin-$Fe^{=}$	15·4	—	—
Alloxantin	—	7·9	—
Alloxantin-$Fe^{=}$	—	9·6	—
Uric acid	6·2	—	—
Uric acid-$Fe^{=}$	11·6	—	—

Table II. *The effect of methylglyoxal, pyruvic acid and* d-*lactic acid.*

The enzyme was activated by 0·5 ml. 0·1 *N* $FeSO_4$ or 16·0 mg. methylglyoxal, 20·0 mg. pyruvic acid, 20·0 mg. *d*-lactic acid, respectively. p_H 7.

	Enzyme		
		Cancer suspension (Philadelphia # 1 rat sarcoma) (ml. 0·02 *N* H_2SO_4)	
Activator system	Rat liver suspension (ml. 0·02 *N* H_2SO_4)	No. 1	No. 2
Initial activity	7·0	7·7	3·4
$Fe^{=}$	6·4	7·6	4·7
Cysteine	8·1	10·8	4·3
Cysteine-$Fe^{=}$	15·2	10·8	8·2
Methylglyoxal	—	2·0	4·0
Methylglyoxal-$Fe^{=}$	—	18·6	7·7
Pyruvic acid	7·1	—	—
Pyruvic acid-$Fe^{=}$	6·3	—	—
d-Lactic acid	6·2	—	—
d-Lactic acid-$Fe^{=}$	6·7	—	—

[1] The pathological description of this transplantable rat sarcoma, Philadelphia # 1, can be found in the article "Ueber die Enzyme in Tumoren," by Waldschmidt-Leitz, McDonald *et al.* [1933].

(1 : 10). The amounts used were: 5·0 ml. cancer suspension; 0·25 ml. liver suspension; 0·5 ml. extract of acetone-ether dry preparation. For activation 2·0 ml. neutral cysteine-HCl solution (containing 20 mg. cysteine-HCl) or 0·5 ml. 0·1 *N* $FeSO_4$, respectively, were added to the enzyme and allowed to stand 1 hour at p_H 7 at 30°. 10 ml. of 1 % arginine carbonate and 5·0 ml. 0·1 *M* glycine buffer of p_H 9·5 were then added and the reaction mixtures allowed to stand 60 minutes at 30°.

Results are shown in ml. 0·02 *N* H_2SO_4, as is customary.

Table III. *The effect of thiolacetic acid.*

The enzyme was activated by 0·5 ml. 0·1 *N* $FeSO_4$ or 20·0 mg. thiolacetic acid, respectively. p_H 7.

Activator system	Enzyme. Cancer suspension (Philadelphia # 1 rat sarcoma) (ml. 0·02 *N* H_2SO_4)
Initial activity	6·0
$Fe^{=}$	10·5
Cysteine	8·1
Cysteine-$Fe^{=}$	14·5
Thiolacetic acid	6·8
Thiolacetic acid-$Fe^{=}$	12·5

Table IV. *The effect of bivalent iron in complex combination.*

(Potassium ferrocyanide, $Fe^{=}$-dimethylglyoxime and αα′-dipyridyl[1].)

The enzyme was activated by 20·0 mg. potassium ferrocyanide; 20·0 mg. dimethylglyoxime; 5 mg. dipyridyl; (A) after 1 hour's standing with dipyridyl, $FeSO_4$ was added and the mixture allowed to stand another hour, p_H 7; (B) the same with cysteine instead of $FeSO_4$; (C) the same with cysteine-$Fe^{=}$; (D) after 1 hour's standing with dipyridyl, and another after addition of $FeSO_4$, cysteine-$Fe^{=}$ was added and the mixture allowed to stand an additional hour.

Activator system	Enzyme: Liver acetone-ether dry preparation (ml. 0·02 *N* H_2SO_4)	Enzyme: Rat liver suspension (ml. 0·02 *N* H_2SO_4)
Initial activity	6·8	7·0
$Fe^{=}$	14·6	6·4
Cysteine	7·1	8·1
Cysteine-$Fe^{=}$	20·4	15·2
Potassium ferrocyanide	3·6	—
Dimethylglyoxime	3·3	—
Dimethylglyoxime-$Fe^{=}$	8·3	—
αα′-Dipyridyl	—	6·2
(A)	—	1·0
(B)	—	5·0
(C)	—	14·4
(D)	—	3·0

From these figures it is evident that ferrous compounds in their rôle as activator for arginase are not interchangeable with bivalent iron in complex combination. It is also shown that ferrodipyridyl (A) inhibits irreversibly the action of arginase.

[1] The authors wish to thank Dr L. Michaelis, of the Rockefeller Foundation for Medical Research, for his kindness in supplying the αα′-dipyridyl.

Table V. *The effect of* p_H *(specificity of activation).*

The enzyme was activated by 0·5 ml. 0·1 *N* $FeSO_4$ or by 20 mg. cysteine-HCl or 16 mg. ascorbic acid ("chinoin") respectively, and allowed to stand 1 hour at p_H 5·0, 5·5, 6·2, 7·0, 8·7, 9·5 at 30° (the p_H was controlled). The activation of arginase was determined as usual at p_H 9·5. (Enzyme influence in ml. 0·02 *N* H_2SO_4.)

	Enzyme. Rat liver suspension					
p_H	5·0	5·5	6·2	7·0	8·7	9·5
Activator system						
Initial activity	—	—	—	4·7	—	—
$Fe^{=}$	6·2	6·4	6·0	5·1	4·0	—
Cysteine	4·5	—	—	6·9	9·4	6·7
Cysteine-$Fe^{=}$	7·7	—	—	9·1	5·7	—
Ascorbic acid-$Fe^{=}$	7·5	—	8·8	8·0	5·4	—
Increase in activation						
$Fe^{=}$	1·5	1·7	1·3	0·4	−0·7	—
Cysteine	0·2	—	—	2·2	4·7	2·0
Cysteine-$Fe^{=}$	3·0	—	—	4·4	1·0	—
Ascorbic acid-$Fe^{=}$	2·8	—	4·1	3·3	0·7	—

SUMMARY.

It has been shown that various intermediate products of metabolism, in combination with ferrous iron, are able to activate arginase. The activation mechanism of this enzyme is probably connected with a specific oxidation-reduction potential and is not due simply to a specific effect of the SH group.

We wish to express our thanks to Doctor Ellice McDonald, Director, for his interest and support throughout this work.

REFERENCES.

Edlbacher, Kraus and Leuthardt (1933). *Z. physiol. Chem.* **217**, 89.
Grassmann, Schoenebeck and Eibeler (1931). *Z. physiol. Chem.* **194**, 124.
Hill and Michaelis (1933). *Science*, **78**, 485.
Purr (1933). *Biochem. J.* **27**, 1703.
Waldschmidt-Leitz, Schäffner, Bek and Blum (1930). *Z. physiol. Chem.* **188**, 29.
—— McDonald und Mitarbeiter (1933). *Z. physiol. Chem.* **219**, 120.
—— and Purr (1931). *Z. physiol. Chem.* **198**, 260.
—— Scharikova and Schäffner (1933). *Z. physiol. Chem.* **214**, 75.
—— Weil and Purr (1933). *Z. physiol. Chem.* **215**, 64.