THE INFLUENCE OF ADRENALINE AND ADRENOCHROME ON OXYGEN CONSUMPTION OF LIVER HOMOGENATES*

by

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AHLGREN[1] reported in 1925 that the reduction of methylene blue by muscle-pulp *in vacuo* was accelerated by adrenaline in concentrations from $1:10^7$ down to $1:10^{15}$ Numerous publications on this subject have since then appeared. AHLGREN's results were confirmed by VON EULER[2] and LILJESTRAND among others, many investigators found, however, no influence of adrenaline *in vitro*, neither on reduction of methylene blue nor on oxygen-consumption of muscle-pulp or other tissue-preparations in the Warburg-apparatus (references in GRIFFITH[3]).

GRIFFITH[3] has surveyed the literature of the last 25 years on this subject. He mentions some 50 publications which reported no influence whatever of adrenaline in varying concentrations, an almost equal number of publications in which an inhibiting effect on methylene blue-reduction or oxygen-consumption is described and about twice as many publications in which stimulation by adrenaline was found from concentrations between $1:10^6$ and $1:10^{15}$.

No explanation has been given of these contradictory results. It seems reasonable to suppose that the differences can, at least partly, be ascribed to the fact that, when experimenting with tissue pulp, tissue-slices or other surviving tissue-preparations, one is never exactly informed about the kind of substrates which are oxidised, nor about the question which of the many chemical reactions that are implied in the methylene blue-reduction or the oxygen-consumption by the tissue-preparations is the rate-limiting reaction. It seems quite possible that adrenaline stimulates some of these reactions and inhibits others. Moreover, adrenaline is a labile substance, which can be transformed in tissues into other compounds possessing biological activity, for instance adrenochrome, and these transformations too might vary in tissue preparations in an uncontrollable way.

We have investigated the influence of adrenaline and of adrenochrome in tissue-systems in which the nature of the substrates oxidised and the rate-limiting reactions of oxygen-consumption could be varied in a controllable way.

For this purpose we used homogenates of rat-liver, which were diluted to concentrations in which they showed no spontaneous oxygen-consumption, and investigated the influence of adrenaline and adrenochrome on the oxygen-consumption obtained by adding various substrates and adding or omitting cytochrome *c* and various co-factors.

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TECHNICAL DETAILS

Cytochrome *c* was prepared from ox-heart. We used the KEILIN AND HARTREES method as modified by POTTER[4]. We obtained a solution of cytochrome which contained 1.3 mg of dry residue per ml. One ml of this solution diluted with 2 ml phosphate buffer-solution, pH 7.4, gave at 550 mμ an extinction of 0.595. Using the extinction constants of cytochrome *c* given by DRABKIN[5], the calculated amount of cytochrome in one ml of our solution is 1.06 mg, that means that the purity of our preparation was about 80%.

Diphosphopyridine nucleotide (DPN) was prepared from bakers yeast. We obtained one gram of DPN from 6 kilogram of yeast. We used the method described by LE PAGE[6] in *Manometric technics and tissue metabolism*, 1951.

Ag_2O was prepared by adding a 10% NaOH solution to a diluted solution of $AgNO_3$, washing the precipitate thoroughly with distilled water then with acetone and drying.

For the *preparation of adrenochrome* we used a modification of VEERS[7] method. 1 mg of adrenaline is dissolved in 10 ml bidistilled water. The solution is shaken with 25–50 mg of Ag_2O for 0.5 to 1 minute. After the red color of adrenochrome has developed, the Ag_2O is centrifuged off. Even at room temperature the red color fades only very slowly. In the Beckmann spectrophotometer we found absorption maxima at 300 mμ and at 485 mμ, minima at 260 mμ and 360 mμ. This spectrum agrees with that of CHAIX, only our maximum at 485 mμ was broader than that described by CHAIX[8].

Liver-homogenates were prepared by homogenizing fresh rat-liver in ice-cold Ringer-phosphate solution in a Waring-blendor. We used Ca-free Krebs-Ringer-phosphate solution.

Oxygen-consumption was measured with the Warburg-apparatus at 38° C. In all the experiments sets of three manometers were used for each determination. The figures in the tables give the average of three values. Half of the maximal deviation from the average in the three readings is designated by ±.

EXPERIMENTAL RESULTS

Influence of adrenaline and adrenochrome on the oxygen-consumption of concentrated homogenates to which no substrate has been added

For these experiments we used 10% and 20% homogenates, which showed an oxygen consumption of about 60 and 150 μl, respectively, in 60 minutes by 3 ml of homogenate. No influence of adrenaline and adrenochrome on oxygen-consumption could be detected.

Influence of adrenaline and adrenochrome on the oxygen-consumption of diluted homogenates + succinate with and without addition of cytochrome c

According to SCHNEIDER AND POTTER[9], in a system of this kind, without addition of cytochrome *c*, the oxido-reduction of cytochrome *c* is the rate-limiting reaction of oxygen-consumption. When cytochrome *c* is added in high concentration, the rate of dehydrogenation of succinate determines the rate of oxygen-consumption. We found no influence of adrenaline and adrenochrome on the oxygen-consumption of these systems.

Influence of adrenaline and adrenochrome on the oxygen-consumption of diluted homogenates + cytochrome c + ascorbate

When ascorbate is added to diluted homogenates in high concentration, the cytochrome *c* in the homogenate is kept continually in reduced state. Under these circumstances the oxido-reduction of cytochrome-oxidase is the rate-limiting reaction for oxygen-consumption.

In this system, as is shown in Table I, adrenochrome stimulated oxygen-consumption, adrenaline had no effect.

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TABLE I

INFLUENCE OF ADRENALINE AND OF ADRENOCHROME ON THE OXYGEN-CONSUMPTION OF DILUTED HOMOGENATES + CYTOCHROME *c* + ASCORBATE

Total volume 2.5 ml: 1.5 ml homogenate, 0.5 ml 0.1 *M* ascorbate, 0.5 ml H_2O or 0.5 ml $6 \cdot 10^{-4}$ *M* KCN, or 0.5 ml adrenaline (adrenochrome-solution), pH 7.4.

Time in minutes	*Conc. of homogenate* %	*O_2 consumption in µl*		
		H_2O	*adrenaline 20 γ/ml*	*adrenochrome 20 γ/ml*
50	2.5	40 ± 1		465 ± 5
50	2.5	35 ± 1		379 ± 4 (KCN added)
30	2.5	26 ± 1	28 ± 1	
30	5	44 ± 2	48 ± 2	

As adrenochrome also stimulated oxygen-consumption when KCN was added its effect could not be ascribed to acceleration of the oxido-reduction of cytochrome-oxidase.

We found that adrenochrome also catalysed the oxygen-consumption of Ringer phosphate solution to which ascorbate was added (Table II).

TABLE II

INFLUENCE OF ADRENOCHROME ON OXYGEN-CONSUMPTION OF HOMOGENATES + ASCORBATE AND OF RINGER-PHOSPHATE-SOLUTION + ASCORBATE

Total volume 2.5 ml: 0.5 ml 0.1 *M* ascorbate, 0.5 ml adrenochrome-solution or 0.5 ml H_2O, 1.5 ml buffer solution or homogenate.

Time in minutes	*Homogenate 2.5% 1.5 ml oxygen-consumption in µl*		*1.5 ml Ringer-phosphate solution oxygen-consumption in µl*	
	adrenochrome 20 γ/ml	*H_2O*	*adrenochrome 20 γ/ml*	*H_2O*
10	133	16	124	10
60	343	62	464	56

The catalytic effect of adrenochrome on the oxidation of ascorbate in Ringer solution could be detected in concentrations as low as 0.15 γ ml (Table III).

TABLE III

RELATION BETWEEN CONCENTRATION OF ADRENOCHROME AND EFFECT ON OXYGEN-CONSUMPTION OF ASCORBATE IN RINGER SOLUTION

Concentration of 1/60 *M* ascorbate, time 20 minutes.

Concentration of adrenochrome (W)	*O_2 consumption (µl)*	*Concentration of adrenochrome (W)*	*O_2 consumption (µl)*
$1.5 \cdot 10^{-6}$	183	10^{-8}	2 ± 5
10^{-6}	86	10^{-11}	5 ± 6
10^{-7}	20	10^{-14}	0 ± 6

Our results confirm those of FALK[10] who described the catalytic influence of adrenochrome on the oxidation of ascorbic acid by molecular oxygen in 1949.

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No effect of adrenochrome or adrenaline was observed on the oxygen-consumption of diluted homogenates to which ascorbate was added in *concentrations which are normally found in liver tissue* (Table IV).

TABLE IV

INFLUENCE OF ADRENALINE AND OF ADRENOCHROME ON OXYGEN-CONSUMPTION OF DILUTED HOMOGENATES TO WHICH ASCORBATE IS ADDED IN CONCENTRATIONS NORMALLY OCCURRING IN TISSUES

Total volume 3 ml: 0.5 ml 0.5 *M* succinate, 0.5 ml 10^{-3} *M* ascorbate, 0.5 ml H_2O or adrenaline or adrenochrome solution, 1.5 ml homogenate.

Time in minutes	*Homogenate %*	*O_2 consumption in µl*		
		H_2O	*adrenaline 15 γ/ml*	*adrenochrome 15 γ/ml*
35	2.5	140 ± 3		144 ± 2
35	2.5	152 ± 2		149 ± 3
35	2.5	59 ± 2	62 ± 2	
35	5	112 ± 4	112 ± 3	

It may be supposed, that although an increased oxygen consumption could not be detected with these small concentrations of ascorbate, yet the ascorbate was catalytically oxidised in the homogenate system by adrenochrome. We argued that in a system in which ascorbic acid catalyses oxygen-consumption, the addition of adrenochrome might cause inhibition of oxidation by destruction of ascorbic acid.

As is shown in Table V, ascorbic acid does not catalyse the oxygen-consumption of homogenates and of diluted homogenates to which succinate has been added as substrate.

TABLE V

INFLUENCE OF ASCORBATE ON OXYGEN-CONSUMPTION OF HOMOGENATE AND OF HOMOGENATE + SUCCINATE

Time in minutes	*Preparation*	*Oxygen-consumption in µl*	
		no ascorbate	*0.5 ml 10^{-3} M ascorbate*
35	homogenate 10%	195 ± 2	193 ± 2
35	homogenate 2.5% + succinate	50 ± 1	47 ± 2

We therefore investigated whether ascorbic acid had a catalytic influence on oxygen-consumption of diluted liver homogenates to which other substrates than succinate were added. We found that the oxidation of lactate by our homogenates was stimulated by ascorbic acid and the oxygen-consumption of the system: homogenate + lactate + ascorbic acid was indeed inhibited by adrenochrome.

The oxygen-consumption of the system homogenate + lactate + ascorbate was small, it was increased by the addition of DPN. The inhibition of the oxygen-consumption of this system by adrenochrome is shown in Table VI. For these experiments Warburg-vessels with two side-arms were used. The lactate was always added to the homogenate in the flask.

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TABLE VI

INFLUENCE OF ADRENOCHROME ON THE SYSTEM: DILUTED HOMOGENATE + LACTATE + DPN-ASCORBATE

In flask: 1.4 ml 2.5% homogenate, 0.5 ml 1/4 *M* lactate
In side-arms: 0.5 ml 10^{-3} *M* ascorbate, 0.5 ml adrenochrome sol. DPN 0.1 ml (1 mg).

Side-arm I	*Side-arm II*	*Oxygen-consumption µl in 20 minutes*	
		Experiment I	*Experiment II*
Ascorbate	DPN + H_2O	79 ± 3	85 ± 1
Ascorbate	DPN + adrenochrome 20 γ/ml	55 ± 1	65 ± 1
H_2O	DPN + H_2O	40 ± 1	26 ± 1
DPN	Ascorbate + H_2O	45 ± 1	108 ± 1.5
DPN	Ascorbate + adrenochrome 20 γ/ml	35 ± 2	50 ± 1
DPN	H_2O	31 ± 1	38 ± 1
Adrenochrome	Ascorbate + DPN	25 ± 3	83 ± 1
H_2O	Ascorbate + DPN	55 ± 2	97 ± 0
H_2O	H_2O + DPN	30 ± 1	27 ± 1

The inhibition of oxygen consumption by adrenochrome is found irrespective of the way in which the components of the system are combined. The solutions in the side arms were introduced in the flasks after the equilibration of temperature in the thermostat. All the experiments show also the stimulation of oxygen-consumption by the addition of ascorbate.

To test our hypothesis that the inhibition of oxygen-consumption by adrenochrome in the above-mentioned experiments was caused by the catalytic action of adrenochrome on the ascorbate in the system, we omitted ascorbate. Table VII shows that in the system homogenate + lactate + DPN adrenochrome had a *stimulating effect* on oxygen-consumption.

TABLE VII

INFLUENCE OF ADRENALINE AND OF ADRENOCHROME ON OXYGEN-CONSUMPTION OF THE SYSTEM: DILUTED HOMOGENATE + LACTATE + DPN

In flask: 1.4 ml 2.5% homogenate, 0.5 ml 1/4 *M* lactate.
In side-arm I: 0.1 ml DPN-sol. (1 mg) + 4 mg nicotinamide.
In side-arm II: 0.5 ml solution of adrenaline or of adrenochrome, or 0.5 ml H_2O.
Oxygen-consumption in µl in 20 minutes

H_2O	*adrenaline 20 γ/ml*	*adrenochrome 20 γ/ml*	*adrenochrome 2 γ/ml*
24 ± 1	17 ± 2	46 ± 1	
31 ± 1	24 ± 1	48 ± 1	
30 ± 2	31 ± 2	50 ± 2	
36 ± 4			33 ± 1
31 ± 1		50 ± 1	36 ± 1
46 ± 2		70 ± 1	53 ± 1
28 ± 4	27 ± 2		

DISCUSSION

Our figures show that adrenochrome, in concentrations from 100 γ to 10 γ/ml, had no detectable effect on the spontaneous oxygen-consumption of liver homogenates in

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Ca-free Ringer-phosphate solution, nor on the oxygen-consumption of diluted homo genates to which succinate or succinate + ascorbate are added. Adrenochrome constantly *inhibits* oxygen-consumption of diluted homogenates to which *lactate* has been added as *substrate* and in which oxygen-consumption is stimulated by *ascorbic acid* + DPN while it *increases* oxygen-consumption in diluted homogenates to which lactate and DPN are added.

In the systems used the oxygen-consumption of adrenochrome itself can be neglected. We found that 50 γ adrenochrome in 3 ml Ringer solution consumed in the Warburg apparatus 5 μl of oxygen in 75 minutes. When adrenochrome was added to 3 ml of Ringer solution which contained 0.5 ml of 10^{-3} M ascorbic acid in the Warburg apparatus, the solution consumed 6.6 μl of oxygen, exactly the quantity calculated for oxidation ot ascorbic acid to dehydroascorbic acid. It is evident that the oxidation of ascorbic acid by adrenochrome plays no part in the oxygen-consumption of the systems used.

This does not, in itself, prove that the oxygen-consumption in the systems to which ascorbate is added, is not partly caused by oxidation of ascorbic acid to products other than dehydroascorbic acid.

Addition of $2 \cdot 10^{-4}$ M ascorbate to homogenate or to the homogenate succinate system does not enhance oxygen-consumption, evidently ascorbic acid is not oxidized in this system in appreciable quantity. On the other hand addition of ascorbic acid to the homogenate lactate or homogenate lactate DPN system increases oxygen-consumption. This may mean that ascorbic acid catalyses oxidation of lactic acid, it might also mean that the oxidation of ascorbic acid to products other than dehydroascorbic acid is catalysed by the system homogenate lactate. However, since homogenate and homogenate + succinate certainly do not show such a catalytic action on the breakdown of ascorbate, it seems more probable that the stimulation of oxygen-consumption of the system homogenate lactate DPN by the addition of ascorbic acid is really a stimulation of the oxidation of lactate by ascorbic acid. The influence of adrenochrome on the oxygen consumption of this system can then be explained as follows: ascorbic acid catalyses the oxidation of lactic acid by homogenate + DPN, adrenochrome oxidizes and inactivates ascorbic acid and thereby inhibits the oxygen-consumption of the homogenate DPN lactate ascorbic acid system.

Our figures also show that adrenochrome *increases* the oxygen-consumption of the system homogenate + lactate DPN.

A similar effect of adrenochrome on the oxidation of lactic acid was described by GREEN AND RICHTER[11]. These authors found that adrenaline catalysed the oxygen-consumption of a system consisting of lactic acid dehydrogenase (which they prepared from pigs heart) + lactic acid + DPN + KCN, and showed that this catalytic effect was in reality an effect of adrenochrome, adrenaline being transformed in this system into adrenochrome. This transformation of adrenaline into adrenochrome evidently does not occur in our homogenate systems, adrenaline had no effect or a slightly inhibiting effect in our experiments (Table VI).

We have, until now, not further analysed the mechanism of the inhibiting and accelerating effects of ascorbic acid and of adrenochrome described in this publication. As adrenaline can be transformed into adrenochrome in living tissues, *our experiments point out a possible cause of the different effects of adrenaline on the oxygen-consumption of tissue preparations, which have been described by different investigators.* In our investi-

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gation we found three sorts of systems in which adrenochrome had respectively *no effect*, an *accelerating effect*, or an *inhibiting effect*, depending upon the use of different normal tissue metabolites as substrates or as catalysing agents for oxygen-consumption.

SUMMARY

1. Adrenochrome catalyses oxidation of ascorbate in Ringer-Krebs solution by molecular oxygen in concentrations between 1 γ and 0.1 γ per ml.

2. Adrenaline and adrenochrome in concentrations from 100 γ to 10 γ pro ml had no influence on the oxygen-consumption of a system consisting of diluted homogenate + succinate + ascorbate in a concentration of 150 γ per ml.

3. Adrenochrome in concentrations from 100 γ to 10 γ per ml constantly *inhibited* the oxygen-consumption of a system consisting of diluted homogenate of rat liver + DPN + ascorbate + lactate.

4. Adrenochrome in concentrations from 100 γ to 10 γ per ml constantly *stimulated* the oxygen-consumption of a system consisting of diluted homogenate + DPN + lactate.

RÉSUMÉ

1. L'adrénochrome à des concentrations comprises entre 1 μg et 0.1 μg par ml catalyse l'oxydation par l'oxygène moléculaire de l'ascorbate dans le liquide de Ringer-Krebs.

2. L'adrénaline et l'adrénochrome à des concentrations allant de 100 μg à 10 μg par ml n'ont pas d'influence sur la consommation d'oxygène d'un système formé d'homogénat dilué, de succinate et d'ascorbate à la concentration de 150 μg par ml.

3. L'adrénochrome, à des concentrations de 100 μg à 10 μg par ml, *inhibe* de façon constante la consommation d'oxygène d'un système formé d'homogénat dilué de foie de rat, de DPN, d'ascorbate et de lactate.

4. L'adrénochrome, à des concentrations de 100 μg à 10 μg par ml, *augmente* de façon constante la consommation d'oxygène d'un système formé d'un homogénat dilué, de DPN et de lactate.

ZUSAMMENFASSUNG

1. Die Oxydation von ascorbinsauren Salzen mit molekularem Sauerstoff in Ringer-Krebslösung wird von Adrenochrom in einer Konzentration zwischen 1 γ – 0.1 γ pro ml katalysiert.

2. Adrenalin und Adrenochrom in Konzentrationen zwischen 100 γ – 10 γ pro ml hatten keinen Einfluss auf den Verbrauch von Sauerstoff eines aus verdünntem Homogenat + Succinat + Ascorbat bei einer Konzentration von 150 γ pro ml bestehenden Systems.

3. Adrenochrom in Konzentrationen von 100 γ – 10 γ pro ml *hemmte* konstant den Sauerstoffverbrauch eines aus verdünntem Rattenleber-homogenat + DPN + Ascorbat + Laktat bestehenden Systems.

4. Adrenochrom in Konzentrationen von 100 γ – 10 γ pro ml *stimulierte* konstant den Sauerstoffverbrauch eines aus verdünntem Homogenat + DPN + Laktat bestehenden Systems.

REFERENCES

1 G. AHLGREN, *Skand. Arch. Physiol.*, 47 (1925) 1.
2 U. S. VON EULER, *Arch. exptl. Pathol. Pharmakol.*, 171 (1933) 186.
3 F. R. GRIFFITH, *Physiological Reviews*, 31 (1951) 151.
4 V. R. POTTER, In *Manometric technics and tissue metabolism*, by W. W. UMBREIT *et al.*, 1951, p. 211.
5 D. L. DRABKIN, *J. Biol. Chem.*, 171 (1947) 409.
6 G. A. LE PAGE, In *Manometric technics and tissue metabolism* by W. W. UMBREIT *et al.*, 1951, p. 215.
7 W. L. C. VEER, *Rec. trav. chim.*, 61 (1942) 643.
8 P. CHAIX, J. CHAUVET AND J. JEZEQUEL, *Biochim. Biophys. Acta*, 4 (1950) 471.
9 W. SCHNEIDER AND V. R. POTTER, *J. Biol. Chem.*, 149 (1943) 217; 177 (1949) 893.
10 J. E. FALK, *Biochem. J.*, 44 (1949) 129, 369.
11 D. E. GREEN AND D. RICHTER, *Biochem. J.*, 31 (1937) 596.

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