ESTERASE ACTIVITY AND CREATIN EXCRETION IN E-DEFICIENT RATS

by

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INTRODUCTION

BLOCH[1] pointed out that the symptoms of vitamin E-deficient animals correspond with those observed in triorthocresyl phosphate (T.O.C.P.) poisoning.

He found that, in agreement with the inhibition of cholinesterase (ChE) by T.O.C.P., the ChE activity in brain and serum of female rats kept on a vit. E-deficient diet for 5–12 months was decreased. This decrease was not found in animals kept for only 1 month on this diet.

HESS AND VIOLLIER[2] confirmed the decrease in ChE activity in plasma of female rats kept on an E-deficient diet during 5–6 months whereasin their experiments no marked decrease in brain ChE was observed.

HOTTINGER AND BLOCH[3] showed that in rabbits, serum ChE on the one hand, brain and liver ChE on the other hand were inhibited *in vitro* by different concentrations of T.O.C.P. Liveresterase was also inhibited by T.O.C.P.

The inhibition of serum ChE and serum lipase in experiments *in vivo* paralleled the degree of toxic signs and the creatinuria.

BLOCH AND HOTTINGER[4] found that the increased creatin excretion in rabbits after T.O.C.P., could be neutralized by administration of *α-dl*-tocopheryl acetate provided that this is given early enough.

So vit. E-deficiency and T.O.C.P. poisoning have the following points in common.

1. Neuromuscular symptoms show an analogue picture.
2. Creatinuria. The creatinuria caused by T.O.C.P. can be prevented by vit. E.
3. Inhibition of ChE.

The question arose whether inhibition of ChE might be the primary cause by means of which many phenomena of T.O.C.P. poisoning and of E-deficiency can be explained.

First experiments were performed in order to find out whether creatinuria is a consequence of inhibition of ChE.

Excretion of creatine and creatinine was studied in rats injected with the well known ChE inhibitor physostigmine.

After this, creatine and creatinine excretion, ChE activity of brain and serum and liveresterase activity were determined in rats in the first stage of vit. E-deficiency when sterility but no neuromuscular symptoms were observed.

In our experiments true- and pseudo-ChE activities were determined separately.

The E-deficient animals were kindly provided by Dr F. WENSINCK.

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MATERIAL

Rats 5–6 weeks of age were given a vit. E-deficient diet of the following composition (ENGEL[5]).

Casein (technical)	900
Amylum oryzae	3450
Brewers yeast	300
Lard	195
Salt mixture (Steenbock 40)	220

Per kg food 20 g cod liver oil was added.

The control rats were given the same diet with so much wheat germ oil added that each rat received 1 mg of vit. E weekly. After 3 months on the E-deficient diet all animals were paired with a normal partner.

Only those animals which proved to be sterile were used for the experiments.

RESULTS

1. *Excretion of creatine and creatinine in normal rats injected with physostigmine*

Four rats, 200 grams of weight were put in metabolism cages and fed a varied diet. 24 hours urine was collected for determination of excreted creatine and creatinine, by the FOLIN method. After a control period physostigmine was injected subcutaneously in a dose which effected convulsions (varying from 0.150–0.250 mg). This dose was given 1–4 × daily during 3 to 5 consecutive days. With intervals of 1–5 days similar series of injections were administered.

Table I shows the average daily excretion in mg/24 hours covering the periods of physostigmine treatment and the intervals. No definite effect of physostigmine treatment on creatine or creatinine excretion is observed under the conditions of the experiment. The spread in the figures is very considerable.

TABLE I

AVERAGE DAILY EXCRETION (mg/24 h) OF CREATINE AND CREATININE IN RATS DURING TREATMENT WITH PHYSOSTIGMINE AND DURING PERIODS WITHOUT TREATMENT

The numbers in brackets indicate the duration of the periods of treatment resp. control periods.

Rats		No phys.	Phys.	No phys.	Phys.	No phys.	Phys.	No phys.
No. I	Creatine	1.7 (6d)	0.6 (4d)	0.1 (1d)	3.0 (5d)	1.2 (4d)		
	Creatinine	8.1	3.6	2.6	6.2	6.1		
No. II	Creatine	2.0 (6d)	0.6 (4d)	0.3 (1d)	4.3 (5d)	1.4 (4d)		
	Creatinine	5.5	4.3	4.0	9.4	11.1		
No. III	Creatine	3.6 (8d)	4.3 (3d)	2.7 (5d)	1.6 (4d)	5.4 (1d)	4.0 (5d)	1.4 (4d)
	Creatinine	6.0	5.4	7.7	5.2	5.0	7.8	10.5
No. IV	Creatine	1.6 (8d)	2.5 (4d)	1.5 (4d)	1.6 (5d)	1.6 (1d)	3.1 (5d)	1.3 (4d)
	Creatinine	4.0	4.8	3.9	2.7	3.7	5.0	5.6

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2. Experiments on E-deficient rats

a. *Excretion of creatine and creatinine*

Excretion of creatine and creatinine (mg/24h) was determined in a number of E-deficient rats and E-supplemented controls.

The E-deficient animals were fed an E-deficient diet during 6 months and were found to be sterile 3 months before the experiment.

The results are summarized in Table II.

TABLE II

EXCRETION OF CREATINE AND CREATINIME (mg/24h) IN E-DEFICIENT AND E-SUPPLEMENTED RATS

Figures occurring in horizontal columns were obtained from the same animal. Creatinine values are placed in brackets.

Non-deficient animals	♂♂ 1	0.1 (6.2)			Mean 0.8 (8.5)
	♂♂ 2	1.2 (11.2)	0.6 (10.5)		
	♂♂ 3	0.5 (5.4)	0.2 (13.9)		
	♂♂ 4	0.5 (8.8)	0.2 (9.0)	0.2 (4.5)	
	♀♀ 5	1.5 (8.8)			
	♀♀ 6	1.4 (8.8)	0.9 (3.3)	1.0 (3.2)	
	♀♀ 7	2.3 (6.4)			
Deficient animals	8	1.4 (6.9)	1.4 (8.6)		Mean 1.3 (6.8)
	♂♂ 9	0.5 (4.9)	1.2 (6.6)	0.3 (2.3)	
	♂♂ 10	0.6 (7.3)	1.6 (8.2)		
	♂♂ 11	4.8 (9.8)			
	♂♂ 12	0.4 (6.7)	0.8 (8.4)	1.2 (4.5)	
	♀♀ 13	0.2 (4.3)	1.0 (7.5)		
	♀♀ 14	2.3 (10.8)	3.6 (8.4)	1.3 (7.1)	
	♀♀ 15	1.1 (4.6)	0.6 (7.0)		
	♀♀ 16	0.7 (7.0)	1.4 (8.0)	0 (6.8)	

The mean creatine excretion of the non-deficient animals was 0.8 mg/24h that of creatinine 8.5 mg/24h (13 observations).

The excretion in the E-deficient animals was creatine 1.3 mg/24h and creatinine 6.8 mg/24h (21 determinations). The difference in creatine and creatinine excretion was not significant (t = 1.6 resp. 1.3).

b. *Activity of true ChE in brain tissue*

The brain of a rat was prepared as described by COHEN, KALSBEEK AND WARRINGA[6]. ChE activity was determined titrimetrically at 24° C with acetylcholine in a final concentration of 0.01 *M* as substrate.

Activity is expressed as μl 0.01 *N* NaOH/60 min/mg dry weight. E-deficiency was effected under the conditions described under 2a.

Results are found in Table III.

Mean activity in vit. E-deficient animals was 95.0, that in the non-deficient ones 94.7.

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TABLE III

ACTIVITY (μl 0.01 *N* NaOH/mg dry weight/h) OF TRUE ChE IN BRAINS OF E-DEFICIENT AND E-SUPPLEMENTED RATS

Non-deficient	♂♂	121	91	100	99		Mean
	♀♀	67	116	71			94.7
Deficient	♂♂	107	72	114	85	91	Mean
	♀♀	94	91	97	104	92	95.0

c. *Activity of true and pseudo ChE in serum*

The blood obtained after decapitation of 2 male or female rats, which had been treated as described under 2a and 2b, was pooled. After clotting the blood was centrifuged during 10 min and the serum collected. ChE activity was determined by the WARBURG method at 37° C.

The main compartment contained 1 ml substrate, 0.5 ml sodium bicarbonate 0.12 *M* and 0.5 ml 0.9% NaCl.

The side bulb contained 0.5 ml undiluted serum.

Acetylcholine, acetyl-β-methylcholine and butyrylcholine (a specific substrate for pseudo ChE according to COHEN, KALSBEEK AND WARRINGA[7]) were used as substrates in a final concentration of 0.008 *M*.

The gas mixture was 95% N_2 + 5% CO_2.

Activity was expressed as μl CO_2/ml serum/h.

The results are found in Table IV.

TABLE IV

ACTIVITY (μl CO_2/ml serum/h) OF TRUE AND PSEUDO ChE IN SERUM OF E-DEFICIENT AND E-SUPPLEMENTED RATS

Substrate		Ac. chol.	But. chol.	Amechol
Non-deficient	♂♂	389	240	130
		398	278	149
	♀♀	856	744	162
Deficient	♂♂	372	235	163
		414	259	173
	♀♀	680	702	202
		704	512	173

In accordance with the experiments of SAWYER AND EVERETT[8] pseudo ChE activity is greater in the females than in the males. No difference in activity of true or pseudo ChE is found between vit. E-deficient and non-deficient rats.

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d. *Liveresterase activity*

The animals used were fed an E-deficient diet during 6–9 months. After decapitation of a rat the liver was ground in a *Latapie*. About one gram was suspended in nine volumes of distilled water. This suspension was diluted 20 times with distilled water.

Of this suspension 1 ml was titrated at 24° C using ethyl butyrate in a final concentration of 0.015 *M* as substrate. The titration was performed in the way described in section 2b. Esterase activity is expressed in μl 0.01 *N* NaOH/min/ml diluted liver suspension.

The data are found in Table V.

TABLE V

LIVERESTERASE ACTIVITY (μl 0.01 *N* NaOH/min/ml liver suspension 1/200) IN E-DEFICIENT AND E-SUPPLEMENTED RATS

Figures in horizontal columns refer to experiments carried out on the same day.

Non-deficient (23 animals)		Deficient (18 animals)	
♂♂	♀♀	♂♂	♀♀
56	—	51	—
47	—	43	—
169	67	35	33
—	109	128	91
177	68	49	42
102	—	25	44
114	47	—	—
—	70	—	—
—	69	—	—
—	101	—	—
—	113	—	—
—	139	—	—
—	58	—	52
—	63	—	47
—	40	—	44
—	62	—	89
—	141	—	80
—	91	—	56
110	58	74	55
Mean ± S.E.: 90 ± 8*		Mean ± S.E.: 58 ± 6*	

* S.E., Standard Error, is $\sigma/\sqrt{n}$

$t = 3.2$ $p << 0.01$[9]

The mean activity in vit. E-deficient animals is 64% of that in non-deficient animals. This difference is statiscally highly significant ($t = 3.2$; $p = << 0.01$).

The difference between non-deficient males and females is not significant ($t = 1.5$; p between 0.1 and 0.2).

In another series of experiments no difference was found in dry weight of livers of E-deficient and normal rats.

DISCUSSION

In the introduction the supposition was put forward that an inhibition of ChE might be the primary effect of vit. E-deficiency. In the foregoing experiments the ChE

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activity in brain and serum was found unaltered in animals which on an E-deficient diet during six months had become sterile.

As BLOCH (l.c.) found that the decrease of ChE-activity occurred in a period of 6–12 months after the beginning of E-deficient feeding, it may be that our animals were still in a state of lower deficiency than were the animals used by BLOCH and HESS AND VIOLLIER.

All the same our experiments show that a state of vit. E-deficiency in rats may occur not accompanied by inhibition of ChE. They therefore seem to indicate that the inhibition of ChE can hardly be considered of primary importance in early E-deficiency.

Creatinuria could not be correlated with inhibition of ChE by physostigmine. A marked decrease in activity of liveresterase was found in our E-deficient animals. This indicates that a relation may exist between E-deficiency and liveresterase activity.

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SUMMARY

1. Physostigmine administered repeatedly in convulsive doses does not produce creatinuria in rats.

2. E-deficient rats excrete normal amounts of creatine and creatinine.

3. The activity of true ChE in brain and true- or pseudo ChE in serum of E-deficient rats does not differ from that of normal animals.

4. Liveresterase activity in E-deficient rats is 64% of the activity found in non-deficient animals.

RÉSUMÉ

1. Si l'on administre à des rats, de façon répétée, des doses de physostigmine donnant lieu à des convulsions l'on n'observe pas de créatinurie.

2. Les rats souffrant d'une déficience en vitamine-E excrètent des quantités normales de créatine et de créatinine.

3. L'activité cholinestérasique vraie du cerveau, ainsi que l'activité ChE vraie ou pseudo du sérum sont les mêmes chez les rats à déficience de vitamine E que chez les animaux normaux.

4. L'activité de l'estérase du foie chez les rats à déficience de vitamine E correspond à 64% seulement de l'activité trouvée chez les animaux normaux.

ZUSAMMENFASSUNG

1. Häufige Physostigminzufuhr in krampferregenden Dosen ruft bei der Ratte keine Creatinurie hervor.

2. Ratten mit Vitamin-E Mangel scheiden normale Mengen Creatin und Creatinin aus.

3. Die wahre Cholinesterase-Aktivität des Gehirns und die wahre und pseudo-ChE-Aktivität des Serums sind bei Ratten mit Vitamin-E Mangel dieselben, wie bei normalen Tieren.

4. Die Leberesterase-Aktivität beträgt bei Ratten mit E-Mangel 64% derjenigen von normalen Ratten.

REFERENCES

1 H. BLOCH, *Helv. Chim. Acta*, 25 (1942) 793.
2 W. HESS AND G. VIOLLIER, *Helv. Chim. Acta*, 31 (1948) 381.
3 A. HOTTINGER AND H. BLOCH, *Helv. Chim. Acta*, 26 (1943) 142.
4 H. BLOCH AND A. HOTTINGER, *Z. Vitaminforschung*, 13 (1943) 9.
5 CHR. ENGEL, *Thesis*, Utrecht (1941).
6 J. A. COHEN, F. KALSBEEK, AND M. G. P. J. WARRINGA, *Biochim. Biophys. Acta*, 2 (1948) 549.
7 J. A. COHEN, F. KALSBEEK, AND M. G. P. J. WARRINGA, *Acta Brev. Neerl.*, 17 (1949) 32.
8 C. H. SAWYER AND J. W. EVERETT, *Endocrinology*, 39 (1946) 307.
9 R. A. FISHER, *Statistical Methods for Research workers*, London (1938).

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