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CHANGES IN SERUM CHOLINESTERASE ACTIVITY DURING ADMINISTRATION OF SUBTOXIC DOSAGES OF ALKYL PHOSPHATES

By Pharmacist Major Laszlo Gyarmati
and Lieutenant Colonel MD Gabor David

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CHANGES IN SERUM CHOLINESTERASE ACTIVITY DURING ADMINISTRATION OF SUBTOXIC DOSAGES OF ALKYL PHOSPHATES

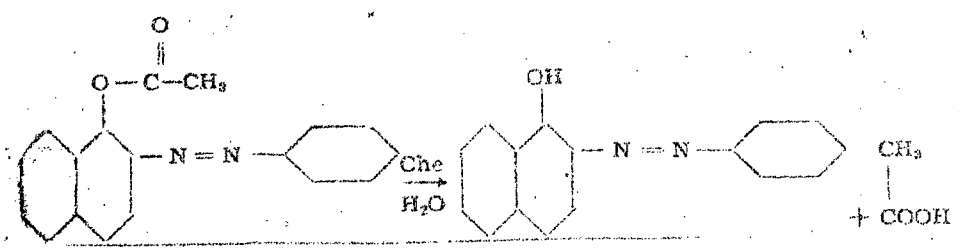
-Hungary-

[Following is the translation of an article by Pharmacist Major Laszlo Gyarmati and Lieutenant Colonel MD Gabor David in Honvedorvos (Army Doctor) Vol XIII, No 1, Budapest, 1961, pages 73-79.]

Insecticides of alkyl phosphate ester type paralyze the specific sible, unlike the effects of physostigmine and its analogs.

Research of the past 15 years have proved that the ChE in red blood corpuscles is different from ChE found in the serum. The former, which shows properties similar to the ChE of the nervous system, is called real or e-type ChE while the latter, non-specific or pseudocholinesterase is called s-type ChE. The physiological role of the s-ChE is not yet clear. It is paralyzed selectively by DFP-type materials and alkyl phosphate esters. The paralysis is 100 times stronger than that of the e-ChE. Hence it can be expected that diagnosing a decrease in s-ChE activity would be a sure sign of the presence of sub-toxic amounts and poisoning.

We worked out a new, simple test to measure ChE activity. It works on the principle that s-ChE changes the yellow 2-azobenzene-1-naphtylacetate to red 2-azobenzene-1-naphtol.



The red hue is measured colorimetrically. The strength of color indicates the activity of the enzyme.

Experimental part and discussion of the results

The activity of the ChE in rats' blood serum after parenteral or peroral DFP poisoning was measured in the following manner.

During superficial ether-anesthesia, blood was obtained from the tail vein; 0.02 ml of the serum of this blood was used in the experiments. Blood was taken just before and after the poisoning, at specified times. The DFP was in aqueous solution and was administered intraperitioneally or intramuscularly or via a stomach sonda, depending on the goal of the particular experiment. DL_{50} of the DFP used was 4.4 mg/1000 g.

In our experiments we found that the extinction of the red color disappears or becomes negligible after a lethal dose was given. E.g. a 300 g rat's extinction was 0.98 before injection, but after 1 mg DFP (i.p.) there was no color whatsoever. Another animal had strong tonus-conus convulsions 50 minutes after the injection, and the original 0.8 extinction fell to 0.17. When animals were given 1 mg DFP through a stomach sonda, the convulsions came after 30 minutes, and the orignal extiction of 1.45 fell to 0.11.

The experiments were followed by sub-toxic doses. Giving 50 and 100 gamma DFP 100 g body weight (this was ca. one eighth and one quarter of the DL_{50}) the rats did not show visible suffering, but their extinction decreased significantly. Control animals were used in all experiments.

Rabbits were given DFP parantericallly or through the skin. We dropped concentrated DFP on the inner earlobe of the rabbits (where the lobe is covered with downy hair only) which was washed off with 3% Na_2CO_3 after 5 minutes. Rabbits' blood was taken either from the ear vein or by heart puncture. (When ear blood was used, the clean, non-poisoned ear was punctured.)

Experimental results are shown in Tables 1-4.

It is important to find out whether the activity of the serum containing paralyzed ChE changes when left standing. Hence the activity was measured at determined intervals. Results are shown in Table 5.

The table shows that ChE activity does not increase, in fact decreases somewhat with time. Hence the measurement does not have to be done immediately after the blood was taken.

Our test can be easily carried out anywhere, even without an instrument, by just using a pipet and a few test tubes. This is due to the fact that with normal serum the red color of the naphtol can be well observed 3-4 minutes after the reagents were added; while paralyzed serum will give color only later, if at all.

We tested the serum of dogs that were made to inhale the vapor of DFP and its analogs. The dogs died. Ten minutes after the clinical death, we tested their blood (by heart puncture). No red color was observed in the reaction.

Likewise, living but poisoned dogs' blood gave no reaction. There were dogs that survived the poisoning, but the ChE activity of their serum decreased significantly, which could be observed with the naked eye.

Our findings confirm several authors who claim that even very low concentrations of DFP (10^{-9}-10^{-10} M = OL) in vitro paralyze the ChE. We found that even 0.005 gamma DFP (i.e., $3.3.10^{-11}$ M = OL) causes a serious decrease in ChE activity. This is one of the reasons why the amount of DFP in the body cannot be estimated from the decrease in ChE activity.

We did not aim to investigate the regeneration or restitution of the ChE. We went as far as 24 hours. At the end of this period, ChE activity in rabbits was equivocally low, maximum 20-25% of the original value. This agrees with the data given in the literature. No such equivocal data was registered with rats. Most rats' ChE activity reached 50-60% of the original value. Those animals that got only a low DFP dose, had a much higher ChE activity after 24 hours of the poisoning than originally. One of us noticed the same behavior in chronically poisoned rats that were given one fifth and one tenth of DL_{50} DFP.

Conclusion

The authors found a new method for measuring ChE activity. They found that activity decreases significantly after only a few minutes when sub-toxic amounts of DFP-type poisons are given. The paralyzed serum does not regenerate within 24 hours. The method is suitable for detecting poisoning quickly under any circumstances.

Table 1.

ChE Activity of Rats after i. p. DFP Poisoning

No.	Rat's weight	Dose gamma/100 g	ChE Activity Extinctions								
			0′	2′	5′	60′	90′	2 h	4 h	6 h	24 h
1	300	25	0,75	—	0,13	—	—	0,14	—	—	0,98
2	230	25	0,78	—	0,38	—	—	0,18	—	—	1,06
3	300	25	0,70	—	0,01	—	—	0,11	—	—	0,83
4	300	25	0,41	—	0,00	—	0,16	—	—	—	1,25
5	230	25	0,58	—	0,47	—	0,16	—	—	—	1,40
6	300	25	0,45	—	0,01	—	0,25	—	—	—	1,40
7	220	50	1,01	—	—	0,00	—	—	—	—	—
8	240	50	1,45	—	—	0,07	—	—	—	—	—
9	300	50	1,14	—	—	0,00	—	—	—	—	—
10	330	100	1,50	0,12	—	—	—	0,04	0,21	—	1,80
11	220	100	1,55	0,12	—	—	—	0,11	0,30	—	1,55
12	300	100	1,10	0,07	—	—	—	0,10	0,19	—	—
13	280	100	0,42	0,20	—	—	—	0,17	0,38	0,68	0,75
14	250	100	0,41	0,22	—	—	—	0,11	0,16	0,53	0,90
15	250	100	0,63	0,10	—	—	—	0,07	0,02	+	—
16	250	100	0,70	0,30	—	—	—	0,20	0,25	0,50	0,85
17	220	100	0,70	0,14	—	—	—	0,20	0,28	0,41	0,40
18	230	100	0,66	0,23	—	—	—	0,19	0,10	0,34	1,00
19	200	100	1,60	0,35	—	—	—	0,13	0,20	0,33	0,38
20	250	100	0,93	—	0,00	—	—	0,04	0,03	—	0,62
21	240	100	1,01	—	0,00	—	+				
22	200	100	1,01	—	0,00	—	—	0,06	0,00	—	0,65
23	240	100	0,93	—	0,04	—	—	0,04	—	—	0,74
24	250	100	0,60	—	5,03	—	—	0,07	0,16	—	0,28
25	190	100	0,95	—	0,11	—	—	0,09	0,15	—	0,52
26	200	100	0,80	—	0,07	—	—	0,05	0,08	—	0,45
27	240	100	1,45	—	0,21	—	—	0,13	0,15	0,31	0,85
28	250	100	0,96	—	5,10	—	—	0,07	0,11	+	
29	220	100	0,89	—	0,06	—	—	0,04	0,07	0,14	5,53
Control Animals											
1	300	—	0,43	—	0,39	—	0,40	—	—	—	0,45
2	300	—	1,00	1,05	—	—	1,10	—	—	—	1,00
3	300	—	0,45	0,45	—	—	—	0,54	0,30	—	0,39
4	300	—	0,74	0,65	—	—	—	0,85	0,75	0,70	0,65
5	190	—	0,86	—	0,85	—	—	1,20	1,13	—	1,18
6	190	—	1,18	—	1,05	—	—	1,20	1,20	—	1,19
7	225	—	1,40	—	1,25	—	—	1,23	1,27	—	1,26
8	180	—	1,24	—	—	—	—	1,19	1,17	—	1,17
9	140	—	1,19	—	—	—	—	1,10	1,12	—	1,11
10	190	—	0,88	—	—	—	—	1,00	1,03	—	5,88

Note: Animals having a cross (+) died durihg the narcosis, but otherwise proved symptom-free.

Table 2

Blood Serum Activity of Rats after Sonda DFP Poisoning

No.	ChE Activity Extinctions				
	0 min.	10 min.	2 hours	4 hours	24 hours
			100 γ dose		
1	0,83	0,15	0,00	0.10	0,14
2	1,12	0,35	0,00	0.23	0,16
2	1,25	0,20	0.10	0,25	0,71
2	1,18	0,22	0,09	0,30	0,70
			200 γ dose		
1	1.04	0,11	0,07	0,15	0,37
2	1,13	0,19	0,09	0,14	0,88
3	1,07	0,10	0,10	0,20	0,65
			1000 γ dose		
1	1,38	0.17	0,17	0,09	0,80
2	1,25	0,15	0,08	0.07	0,83
3	1,30	0,15	0,06	0,07	—
4	1,27	0,14	0,10	0,08	—
5	1,11	0,17	0,08	0,07	0,59
6	1,30	0,14	0,05	0,06	0,62
7	1,16	0.11	0,08	0,05	0,48
			Control Animals		
1	1,24	1,17	1,19	1,17	1,17
2	1,19	1,10	1,10	1,12	1,11
3	0,98	0,98	1,03	1,00	0,96

Note: Weight of rats was between 130 and 250 grams.

Table 3

ChE Activity of Rabbits after 500 gamma/kg i. p. DFP Poisoning

Nos.	ChE Activity Extinctions					
	0 min.	5 min.	2 hour	4 hours	6 hours	24 hours
1.	1,10	0,22	0,17	0,18	—	0,26
2.	1,13	0,10	0,12	0,12	—	0,11
3.	0,95	0,05	0,14	0,12	—	0,11
4.	0,66	0,12	0,12	0,22	—	0,33
5.	0,61	0,23	0,11	0,23	—	0,29
6.	0,68	0,00	0,08	0,12	0,14	0,18
Control Animals						
1	1,07	1,09	1,10	1,05	—	1,00
2.	0,51	0,52	0,52	0,52	—	0,53

Note: Rabbits 1 through 3 were earlobe vein punctured, all others were heart punctured.

Table 4

ChE Activity of Rabbits after Skin DFP Poisoning

No.	ChE Activity Extinctions			
	0 minute	30 minutes	4 hours	24 hours
1.	0,60	0,10	0,07	0,12
2.	0,75	0,03	0,00	0,06
3.	1,08	0,99	0,10	0,32
4.	0,96	0,10	0,06	0,28
5.	0,62	0,08	0,03	0,26
Control	0,98	1,00	0,97	1,02

Dose: Two drops of concentrated DFP