C 21 BIOCHEMICAL CHANGES IN BLOOD, MUSCLE AND LIVER OF PIGS AND LAMBS POISONED WITH SOME ORGANOPHOSPHORUS COMPOUNDS

V.Pesic, Ljiljana Radovic and A.Oreskovic

In Examining toxic effects caused by organophosphorus inhibitors our aim was to obtain a wider understanding of biochemical changes that appear in an organism intoxicated with these poisons.

If we start from the basic biochemical disturbance which occurs in poisoning with organophosphates, i.e. from inhibition cholinesterase and collection of acetylcholine in the central and peripheral nervous systems, then it can be assumed that disturbances may also appear in other metabolic systems. For example it is known that increased quantity of the acetylcholine leads to disturbances of mutual regulation of vegetative nervous centres in the hypothalamus and functioning of the anterior lobe of hypophysis, which hormones regulate secretion of other endocrinous glands including the cortex of the adrenal glands. It has been proved further that acetylcholine develops hyperadrenalinemia (FUKUYAMA,1963) and hypercalemia (HAZARD and DELGA, 1954), and by putting out of order breathing and circulation to cause also stressor reactions.

In our experiments we have examined changes of glucose quantity in the blood serum, glycogen in the liver and lactic acid in meat of pigs and lambs poisoned with lethal and sublethal doses of Tabun. There is scarce information in literature on this and similar problems. For example, GOLD, WELLER and FREEMAN (1957) had proved an increase of glucose in blood of dogs after poisoning with Sarin and Parathion. In our establishment VUCOVIC (1963) also determined a hyperglycemy in blood of rabbits and horses poisoned with Armin and Sarin.

According to the NEYMANS' (1950) finding the process of decomposition of glycogen develops very quickly so that in one minute 16 mg% are decomposed, and it happens with glycogen from liver which influences directly an increase of glucose concentration in blood.

Experimenting with DFP on an isolated muscle of a rat ZAHAROVA and REZENGART (1949) proved thad lactic synthesis was inhibited under influence of this poison. Contrary to them GOLD, WELLER and FREEMAN (1957) have found out stronger increase of lactic acid concentration in blood of dogs after poisoning with Parathion and Sarin.

Materials and Methods

1. Glucose was determined in the serum applying the enzymons ultramicro method given by KESTON (1956) and (TELLER,1956) modified according to SANZ.

2. Glycogen was determined gravimetrically by extraction with threechlorine acetic acid and then settled by help of ethanol.

3. Lactic acid was determined both in light and dark muscles and the method was based on oxidation of lactic acid in acetaldehyde which further was fixed to bisulphite and the surplus of the bisulphite was determined iodometrically.

Results

Glucose in the blood serum of pigs and lambs was determined after application of the poison in the form of a slow intravenous infusion. The poison had been dissolved in the saline. Glucose concentration was estimated after 30, 60 and 120 minutes counting from the start of the infusion. The obtained results are shown in the Table 1.

Table 1. Glucose concentration in blood serum (mg%) of lambs and pigs after intravenous infusion of Tabun

Species	Dose	Normal	30'	60'	120'
Lambs	65-90 g/kg	103 ± 11,4	325 ± 26,9	406 ± 32,5	477 ± 80,7
Pigs	80-100 g/kg	98 ± 6,5	139 ± 8,0	175 ±16,8	198 ± 27,9

Each value in the Table represents a mean value for 4 animals. Compared to the normal, glucose concentration in the lambs' serum, 120 minutes after beginning of the infusion, has increased more than four times, but for pigs this increase is about two times. The serum of the animals was also analysed after 24 hours after infusion and it was found that some animals the hyperglycemy was still persisting while with others glucose had withdrawn to the normal level or it even fell below normal. These experiments have proved that a stronger hyperglycemy does not occur before 30 minutes after the beginning of the infusion.

Glycogen and lactic acid were determined in the liver and muscle one to three hours after death of the animals. In the Table 2 are given the obtained results which represent mean values (statistically worked out) for 4 animals.

Table 2. Concentration of glycogen in liver and of lactic acid in muscle of nonpoisoned animals and those poisoned with 15 DL_{50} of Tabun applied intravenously

Species and dose	Glycogen in liver mg/g	Lactic acid in mg/gr	
		Light muscle	Dark muscle
Lambs not poisoned	8,5 ± 3,6	5,2 ± 1,0	4,4 ± 0,6
Lambs poisoned with 15 FL_{50}	5,0 ± 2,5	3,8 ± 0,9	3,6 ± 1,0
Pigs not poisoned	78,4 ± 8,7	6,0 ± 0,8	3,3 ± 0,4
Pigs poisoned with 15 DL_{50}	45,7 ± 11,0	4,4 ± 0,7	3,1 ± 0,3

In both species the contents of glycogen in the liver was reduced by 42% after poisoning with Tabun. If pigs and lambs are compared then big differences in the contents of glycogen are evident between the two groups of animals. Besides their difference as species, it has also been a result of different feeding, for lambs were lean, and it is also a reason for a big standard error that has been obtained when working out data for lambs.

It is also interesting that a fall of glycogen concentration in the liver depends on the length of life of the poisoned animal. Insofar as the animal lives longer from the moment of the application of the poison, to that degree the fall of glycogen concentration is greater.

The results for lactic acid show, that in the light muscle (m.longissimus dorsi) its concentration is higher than in the dark one (m.quadriceps femoris) and that with poisoned animals of both species a certain reduction has been noted.

Discussion

The hyperglycemy that appears with pigs and lambs poisoned with Tabun undoubtedly shows that also in this case of poisoning with organophosphorus compounds an increased glycogenolysis occurs. Namely, this shows that in poisoning with Tabun appear changes in the metabolism of those systems in wich adrenal gland has considerable share in their regulation. So, FUKUYAMA (1963) has found that the acute poisoning with organophosphates lead to stimulation of sympathetic nervous system, faster release adrenaline and noradrenalinefrom adrenal glands and increase his concentrations in blood. As the adrenaline is a specific agent of the phosphorylasis, hence the hyperglycemy appears.

It has to be pointed out here that only glycogenolysis in the liver influences the concentration of glucose in blood and leads to hyperglycemy reaction. In other tissues, however, glycogenolysis raises the concentration of glucose -6-phosphate wich is not passing into blood.

Simultaneously with the increase of glucose in blood an increase of lactic acid was also expected, both as a result of decomposition of glycogen and as stronger contractions that follow every poisoning with anticholinesterasis compounds.

In our experiments a reduction of lactic acid concentration in muscle had occured. ZAHAROVA and ROZENGART 1949) was found such reduction. They have established out that DFP caused an inhibition the synthesis of lactic acid in the isolated muscle of a rat.

The results of our examination show that in poisoning with organophosphates, besides basic changes that are caused by these compounds, inhibition of cholinesterase and collection endogenous

acetylcholine, that also other metabolic processes are put out of order which are closely connected with the nervous and hormonal regulation organism.

REFERENCES

1. Gold A.L., J.M. Weller, G.Freeman (1957)
   Am. J. Physiol. 188, 321-26
2. Zaharova A.V., R.I.Rozengart (1949)
   Biohimija, 14, 67-69
3. Vukovic D. (1963)
   Biblioteka VMA - Beograd
4. Fukuyama G., P.Adio (1963)
   Arch.int. Pharmacodyn. 146, 56
5. Neymans H.V.D. (1950)
   Arch.int. Pharmacodyn. 83, 386
6. Hazard R., J.Delga (1954)
   J. Physiol. 46, 585.