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NEW RESULTS OF OUR SUPEROXIDE DISMUTASE STUDIES AND FUTURE PLANS IN THIS FIELDS

Our current studies relating to changes in superoxide dismutase activity can be divided into four groups:

a) In joint work with the Department of Paediatrics of the University Medical School in Szeged, we have studied and compared the superoxide dismutase activities, other enzymes of the oxidative metabolism and the lipid peroxidation in some genetically well-defined diseases. In this group we have examined, and continue to examine, the activities of the antioxidant enzymes in children with cystic fibrosis or with Duchenne muscular dystrophy.

b) In other joint work with the Department of Paediatrics of the University Medical School in Debrecen and the Children's Hospital in Szeged, we examine the effects of D-penicillamine and of riboflavin in neonatal hyperbilirubinaemias. Here too studies have been made of the effects on the antioxidant enzymes, but more recently we have been investigating the activities of delta-aminolevulinic acid synthetase and haeme oxygenase.

c) We cooperate with the Department of Public Health and Epidemiology of the University Medical School in Szeged in clarifying the mechanism of molecular action of the well-known dipyridyl herbicide paraquat in various living organisms. An account of some these investigations will be presented in our other lecture.

d) We deal with the ever more important oxidative metabolism changes in human and experimental diabetes. We shall give a brief survey of our results in this field and of our future ideas.

Materials and methods

Venous blood was taken from cubital or umbilical vein and heparin was used as anticoagulant. The red blood cells (RBC) were separated by centrifugation and washed 2-3 times with isoto-

nic saline solution. Following this, the RBC were haemolysed in twice their volume of distilled water by freezing and thawing and were separated from intact RBC and debris by centrifugation. Haemoglobin interferes with enzyme activity measurements and was removed from the haemolysates by chloroform/ethanol treatment. Aliquots of the initially separated plasma and the final supernatants of the haemolysates were used for measurements.

The DMD patients were children of both sexes, aged 6-12 years and had been receiving vitamin E therapy for years. The normal control values are the means for a group of 10 children ranging in age from 4 to 12 years.

The adult control human blood samples were obtained from the Blood Donor Center of the University Medical School of Szeged, while the diabetic human blood samples originated from the Diabetic Station of the University Medical School of Szeged.

About 2000 registrated diabetics in a population of about 170 000 at Pécs, 48 individuals of various sexes and ages with diseases of different aetiologies, treated with insulin or others were selected in accordance with the aims of the examinations. In all diabetics the blood sugars were determined in the fasting state. One of the bases of the classification was the blood sugar level. The washed diabetic RBC were haemolysed, and the enzyme activities were determined from the aliquots of the haemolysates.

The blood glucose was always determined by the GOD-PERID (Böhringer, FRG) test. The examined patients were children into the following three groups (1) 3.9-6.0 (n = 10), (2) 6.1-11.0 (n = = 18), (3) above 11.0 mM/l (n = 20) blood glucose.

The cystic fibrosis (CF) children were aged 1-12 years and were of both sexes, and the obligate heterozygous parents were also of both sexes. Some of the CF children had been participating in continuous oral vitamin E treatment since the diagnosis of their disease.

In the animal experiments for diabetes rats of both sexes from the CFY strain were used. Comparisons were generally made between the data on individuals of the same sex, about the same weight and age. Diabetes was induced by i.v. administration of streptozotocin (70 mg/kg) or alloxan (50 mg/kg) in distilled wa-

ter solution. After the injections the rats were fed standard laboratory diet, with water and libitum. Of the rats treated with diabetogens, only those in which glucose could be detected in the urine were regarded as diabetics, and only these were used in the subsequent experiments. The rats were starved for 12 hours before they were decapitated and exsanguinated. Their tissues were homogenised at 0°C in a glass Potter homogenizer.

In general 1 g or less of wet tissue weight was homogenized in 1 : 10 ml ratio (or a proportionally chosen amount) of 0.005 M phosphate buffer (pH 7.2) and the supernatant from the centrifuged homogenisates were used for determination of enzymatic activities.

For toxicological examinations CFLP mice of both sexes, with weights of 21-36 g were used. The mice were kept on normal feed and received water as the rats.

The RBC superoxide dismutase (SOD, EC 1.15.1.1.) activity was estimated from the extent of the inhibition of the superoxide (O_2^-) - dependent epinephrine - adrenochrome transformation. 1 unit of SOD can be regarded as the amount of enzyme that causes a 50% inhibition in the extinction change (min. as compared to the control) M a t k o v i c s et al. [11].

Catalase (C-ase, EC 1.11.1.6.) activity was measured by the method of Beers and Sizer modified by M a t k o v i c s et al. [11]. The extent of H_2O_2 consumption, which depends on the amount of enzyme, is measured in a given time under fixed conditions.

Peroxidase (P-ase, EC 1.11.1.7) activity of the haemolysates were measured by the method of Maehly et al. described by M a t k o v i c s et al. [11] The method is based on the spectrophotometric determination of P-ase activity dependent on quaiacol - tetraquaiacol transformation.

The glutathione peroxidase activity (GP-ase, EC 1.11.1.9) was measured by the combined method of C h i u et al. [3] with cumene hydroperoxide substrate. The reduced glutathione residue was measured by the method of S e d l a k et al [21].

The lipid peroxidation (LP) was determined by the method of P l a c e r et al. [19].

The protein contents were measured spectrophotometrically by the method of L o w r y et al. [9].

The reagents used were of the purest quality, and were used without further purification.

The results were subjected to statistical evaluation with the Student t test. All numerical data are given as mean ±SD. In the enzyme activity and LP measurement the difference between duplicate determinations were never in excess of 5%.

Results and discussion

I like to make the following additions to section (a) in the first part of the Summary:

Our colleagues with whom we collaborate in the Department of Paediatrics at the University Medical School in Szeged are K. Gyurkovits, A. László and A. Megyeri. Our joint work began about 3 years ago, and is planned to continue in the future too. A few words will be said about this at the end of this section, after the account of the details.

1. We first compared the RBC antioxidant enzyme (AOE) activities and lipid peroxidation values in children with Duchenne muscular dystrophy (DMD) and in healthy volunteers of the same age. The literature background for our studies was as follows:

i) A number of examinations had been performed on muscle biopsy material in DMD cases, the AOE activities being compared with the values for normal muscle biopsy material. The same applied to the LP values. Such measurements were made in a chicken muscular dystrophy model, P e r k i n s et al. [18], and on human material, K a r et al. [6].

ii) The AOE and LP results relating to plasma and RBC haemolysates were fairly contradictory: in one case RBC membrane differences were demonstrated, while in another no differences were found concerning the members of the AOE system, B u r r i et. al. [1] S o m e r et al. [22].

Our results are ilustrated in the following Tab. 1.

It is clear that the LP values and the AOE activities of the RBC haemolysates in DMD are significantly higher than in healthy children of the same age, M a t k o v i c s et al. [13].

The other hereditary disease examined was cystic fibrosis (CF) (also known as mucoviscidosis), which was similarly inves-

Table 1

TBA-reactive products (Lipid Peroxides), catalase and superoxide dismutase activities in the red blood cells of DMD cases and healthy individuals

Produkty TBA-reaktywne (nadtlenki lipidów), aktywność katalazy i dysmutazy ponadtlenkowej w krwinkach czerwonych chorych z dystrofią mięśniową Duchenne'a i osobników zdrowych

Assay	Controls (n = 10)	DMD (n = 22)	p
SOD U/g prot. mean±SD	1 266.0±237.7	7 131.9 ±4 341.6	$p < 0.0005$
C-ase BU/l haemol.* mean ±SD	$8.04 \times 10^3 \pm 0.78 \times 10^3$	$22.2 \times 10^3 \pm 7.1 \times 10^3$	$p < 0.0005$
TBA-reactive plasma products (nmol MDA/l plasma) mean±SD	$15.46 \times 10^3 \pm 1.50 \times 10^3$	$15.45 \times 10^3 \pm 9.17 \times 10^3$	$p < 0.05$
TBA-reactive products from haemolysates (nmol MDA/l haemol.) mean±SD	$201.9 \times 10^3 \pm 18.9 \times 10^3$	$310.7 \times 10^3 \pm 115.1 \times 10^3$	$p < 0.005$

*BU, Bergmeyer units.

tigated in collaboration with our peediatric colleagues. Here, fewer literature data were available on the oxygen metabolism change, Feigal et al.[4], Campbell et al. [2].

The first of these authors demonstrated that in CF the Ca^{2+} uptake in the mitochondria is changed, and the O_2 requirement is higher. In the other reference it was shown that the O_2 supply to the tissues is impaired because of the altered fatty acid composition of membranes.

Our own results are outlined in the next Tab. 2. This gives the SOD, C-ase and LP results not only for the ill children, but also for their heterozygous parents. In general it may be said that the data for the CF children and their parents are comparable, and in both cases are close to the data for healthy adults.

Table 2

Superoxide dismutase and catalase activities and TBA-reactive products (lipid peroxides) in human blood (plasma and RBC) of healthy volunteers, CF cases and their parents (controls from different age groups)

Aktywność dysmutazy ponadtlenkowej, katalazy i produkty TBA-reaktywne (nadtlenki lipidów) we krwi ludzkiej, osoczu i krwinkach czerwonych zdrowych ochotników ludzi z mukowiscidozą i ich rodziców (kontrole z różnych grup wiekowych)

Assay	Values			Obligate heterozygote parents (from 25-30 years) (n = 13)
	Age group I (from 4-12 years) (n = 10)	Age group II (from 20-55 years) (n = 30)	CF homozygotes (n = 8)	
SOD U/l haemol. mean ± SD	$293.6 \times 10^3 \pm 51.3 \times 10^3$	$716.5 \times 10^3 \pm 92.5 \times 10^3$	596.67×10^3 61.97×10^3	$599.35 \times 10^3 \pm 60.80 \times 10^3$
SOD U/g prot. mean ± SD	1 266.0 ± 273.7	2 047.1 ± 412.2 (n = 52)	1 808.1* ± 186.8	1 8 16.2* ± 182.3
C-ase BU/l haemol. mean ± SD	$2.68 \times 10^3 \pm 0.78 \times 10^3$	$2.05 \times 10^3 \pm 0.68 \times 10^3$ (n = 20)	$5.2 \times 10^{3*} \pm 0.3 \times 10^3$	$5.5 \times 10^{3*} \pm 0.4 \times 10^3$
TBA-reactive plasma prot. (nmol MDA/l plasma)	$5.46 \times 10^3 \pm 1.5 \times 10^3$	$10.3 \times 10^3 \pm 3.9 \times 10^3$	$7.9 \times 10^3 \pm 1.5 \times 10^3$	$7.5 \times 10^3 \pm 2.0 \times 10^3$
TBA-reactive products from haemol. (nmol MDA/l haemol.) mean ± SD	$201.9 \times 10^3 \pm 18.9 \times 10^3$	$67.4 \times 10^3 \pm 15.9 \times 10^3$	$68.8 \times 10^{3*} \pm 11.2 \times 10^3$	68.3×10^3 12.8×10^3

The SOD and C-ase activities of CF children are significantly higher than those of healthy children, whereas the LP is lower, The increased activities of the two enzymes may be a consequence of the partial anoxia, while the LP decrease may be related to the increased viscosity of the blood in this disease.

We consider that the high AOE activities, together with the partial anoxia, may be correlated with the rapid aging of the CF children M a t k o v i c s et al. [14, 15].

In a few cases we have examined the RBC AOE system in Down's syndrome, but our results so far are in contradiction with the literature data. We have found lower AOE activities.

We are continuing with examination of the carbonic acid anhydrase activity in CF, and are acquiring new data on Down's disease.

Part (b) is supplemented as follows: From the Department of Paediatrics at the University Medical School in Debrecen, L. Lakatos and his colleagues reported in 1976 that large doses of D-penicillamine (D-PA) (Metalcaptase^{R}, Roche) induce a rapid fall of the high billirubin level in premature infants and neonates with hyperbilirubinaemia. The oxygen treatment of prematures (which among others serves to lower the hyperbilirubinaemia) may be followed by retrolental fibroplasia, which can result in blindness. This complication is prevented if D-PA is administrated in parallel with the oxygenation L a k a t o s et al. [7, 8].

The question arises of how D-PA acts. It was first believed that it plays a role as a metal-chelating agent in the metabolism. However, this has not yet been conclusively proved by any authors. Our studies to date permit the following remarks about the effect of D-PA:

i) D-PA lowers the LP, primarily as a membrane protector, in the liver of newborn rats. In adult rats it increases the activities of the AOEs in most organs M a t k o v i c s et al. [12].

ii) D-PA enhances the activity of haeme oxygenase O r o s z l á n et al. [16].

Here we plan to study the porphyrin metabolism enzymes, and primarily delta-aminolevulinic acid synthase in the near future.

In joint work with the Children's Hospital in Szeged, we have investigated the autocatalytic effect of riboflavin (vita-

min B_2, Beflavin^R, Roche) observed in hyperbilirubinaemia. It was reported by L. P a t a k i, et al. [17] that exchange transfusion can be avoided in hyperbilirubinaemic prematures and neonates if the "blue light" treatment is combined with the administration of riboflavin. These results are of importance, partly as concerns the curing of ill neonates, and partly for reasons of economy. Here too we have compared the AOE activities and the LP in children treated with exchange transfusion, with blue light, with exchange transfusion + blue light, and with blue light + riboflavin. These examinations are also continuing.

The work in point (c) is common research with K. Barabás. This has been going on for about 5 years, and relates to the effect of Gramoxon^R (which contains paraquat as active ingredient) on mammals, fish and amphibia.

T a b l e 3

Most important measured parameters of human diabetics and controls blood

Najważniejsze parametry oznaczane we krwi diabetyków i osób zdrowych

Parameters	Controls	Diabetics	p
Glucose mg/100 ml blood mean±SD	81.5±10.4	210.3±54.7	-
Protein mg/ml plasma mean±SD	89.4±4.6	78.3±15.1	-
Protein mg/ml haemolysates mean±SD	589.8±131.5	501.2±172.9	-
GP-ase U/ml haemolysates mean±SD	6.43±0.65	16.45±0.58	$p < 0.01$
LP nM MDA/ml haemol. mean ± SD	242.8±76.0	615.0±45.0	$p < 0.01$
C-ase BU/ml haemolysate mean±SD	2.29±0.78	2.15±0.28	-
SOD U/ml haemolysates mean±SD	755.4±61.6	23.0±3.3	$p < 0.002$

Table 4

Enzyme activities and lipid peroxidations of organ homogenates from diabetic and controls rats

Aktywność enzymów i peroksydacja lipidów w homogenatach tkankowych szczurów z cukrzycą i kontrolnych

Organs	SOD U/g w.t.w. mean ± S.D.		P-ase U/g w.t.w. mean ± S.D.		C-ase BU/g w.t.w. mean ± S.D.		LP nM MDA/g w.t.w. mean ± S.D.	
	Controls	Diabetics	Controls	Diabetics	Controls	Diabetics	Controls	Diabetics
Liver	4 000±600	2 500±300	0.0	200±19	4.8±0.4	15.9±1.2	33.2±1.6	144.3±12.2
Kidney	1 200±151	327±41	0.0	0.0	0.4±0.04	0.93±0.10	22.4±1.8	6.1±0.6
Spleen	560±50	272±36	963±91	2 810±205	2.4±0.2	0.32±0.04	19.5±1.2	8.4±0.9
Testes	960±63	308±31	407±41	100±9.4	0.4±0.0	0.9±0.02	-	-
Whole brain	240±24	185±22	120±10	160±12	0.04±0.004	0.04±0.01	44.0±2.9	87.3±6.5
Lung	210±20	241±26	872±86	1 070±100	0.3±0.02	0.21±0.02	-	-
Pancreas	310±31	128±10	136±13	280±30	0.19±0.01	0.12±0.01	-	-
Heart muscle	480±47	263±29	2 690±300	1 583±96	0.24±0.02	0.44±0.05	-	-
Skeletal muscle	300±27	223±30	105±10	220±24	0.11±0.01	0.16±0.01	-	-
Haemolysate*	696±68	525±50	11 666±1 000	1 172±131	2.67±0.31	4.14±0.39	-	-

*U/ml.

The fish AOE investigations were joint work, in part with J. Nemcsók et al. in the Department of Biochemistry of our University, and also with the team of Prof. Leyko in the Department of Biophysics at the University of Lodz in Poland. I do not wish to go into details here on this, I presume that similar topics will be discussed in other lectures, or that we shall we able to talk about our results separately.

To turn to section (d), our results to date are presented in Tab. 3 and Tab. 4. They deal with the results of our human blood examinations (Tab. 3). These relate in part to experimental dia-

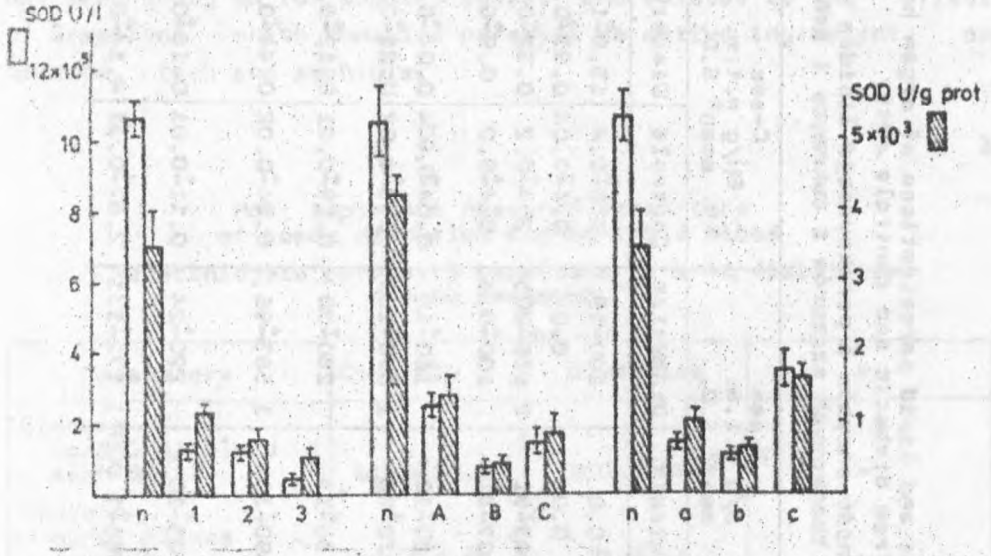

Fig. 1. SOD activities in U/I and U/g protein units (means ±SD). Actual blood glucose levels during treatment: 1) 3.9-6.0 (n = 10); 2) 6.1-11.0 (n = 18); 3) above 11.0 mmol/I (n = 20). Period since diagnosis: A)0-5 (n = 15); B) 5-10 (n = 17); C) above 10 years (n = 16). Therapy: a) insulin + diet (n = 22); B) oral antidiabetics, mainly of sulphanylurea type (n = 23); c) diet carbohydrate restriction (n = 3). Normal values relate to haemolysates of heparin-containing blood from the Blood Bank in Szeged (accuracy of enzyme determinations: ±5%)

Aktywność SOD w U/I oraz U/g białka (średnia ±SD). Poziom glukozy w czasie doświadczenia: 1) 3,9-6,0 (n = 10); 2) 6,1-11,0 (n = 18); 3) powyżej 11,0 mmol/l (n = 20). Czas stwierdzenia choroby A) 0-5 (n = 15); B) 5-10 (n = 17); C) powyżej 10 lat (n = 16). Terapia: a) insulina + dieta (n = 22); b) antybiotyki doustne,głównie sulfanylomocznikowe (n = 23); c) dieta bezcukrowa (n = 3). Wartości kontrolne otrzymano używając hemolizatów krwi pobieranej na heparynę w Stacji Krwiodawstwa w Szeged (dokładność oznaczeń enzymów: ±5%)

betes material (Tab. 4), these were rat experiments, where alloxan and streptozotocin were used as diabetogenic agents. From these it is quite clear that in both cases the AOE defence is lowered. If we take into account the newer results, which strikingly demonstrate the importance of oxygen radicals in experimental diabetes (alloxan ⟶ dialuric acid + O_2^-), H o u é e-L e v i n et al. [5], R o b i n s et al. [20], significance must be attributed to our human results connected with the decreased antioxidant defence. An example is provided here by our joint experiments with J. S t r e n g e r [23]. Figure 1 shows the decrease in one of the AOEs in parallel with the increase in the glucose concentration of the RBC haemolysates, also illustrated is the correlation between the time of diagnosis of the diabetes and the SOD activity of the haemolysate, together with the correlation between the diabetes treatment and the haemolysate SOD. Figure 2 presents the C-ase reactions as a function of the above conditions, and Fig. 3 the P-ase changes.

Figure 4 depicts the SOD decrease in human material as functions of the diagnosis and treatment time.

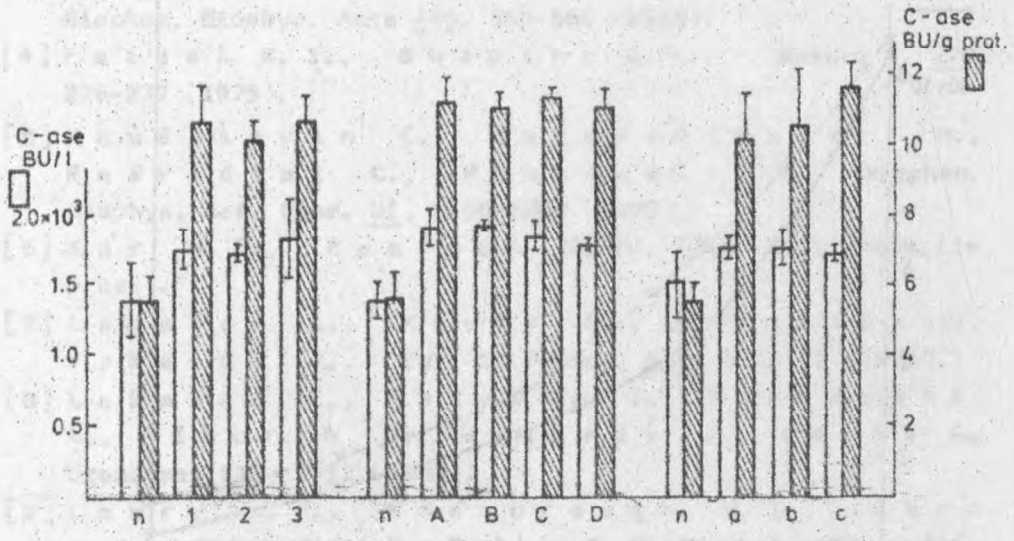

Fig. 2. C-ase activities in BU/l and BU/g protein units. For notes, see Fig. 1

Aktywność C-azy w BU/l oraz BU/g białka. Objaśnienia jak do rys. 1

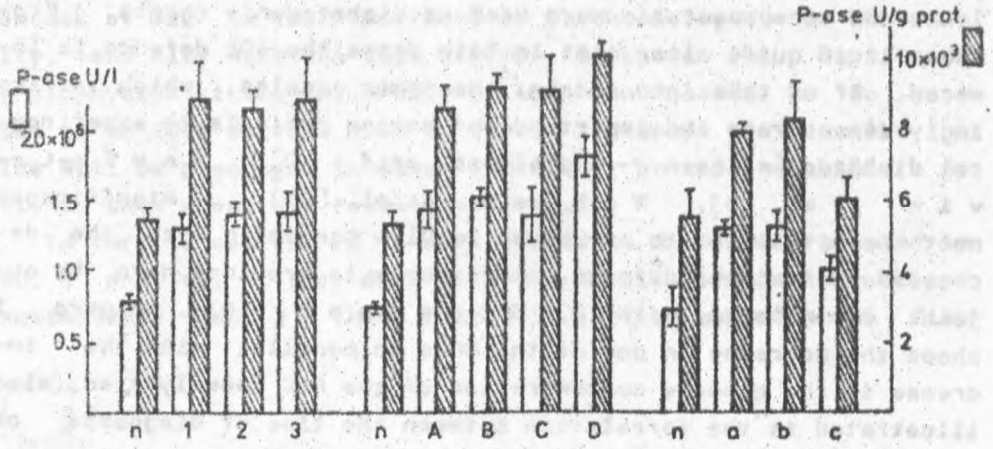

Fig. 3. P-ase activities. For notes, see Fig. 1

Aktywność P-azy. Objaśnienia jak do rys. 1

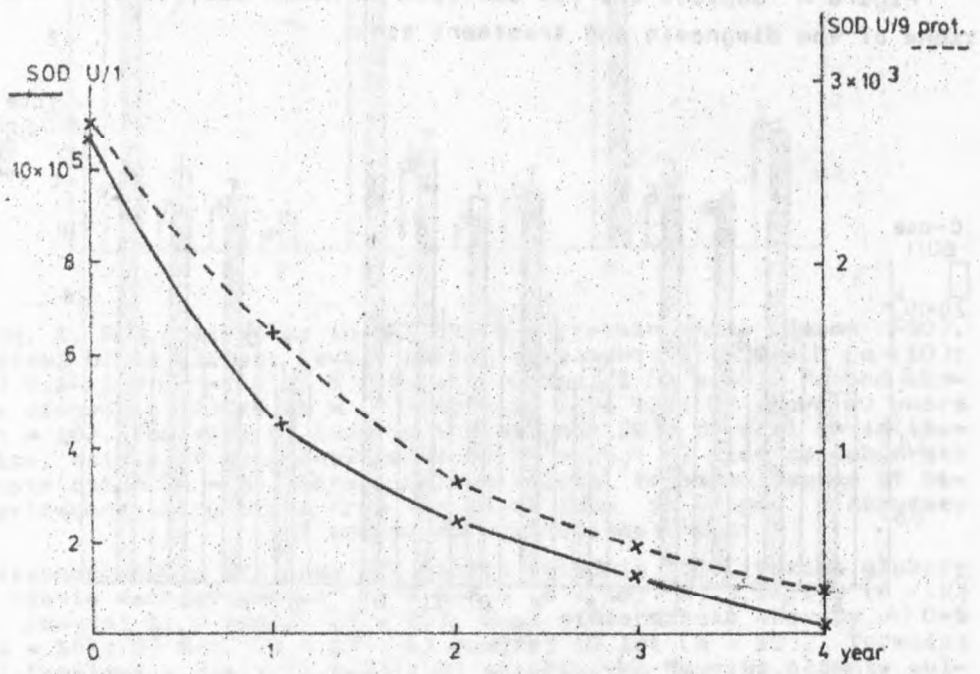

Fig. 4. SOD activities as a function of the period since diagnosis and commencement of treatment

Aktywność SOD w funkcji czasu, który upłynął od diagnozy i początku doświadczenia

It should also be mentioned here that, in cooperation with oxygen radicals, the anthracycline antitumour agent adriamycin becomes cardiotoxic, with scavengers (DMSO and ascorbic acid) the antitumour effect remains unchanged, while this toxicity of the drug is diminished Marián et al. [10].

Finally, I should like to express my thanks to our hosts, and particularly to Professors Krajewski and Leyko, for providing me with the opportunity to attend. My thanks are also due to all those who take part in our joint work, including those who may not have been mentioned by name.

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NOWE WYNIKI BADAŃ WŁASNYCH DYSMUTAZY PONADTLENKOWEJ I ZAMIERZENIA BADAWCZE

Praca podsumowuje badania aktywności dysmutazy ponadtlenkowej w przypadkach wybranych chorób dziedzicznych i cukrzycy oraz molekularnych mechanizmów działania parakwatu w różnych warunkach, a także D-penicyloaminy i ryboflawiny w hiperbilirubinemiach.