BIOCHEMISTRY AND BIOPHYSICS

THE INFLUENCE OF COPROPORPHYRIN AND PROTOPORPHYRIN ON TISSUE RESPIRATION

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Biologic oxidation occupies a prominent position in the complex system of metabolic changes. The majority of oxidative enxymes are hemoporphyrin complexes. The structure and physicochemical properties of porphins, first established by M. V. Nentsky, have now been relatively well investigated [1]. The questions of synthesis and the role of porphyrins in the organism under normal and pathologic conditions have received considerably less attention [4].

Of great interest is the problem of the relation of natural porphyrins to the action of oxidative enzymes possessing a hemoporphyrin structure, and to the whole complex of oxidation-reduction enzymes concerned with the final stages of biologic oxidation.

I. E. Elpiner, L. A. Blumenfeld, and S. E. Krasovitskaya showed that under certain conditions protoporphyrin IX acts as a good hydrogen carrier (acceptor and donor) [5].

Klyuver (cited by A. M. Charny) demonstated the presence of coproporphyrin in the white matter of the brain, where there is no cytochrome and cytochrome oxidase under physiologic conditions. A. M. Charny supposed that in these parts of the brain "porphyrins could be regarded as an oxidation-reduction system" [3]. Yu. K. Smirnov [2] brought to lignt appreciable changes in porphyrin metabolism associated with diseases of the nervous system. It has been established that the presence of iron-containing porphyrin is required for the growth of Hemophilus influenzae. The growth of these bacteria was also enhanced by the addition of protoporphyrin to the medium, while simultaneous presence of hematoporphyrin inhibited this growth [7].

S. Granick and H. Gilder [7] consider that the ability to include iron in porphyrin in the process of biosynthesis is determined by the presence of vinyl groups in the structure of porphyrin. However, the formation of a complex with a specific protein, mediated by the propionic groups, is evidently possible for all natural porphyrins.

Both isomers of coproporphyrin are natural porphyrins, devoid of vinyl groups. It is thought that under certain conditions coproporphyrin acts as a regulator of the rate of oxygen utilization by cells [7].

The effect of coproporphyrin and protoporphyrin on the rate of oxygen utilization by the tissues (animal) has not, as far as could be discovered, been confirmed.

The present work presents data on the effect of coproporphyrin and protoporphyrin on liver, brain and kidney tissue respiration.

EXPERIMENTAL

Experiments were performed on male and female rats weighing from 120 to 160 g; the animals were on the usual laboratory diet. Altogether 57 experiments were carried out.

Determination of the rate of oxygen utilization by the tissues was performed in the Warburg apparatus in an oxygen atmosphere at bath temperature of 38° with variations of ± 0.015° at 80 oscillations of the manometers per 1 minute. Examination of the brain was performed on macerated tissue with parallel determination of solid residue. Slices were prepared from liver and kidneys; these were dried to constant weight at 100-105° after the experiment. The material was weighed on analytical balances. Phosphate buffer of pH 7.0-7.35 was used as medium. The amount of coproporphyrin and protopotphyrin added to the medium was calculated to give a concentration of 0.1 and 1.0 γ in the sample.

Protoporphyrin was prepared from crystalline hemin obtained from the blood of donors by the Nentsky method. Coproporphyrin was isolated from the urine of rabbits subjected to lead poisoning; isomers 1 and 3 were not separated. Purification of coproporphyrin was carried out according to Fischer's method. The purity of the preparations and the concentration of the main solutions were checked by means of a Beckman spectrophotometer.

The protoporphyrin and coproporphyrin were obtained and their purity and concentration checked by L. A. Blumenfeld and S. E. Krasovitskaya.

The study of the effect of protoporphyrin and coproporphyrin on tissue respiration was carried out parallel with the determination of normal oxygen utilization by the tissues of the same animal. In all cases each determination was performed on three parallel samples. The average data obtained in these three determinations are given in the tables. The rate of oxygen utilization by the tissues is expressed as Q_{O_2}

RESULTS

Tables 1, 2, and 3 show that under the experimental conditions the oxygen utilization by rat tissues varied individually over a considerable range.

Thus, the Q_{O_2} value for kidneys varied in individual cases from 11.1 to 22.5, the Q_{O_2} value for liver - from 4.0 to 9.9 and the Q_{O_2} value for brain - from 3.8 to 6.4 while variations in the parallel samples were insignificant.

Results of the study of the effect of coproporphyrin and protoporphyrin on tissue respiration of rat brain, liver, and kidneys given in Table 1 indicate that the presence of these porphyrins in the medium in doses of 0.1 γ and 1γ exerts no appreciable influence on the utilization of oxygen by the tissues.

All the Q_{O_2} values for tissues under investigation in the presence of coproporphyrin and protoporphyrin are within the limits of usual individual variations in the utilization of oxygen. Thus, the Q_{O_2} value for kidney varied between 12.8 and 22.7 on the addition of coproporphyrin, and between 15.4 and 22.7 on the addition of protoporphyrin; the Q_{O_2} value for liver under the same conditions varied from 6.6 to 9.7 (with added coproporphyrin) and from 6.1 to 9.7 (with added protoporphyrin); in the case of brain the individual variations in the Q_{O_2} value were from 3.8 to 5.3 in the presence of coproporphyrin and from 4.2 to 5.6 in the presence of protoporphyrin.

Taking into account the reported formation of porphyrin-serum albumin complexes [6], it was considered interesting to elucidate the effect of coproporphyrin and protoporphyrin on tissue respiration in the presence of soluble protein in the medium. With this aim in view in a series of experiments from 0.15 to 0.2 ml plasma was added to the medium; the plasms was obtained from the blood of the same rat whose organs were being investigated. The results (Table 2) show that under these conditions there was also no change in the amount of oxygen taken up by the the brain, liver, and kidney tissue. The Q_{O_2} value for all these tissues showed variation within the usual limits both in each individual experiment and from experiment to experiment.

Table 3 gives the results of the investigation of the effect of protoporphyrin on tissue respiration in the presence of iron and on the addition of iron and plasma to the medium (the iron was added as a solution of reduced iron in 0.1N solution of hydrochloric acid calculated to give a concentration of 2.8 γ in the sample). Under these conditions once again there was no evidence of any influence of protoporphyrin on the oxygen

uptake of the tissues. Therefore, the experiments show that under experimental conditions in vitro (tissue slices and homogenates) coproporphyrin and protoporphyrin exert no influence on the action of oxidation-reduction enzymes in rat liver, kidney, and brain tissues.

TABLE 1

Rate of Oxygen Utilization by Tissues in the Presence of Coproporphyrin and Protoporphyrin

Tissue	No. of animal	Medium			No. of animal	Medium		
		Buffer solution	Buffer solution + coproporphyrin			Buffer solution	Buffer solution + coproporphyrin	
			In the dose of 0.1γ	In the dose of 1γ			In the dose of 0.1γ	In the dose of 1γ
Brain	1	4.5	4.6	3.9	6	4.8	5.3	5.4
	2	3.8	3.8	4.0	7	5.5	5.5	5.5
	3	4.7	4.6	5.3	8	5.5	5.6	5.6
	4	4.2	4.7	4.3	9	5.2	5.4	5.5
	5	4.4		4.8	10	4.9	4.6	4.2
Liver	11	9.9	9.7	9.4	16	9.4	9.7	9.4
	12	9.4	8.1	9.3	17	6.0	7.1	7.1
	13	8.7	8.6	7.2	18	5.2	5.3	5.6
	14	7.4	7.8	8.3	19	7.1	6.9	6.8
	15	8.0	6.6	7.8	20	6.0	5.2	5.8
Kidneys	21	19.4	19.0	18.9	26	20.0	19.2	21.9
	22	13.6	14.0	12.8	27	18.1	19.5	20.0
	23	22.5	22.7	22.0	28	22.6	17.8	22.4
	24	—	16.0	15.8	29	22.6	20.1	22.7
	25	17.2	—	17.4	30	16.0	16.0	15.4

TABLE 2

Q_{O_2} of Tissues in the Presence of Plasma, Coproporphyrin, and Protoporphyrin

Tissue	No. of animal	Medium		
		Buffer solution and plasma	Buffer solution, plasma, and coproporphyrin 1.0γ	Buffer solution, plasma, and protoporphyrin 1.0γ
Brain	31	4.1	4.3	5.3
	32	6.4	4.2	4.8
	33	4.6	5.3	4.5
	34	4.1	3.7	3.7
	35	4.3	4.6	4.0
	36	4.7	4.3	5.2
Liver	37	4.5	5.0	4.6
	38	5.4	4.6	3.9
	39	8.2	7.3	7.2
	40	4.0	3.0	4.5
	41	—	3.3	5.4
	42	5.6	—	5.3
Kidneys	43	20.3	13.4	23.7
	44	11.1	13.9	12.6
	45	12.2	14.4	13.4
	46	15.4	16.8	15.0
	47	17.1	15.2	17.6
	48	—	—	14.9

TABLE 3

Q_{O_2} of Tissues in the Presence of Plasma, Iron, and Protoporphyrin

Tissue	No. of animal	Medium			
		Buffer solution	Buffer solution and iron	Buffer solution, iron, and protoporphyrin	Buffer solution, plasma, iron, and protoporphyrin
Brain	49	5.2	5.6	5.4	5.2
	50	5.8	5.7	6.1	5.4
	51	4.8	5.2	5.1	5.5
Liver	52	8.6	6.7	7.5	7.1
	53	9.5	9.7	8.9	8
	54	8.0	7.9	7.5	8.6
Kidneys	55	12.9	13.5	14.9	15.0
	56	16.0	—	15.9	15.0
	57	18.0	16.6	17.1	16.2

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