These results suggest that during photooxidation the first event to take place is destruction of tryptophan residues, which are evidently on the surface of the protein globule. This leads to partial disturbance of the conformation of the enzyme molecule, accompanied by a fall in its activity, and it makes the histidine residues accessible for oxidation. The essential role of histidine residues in the manifestation of enzymic activity of the asparaginase of *E. coli* has been demonstrated by photooxidation, chemical modification [7], and by the use of a kinetic approach [8].

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CLASSIFICATION OF XENOBIOTICS BY LOCALIZATION OF THEIR ACTION ON MITOCHONDRIAL ENZYME SYSTEMS

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The study of the action of toxic agents on energy metabolism in biological objects is finding ever-increasing application in different branches of toxicology and pharmacology. These investigations have proved productive not only from the theoretical (to study the structure and functions of the respiratory chain and the mechanism of action of xenobiotics), but also from the practical point of view (for the rapid assessment of the toxicity of new compounds, establishing health rules, and so on) [1, 6, 9, 11-14, 16, 17, 19].

Analysis of existing data on the action of some hundreds of xenobiotics, of varied structure, on respiration and oxidative phosphorylation has led to the discovery of correlation between the chemical structure of the substance and the character and location of its action on molecular targets of the mitochondria. The results of one such analysis of personal data (over 100 compounds) and of data in the literature are given below.

EXPERIMENTAL METHOD

Experiments were carried out on liver mitochondria from male noninbred albino rats. Mitochondria were isolated and respiration recorded by a polarographic method as described in [8]. The test compounds were introduced into the spectrophotometer cell containing mitochondria in a volume of 1-10 μl. When nonaqueous solvents (ethanol, dimethyl sulfoxide, acetone) were used a control was set up with the pure solvents. Succinic and β-hydroxybutyric acids in the form of neutralized solutions were used as oxidation substrates.

EXPERIMENTAL RESULTS

The different stages of the complex and multistage process of oxidative phosphorylation,

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TABLE 1. Classification of Toxic Substances by Character and Localization of Their Action on Enzyme Systems and Mitochondrial Complexes

Serial	Character of action	Classes of compounds and separate substances	Highly specific agents
1	Inhibition of electron transfer at the level of:		
1.1	NADH dehydrogenase	Saturated acyclic and cyclic hydrocarbons; alcohols; aldehydes (except formaldehyde); aromatic compounds including polycyclic aromatic hydrocarbons; diphenyls; cyclodiene hydrocarbons; aromatic halide derivatives of hydrocarbons; dithiocarbamates; pyridines and other heterocyclic compounds; steroids; fat-soluble vitamins; organophosphorus compounds; O_3; lead, etc.	Rotenone; pyricidin
1.2	Succinate dehydrogenase	Malonic and oxaloacetic acids; phenols with $pK \leq 7.5$; bathophenanthroline; thenoyltrifluoroacetone; norbormide	Malonic and oxaloacetic acids
1.3	Respiratory chain before cytochrome oxidase	Gladiolic acid; N,N-Di-(chloroethyl)-p-aminophenylacetic acid; bathophenanthroline; lead; zinc; antimycins	Antimycin A; zinc
1.4	Cytochrome oxidase	CO; sulfites; sulfides; azides; cyanides; phosphine; formates; aromatic and aliphatic nitriles (except unsaturated)	Cyanides; azides
2	ATPase inhibitors	Alkylating compounds (nitrogen mustards); dicyclohexylcarbodiimide; aflatoxins; citroveridin; triethyltin; dibutyltin sulfide; bis(tributyltin) oxide; formaldehyde	Oligomycin; bis(tributyltin) oxide; citroveridin
3	Inhibitors of transmembrane transport		
3.1	Inorganic phosphate	Thiol poisons (cadmium, mercury, and their inorganic and organic compounds); maleimides; nitrogen mustards, etc.; tetradifon; fuscin	Organomercury compounds
3.2	Adenine nucleotides	Opiates; bongkrekic acid; agaric acid; atractyloside	Atractyloside
3.3	Cations	Alkylators; local anesthetics; formaldehyde; complex compounds of cobalt and lanthanum	Hexamine-cobalt chloride
3.4	Substrates	Fluorocitrate; fuscin; bathophenanthroline; trialkyl compounds of tin; avenocyolide	Substrate analogs (butyl malonate, for example)
4	Uncouplers		
4.1	Protonophores	Halogen derivatives of aliphatic and unsaturated hydrocarbons, phenols, diphenyls; aromatic acids; nitro- and amino-compounds; benzothiazoles; dithiocarbamates; heterocyclic compounds	2,4-Dinitrophenol; tetrachlorotrifluoromethyl benzimidazole
4.2	Ionophores	Valinomycin; nigericin; gramicidin	Valinomycin; nigericin

starting from detachment of a proton and electron from the substrate and ending with incorporation of inorganic phosphate into the ADP molecule, are by no means equally sensitive to the action of toxic substances (Table 1; Fig. 1).

The most frequent causes of a disturbance of this process are inhibition of NADH-dehydrogenase and uncoupling of respiration and phosphorylation by protonophores: The action of most toxic substances is localized at these points. Inhibitors of succinate dehydrogenase (SDH) and ATPase and also inhibitors of the cytochrome factor of the respiratory chain and, in particular, of cytochrome oxidase, are much less common. Ionophores of the valinomycin or nigericin type, acting as uncouplers, are found extremely rarely (mainly among antibiotics).

It must be pointed out that no absolutely specific inhibitors of a particular enzyme or enzyme complex exists ("everything acts on everything"), and when speaking of inhibitors of a particular region of mitochondria their relative specificity is implied or, in other words, concentration relationships. Armin, for example, inhibits oxidation of NAD-dependent substrates by 50% in a concentration of $(1.6 \pm 0.28) \times 10^{-6}$ M, whereas succinic acid does so in a concentration of $(2.7 \pm 0.33) \times 10^{-5}$ M; the terminal region of the respiratory chain is even less sensitive to the action of this inhibitor: $C_{i50} = (8.2 \pm 2.2) \times 10^{-2}$, so that this compound can be classed as an NADH-dehydrogenase inhibitor. Meanwhile bis(tributyltin) oxide inhibits ATPase 2000-5000 times (!) more effectively than electron transport in different regions of the respiratory chain, and we have therefore classed it among the ATPase inhibitors. These examples also show that the degree of specificity may differ within wide limits, and on that account some compounds (classes of compounds), whose action is not distinguished by any marked specificity, are placed in different sections of the table at the same time (lead, aromatic acids, alkylating compounds, etc.). In general, in our opinion, relative specificity of an inhibitor can be implied only if the active concentrations for

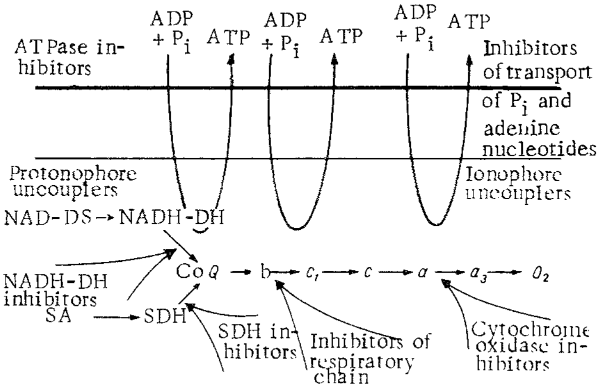

Fig. 1. Diagram of oxidative phosphorylation and points of application of action of different classes of toxic substances. NAD-DS) NAD-dependent substrates; NADH-DH) NADH dehydrogenase; SA) succinic acid; SDH) succinate dehydrogenase; CoQ) coenzyme Q (ubiquinone); b, c_1, c, a, a_3) cytochromes of respiratory chain; P_i) inorganic phosphate.

the "specific" and "nonspecific" regions differ by not less than one order of magnitude. The very high sensitivity of the NADH-dehydrogenase stage of the respiratory chain to the inhibitory action of compounds which differ widely in their nature, but are mainly organic, does not seem to be accidental. The fact is that inhibition of NADH-dehydrogenase sharply increases the store of reduced pyridine nucleotides in the mitochondria, and this is facilitated, in particular, by the activation of SDH which takes place under these conditions [2-5]. In turn, a very high NADH level in the mitochondria may, with the participation of a reversed transhydrogenase reaction and the isocitrate shuttle mechanism [10, 18], lead to an increase in the NADPH reserves in the cytosol compartment of the cell, and may thereby promote activation of destruction of the inhibitor in the system of NADPH-dependent microsomal oxidases.

It is very important to note that the two most specific (and at the same time competitive) inhibitors of SDH, namely malonic and oxaloacetic acids, are natural metabolites, and they may thus perform regulatory functions in the cell, in good agreement with existing views on the special role of succinic acid in the energy and general metabolism of the cell [4, 5].

The high resistance of the terminal region of the respiratory chain, which is inhibited only by a restricted group of relatively simple inorganic compounds, to the action of toxic substances also is not noteworthy. This may be due to the fact that the terminal region of the respiratory chain is the phylogenetically oldest formation, whereas the dehydrogenase regions, especially the NADH-dehydrogenase region, are substantially later "adjustments."

Finally, the extremely wide spectrum of compounds possessing the properties of protonophore uncouplers, a fact which agrees fully with views on the chemiosmotic mechanism of coupling [10, 15], must also be noted. On the other hand, uncoupling by transmembrane ion transport requires highly specific carriers of biogenic origin (ionophores).

In our view the general rules described in this paper allow the investigator, while still at the experimental planning stage, to postulate which component of the complex polyenzymic structure of the mitochondria will suffer the greatest injury as a result of the action of a toxic substance of a particular chemical structure that is to be studied, and they will also help with the search for substances useful as specific antidotes in the treatment of poisoning.

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CYCLIC NUCLEOTIDES (cAMP AND cGMP) IN THE BLOOD PLASMA OF DOGS DURING EXTRACORPOREAL CHARCOAL HEMOPERFUSION

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Until recently no satisfactory explanation was forthcoming for the fact that cyclic nucleotides (CN), which are intracellular mediators of the action of hormones and other biologically active substances, are constantly present also in extracellular fluids — in lymph, saliva, urine, and blood plasma. It has been suggested that the outflow of CN into the extracellular fluid regulates their content in the cells, i.e., extracellular CN were regarded as ballast substances [6]. In fact, stimulators of intracellular cAMP formation such as isoproterenol cause an increase in the concentration of the nucleotide in blood plasma and urine [12]. The cGMP concentration is similarly increased by cholinergic agents [8]. However, it was shown more recently that an increase in the CN concentration in cells is not always accompanied by their outflow into the surrounding medium. For instance, the CN phosphodiesterase inhibitor papaverine, which raises the intracellular cAMP level, does not change the rate of its liberation from erythrocytes [7]. CN transport from cells likewise is not a passive, but an active, energy-dependent process, which requires the presence of ATP and is inhibited by inhibitors of oxidative phosphorylation [5].

The view also is held that CN synthesis takes place not only inside, but also outside the cell, on the outer side of the membrane [7].

Metabolism of CN in blood plasma may follow several pathways. Some CN is excreted unchanged with urine, some is hydrolyzed by plasma phosphodiesterase [3] and also by the phosphodiesterase of the liver and kidneys [4]. Meanwhile the CN concentration in blood plasma is very constant under normal conditions and long-lasting changes in it are observed only in pathological states [11].

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