the 20th-30th day the threshold of cardiac pacing stabilized at a sufficiently low level, on average 0.6 V. Such a low pacing threshold was observed in only one case when electrodes of the control group were used. Saturation of a porous electrode with glucocorticoids immediately before implantation thus significantly lowers the pacing threshold.

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INVESTIGATION OF THE MULTICOMPONENT DETOXICATION SYSTEM IN THE ALBINO RAT LIVER

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KEY WORDS: microsomal monooxygenases; cytochrome P-450; cytochrome b_5; factor analysis.

In a series of investigations Sarkisov has developed the view that any pathological process is based on injury to intracellular membrane structures [8, 9]. Membrane structures, however, are supramolecular complexes of enzymes and information macromolecules functioning as integrated systems [6]. During the formation of responses to external stimuli the number of actively "working" structural units in these systems varies within the limits of the available reserve, but under certain conditions their number increases because of intensification of synthesis of new molecules [7]. The formation of responses of integrated biochemical systems to an external stimulus is thus coupled with corresponding changes in their components.

One typical example illustrating this view is functioning of the multicomponent system for detoxication of xenobiotics, which includes the polyenzymic complex of the endoplasmic reticulum of cells which is functionally connected with phospholipids of microsomal membranes. This balanced biochemical system ensures homeostasis provided that the increase in the intensity of toxic action or its duration does not cause failure of this protective mechanism [10].

The problem of assessing the reliability of the complex integrative system, taking account of the wide range of possible changes in its components, accordingly arises, and to study it was the aim of the present investigation. It was considered that the study of the pattern of function of a multicomponent system would enable the closest analysis to be made of the most informative parameters, the structure of correlations between them, and its modification under the influence of external factors. One approach to the solution of this problem may be by the use of factor analysis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 300 ± 30 g. Before the beginning of the experiments the animals were kept on a standard diet. Activity of the microsomal monooxygenase system, forming a component of the polyenzymic complex of the smooth endoplastic reticulum of the hepatocytes, was investigated. Phenobarbital, injected intraperitoneally in a dose of 80 mg/kg daily for 3 days, was used as inducer of microsomal monooxygenases. The compound SKF-525A (β-diethylaminoethyl-diphenylpropyl acetate), injected intraperitoneally in a single dose of 80 mg/kg, was used as the inhibitor. The animals were decapitated 24 h after injections of the inducer and 18 h after injection of the inhibitor, after which microsomes were isolated from the perfused liver tissue. Cytochromes P-450 and b_5 were determined by the method in [2]. Total NADPH- and NADH-dependent monooxygenase activity was measured by the method in [13]. The concentration of SH-groups in microsomal proteins was determined by the method in [12]. Protein was estimated spectrophotometrically

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TABLE 1. Effect of Phenobarbital and SKF-525A on Monooxygenase Enzyme System in Microsomal Fraction of Albino Rat Liver (M ± m)

Parameter studied	Intact animals ($n=14$)	Animals receiving phenobarbital ($n=6$)	Animals receiving SKF-525A ($n=8$)
Cytochrome P-450, $D_{450-490} \cdot 10^{-2}$/mg protein	1,39±0,12	3,21±0,22**	0,84±0,13*
Cytochrome b_5, $D_{425-408} \cdot 10^{-2}$/mg protein	2,19±0,12	1,83±0,26	1,70±0,15**
Microsomal protein, mg/ml	3,17±0,215	4,20±0,19**	2,97±0,22
NADPH-dependent oxygenase activity, nmoles/mg protein	51,0±2,94	60,0±2,78*	30,0±2,72*
NADH-dependent oxygenase activity, nmoles/mg protein	56,28±2,75	57,12±1,50	68,75±3,17*
SH-groups in microsomal fraction, mg/kg	1,10±0,057	0,98±0,071	1,06±0,57
Weight index of liver	0,0378±0,0019	0,0455*±0,0026	0,0374±0,0006

Legend. *P ≤ 0.05, **P ≤ 0.001. D) Optical density (in relative units).

TABLE 2. Structure of Correlations between Parameters Characterizing State of Detoxication System (values of factor loadings for two principal components and their dispersions)

Parameter studied	Intact animals		Animals receiving phenobarbital		Animals receiving SKF-525A	
	1st factor	2nd factor	1st factor	2nd factor	1st factor	2nd factor
Cytochrome P-450	0,81	—0,30	0,74	—0,22	0,44	—0,34
Cytochrome b_5	0,73	0,35	0,14	0,27	0,74	—0,40
Microsomal protein	—0,66	—0,68	—0,96	0,20	—0,66	0,63
NADPH-dependent oxygenase activity	0,66	—0,54	—0,67	—0,35	0,86	0,46
NADH-dependent oxygenase activity	0,32	—0,41	0,57	0,77	0,72	0,63
SH-groups of microsomes	0,88	0,36	0,96	—0,13	0,88	0,083
Weight index of liver	—0,19	0,63	—0,54	0,84	—0,035	0,13
Dispersion (informativeness of factor), %	38	26	48	21	40	28

according to optical density at 220 nm. The weight index of the liver also was monitored. For statistical analysis one version of factor analysis was used, namely the method of chief components [1]. The appropriate calculations were done by computer using standard programs.

EXPERIMENTAL RESULTS

The results of a comparative study of several biochemical parameters in hepatocyte microsomes of intact rats and of experimental animals with an activated and inhibited monooxygenase enzyme system are given in Table 1. During activation of the system by phenobarbital, in agreement with data in the literature [5], an increase in the concentration of cytochrome P-450, in NADPH-dependent monooxygenase activity, in the microsomal protein concentration, and in the weight index of the liver was observed. On inhibition of the system by SKF-525A concentrations of cytochromes P-450 and b_5 and activity of NADPH-dependent microsomal monooxygenase were reduced.

To discover latent correlations to determine the structure of relationships between the parameters studied, the results were subjected to factor (component) analysis. The model of analysis of the chief components [1] was designed to take into consideration the fact that the most significant (useful) information contained in the original multidimensional mass of data is proportional to the sum of the total dispersion of the sample [3, 4]. Consequently, estimation of dispersion of the chief components (factors) may provide a quantitative measure of their informativeness. Under these circumstances the structure of the chief components (linear combinations of random values with large dispersions) is itself determined by the magnitude of the factor loadings, which are coefficients of correlation between the original parameters and the chief component value. The direction of analysis of the experimental data was reduced to estimation of values of dispersions and factor weightings, followed by their interpretation within the limits of observation groups in the control and during "disturbances" of the test system by induction or inhibition. Of the results for dispersions of the chief components (factors) the most interesting of all the possible factors were the first two, whose information capacity was about 64-69%, and remained basically unchanged under the influence of the substances in the doses used in these experiments (Table 2). So far as the factor loadings of the parameters are concerned, these did undergo significant changes.

In intact animals both factors in general reflect the state of the NADPH-dependent electron transport chain in membranes of the endoplasmic reticulum, but with different degrees of

interdependence of the individual parameters of the system. The first factor, in particular, combined cytochromes P-450 and b_5, NADPH-dependent oxygenase activity, and the number of SH-groups. In the presence of direct correlations, this combination was not accidental. Cytochromes P-450 and b_5 are functionally closely connected. They can form hemoprotein complexes, as a result of which the cytochrome P-450 is stabilized in a catalytically active conformational state, leading to an increase in the velocity of the reactions catalyzed by it [11]. Correlation discovered between cytochrome P-450 and SH-groups and the combination of these parameters into the first factor, carrying maximal information of the state of the detoxication system, can evidently be explained on the grounds that superoxide $O_2^{\cdot}$ anions are formed during transformation of the xenobiotics through the action of microsomal monooxygenases because of decomposition of the oxygenated ferrocomplex of cytochrome P-450. Under these circumstances $O_2^{\cdot}$ radicals can induce oxidation of SH-groups and initiate lipid peroxidation [14]. The second factor combines the most integral parameters: total NADPH- and NADH-dependent microsomal oxygenase activities, protein content, and weight index of the liver.

As a result of induction of the microsomal monooxygenase system by phenobarbital (Table 2) functional correlations were transformed: Individual correlations disappeared or new ones appeared, the character of the correlations and the degree of coupling between them were modified, against the background of activation of the system as shown by levels of cytochrome P-450, NADPH-oxygenase activity, microsomal protein concentration, and weight index of the liver. These changes can be interpreted as transition of the system to a new level of function.

When the inhibitor SKF-525A was used, the most typical results were a change in the character (sign) of correlations, a decrease in the factor loading of cytochrome P-450 and an increase in the factor loading of SH-groups, and reduction of significant correlation between components of the system and the change in their closeness. This means that the inhibitor disturbed the synchronously functioning detoxication system and weakened its power. Since the informativeness of the factors was not significantly changed under these circumstances it can be postulated that under the conditions of the investigation inhibition did not induce profound irreversible disturbances of this system.

The use of factor analysis thus enabled the dominant and most informative parameters determining the level of function of the system as a whole to be distinguished from other components of the microsomal monooxygenase system: the concentrations of cytochromes P-450 and b_5, which correlate closely with the concentration of SH-groups in microsomal proteins. External factors causing activation or inhibition of this system led to reorganization of correlations between its structural units. The biological meaning of these reorganizations calls for special study. It can be tentatively suggested that the closeness and transformation of correlations between elements composing the integrated system are among the quantitative characteristics of the intensity of its function and, together with other parameters, they can serve as a measure of reliability under the influence of unfavorable factors. It is evidence that as long as a certain degree of integration is preserved, expressed as the number, value, and direction of correlations in a multicomponent system, it will remain capable of functioning at the appropriate intensity and of restoring its own activity when changed by external forces.

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MORPHOGENESIS OF PNEUMONIA DURING ALTERED IMMUNITY

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The great diversity of connections between immune and inflammatory reactions makes it difficult to draw a sharp line between them and to evaluate their interdependence [5]. The aim of this investigation was to study the effect of various changes in the immune response on the development of inflammation of the lungs.

EXPERIMENTAL METHOD

Pneumonia lasting 2 weeks was induced in 30 Wistar rats, divided into five groups (with six rats of the same weight and sex in each group) by introduction of a foreign body (Kapron thread 0.4 mm in diameter and 3 cm long) into the trachea by the method in [3]. Additionally, animals of group 1 received subcutaneous injections of 25 U of heparin twice a day every day for 3 weeks (1 week before and 2 weeks after introduction of the thread). Animals of groups 2 and 3 received intramuscular injections of cyclophosphamide in a dose of 0.6 mg on alternate days and phytohemagglutinin (PHA) in a dose of 5 mg once every 5 days respectively for 3 weeks (1 week before and 2 weeks after introduction of the thread). Animals of group 4 received a single subcutaneous injection of 1.5 ml of sterile mineral oil 5 days before introduction of the thread into the trachea. According to data in the literature [1] this inhibits complement-dependent reactions (heparin) and leads to various kinds of stimulation (PHA, mineral oil) and to inhibition of immunity (cyclophosphamide). Group 5 (control) consisted of six rats with pneumonia for 2 weeks. Sections of lung tissue were stained with hematoxylin and eosin, by Van Gieson's method, and for DNA by Feulgen's and RNA by Brachet's methods, for glycoproteins (GP) by the PAS reaction, and for glycosaminoglycans (GAG) by the method of Hale and Muller. The density of cellular infiltration was studied morphometrically by the dot counting method [2]. Titers of heterophilic agglutinins (by a modified Paul–Bunnell method) and of antistaphylococcal antibodies (passive hemagglutination test) were determined in blood sera. The cellular immune response was estimated from the difference between limb volumes before and 48 h after injection of 0.2 ml of standard *Neisseria catarrhalis* allergen into the footpads. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Inflammation of the lungs of 2 weeks' duration was characterized by the development of acute catarrhal bronchitis with moderate peribronchial exudation. In some cases acinar bronchopneumonia developed. There was a moderate microfocal interstitial reaction in the form of thickening of the alveolar septa by edema and concentrations of macrophages and lymphocytes around single vessels. Histochemically an increase in the content of DNA (nucleus), RNA, GAG, and GP (cytoplasm) was observed in the epithelium of most bronchi, the vascular endothelium, and septal cells, pointing to the development of an anabolic type of tissue reaction.

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