ASSESSMENT OF THE STATE OF CELL AND TISSUE FUNCTION BY THEIR ABILITY TO ELIMINATE FOREIGN MATERIALS

S. N. Romanov, R. A. Artsishevskaya, L. I. Misnik, and L. S. Puzina

UDC 612.015.34-087.5

A method of assessing the state of cell and tissue function on the basis of their ability to eliminate previously adsorbed dye is suggested. The theoretical basis of the method is the property of cells to actively eliminate their metabolic products. It was shown experimentally that the liberation of dye (desorption) is accompanied by the expenditure of energy. If glycolysis in the cell is inhibited, its powers of elimination are depressed. Thyroid hormone stimulates the excretory function of the cell; the presence of an MOP sarcoma in rats paralyzes this cell function.

KEY WORDS: desorption of dye; state of cell function; vital staining.

Of all variants of the method of vital staining of tissues, the one most commonly used and for the greatest benefit is that of staining the tissues in vitro, followed by quantitative estimation of the bound dye, as developed in D. N. Nasonov's school. By a combination of this method with microscopy of the intravitally stained tissues, the general principles of the response of living matter to external factors have been successfully studied [5, 6]. However, this method has one very important disadvantage: the state of function of cells and tissues has been studied after their isolation from the organism (in vitro), and the results therefore do not always reflect the state of the tissues in situ. To overcome this disadvantage attempts have been made to stain the tissues in situ, and then to kill the animals and to determine the quantity of dye bound by various tissues [1, 3, 7]. Unfortunately, after many attempts to use this variant of the technique to assess the state of cell function, the writers were forced to conclude that it is absolutely unsuitable for this purpose. The quantity of dye bound after intravenous injection is determined not by the state of the cell, but by the reactivity of the reticulo-endothelial system [2], the velocity of the blood flow of the given organ, the permeability of the blood vessels, and other factors that it is virtually impossible to take into account. There are no grounds for considering that under given conditions the intensity of staining of the cells depends on their state of function. In the writers' opinion, to assess the state of cell function, the method of desorption of a previously bound dye by the cells could be very informative.

The theoretical basis of the method is as follows. In the cell, just as in an integral system, metabolism takes place, and this requires two processes of equal importance: the first is the selective intake of substances for synthesis of the cell components, the second the selective elimination of metabolic products, without which prolonged existence of the cell would be impossible.

It is suggested that the intensity of elimination not only of metabolic products, but also of a foreign dye, from the cell could be used as a measure of its functional activity. The higher the functional activity of the cell, the more rapidly it would eliminate metabolic products or foreign dyes; conversely, the more depressed its activity, the lower the rate of elimination. Elimination of foreign materials from cells is an active process, requiring the expenditure of energy. These suggestions have been tested experimentally.

Institute of Cytology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. G. Baranov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 1, pp. 88-90, January, 1975. Original article submitted February 15, 1974.

TABLE 1. Changes in Degree of Staining (Sorption) and Intensity of "Decolorizing" (Desorption) of Tissues of Various Organs in Mice Receiving an Excess of Thyroid and in Rats during Development of Sarcoma MOP (in % of control)

Experimental conditions	Time of action (days)	Cortex		Cerebellum		Kidney		Liver	
		sorption	desorption	sorption	desorption	sorption	desorption	sorption	desorption
Increased thyroid content in mice produced by daily feeding with 100 mg thyroid extract	1/2	86	179*	79*	204*	41*	160*	65*	128*
	2	98	184*	84*	252*	78*	101	109	90
	5	128*	108	98	173*	52*	171*	136*	114
	10	119*	109	85	66*	60*	49*	133*	73*
	15	84*	206*	75*	206*	57*	113	80	115
Transplantation of MOP sarcoma into rats		Left hemisphere		Liver		Kidney			
	5	82	85*	83*	73*	58*	69*		
	23	56*	65*	63*	71*	66*	64* left		
						100	48* right		

* Differences between experiment and control significant for which $P < 0.05$.

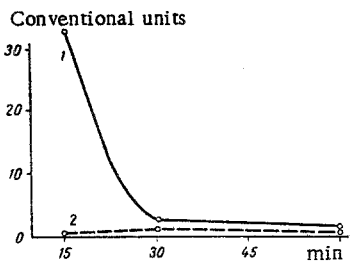

Fig. 1

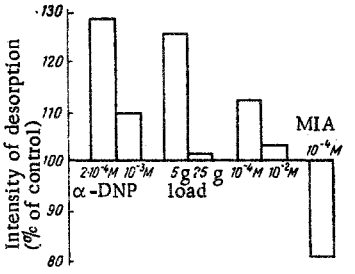

Fig. 2

Fig. 1. Rate of elimination of dye by living (1) and dead (2) muscles. Abscissa, time (in min); ordinate, rate of elimination of dye (in conventional units).

Fig. 2. Change in intensity of elimination of dye by muscles under the influence of various factors.

EXPERIMENTAL METHOD

The paired sartorius muscles of frogs were stained in vitro with neutral red in a concentration of 0.05% for 30 min. One frog was then killed either with boiling Ringer's solution or with hot steam. The experimental and control muscles were then incubated ("decolorized") in Ringer's solution at a strictly controlled temperature (25°C). The quantity of dye eliminated into the surrounding solution during the period of incubation was determined photometrically. The muscles, after rinsing, were placed in acidified alcohol to extract the remaining dye. The intensity of desorption (in %) was determined by the equation:

$$D = \frac{a}{A + b} \cdot 100,$$

where a is the quantity of dye eliminated into the surrounding solution, and b the quantity of dye extracted by alcohol. To investigate the role of respiration and glycolysis in the excretory function of the cell the corresponding inhibitors – KCN and monoiodoacetate (MIA) – were used; the muscles were kept for 10-15 min in solutions of these inhibitors before "decolorizing." At the same time, the effect of mechanical loading on the intensity of elimination of the dye was studied, for which purpose weights of different sizes were suspended from the muscle at the time of incubation.

EXPERIMENTAL RESULTS

The dead muscle virtually did not eliminate the previously bound dye (Fig. 1), whereas the living muscle excreted most of the dye in the course of 30 min. If the muscle was kept before "decolorizing in

Ringer's solution with the addition of 2×10^{-4} M α-dinitrophenol (α-DNP) for 20 min, the intensity of elimination of the dye (desorption) was increased; the same effect was observed after the action of a 5-g load (Fig. 2). Higher concentrations of α-DNP, or increasing the load to 25 g, reduced the intensity of desorption virtually to the control level. If the muscles were kept in MIA (10^{-4} M) for only 10 min before "decolorizing," i.e., if glycolysis was blocked, desorption was sharply reduced. The results of this experimental verification of the method show that the ability of cells to eliminate dye is a very important index of their functional activity and that the excretory function of the cell is an active process requiring the expenditure of energy, and also that glycolysis is probably the chief source of energy for this cell function.

The functional state of tissues of various organs taken from mice receiving thyroid extract for a long time was investigated by the desorption method [4]. The tissues were stained in situ, and the dye was injected intravenously. The organs stained in situ were dissected and then incubated ("decolorized") in Ringer's solution with glucose at 37°C for 1.5 h. The results showed (Table 1) that an excess of thyroid hormone in the body sharply stimulated the excretory ability (desorption) of cells of the cerebral cortex, cerebellum, and kidneys. Desorption by liver cells was virtually unchanged, indicating that the hormone acts selectively on different organs. It is important at this stage to note that thyroid activates the functional activity of nerve cells particularly intensively. Meanwhile, the desorption method was also used to assess the state of function of various cells and tissues of rats with tumors (transplanted MOP sarcoma). In these cases the excretory power of the cells of the cerebral hemispheres, liver, and kidneys was paralyzed, as early as on the fifth day after transplantation of the tumor.

The results of this experimental verification of the desorption method as applied to the state of function of the tissues in animals loaded with excess thyroid and during the development of tumors suggests that the method could provide objective and very valuable information as regards both the functional activity of the cells and their behavior in various processes taking place in vivo. This, in turn, enables the method of desorption of vitally bound dyes to be recommended as a means of assessing the functional state of cells under different conditions of their existence.

LITERATURE CITED

1. Ya. I. Azhipa, Pat. Fiziol., No. 3, 77 (1961).
2. N. N. Anichkov, The Reticulo-Endothelial System [in Russian], Moscow–Leningrad (1930), p. 1.
3. E. M. Gramenitskii, Gig. i San., No. 11, 20 (1956).
4. L. I. Misnik, Zdravookhr. Belorussii, No. 1, 24 (1968).
5. D. N. Nasonov, Local Response of Protoplasm and Spreading Excitation [in Russian], Moscow–Leningrad (1959).
6. D. N. Nasonov and V. Ya. Aleksandrov, The Response of Living Matter to External Factors [in Russian], Moscow–Leningrad (1940).
7. S. N. Romanov, in: Problems in Cytology and General Physiology [in Russian], Moscow–Leningrad (1960), p. 254.