BIOASSAY OF BURN TOXIN IN MICE WITH BLOCKED RETICULOENDOTHELIAL SYSTEM

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Bioassay as a method of studying biologically active substances has the unique ability to evaluate an effect produced in the most general form. On this basis a method of determining toxicity in mice with blocked reticuloendothelial system (RES) has been developed and is being increasingly applied [2, 8, 9, 11].

Monocytes have been ascribed a special role in resistance of the organism to external pathogenic influences [1, 3]. Conversely, by injecting into the bloodstream substances which are selectively accumulated in cells of the RES in experimental animals it is possible to induce a state of increased sensitivity to various toxic factors. In this case toxicity is expressed as an unmitigated "all or nothing" reaction and it can be estimated quantitatively.

In the investigation described below bioassay on mice with blocked RES was used to study a high-molecular-weight burn toxin.

EXPERIMENTAL METHOD

Wistar rats weighing 100-200 g were subjected to a flame burn of the skin from a swab soaked in alcohol (area of burn 15-20% of the body surface, exposure 45-50 sec). Extracts of normal and burned skin were prepared by the method described previously [5]. Products of physicochemical fractionation of burned skin extracts (BSE) were obtained by salting out with ammonium sulfate (60-75% saturation, preparation No. 11) or by preparative electrophoresis in 5% polyacrylamide gel (the fraction with mobility of 0.43 conventional unit relative to the buffer front, preparation No. 1). Purified high-molecular-weight toxin was isolated from BSE by means of immunosorbents containing antibodies against the toxic component (preparations Nos. 2-4, 8). The toxin mixed with burn antigens was obtained by means of immunosorbents containing antibodies against burn-specific components (Preparation No. 12). A full description of the immunochemical method of isolating the burn toxin is given in [4]. The protein concentration in the preparations was determined by Lowry's method. Bioassay was carried out on noninbred albino mice weighing 18-20 g. Substances blocking the RES, namely C 11/1431a ink (from Gunter Wagner, West Germany) with a particle size of 20-50 nm, or colloidal trypan blue dye, were injected into the caudal vein in a single dose of 0.25 mg/g body weight. The test preparations were injected intraperitoneally into the animals in a volume of 1 ml, 30-40 min later. Toxicity was estimated from the number of mice dying in the course of 72 h.

The data were analyzed by the probit method [7] after equalization of the series by the method of least squares. With different numbers of animals in the groups, "weighting" factors were used for the calculations [6].

EXPERIMENTAL RESULTS

Bioassay of 18 preparations was carried out on 1135 mice (Table 1). An integral relationship was established between the protein concentration in the preparation and the frequency of death of the experimental mice. Spearman's coefficient indicates the presence of strong correlation between dose and effect, which was significant for all preparations. After transformation, the dependence can be described by the linear equation: $y = A + Bx$, where x is the logarithm of dose (in μg protein) and y denotes probits. Estimation of linearity of the equations thus calculated, by the chi-square test, shows it to be highly significant for 13 preparations. This confirms that the characteristics of dose and effect chosen are adequate.

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TABLE 1. Correlation Analysis of Dose-Effect during Study of Toxic Preparations from Rat Skin

No.	Preparation	No. of doses	No. of mice	Coefficient of equation		LD_{50}, μg protein	Spearman's coefficient	χ^2
				A	B			
1	Fraction 0.43 of BSE	3	20	3.608	1,122	17,4	1,000*	0,0009*
2	Toxin stabilized with albumin**	4	50	1,499	2,560	23,4	1,000*	0,0252*
3	Freshly prepared toxin	3	55	3,548	0,998	28,5	1,000*	0,0074*
4	The same	2	20	—5,053	6,750	30,9	—	—
5	BSE	7	111	0,886	2,386	53	0,830*	11,32
6	The same	6	102	2,477	1,387	66	0,886*	2,771*
7	The same	5	25	2,296	1,344	103	0,950*	0,4308*
8	Toxin from BSE stored for different times	4	80	0,073	2,297	140	1,000*	0,3170*
9	BSE	4	64	0,659	1,998	149	1,000*	0,1537*
10	The same	6	65	—4,616	4,393	155	0,943*	4,30
11	Fraction of 60-75 BSE	4	86	0,372	1,990	212	1,000*	4,33
12	Toxin containing burn antigens	4	88	—3,351	2,934	704	1,000*	0,4160*
13	Extract of normal skin	4	56	1,692	1,119	905	1,000*	0,9696*
14	BSE	4	49	2,980	0,679	945	0,650	3,836
15	Extract of normal skin	3	66	—0,554	1,840	1045	0,500	0,5350*
16	BSE	4	106	3,252	0,560	1310	0,800	4,20
17	The same	2	40	—7,413	3,200	7580	—	—
18	" "	3	52	4,237	0,195	8200	0,200	2,451*

Legend. *$P \leq 0.05$; **bovine serum albumin, 0.1% solution.

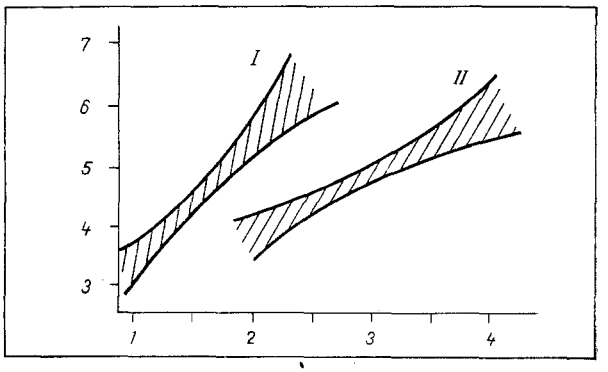

Fig. 1. Dose-effect dependence for preparations with high (I) and low (II) toxicity from skin of burned and normal rats. Abscissa, log of dose (in μg protein/mouse); ordinate, fraction of dying mice (in probits). Shaded area corresponds to y ± m.

The quantitative expression of toxicity satisfies the requirements of biological standardization and enables activity of preparations, including those prepared under different conditions, to be compared.

On the basis of the results of bioassay the preparations could be classed either in the highly toxic group (Nos. 1-11, LD_{50} from 0.017 to 0.2 mg) or the group with low toxicity (Nos. 12-18 LD_{50} from 0.7 to 8.2 mg) (Fig. 1). Regions corresponding to these preparations did not overlap, reflecting essential differences between the groups. The gradient of the straight line (B in the equation of the dose–effect dependence) was significantly greater for preparations of the first than of the second group. An increase in the dose of protein leads to an increase in magnitude of the toxic effect. It can be postulated that toxicity of the skin extracts was due to the action of at least two factors. One of them, which is stable but relatively inert, is present in extracts from both burned and normal skin. The other, burn toxin proper, arises as a result of the action of heat, possesses extremely high activity, and determines the qualitative difference between burned and normal skin. If for some reason or other burn toxin is destroyed the toxicity of the burned skin is sharply reduced, and it corresponds to the activity of normal skin.

Incidentally, if the content of the "normal" toxin increased in burns, the coefficients B in the first and second groups of preparations would be about equal, and the dose–response lines would be parallel. An example of this kind is dependence of the number of dying mice on the area of the burn, as Lawrence [10] found.

The present experiments showed that the toxic properties demonstrable in burn toxin depend most of all on the degree of purification of the extracts and on individual reactivity of the animals used as biological test objects. The length of keeping time of the purified preparations also has a marked influence on their activity. Meanwhile, the "all or nothing" nature of their action does not exhaust the wide range of pathological effects of burn toxin. In some experiments (results not given in Table 1), for instance, the experimental animals developed all the signs of toxic action: conjunctivitis, diarrhea, adynamia, seizures, but only during the first few hours after injection of burn toxin. Later these disorders disappeared and the animals did not die, either during the first 72 h or later. This phenomenon has not yet received a satisfactory explanation and it calls for further study.

Bioassay on mice with blocked RES thus provides additional opportunities for analysis of the factors of burn toxemia. The high toxicity of burned skin is due to a high-molecular-weight protein toxin. Meanwhile quantitative analysis of the dose–effect suggests the existence of a toxic factor in normal skin also, which differs from burn toxin in the conditions of its appearance and in the level of its activity.

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