great, the TC labeled with it become sensitive to exposures at the subthreshold level for unlabeled cells, and in turn this makes interpretation of the results more difficult [2]. Of all the radioactive labels used, ^{3}H-thymidine is least toxic for cells [1, 4] and, consequently, it is the most suitable label for the study of the genuine antitumor properties of Mph, unconnected with the effect of the isotope. The main disadvantage of labeling with ^{51}Cr, besides its toxicity, is the high spontaneous release of the isotope into the medium, which makes the estimation of lysis impossible when labeled TC are cultured in vitro for longer than 20 h.

It can be concluded from the facts described above that the technique for detection of lysis of ^{3}H-thymidine-labeled tumor cells, in the version described above, possesses high sensitivity for determination of MAF-induced cytolytic activity of Mph. This method can be used in other systems for analysis of tumor cell destruction under conditions requiring long-term culture in vitro.

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POSSIBILITIES OF PAIRED COMPARISON OF RECEPTOR BINDING PARAMETERS OBTAINED IN A SINGLE EXPERIMENT

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Radioligand methods of analysis have now achieved wide popularity. The concrete aim of many of the investigations conducted by these methods is to compare different groups of animals with respect to receptor binding parameters. In order to analyze the results by the use of existing statistical methods, both parametric and nonparametric, a definite number of values of the parameter to be studied must be obtained in each group. The most adequate approach is to determine these values in every animal. However, this is impossible in some cases, due mainly to the insufficiency of biological material obtained from each animal, when small brain structures are used, for example. The only way of carrying out the measurement in such a case is to pool the biological material in the group [6, 7]. This method can be used and its use is justified also in preliminary investigations.

With such an approach, to obtain the necessary number of values of parameters to be studied, the procedure often adopted is to repeat experiments of a similar kind, using the same pooled material. In that case, however, not every new experiment yields additional information about the object studied, but simply enables the error of the method to be assessed.

In experiments of this type, to obtain one or two extrapolation characteristics, many measurements are required. For instance, to determine two equilibrium parameters of receptor binding (K_D and B_{max}), traditionally done on a Scatchard plot, several scores of measurements

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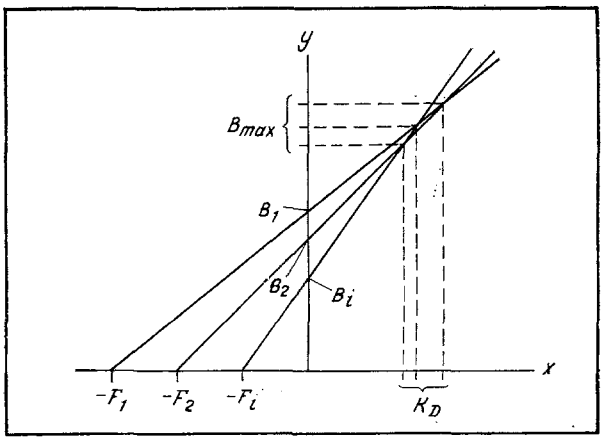

Fig. 1

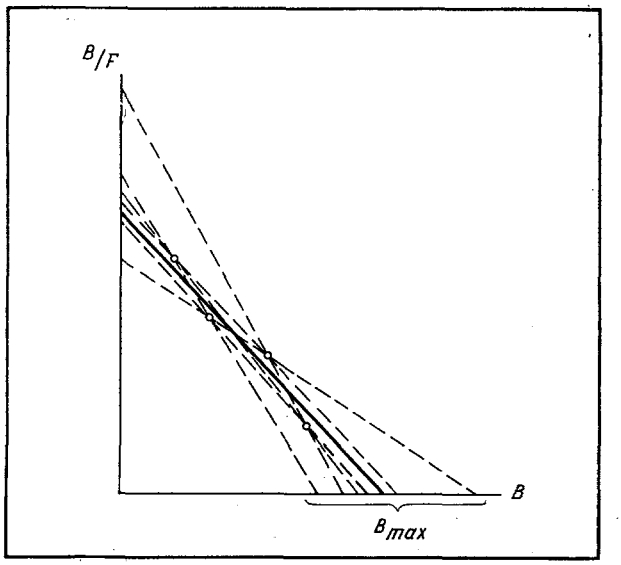

Fig. 2

Fig. 1. System of rectilinear Cornish-Bowden coordinates as used to analyze receptor binding.

Fig. 2. Experimental points and possible alternative ways of drawing straight lines through them on a Scatchard plot. Continuous line denotes graph obtained by method of least squares.

(n) of specific binding of the ligand in different concentrations are needed. Repetition of such experiments is extremely laborious and expensive. To this it may be added that sometimes, if the quantity of biological material available for the investigator is insufficient, he will be unable to undertake a statistical analysis of the results, even by repeating analogous experiments.

For the reasons given above, the use of methods of comparing control and experimental groups on the basis of extrapolation parameters obtained from a single pair of experiments is justified and necessary.

Analysis of receptor binding by the Cornish-Bowden method [1, 3, 4] provides such a facility. The essence of the method is as follows.

Straight lines are drawn in the plane of coordinates (Fig. 1) through the points $(-F_i 0_i)$ and $(0_i B_i)$, where F_i and B_i are the i-th measured concentration of free and bound ligand. The abscissa and ordinate of the point of intersection of each pair of such straight lines give values of K_D and B_{max}, respectively. The equation for the straight lines thus obtained is actually as follows:

$$y = \frac{B_i}{F_i} x + B_i. \tag{1}$$

Let us find the coordinates of the point of intersection of, for example, the first pair of straight lines:

$$\begin{cases} y = \dfrac{B_1}{F_1} x + B_1 \\ y = \dfrac{B_2}{F_2} x + B_2. \end{cases} \tag{2}$$

By solving the system of equations we obtain:

$$x = \frac{B_2 - B_1}{\dfrac{B_1}{F_1} - \dfrac{B_2}{F_2}} \tag{3}$$

TABLE 1. Binding Parameters of δ-Opiate Receptors of Two Groups of Rats, Analyzed by the Cornish-Bowden Method

No. of point	Group I		Group II		Number of inversions	
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
1—2	50.40	7125	5.02	1505	+	+
1—3	35.62	5118	8.27	2193	+	+
1—4	5.45	1025	4.60	1415	+	—
1—5	9.76	1609	6.57	1832	+	—
1—6	8.94	1497	4.73	1444	+	+
1—7	6.13	1116	4.30	1352	+	—
1—8	8.11	1386	4.68	1433	+	—
2—3	27.02	3994	18.18	4039	+	—
2—4	2.56	717	4.34	1374	—	—
2—5	6.75	1279	7.46	1975	—	—
2—6	6.54	1250	4.63	1430	+	—
2—7	4.33	955	4.08	1325	+	—
2—8	6.33	1222	4.59	1421	+	—
3—4	—0.49	394	0.95	828	—	—
3—5	3.80	955	4.95	1573	—	—
3—6	4.28	1017	2.82	1177	+	—
3—7	2.63	802	2.59	1134	+	—
3—8	4.70	1073	3.26	1258	+	—
4—5	—25.52	2263	95.52	15567	—	—
4—6	41.52	4855	5.06	1491	+	+
4—7	7.34	1225	3.84	1294	+	—
4—8	13.54	1883	4.79	1446	+	+
5—6	5.77	1171	0	783	+	+
5—7	1.40	692	0.65	887	+	—
5—8	5.75	1169	2.05	1110	+	+
6—7	—0,83	491	1.72	1023	—	—
6—8	5.74	1168	4.44	1404	+	—
7—8	—40.58	—3079	11.21	2235	—	—
Median	5.95	1170	4.591	1425.5	7	7

Legend. No. of point denotes serial numbers of two straight lines intersecting at that point. For the number of inversions, 7 corresponds to $P < 0.01$. Values of K_D given in nM and of B_{max} in fmoles/g wet weight of tissue.

$$y = \frac{B_1 B_2 \left(\frac{1}{F_1} - \frac{1}{F_2}\right)}{\frac{B_1}{F_1} - \frac{B_2}{F_2}}. \tag{4}$$

Equilibrium receptor binding is described on a Scatchard plot by the equation:

$$\frac{B}{F} = \frac{B_{max}}{K_D} - \frac{1}{K_D} B. \tag{5}$$

Rewriting Eq. (5) for the first pair of points, we have:

$$\frac{B_1}{F_1} = \frac{B_{max}}{K_D} - \frac{1}{K_D} B_1; \tag{6}$$

$$\frac{B_2}{F_2} = \frac{B_{max}}{K_D} - \frac{1}{K_D} B_2. \tag{7}$$

K_D and B_{max} can be expressed by means of Eqs. (6) and (7). We obtain:

$$K_D = \frac{B_2 - B_1}{\frac{B_1}{F_1} - \frac{B_2}{F_2}}; \tag{8}$$

$$B_{max} = \frac{B_1 B_2 \left(\frac{1}{F_1} - \frac{1}{F_2}\right)}{\frac{B_1}{F_1} - \frac{B_2}{F_2}}. \tag{9}$$

There is no doubt that Eqs. (3) and (8) and also Eqs. (4) and (9) are equivalent.

Here it follows that the abscissa and ordinate of the point of intersection of the straight lines in Cornish-Bowden coordinates determine the values of K_D and B_{max}, respectively. Thus, n of the measurements of specific binding made determined $N = n(n-1)/2$ values of K_D and B_{max}. On a Scatchard plot this would correspond to taking all possible straight lines through

any pairs of points and determining N values for K_D and B_{max} from one extrapolation experiment (Fig. 2). The N values of K_D and B_{max} thus obtained can be used to compare control and experimental groups of animals by the use of statistical methods. In the case of comparison by Student's t test the number of degrees of freedom will be determined from the number N of values of the parameters studied.

However, to use parametric statistical methods, the following assumptions must be observed [5]: 1) the distribution of errors of random measurements is normal; 2) the element of error is determined by one variable; 3) the corresponding weights of all measurements are known. Since it is by no means evident that all these demands are observed during the conduct of most experiments, according to Cornish-Bowden [5] it is not the arithmetic mean that is regarded as the value of the parameter studied, but the median; paired series of values of K_D and B_{max} must be compared by nonparametric statistical methods, of which the most convenient is the sign test [2].

One of our experiments to study parameters of specific binding of ^{3}H-D-ala-2-enkephelin-5-D-leucine (a high-affinity ligand of δ-opiate receptors, in the striatum of different groups of rats will serve as an example of the use of this method. To perform one experiment, the striatum of no fewer than four animals of each group had to be pooled. The study of the parameters of specific binding in this pooled material by the use of a Scatchard plot gave values for K_D and B_{max} of 5.95 nM and 1160 fmoles/g wet weight of tissue and 3.97 nM and 1380 fmoles/g respectively for the animals of groups I and II. The same parameters, determined by the Cornish-Bowden method as the median of the corresponding series, were 5.95 nM and 1170 fmoles/g and 4.59 nM and 1425.5 fmoles/g wet weight of tissue. Comparison of the series of values of K_D and B_{max} for animals belonging to different groups by the signs test (Table 1) showed a significant difference ($P < 0.01$) between the series of these values. It thus follows from this example that animals of groups I and II differed from each other statistically significantly with respect both to K_D and to B_{max}.

In conclusion, it must be emphasized once again that the above approach enables repetition of analogous experiments on pooled biological material to be avoided, while still allowing the possibility of statistical analysis of the results of receptor binding, so that the work is made less laborious and less expensive, and pooled biological material obtained from very small groups of animals can be studied. Another advantage of this method is the comparative simplicity of analysis of the results.

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