A METHOD OF PAIRED CONSECUTIVE COMPARISONS FOR USE IN MORPHOLOGICAL RESEARCH

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UDC 611-08:519.24

A simple method of quantitative analysis of morphological pictures is suggested which does not require direct measurement of structures and is particuarly suitable for the objective study of morphological changes produced experimentally by a given factor. The method is based on comparison of one feature or property of the process studied in each of m coded micropreparations consecutively with the analogous feature or property in all the remaining m-1 preparations.

The suggested method is an attempt to carry out an objective analysis of morphological structures in two comparable groups. It is based on the comparison of one particular feature or property of the morphological picture to be studied in each of m-coded micropreparations* consecutively with the analogous feature or property in all the remaining m-1 preparations. The morphological picture is formalized by comparing the preparations separately by each of a number of features. Since only two preparations are compared at one time, it is easier to assess the differences precisely. During evaluation of the preparations, that in which the preparation in which the feature is less marked or absent scores one +, while the preparation in which the feature is less marked or absent scores one –. If the feature is equally expressed, both preparations conventionally score one +. If some doubt regarding the score remains after comparison of two preparations, the evaluation is repeated a short time later. If the assessment does not agree with that made previously, in order to ensure that the total number of points is kept the same for all the preparations, which is important for subsequent statistical analysis of the results, each of the two preparations must receive a mean score: one 0.5+, the other 0.5–.

Each of the m preparations receives m-1 points for one feature, and within this number all combinations of plus and minus signs are theoretically possible (a total of m combinations). A score consisting entirely of plus signs is obtained by the preparation in which this feature is expressed to the maximum degree, and a score consisting entirely of minus signs by one in which it is expressed to the minimal degree or is absent; the remaining preparations have intermediate scores between these extremes. The method allows ranking of the preparations in the order of expression of each qualitative or quantitative feature of the phenomenon investigated.

The suggested method of paired consecutive comparisons can be used to investigate differences between different groups of cases. It is particularly suitable for the objective analysis of changes produced by experimental factors.

The total number of comparisons of m preparations is equal to the total number of combinations of m elements taken 2 at a time, and can be expressed by the formula: $C_m^2 = m!/2!(m-2)$ or $C_m^2 = m(m-1)/2$.

A test of the reliability of the method in connection with a study of bone regeneration during exposure to certain factors (see below) showed a high level of reproducibility of the results of repeated evaluations

* The micropreparations must be coded so that the investigator does not know until the end of the analysis to which of the two compared groups each of the preparations belongs.

Central Institute of Traumatology and Orthopedics, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 5, pp. 118-122, May, 1970. Original article submitted June 30, 1969.

TABLE 1. Assessment (in points) of Micropreparations of Callus in Rats with Fractures of the Tibia (28 days after operation) Based on the Feature "Relative Areas of Bands of Connective Tissue and Cartilage in Callus" (see correlation between areas of bands in different cases in Fig. 1)*

Series	Animal No.	Assessment	No. of corresponding to score in points and reflecting % of + signs in it	Ranks corresponding to area of "bands" and to their scores in points	Areas occupied by "bands" after magnification of preparations by 20 times (in mm²)†
Control	$K_4$5	++————————	0,222	2	350
	$K_4$4	+————————	0,111	1	0,0
	$K_4$3	++—+—+—++	0,667	6	850
	$K_4$2	+++++++—++	0,889	8	1 975
	$K_4$1	+————————	0,111	1	0,0
Experimental	$T_4$5	++——+———	0,333	3	600
	$T_4$4	++++—+—++	0,778	7	1000
	$T_4$3	++———+—+	0,445	4	675
	$T_4$2	+++++++++	1,0	9	3 625
	$T_4$1	++———+—++	0,556	5	800

*Animals of the experimental series received injections of tetracycline.

†Technique of measurement of areas: the micropreparations were fitted into a photographic enlarger and their images were thrown on to paper with a magnification of 20 times. The outlines of the "bands" were traced in pencil. The areas of the figures thus obtained were measured by a special grid placed on the paper. The grid consisted of a sheet of transparent plastic on which black dots, each corresponding to an area of 4 mm², were marked. Measurement of the area involved counting the dots above it.

of the same preparations by the same and by different investigators. The results of statistical analysis of these evaluations were identical. The reliability of the method also was tested by comparing the results of assessment of certain features with the results of their direct measurement. Areas occupied by newly formed bony structures in the region of an experimental bone injury and areas of bands of connective tissue and cartilage in callus were estimated (Table 1; Fig. 1). The comparison showed that the ranks corresponding to the scores obtained by the areas and to the results of their measurement were the same.

If differences were revealed by the suggested method, then they do in fact exist. If, however, no difference was established, this still does not mean that a difference cannot be present. It is perfectly possible that the difference can be found by direct measurement of the structures. The limits of the possibilities of the method lie within the limits of ability of an investigator, equipped with a microscope, to detect a difference. An essential factor for the successful use of the method is the precise formulation of the characteristics of the feature being compared, so that the possibility of a difference of interpretation cannot arise.

The task of comparison is made easier and the accuracy of the method is increased if the preparations are made and stained as identically as possible. If the number of pieces of tissue to be investigated is small, the method of embedding them simultaneously in the same block of paraffin wax, as suggested by Petrukhin and Gaidamakin [1], can be used.

When comparing micropreparations it is useful to have a binocular loupe giving several different magnifications, and also a comparing microscope, and to photograph total preparations (for example, sections through the whole of the callus). These measures give a survey of the whole of the region of interest and facilitate the task of comparison.

Nonparametric criteria of difference, such as Wilcoxon's criterion and the more sensitive (and powerful) X criterion of Van der Waerden, by means of which the central tendencies of two series of numbers are compared, can be used as the mathematical system which is essential for analysis of the scores obtained by the two groups of cases (the experimental and control series, for example). The technique of use of these methods and their logic have been described, in particular, by Urbakh [2].

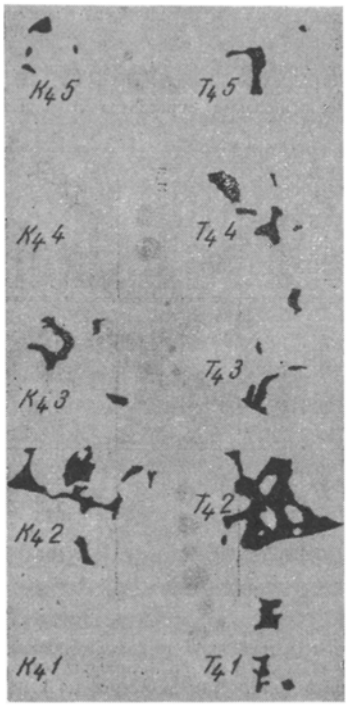

Fig. 1

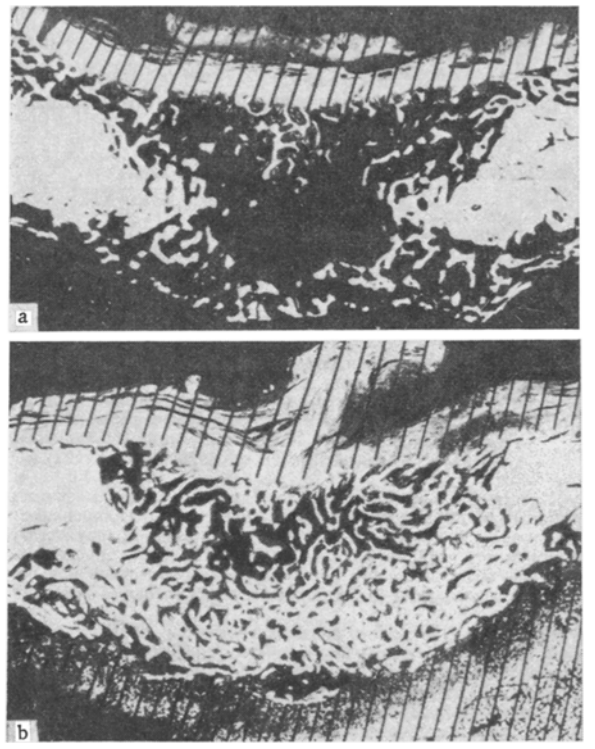

Fig. 2

Fig. 1. Relative size of areas occupied by bands of connective tissue and cartilage in histological sections of callus following a fracture of the tibia in rats 28 days after operation. Outlines of "bands" drawn on paper (by projecting images of micropreparations through photographic enlarger giving magnification of 20 times) and corresponding areas colored in ink. Photographs taken with identical enlargement. On left: control data; on right: experimental (injections of tetracycline). Bands K_41 and K_44 absent in rats.

Fig. 2. Histotopographical sections passing through the center of an experimental circular defect of the rabbit femur (14 days after operation). The bone marrow and periosteal growths of connective tissue were shaded on the original photomicrographs. Van Gieson, 15×. a) Control; b) experiment (injections of anabolic steroid). In the comparison of Fig. 2, a and b on the basis of degree of filling of the bone defect with newly formed bony structures, the point awarded was – for a and + for b. Conditions of photography: micropreparations stained by Van Gieson's method and inserted into a photographic enlarger instead of negatives, and then photographed directly on No. 7 photographic paper after enlargement by 15 times.

Before these criteria are used, the score obtained by each case must be converted into a number, e.g., that expressing the relative proportion of plus signs which it contains (Table 1).

The informativeness of the score can be increased by using a system of triple assessment of the two preparations to be compared: 1) to begin with the presence (awarded a +) or the absence (awarded a –) of the feature is determined; 2) the degree of expression of the feature is then assessed as described earlier. In the absence of the feature in one of the preparations it is awarded a second –. If the feature is expressed equally, both preparations receive a second +; 3) the degree of difference is then determined; if the feature in one of the preparations is expressed much more (the level of "significance" of the differences is established as the investigator wishes) than in the other, the first is awarded a + and the second a –; if the differences are small both preparations are awarded +; the preparation in which the feature is absent is awarded a third –. The evaluation by item 3 has the disadvantage of being to some extent subjective.

However, it is less subjective that the assessment in points of the absolute degree of expression of a morphological feature, for it requires the retention of only one level of differences in the memory.

After comparison of the two preparations each thus receives three points. If m preparations are compared, the assessment of each of them on the basis of one feature will include 3(m-1) points.

The suggested method (in its simpler form) was used by the writer to investigate the reparative regeneration of bone under the influence of certain physiotherapeutic factors (superhigh frequency, inductothermy), of anabolic steroids, and of tetracycline. For the quantitative analysis of the morphological pictures, in each case one section corresponding to the center of the experimental bone defect was chosen from the series of histological sections by a special method (when studying regeneration in the region of a standard circular defect of the femur in rabbits), or the section of callus with the largest area was chosen (when studying a subcutaneous fracture of the lower third of the tibia in albino rats).

The following features were subjected to quantitative analysis: the degree of osteogenic differentiation of the connective-tissue cells, the degree of functional activity of the osteoblasts (determined from their size and their degree of plasmatization) on the surface of the newly formed bony structures, the degree of filling of the bond defect with newly formed bony structures, the degree of endosteal and periosteal osteogenetic reaction near the bone defect, the degree of spread of necrosis of osteocytes in the cortical layer of the bond fragments, and the relative size of the bands of connective tissue and cartilage in the callus.

Analysis of these features was carried out on histological sections (Table 1; Fig. 1). To assess the degree of filling of the bond defect by newly formed bony structures photomicrographs of total sections also were used (Fig. 2a, b).

It is claimed that the method can be used to study changes not only in bone, but also in other tissues. It can also probably be used to assess the intensity of various histochemical reactions and also for the analysis of roentgenograms of bones.

LITERATURE CITED

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2. V. Yu. Urbakh, Biometric Methods [in Russian], Moscow (1964).