

striations (Figure 2E, arrows). These features may reflect 2 different modes of regeneration, which may concomitantly occur under our experimental conditions²⁰ (for review of muscle regeneration, see²¹⁻²³).

There remained relatively intact muscle fibres in the outer zone of the mass 3 to 12 days after transplantation (Figure 2B-F). These apparently intact muscle fibres seemed to be subjected to degenerative and regenerative processes 3 weeks after operation (Figure 2G). In some other transplants at this time, although the original muscle fibres were still definitely preserved, myoblasts or initial myotubes were scarcely found. From these findings it is likely that such slender fibres, especially those at the outer zone, may represent either less dedifferentiated fibres or simply atrophied fibres associated with temporary denervation¹⁶. Tissue reconstitution was practically complete 3 weeks after transplantation (Figure 2G). Infiltration of lymphocytes was not marked at any stage after transplantation.

Between 120 and 150 days after operation, the muscle suffered a 15% loss in number of fibres compared with the normal EDL muscle of contralateral side. Our transplant seems to be a fairly complete structural replica of the muscle removed (Figure 2K). In contrast with the results by other investigators^{13,14}, the tissue reconstitution of our transplant took place, keeping its internal architecture apparently intact. This may provide favorable conditions for studying the mechanisms of muscle regeneration or reconstitution²⁴.

Zusammenfassung. Bei Mäusegeschwistern führte homologe Transplantation kleiner Muskeln in toto zur Wiedererlangung ihrer vollen Funktion unter nervöser Kontrolle.

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²¹ G. C. GODMAN, in *Frontiers in Cytology* (Ed. S. L. PALAY; Yale University Press, New Haven 1958), p. 381.

²² E. J. FIELD, in *Structure and Function of Muscle* (Ed. G. H. BOURNE; Academic Press, New York 1960), vol. 3, p. 139.

²³ E. H. BETZ, H. FIRKET and M. REZNIK, *Int. Rev. Cytol.* 19, 203 (1966).

²⁴ B. M. CARLSON, *The Regeneration of Minced Muscles, Monographs in Developmental Biology* (Ed. A. WOLSKY; S. Karger, Basel 1972), vol. 4.

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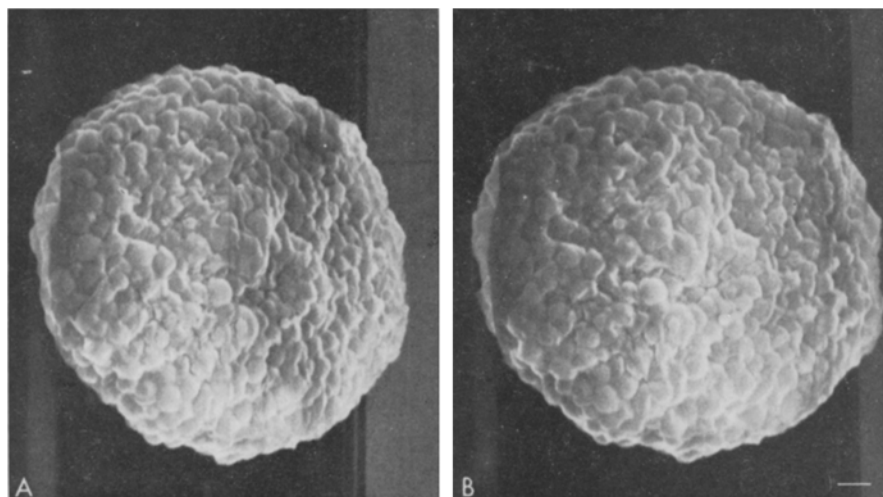
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Viewing Stereopair Micrographs: Facilitated Alignment through Use of Color Filter

Stereopair micrographs are readily obtained with the scanning electron microscope. They are particularly useful at high magnifications, where small surface features are not always easily interpreted from single micrographs. Fuller advantage can be taken of the high resolution obtained with this instrument if some further optical magnification is used while viewing the prints. A commercially available viewer (Wild Heerbrugg ST 4 mirror stereoscope) in fact, affords a choice of 3× and 8× oculars as adjuncts to the basic nonmagnifying unit. With some images, particularly where a nearly-repeating pattern is present, proper alignment of the images with

respect to each other becomes progressively more difficult as greater magnification is used because reference features are easily lost. Eye strain rapidly develops, perhaps because of involuntary efforts at focusing the images by eye.

It has been found that insertion of a lightly colored filter on one side of the optics greatly facilitates proper alignment by reducing the confusion between the two images and by emphasizing the individuality of the two members in paired reference features without interfering with perception of the three-dimensional effect. The filter can be removed after alignment.



Rat peritoneal fluid mast cell air-dried from 95% ethanol after fixation in mixed aldehydes and post-fixation in OsO_4 . Cambridge Stereoscan S4-10 SEM, secondary electron mode, 5,000× direct magnification. (These are contact prints.) Tilt angles: A = 42°; B = 28°. Marker = 1 μm .

Light yellow (Ednalite K2), light green (G1) and light blue (No. 80) photographic filters have proved more satisfactory than deep red (R2), but this may be a personal matter and possibly a function of one's color vision. An example of a stereopair for which this approach has proven successful is given in the Figure. This method has been found equally useful for sectioned material.

Résumé. On propose l'emploi d'un écran coloré, disposé devant un des oculaires, comme aide à la superposition d'images paires pour l'observation stéréoscopique d'objets examinés en microscopie à balayage. Le filtre permet un alignement facile et rapide, ce qui réduit

appréciablement la fatigue et les maux de tête que beaucoup ressentent au cours de ces manipulations.

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A New Method in Cytospectrophotometry

Classical cytospectrophotometry measures quantitatively the various chemical components existing in a given part of the cell. Unlike the classical methods, the method used here reveals the changes occurring in the proportions in which these different chemical components exist within the cell. As an example, the changes shown are induced by X-rays in the rat lymphocyte nucleus. One starts from the premises: 1. The lymphocytes of the same age, from the peripheral circulation, have the same chemical nuclear composition, i.e. the proportions of the nucleic acids, nucleoproteins, are the same in the all nuclei. 2. These proportions determine the colour of the nucleus in a panchromatic staining made with a complex stain, like Giemsa, in which the colouring substances (Eosine, Azur and Methylblau), have different affinities for the nuclear components. 3. By any chemical, physical or biological aggression (here a physical one), which provokes defense reactions, the proportions of different nuclear components must change, and these changes are revealed by changes in colour.

Thus, by determining with the 'Tristimuli' method^{1,2} the coordinates of 1 lot of lymphocyte nuclei panchromatically stained, one obtains in the colours triangle a rectangular-shaped confidence area, with the basis: $x_{med} \pm n\epsilon$ and the height: $y_{med} \pm n\epsilon$, in which reside, statistically significant, the great majority of the nuclei.

The measurements were made with a microspectrophotometer built by an adequate assembling of a Reichert Zetopan microscope with a Beckman B spectrophotometer, a Leitz Panphot microphotodevice and a Graphi-spot Sefram recorder. A group of 24 white mice, unselected as concerns age, sex or pathological antecedentes, was utilized. The programme of blood drawing from the tail vein and irradiation of the mice is presented in the Table.

In the unirradiated stage, 2 smears were made to check whether the injuries provoked to the tail do not induce unspecific chromatic changes; this was not the case. To avoid staining artefacts, the samples were chemically fixed immediately after smearing, and were stained, all together, in the same bath up to the 10th day. Microspectrophotometric curves were plotted from $\lambda = 400$ nm to $\lambda = 760$ nm for 2 different lymphocytes on each sample. The area explored in the nucleus was round with a diameter of about $3.5 \mu\text{m}$. When the peaks differed with more than $\lambda = 5$ nm, 2 supplementary curves for 2 other elements were plotted and introduced in the mean value. The transmission was calculated as function of the blank plotted in a free area in the neighborhood of the measured elements. The chromatic coordinates were calculated in conformity with the method of 10 selected ordinates.

Results. 1. The colour of Giemsa stained mouse lymphocytes occupies in the colour triangle a rectangular-shaped confidence area with the coordinates:

$$x \pm 3\epsilon = 0.2684 \pm 0.0102$$

$$y \pm 3\epsilon = 0.1782 \pm 0.0192$$

2. Irradiation with X-rays, 55 KV and 250 R, provokes its shift to the coordinates:

$$x \pm 3\epsilon = 0.2583 \pm 0.0105$$

$$y \pm 3\epsilon = 0.1563 \pm 0.0147$$

3. A new irradiation with the same dose maintains the size, direction and sense of the shift, but reduces the dispersion

$$x \pm 3\epsilon = 0.2583 \pm 0.0099$$

$$y \pm 3\epsilon = 0.1530 \pm 0.0087$$

4. The visual efficiency (the green stimuli value Y) also suffers a shift:

$$\text{before irradiation: } Y \pm 3\epsilon = 0.256 \pm 0.0054$$

$$\text{after 250 R: } Y \pm 3\epsilon = 0.222 \pm 0.0048$$

$$\text{after 500 R: } Y \pm 3\epsilon = 0.200 \pm 0.0021$$

The animals being unselected, the chromatic shift is induced only by irradiation and is specific to it. In our experiment the type of cell and of aggression, the stain, and the time delay between aggression and measurement have been taken without any optimization study; such a study could have determined: 1. the cell with the most significant reaction for every kind of aggression, 2. the stain with the most sensitive colour variation for every type of change occurring in the nuclear composition, and 3. the delay for which the chromatic shift has its maximal value.

Discussion. The capital letters stand for the nuclear components of a well-defined kind of cell, e.g. A_1 = the amount of DNA in the nucleus of lymphocyte No. 1. B_2 = the amount of B type protein in the nucleus of lymphocyte No. 2, we have:

$$A_1 + B_1 + C_1 + \dots + N_1 = O_1$$

$$A_2 + B_2 + C_2 + \dots + N_2 = O_2$$

$$\dots$$

$$A_n + B_n + C_n + \dots + N_n = O_n$$

¹ *The Science of Colour* (The Committee on Colourimetry of the Optical Society of America; Thomas Crowel Co., New York 1953).

² A. C. HARDY, *Handbook of Colorimetry* (The Technology Press M.I.T., Cambridge, Mass. 1936).