amplitude of a single frequency at two different points at known distances from the fibre. As an illustration, an experiment performed by HÅKANSSON has been worked out. The action potential has been recorded at 8 different distances (80–496 μ) from the centre of a frog muscle fibre in Ringer solution (Fig. 1). The fibre is considered infinitely thin in relation to the electrode distance. For the 2 nearest points (80 and 96 μ) this means an error, which is estimated at as much as 10% for the highest frequencies. With increasing y_0 the error falls rapidly. During the experiment the value for the velocity was determined at 2·0 m/sec.

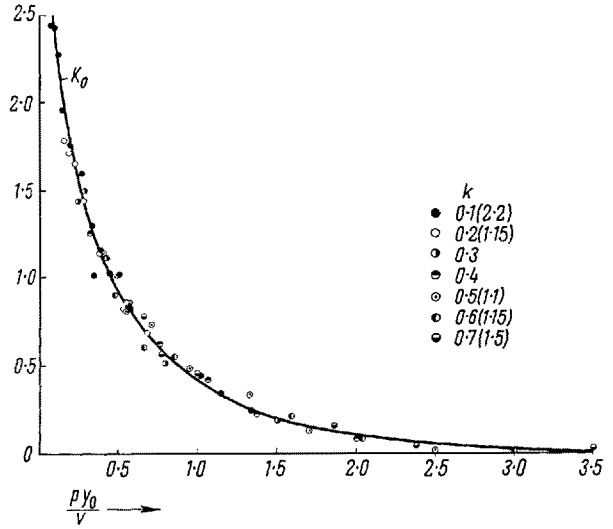

Fig. 2. – Full drawn line: the graph of the function K_0 (py_0/v). The number k is related to frequency p by $k = p/2\pi \cdot 4250$. A series of similar dots represents the decrease of a certain frequency (p) with increasing distance (y_0). In brackets: the factor necessary for curve fitting of the amplitude values.

The amplitudes of the curves (Fig. 1) have been measured at c:a 50 equidistant points on each. The absolute value of integral (2) has been calculated for seven frequencies in the range 425–2975 cps by means of a digital computer. Simpson's rule has been applied. The decrease of the amplitude for each frequency with increasing y_0 has to follow the K_0. The values for each frequency have thus been multiplied by a common factor so as to fit the same K_0. These factors are given in brackets in Figure 2. By means of this adjustment, the decrement curve for every frequency is brought into overlapping continuity with the following, and they all seem to follow a K_0 function well. However, the scale of K_0 that fits best is obtained by putting $v = 2{\cdot}57$ m/sec instead of 2·0 found at direct measuring. This discrepancy may be explained by a.o. the size of the electrodes (30–50 μ tip diameter), which reduces the exactness of the position determinations. The example demonstrates a consequence of LORENTE DE NÓ's field equation, namely that the decrement of the action potential is determined not only by the geometry at recording but also by the impulse shape and velocity.

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Zusammenfassung

Eine Modifikation der Feldgleichung von LORENTE DE NÓ wird einer Fourier-Transformation unterworfen. Dabei zeigt sich, dass das Dekrement einer jeden Frequenz eine K_0-Funktion in ihrem eigenen Maßstab darstellt. Die theoretische Dekrementkurve stimmt gut mit der experimentell ausgearbeiteten überein.

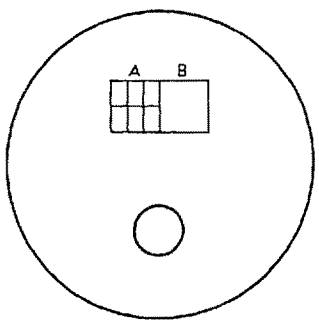
Determination of the Influence of Size in the Differentiation of an Isolate

Introduction. The significance of size in tissue differentiation is well known from various experiments.

DRAGOMIRROW[1] observed a failure of the pigmented epithelium of the eye to regulate into a cup if the isolate fell below a certain size. LOPASCHOV[2] found that an increase in the amount of head mesenchyme of an amphibian gastrula gave rise to a complexity of differentiation, whereas, one or two fragments developed into striated muscles. WEISS and AMPRINO[3] also found that below a certain size, there would be no differentiation of the prescleral mesenchyme into cartilage in the chick.

Results of ANDRES[4] and BERRILL[5] and others also show that the degree of differentiation is dependent upon the mass of tissue.

GROBSTEIN[6,7] and GROBSTEIN and ZWILLING[8] made an extensive study of the nervous tissue differentiation in the mouse embryonic shield and in the chick blastoderm. They found a decrease in the percentage of neural differentiation with a decrease of size of the explants. GROBSTEIN[6] observed that, when the fragmentation was carried out to 1/16 parts, differentiation was virtually eliminated with a 'dispersed cluster' and the percentage of neural differentiation was increased when these 1/16 parts were made into a 'close cluster'.



Mid gastrula of *Triturus alpestris* (A) represents the area which was subsequently cut into smaller bits and these are represented by horizontal and vertical lines. (B) represents the same area on the other side of (A). It was not cut into smaller parts and treated as the control.

It was decided to conduct experiments similar to GROBSTEIN and ZWILLING's[8] with amphibian presumptive neural plates by cutting a definite part of it into 1/2, 1/4, and 1/6 to determine the critical mass of these isolates to undergo neural differentiation. Some of these parts were left intact as controls.

Technique and experiments. A square piece of the presumptive neural plate (0·5 × 0·5 mm) was excised from *Triturus alpestris* gastrulae with rounded blastopore (comparable to 13C stage of *T. pyrrhogaster*, OKADA and ICHIKAWA[9]) as shown in the Figure (A). It was carefully

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[2] G. LOPASCHOV, Biol. Zbl. *55*, 606 (1935).
[3] P. WEISS and P. AMPRINO, Growth *4*, 245 (1940).
[4] G. ANDRES, J. exp. Zool. *122*, 507 (1953).
[5] N. J. BERRILL, *Growth and form* (Oxford Univ. Press, England 1945).
[6] C. GROBSTEIN, J. exp. Zool. *120*, 437 (1952).
[7] C. GROBSTEIN, Ann. N.Y. Acad. Sci. *60*, 1095 (1955).
[8] C. GROBSTEIN and E. ZWILLING, J. exp. Zool. *122*, 259 (1953).
[9] YO. K. OKADA and M. ICHIKAWA, Jap. J. exp. Morph. No. 3 (1947).

freed from underlying mesoderm and then cut at random into 1/2, 1/4, and 1/6 parts. A similar square piece from the other side (Fig. B) was left intact as control. Sterile Holtfreter buffered (after DEUCHAR[10]) was used throughout the experiments and this contained a mixture of 15,000 i.u./l of Penicillin and Streptomycin. These small isolates, together with the controls, were cultured for 48 h. During this period, some of the isolates lost quite a number of cells and were rejected. The healthier ones were wrapped up either with *Triturus* or with *Xenopus* ectoderm and cultured for another 72 h. *Xenopus* ectoderm was mostly used in these experiments. The materials were fixed in Bouins. Sections were cut at 10 μ and stained either with celestine blue or with celestine blue and eosin.

Results and discussion. The amount of differentiation of the isolates varied from neural palisade to neural tube. This is shown in the following Table.

Size of the isolate	Total number of cases	Number of cases with differentiation	% of different pieces
1	7	7	100·0
1/2	21	18	86·0
1/4	31	22	71·0
1/6	25	17	68·0

It is clear from the small series of experiments that neural differentiation gradually dropped with the decrease in the size of the isolate down to the limiting value of 1/6. This size allowed the isolate to remain viable and undergo differentiation. Fragmentation beyond 1/6 was rather difficult as nearly all the fragments disintegrated soon after they were made.

GROBSTEIN[7] observed that with the mouse and chick materials 1/8 was the 'critical mass' and 1/16 part never achieved this when left alone, but underwent neural differentiation when combined in 'close cluster'.

It seems from the present experiments that, in *T. alpestris* material, 1/6 is the 'critical mass' to undergo neural differentiation.

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Department of Zoology, Bangabasi College, Calcutta (India), March 31, 1959.

Résumé

On a détaché de l'ectoderme présomptif neural de migastrulae de *Triturus alpestris* des lambeaux de grandeur définie qui ont été sectionnés au hasard en fragments d'une demi, d'un quart et d'un sixième, puis emballés dans de l'ectoderme de *Xenopus* ou *Triturus* et mises en culture dans la solution d'Holtfreter. Il a été constaté que le % des cas où une différenciation neurale s'est produite décroît avec la taille du fragment.

[10] E. DEUCHAR, J. exp. Biol. *30*, 18 (1953).

The Effect of Age on the Responses of Animal and Plant Tissues to Metabolic Inhibitors

An observation that the respiratory responses of tissues to metabolic inhibitors showed similar changes with age in plants and animals led to a series of experiments summarised below.

(i) Respiration rates of slices from the brains of 1–3-day and 14–17-month old rats were determined in the presence and absence of different metabolic inhibitors (Table I and II). The rats were killed by a blow on the neck, the brain dissected out, halved, and weighed. Each half was cut into thin slices and placed in one of a pair of Warburg vessels containing Krebs-Ringer solution[1] plus 2% glucose, and one of which contained in addition the dissolved inhibitor. Oxygen uptakes were determined at 37·2°C by conventional methods[1]. Studies using cyanide were carried out according to ROBBIE[2]. Two sets of experiments were performed, one in 1956 using 14-month old rats (Table I) and one in 1958 using 17-month old rats (Table II).

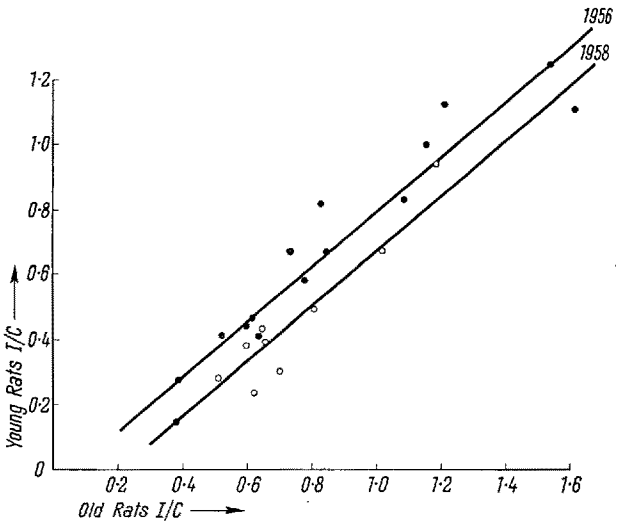

Figure 1.—The ratio (I/C) of respiration rate of young and old rat brain in presence (I) or in absence (C) or various respiratory inhibitors.

A statistical analysis of the differences in oxygen uptake for control (C) and inhibited (I) units was considered. The variability of this difference over the set of inhibitors and concentrations used, was greater for young rats than for old rats. Consequently separate within-animal standard errors, based on 23 degrees of freedom, are presented for testing the overall differences ($C - I$). The mean values for all 24 trials are presented in Table III. The presence of inhibitors reduces markedly the oxygen uptake of young rat brain tissue. This contrasts with the much smaller and non-significant effect of inhibitors on old brain, where the mean oxygen uptake is much lower than that of young rat brains.

When the ratio of 'inhibitors' to 'control' respiration rates (I/C) was calculated for each concentration of each inhibitor for old rats (denoted by x) and for young rats (denoted by y) and regression lines of the form $y = a + bx$ were fitted for the 1956 and 1958 sets of data (Fig. 1), it was found that a pooled analysis could

[1] W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric Techniques* (Burgess Publish. Co., Minneapolis 1957).

[2] W. A. ROBBIE, *Methods in Medical Research* (Edit. V. R. POTTER, Year Book Pub., Chicago 1948).