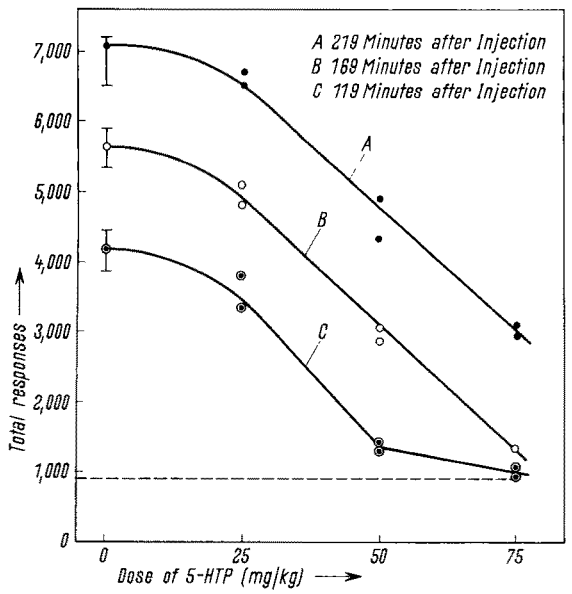

Relationship of dose of 5-hydroxytryptophan and amount of responding in one pigeon. Each curve presents the number of responses made in the indicated times after injection. Values are adjusted in terms of the pre-injection levels of responding. Each point represents a single dose, except, in the case of the control and saline values where the median and inter-quartile range are given for 6 sessions. Control sessions were taken from the days preceding each 5-HTP injection

Two groups of pigeons were used; one, in which the 5-HTP was administered orally ($n = 4$) and the other, a group in which the compound was injected intramuscularly ($n = 4$). The results of a typical experiment on a single pigeon in the latter group are shown in the Figure, where the abscissa axis represents the dose of 5-HTP administered (mg/kg) and the ordinate axis the total number of responses made by the bird in a specific interval after injection. The control data was calculated by determining the median and quarter percentile values of data obtained on days when saline or no 5-HTP was administered. Single doses of 25, 50, and 75 mg/kg of 5-HTP were injected into the breast muscle over a period of several months with each dose given at least twice. The

When the 5-HTP was administered orally, the same type of relationship was obtained except that higher doses of 5-HTP were required to produce a decrease in pecking. The appearance of the behavioral effect of the 5-HTP injection occurred later than when administered intramuscularly.

Studies are now under way in which serotonin, 5-hydroxytryptophan plus Marsilid, and serotonin antimetabolites are being administered to pigeons under similar conditions in an attempt to determine if the behavioral effects observed are central, peripheral, or both.

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Zusammenfassung

Bestimmung der Verhaltensänderung bei Tauben, durch Injektion von 5-Hydroxytryptophan, der Vorstufe von Serotonin hervorgerufen. Mit steigender Dosis von 5-Hydroxytryptophan wird eine entsprechende Frequenzverminderung des Pick-Verhaltens ausgelöst.

Autoradiographic Study of Cartilage Differentiation in Organ Culture

Recent work has shown that radio-sulphate is taken up specifically by cells and tissues known to contain large amounts of sulphomucopolysaccharides[1]. Studies on young embryos have not only demonstrated a high activity in embryonic cartilage, but have suggested a specificity of precartilaginous cells in high incorporation of the isotype[2]. The present note describes the situation in explanted embryonic somites differentiating in the conditions of organ culture.

Materials and Methods. Most of the experiments used chick embryos of $4^1/_2$ days incubation (stage 24–25[3]), but some 11-day mouse embryos were also employed. Clean isolation of the somitic material was assisted by trypsin treatment[4]. The liquid culture medium was composed of

Results. The specimens which were labelled with radiosulphate for 16 h gave sufficient density of grains for a qualitative description to be based on a subjective inspection of the autoradiographs. After labelling for 2·5 h, the activity was not high enough to make a definite estimation, although the results were generally similar to those from the specimens with 16 h labelling. No essential difference was noted between the cultures of mouse and chick somites.

In the specimens labelled for 16 h immediately after the isolation of the somites, and then fixed, marked accumulation of the isotope in the chondrogenic blastema was already quite indisputable, although a fairly high activity was found all over the tissue fragment (Fig. 1). In the chondrogenic area the grains were mainly distributed in the cytoplasm of the precartilaginous cells and in the intercellular space, while the activity in the nucleus was very doubtful. It is noteworthy that the cytoplasmic activity was estimated to be relatively higher than intercellular activity. The number of the grains per single precartilage cell was counted as 3·2 times as many as those per single non-chondrogenic cell in the somites. On observation by conventional histological methods, however, the chondrogenic area of the present specimens appeared merely as a compact assembly of undifferentiated precartilaginous cells, no metachromatic substance being detectable after staining with toluidine blue. Thus, the selectivity of the precartilaginous blastema for radiosulphate, which was manifested both in the intact animal[7] and in the dispersed tissue culture[8], can be well confirmed in the condition of organ culture. The specificity of the precartilage cells has also been demonstrated by an immunological approach, in which the positive reaction of these cells to fluorescent labelled antiserum against the differentiated cartilage has been observed.

After another 48 h' cultivation in non-radioactive medium (total culturing time, ca. 64 h), cartilage with metachromatic matrix was developed; and the radiosulphate taken up in the initial labelling was well maintained in this tissue (Fig. 2). On the other hand, a slight loss of activity was noted in the non-chondral tissues. As a result, the localization of radiosulphate in the cartilage appears to be very precise. Observation under high magnification revealed that the activity of the intercellular material was very much intensified in comparison with the previous stage, whereas the intracellular activity became lower. This must indicate a shift of the isotope from the interior of the precartilage cells into the intercellular matrix of the newly differentiated cartilage.

In some cases, cultivation in non-radioactive medium was prolonged up to 96 h after labelling. The autoradiography of these cases shows the preservation of radiosulphate in the cartilaginous matrix, only a very small number of grains being found on the cells. But, as a general impression, the density of grains per unit area of the matrix appeared less than that in earlier stage. The swelling of the intercellular matrix caused by the deposition

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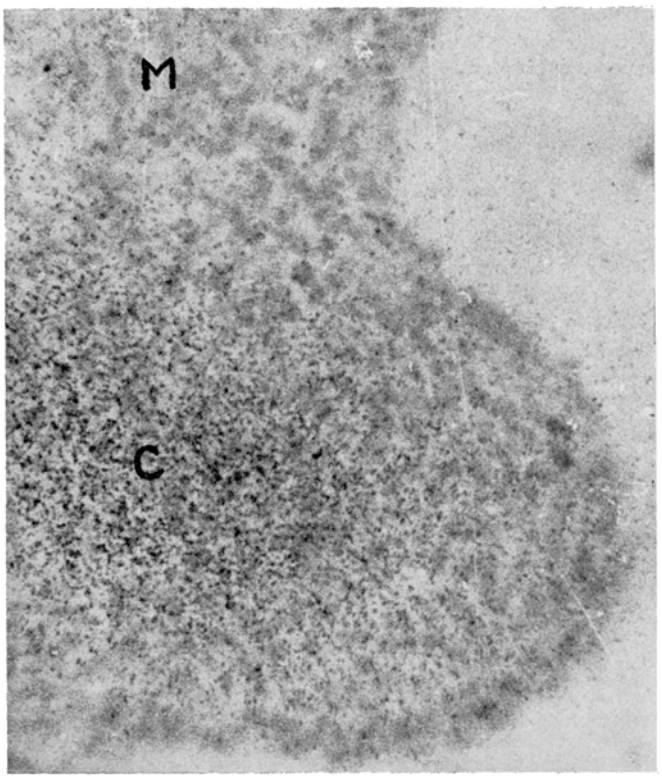

Fig. 1. – Autoradiograph of the culture fixed immediately after labelling in the initial stage of culturing. High uptake of radiosulphate is seen in the chondrogenic blastema, the grains being mainly distributed over intracellular sites (Haematoxylin and diluted eosin, chick)

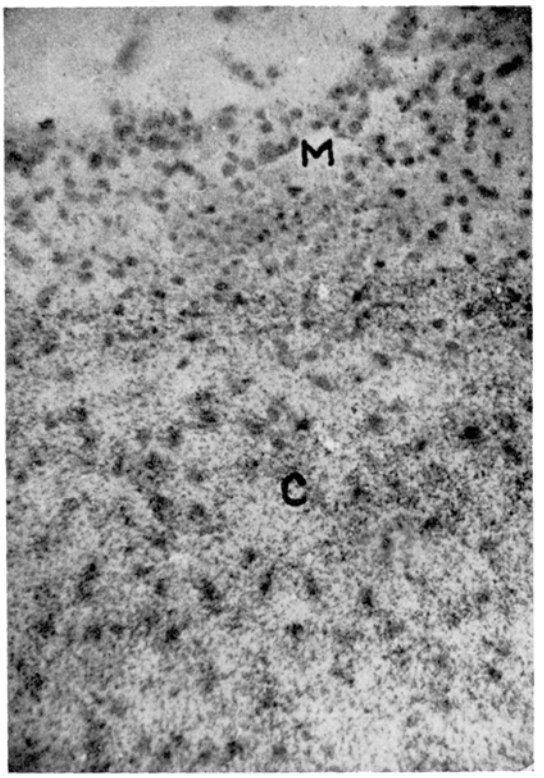

Fig. 2. – Autoradiograph of the culture fixed 48 h, after labelling in the initial stage. Note that the intercellular activity is higher than in the previous stage shown in Figure 1 (Haematoxylin and diluted eosin, chick)

C: Precartilaginous (in Fig. 1) or cartilaginous area (in Fig. 2). *M:* Myogenic area

of newly synthesized ground substance would bring about the dispersal of the grains per unit area; thus the actual decrease in the total activity in the cartilage may not be as great as suggested by the grain density.

Mesonephric tissue also picked up some radiosulphate under organ culture conditions, and the activity was still preserved after 2 days' culturing in non-radioactive medium, by which time typical mesonephric tubules were formed. The activity of this tissue, however, was less than that of even the non-chondral components of somites, and far below that of the chondrogenic tissue. This fact demonstrates that the specificity of the uptake of radiosulphate by different kinds of tissue is well maintained in isolated tissue fragments.

Discussion. The uptake of radiosulphate by cells and subsequent movement into matrix have been previously shown in the differentiated cartilage of the adult mouse[10] and of the late chick embryo[11]. The present results indicate that a similar shift of tracer takes place in the initial differentiation of precartilage into cartilage with metachromatic matrix.

No metachromatic substance is detectable in the precartilaginous tissue, and it therefore seems likely that the substance into which the isotope is incorporated is a precursor of the sulpho-mucopolysaccharides of the future matrix. The existence of such a precursor has been suggested from the results of PAS staining[12], while it has also been claimed that chondroitin sulphate does not appear until after cartilage becomes histologically differentiated[13].

Acknowledgments. The author wishes to thank Prof. C. H. WADDINGTON for suggesting this problem and for his encouragement; he is also indebted to Dr. J. L. SIRLIN for introducing him to the technique of autoradiography. The author has been the holder of a Macauley Fellowship from Edinburgh University, which has made his research in the U. K. possible.

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Institute of Animal Genetics, Edinburgh, August 17, 1959. * On leave of absence from the Zoological Institute, Faculty of Science, University of Kyoto (Japan).

Zusammenfassung

Aufnahme und Verhalten von ^{35}-S-Sulfat wurde in einer Organkultur sich differenzierender Somiten von Hühner- und Mäuse-Embryonen untersucht. Die Vorknorpelzellen zeigten den grössten Isotopeneinbau im Vergleich zu andern mesodermalen Zellen. Nach Vollendung der Knorpeldifferenzierung fand sich das von den Vorknorpelzellen aufgenommene Isotop in der interzellulären Matrix.

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PRO LABORATORIO

An Adjustable Synchronized Electron Flash for Phase Contrast Micrography

The growing use of phase contrast microscopy for the study of brain tissue made a difficulty which was felt in the photographic recording of the pictures obtained. In this form of microscopy, the preparations studied are often not fixed and usually show intense Brownian movement, which may make an extremely short exposure time indispensable. However, this demand is very difficult to meet, since with the same source of light, the pictures have a lower light intensity than those of common microscopy with diaphenous light. Phase contrast microscopy therefore often requires flash exposure with extra strong light intensity. The commercially available flash apparatus for micrography are not suitable for this purpose, because the exposure time cannot be regulated

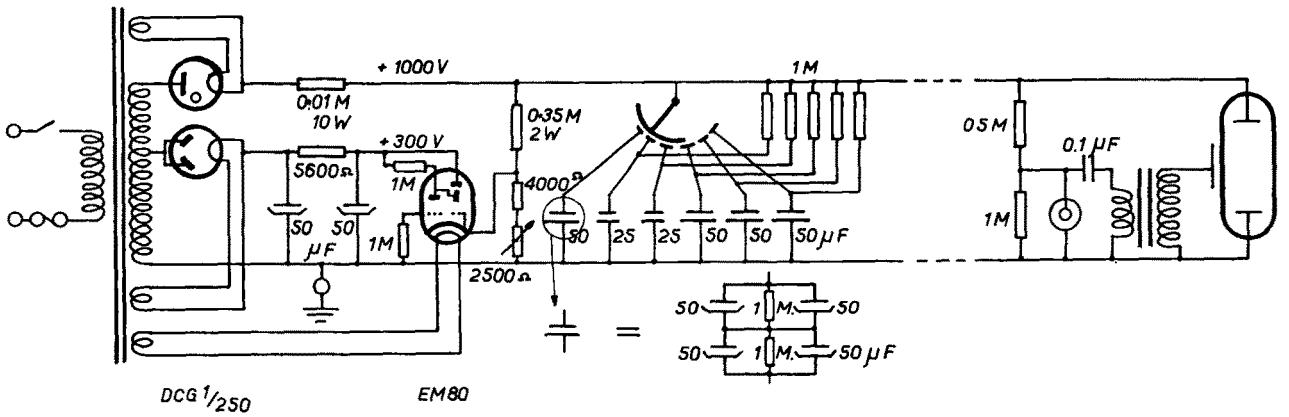

Fig. 1.—Wiring scheme of an electron flash for phase contrast microscopy

A double rectifier delivers two voltages one of + 1000 V and another of + 300 V with regard to a common negative pole. Between the output of + 1000 V and earth, a set of condensors is connected with a total capacity of 50 μF. With the aid of a switch, one to six condensors of 50 μF can be added in parallel. To prevent sparks, which occur when unloaded condensors are added to the original set by means of the switch, the positive pole of each condensor is connected via a resistor of 1 MegOhm with the wire of the + 1000 V. In this way all condensors are charged to + 1000 V, except very shortly after the discharge.

Between earth and the sparking plug of the electron flash bulb, the secondary coil of a high voltage transformer is connected. The primary coil of this transformer is connected in series with a condensor of 0·1 μF and in parallel with a resistor of 1 MegOhm, which is part of a resistor bridge of 1·5 MegOhm between 0 and + 1000 V. One of the poles of the condensor has now in this way a potential of 2/3 × 1000 V. The resistor of 1 MegOhm can be short-circuited through a switch, at which moment the potential of this condensor drops suddenly from + 1000 V to 0 V. This discharge causes sufficient potential to ignite the flash bulb. In this circuit, the discharge of the condensors causes an amount of light which is approximately proportional to the total capacity of the condensors.