does not appear, therefore, to be associated with a measurable rise in brain amine levels.

The data presented here show that monoamine oxidase inhibitors elevate norepinephrine and serotonin brain levels and suppress the tonic extensor component of electroshock seizures in rats. In contrast, reserpine releases brain amines and facilitates electroshock seizures. The results do not permit the conclusion that the effects of the drugs are attributable to changes in serotonin or norepinephrine, but suggest the possibility that a substance released by reserpine and metabolized by monoamine oxidase is involved[11].

Further studies have demonstrated that JB 516 and JB 807 suppress the tonic extensor phase of convulsions evoked by intravenously administered pentylenetetrazol in mice. A detailed account of these results will be published elsenwhere.

The possible clinical usefulness of monoamine oxidase inhibitors in epilepsy is now under investigation.

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Laboratory of Chemical Pharmacology, National Heart Institute, U. S. Department of Health, Education, and Welfare, Bethesda (Maryland), December 11, 1958.

Zusammenfassung

Inhibitoren der Monoaminoxydase haben eine antikonvulsive Wirkung, die mit einer Erhöhung des Serotonin- und Noradrenalingehaltes im Gehirn verbunden ist. Da anderseits Reserpin durch Freisetzung zu einer Verminderung dieser Amine im Gehirn führt und die Krämpfe verstärkt, ist es wahrscheinlich, dass gewisse physiologisch aktive Amine bei der Entstehung experimenteller Konvulsionen eine entscheidende Rolle spielen. Die Resultate stützen die Annahme, dass gewisse Formen von Epilepsie auf eine lokale Störung im Stoffwechsel der Gehirnamine zurückzuführen sind.

[11] In preliminary experiments, harmaline, a reversibly acting monoamine oxidase inhibitor [12] was found to raise the brain levels of amines and block the tonic extensor phase of electroshock seizures in rats.

[12] S. Udenfriend, B. Witkop, B. G. Redfield, and H. Weissbach, Biochem. Pharmacol., in press.

* Fellow, Life Insurance Medical Research Fund.

Regeneration of Cartilaginous Matrix from the Dissociated Chondrocytes *in vitro*

Recently, it has been well established that the constituent cells of a precartilaginous blastema can, after dissociation, reaggregate into a chaotic assemblage and finally differentiate into organised cartilage[1]. However, if the dissociation of the cells is done in the stages before the onset of visible differentiation of cartilage, the second process, that is, the transformation of the initial reaggregates into organised cartilage, can be considered as essentially the same process as the differentiation of cartilage from the intact precartilage[2]. This provides, therefore, little direct information as to the relative importance of the intercellular matrix and the constituent cells in the maintenance of the tissue architecture. It seemed to the present author that useful information on this point might be obtained by studying the process of tissue reconstitution from differentiated chondrocytes after freeing them from the already deposited matrix. In this paper, the results of an experiment along these lines will be described.

Experimental. Suspensions of chondrocytes were made from femora and tibiae of chick embryos after 8 days' incubation. In this material, a considerable amount of cartilaginous matrix, which gives strong metachromatic staining with toluidine blue, is deposited intercellularly. The demarcation of diaphysial and epiphysial zones is quite clear in femora, and hypertrophy of chondrocytes is conspicuous.

The procedure of making cell suspension by trypsin digestion was essentially the same as that established by Moscona[3] except for slight modification[2]. With the present material, however, much more prolonged treatment by trypsin than that applied to the precartilaginous material was necessary, in order to obtain a soft mass which could then be pipetted with a small-bore pipette or syringe to make a cell suspension. Approximately 30 min pretreatment in Ca- and Mg-free saline, and a further one hour's incubation in the trypsin solution, proved adequate. The periosteum, which was developed as a thin conical cylinder around the diaphysis of the femur, was so resistant to enzymatic digestion that it remained as a hard sheath, even after such prolonged treatment. The final suspension did not, therefore, contain any cells of this tissue.

The description of the sequence of events which will be given below is based on 34 specimens fixed in various steps of the experiment, together with some smeared preparations of the discrete cells of the suspension. For the histological examination, alternative sections were made, half being stained with haematoxyline (Meyer or Heidenhain) and the other half stained with toluidine blue for the detection of cartilaginous matrix.

The observation of the smeared preparations of the discrete chondrocytes showed only the simple spherical cells which are commonly found in cell suspensions of any kind of embryonic tissue, without showing any structural characteristics of the initial chondrocytes, i. e. hypertrophic and flattened cells (Fig. A). Mortality of cells in the suspension was much higher than in suspensions made from earlier embryonic stages. A higher mortality of discrete cells obtained from older tissue was also described for nervous tissue[4]. The fatal effect of prolonged trypsin treatment and trauma by vigorous pipetting are likely to be the reasons for the high percentages of damaged cells.

Reaggregation of the discrete cells was tried in two ways: (1) The cultivation of the very slimy soft mass obtained after centrigugation of the suspension, or (2) the cultivation of a dense suspension within a hollow ground slide or solid watch-glass filled with the liquid medium. A mixture of modified Tyrode[5], horse serum, and embryo extract in equal proportions served as the culture medium.

During 24 hours' cultivation within the liquid medium, the isolated cells assembled spontaneously into small reaggregates, whilst the slimy aggregates formed by centrifuging lost their stickness and became more or less firm, probably owing to the contraction of a very viscous substance which surrounds the intact cells and appears first after centrifuging. These initial aggregated masses

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[2] T. S. Okada, Exp. Cell Res. *16*, 437 (1959).

[3] A. Moscona, Exp. Cell Res. *3*, 535 (1952).

[4] M. Cavanaugh, Exp. Cell Res. *9*, 42 (1955).

[5] M. Jones and S. L. Bonting, Exp. Cell Res. *10*, 631 (1956).

were colonies of loosely packed spherical precartilage-like cells; the intercellular space gave no metachromatic staining with toluidine blue (Fig. *B*). Neither hypertrophied nor flattened cells were found in this stage. The periphery of such reaggregate was already surrounded by one or two layers of flattened mesenchymal cells and was reminiscent of perichondral organisation.

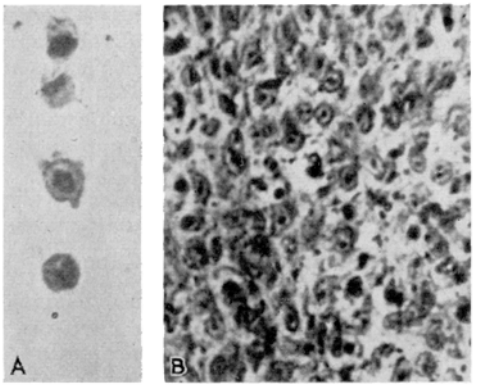

Fig. A.—Smear of a cell suspension of the chondrocytes from 8-day embryo (Meyer Haematoxyline)

Fig. B.—Initial reaggregate of the dissociated chondrocytes, showing the assemblage of spherical cells (Heidenhain Haematoxyline)

Fig. C.—Regeneration of matrix after 2 days' culturing on lens paper (Toluidine blue)

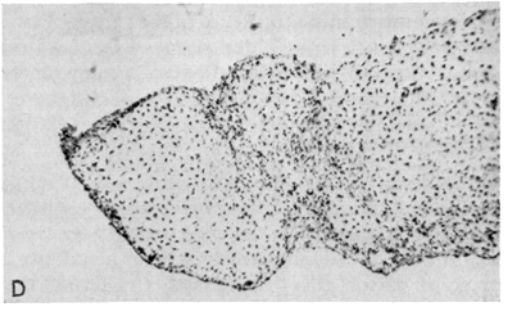

Fig. D.—Colony of cartilaginous nodules formed after 3·5 days' culturing on lens paper (Heidenhain Haematoxyline)

After further cultivation of the reaggregates in fesh liquid medium for 24 hours, several small foci of chondrification began to appear; here the intercellular space gave faint metachromatic staining with toluidine blue. At this stage the reaggregates were transferred to lens papers placed over the liquid medium[6]. In this culture condition chondrification proceeded quite well. Sections of the cultures fixed after 36 hours' cultivation upon lens paper showed the production of conspicuous amounts of metachromatic substance within the intercellular spaces (Fig. *C*).

Cultures on lens paper lasted for 3·5 days without changing medium. At this terminal stage, all cultures were fixed, and on examination were found to be colonies of cartilaginous nodules surrounded by a sheath of flattened mesenchymal cells, which now can be safely called perichondrium (Fig. *D*). All chondrocytes had regained their original structural characteristics.

Discussion. The results described above demonstrate that the isolated chondrocytes of fully differentiated cartilage, which were obtained after complete trypsin digestion of the intercellular matrix, are able to reaggregate and to re-establish the original histological architecture of cartilage by regenerating the intercellular matrix. It is noteworthy that this process of the matrix regeneration is always accompanied by some structural changes in the constituent cells; the simple spherical form which chondorcytes assume in the initial reaggregates, changes and is replaced by the original cellular structure. The loss of the structural characteristics of chondrocytes, accompanied by the disappearance of the intercellular matrix, has been recorded in the culture of cartilaginous fragments in a medium containing excess amounts of Vitamin A[7]. In these cases, however, both the recovery of synthetic activity in regeneration of matrix and the return of original cellular structure were reported as very incomplete, after transferring into normal medium[8], whereas in the discrete chondrocytes here described, the recovery was found to be nearly complete both of their morphology and of their function of secreting the intercellular matrix. Therefore, the altered chondrocytes appearing in these two different experiments must be considered as strikingly different from each other in their capacity for differentiation.

In the sequence of the events described in this paper, no metachromatic substance was found in the intercellular regions of the cell assemblages fixed soon after reaggregation of the dissociated cells. This fact suggests that the contiguity and stability of the chondrocytes in the initial reaggregate may not be maintained by the same cementing substance as that filling up the intercellular spaces of the original cartilage.

Since the metachromatic matrix always appeared in aggregates fixed at a later time in the course of the experiments, one has the impression that mutual assembly of the discrete cells may be the prerequisite condition for the recovery of their synthetic activity. At present, nothing definite can be said on this point, as we do not know yet whether or not the dissociated chondrocytes, had they been cultured in such exprimental conditions as to prevent their reaggregation, could regain their cellular activity of secreting the matrix substance as such. One of the specimens which was fixed at 24 h after culturing the centrifuged slimy mass may, however, have some bearing on this point. In this reaggregate so many damaged cells were incorporated, that healthy cells were singly dispersed among them, except for one locus, where 10–15 healthy cells were assembled, and it was just in this region that chondrification was observed to have began. This observation, although far from conclusive, seems to suggest that there is a limiting minimum mass, below which the regeneration of matrix and re-establishment of cartilaginous tissue architecture will not occur[9].

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Zusammenfassung

Aus 8 Tage alten Hühnerembryonen wurden durch Verdauung der intrazellulären Matrix des differenzierten Knorpelgewebes dissoziierte Chondrozyten gewonnen. Diese Chondrozyten schlossen sich wieder zu Kolonien lose gepackter sphärischer Zellen zusammen. Durch Regeneration der interzellulären Matrix bildete sich in den Organkulturen solcher Reaggregate die ursprüngliche Knorpel-Architektur wieder aus.

Normal Count and Physiological Variability of Rabbit Blood Basophils[1]

Rabbit blood contains an exceptionally great number of basophil granulocytes. Basophil countings from rabbit blood smears have been performed by various authors, yielding highly varying results. Using a direct chamber counting method[2], the present study of the basophil count in rabbit blood under physiological variations was undertaken.

Methods. 159 six-months-old white rabbits of both sexes and of known homogeneous breed were used. They were separately caged, at a temperature of 20° ± 1°C, fed on oats, hay, and sugar beets. The animals were carefully handled and trained to accommodate in blood-sampling boxes. For blood sampling, the ear was tapped lightly by finger to stimulate blood circulation, and a prick with a 14-gauge needle or a small incision in one of the ear veins usually secured a free flow of blood drops. After discarding the first drop, 20 µl of blood was drawn into a standard blood pipette, and quickly transferred into a glass tube containing 180 µl of a modified Moore & James toluidine blue solution[3]. The tube was shaken until complete hemolysis of the red cells. A solution of 0·05% toluidine blue in 50% watery propylene glycol was used in few cases. The diluted blood was then transferred by capillarity to Fuchs-Rosenthal counting chambers (0·2 mm deep) and the cells allowed to settle. Counting slides were kept in a covered Petri dish floored with a damp filter paper to avoid evaporation. Two chambers of 16 mm² area each were counted for basophils, and two big squares of 1 mm² area each for obtaining total leucocyte counts. A binocular microscope with × 10 oculars and a × 10 achromatic plane objective was used; a blue filter contrasted the metachromatically stained basophils against the faintly blue-stained leucocytes of different types.

Results. Figure 1 presents distribution curves, as well as mean values ± standard error, standard deviations and range of 9 a.m. basophil counts (absolute and differential) of the first blood samples ever taken from all rabbits under study. The curves are more or less positively skewed[4].

A very slight variation in the morning count from day to day was encountered (Table). A study of diurnal variation in the number of basophils in 58 male rabbits, however, revealed a statistically significant ($P < 0{\cdot}001$) increase in late afternoon (3 p.m.) as compared to the comparatively low morning (9 a.m.) count (Fig. 2).

[1] Aided by grants from the Danish Rheumatism Association and Eli Lilly and Co., Indianapolis, Ind., U.S.A., to Dr. ASBOE-HANSEN.

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[4] The advice and help of Miss HANNE BACKHAUSEN with statistical analysis are gratefully acknowledged.

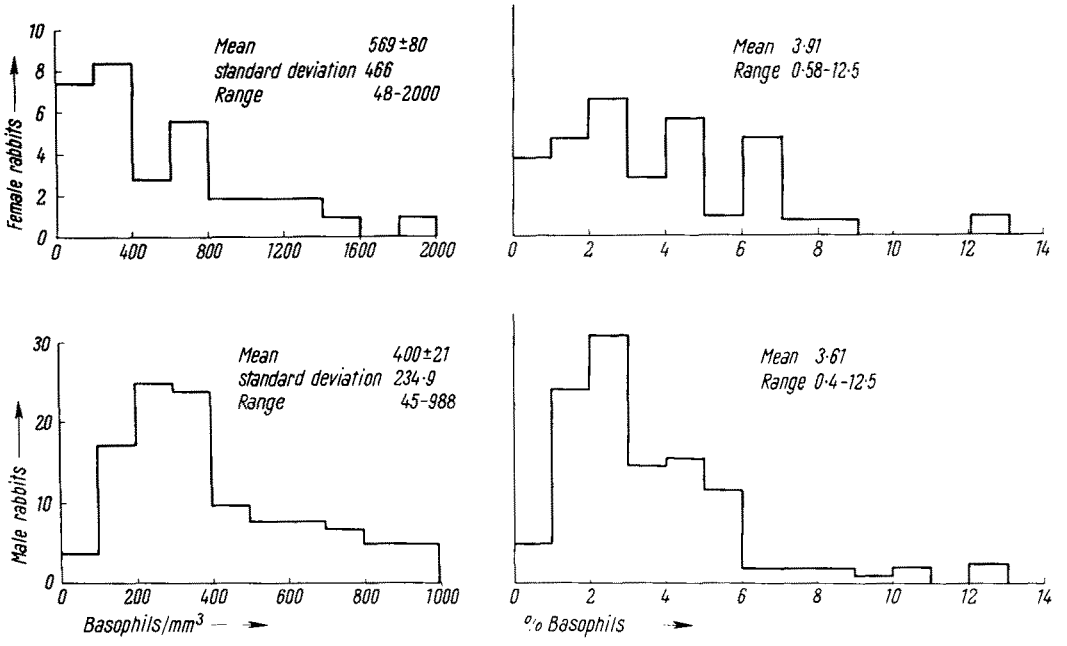

Fig. 1. – Distribution curves of first absolute and relative basophil leucocyte morning (9 a.m.) counts ever taken from 116 male and 43 female adult rabbits. Mean ± standard error, standard deviation, and range are indicated.