(Armour & Co.) (100 γ/ml in 0,03 *M* Phosphatpuffer von pH 7,0), so gelang es, die Stacheln abzubauen, ohne dass die Lysozym-resistentere Sporenmembran angegriffen wurde; die Sporen erschienen nach der enzymatischen Behandlung unbestachelt, was als Beweis für die Entstehung der Stacheln aus der Hyphenmembran gewertet wurde (Abb. 2b).

Wie weitere, noch nicht abgeschlossene Untersuchungen zeigten, existiert neben diesem Modus der Stachelbildung ein zweiter, bei dem die Stacheln Auswüchse der *Sporen*membran sind. Nach Freiwerden der Sporen bleiben leere, bestachelte Hüllen zurück. Hierbei könnte es sich um Sporen mit mehreren, mindestens aber mit *zwei* Membranschichten handeln. Eine sichere Entscheidung über die Membranverhältnisse werden Ultradünnschnitte durch verschiedene Sporentypen ergeben.

Dank gebührt meinem Institutsdirektor, Herrn Prof. Dr. BORRISS, für die anregenden Diskussionen, Herrn E. FISCHER für seine Unterstützung bei der Anfertigung der elektronenoptischen Aufnahmen, sowie Fräulein BOLDUAN für ihre gewissenhafte Mithilfe.

F. MACH

Botanisches Institut der Ernst-Moritz-Arndt-Universität zu Greifswald, 7. Mai 1959.

Summary

It is demonstrated, by electron-optical photography in a Streptomyces strain of the morphological section 'spira', that the spines of the spores arise from the membrane of the aerial mycelium hyphae and can be decomposed by lysozyme.

Localisation of Alkaline Phosphatase in the Development of the Vertebral Column in Chick

The association of the alkaline phosphatase in the mechanism of bone formation has been shown to be an important feature (MOOG[1] and LORCH[2]). A follow-up of the alkaline phosphatase reaction in the development of the vertebral column of chick embryos will be worthwhile because no detailed histochemical study of phosphatase in the chick vertebral column has so far been made, although MOOG[1] gives a general account of phosphatase in embryogenesis of the chick. It is important to bear in mind the changes of phosphatase reaction in the sclerotogenous tissues undergoing histogenesis and forming the elements of the vertebral column.

This study is based on White Leghorn eggs. A series of embryos ranging from 24 h to 17 days of incubation were collected. The total number of embryos studied was 32. They were fixed in chilled 80% alcohol. Serial sections were made of 10 μ thickness. For the alkaline phosphatase reaction of the cell, a modified technique of Gomori was followed, comparing each reaction with controlled slides.

Positive homogeneous reaction in the sclerotogenous cells. In the beginning of the development of the vertebral column, the sclerotogenous cells show a positive homogeneous reaction of phosphatase activity. In the embryos of 24 h and 48 h, the transverse sections show that the whole field is uniformly positive in phosphatase localisation. The cells concentrate round the notochord to form the perichordal tube which is positive in the enzyme reaction. Further development of the perichordal tube is possible by subsequent accumulation of the sclerotogenous cells round the notochord. At this stage, the cells of the centrum are highly positive in reaction; they seem to be more reactive to this enzyme than those of the neural arches of the two sides. The cells of the basidorsal at the top may be slightly less in enzyme reaction than at its base. The area of the future supradorsal gives a feeble reaction.

Diminution of the phosphatase reaction. The changes of the alkaline phosphatase reaction in the cells of the developing centrum and arches become detectable next in the embryos of sixth and seventh day of incubation when the sclerotogenous cells are transformed into protochondrium. The development of the centrum and arches has now become more well defined by the cell aggregations. The most noticeable change in the conversion of the osteogenous cells is the evidence of a decrease of the phosphatase activity in them. At the periphery of the developing vertebra, the surrounding cells continue to show rich phosphatase accumulation. Decrease of phosphatase reaction in the future vertebral elements is well marked. The positive reactions of the peripheral cells are also clear.

Furthermore, the centrum proper, the fibrous arch elements of the neural arch, the fibrous crescent shaped intercentrum and the packing cells continue to be rich in the phosphatase activity.

Whenever there is a secondary plastering of cells, these cells are invariably positive in reaction. The notochord cells, at this stage, do not show any phosphatase activity. In an embryo of 10 days, sections demonstrate the weak nature of the phosphatase activity.

Revival of phosphatase activity during calcification. — In the general background of a reduced phosphatase reaction of the cellular matrix of the centrum and the arches, a number of reactive cells appear. The peripheral cells continue to be positive in the enzyme activity. This marks a transformation of the sclerotogenous cells and a revival of the phosphatase activity in the different elements of the vertebral column. The appearance of phosphatase reaction in the cells coincides with the process of calcification and marrow formation. The reappearance of phosphatase has been observed to be largely extracellular in nature. Gradually the number of reactive areas increases and at the 7th day of incubation large number of cells accumulate alkaline phosphatase round them. It shows that the phosphatase activity is noticed around the cells at a time when they are undergoing a process of calcification. Phosphatase-laden osteoid gradually achieves thickness. A number of reactive cells appear among the negative ones in the centre of the centrum and arches of the vertebral column.

Discussion. — The important feature of this study centres round the phase-variation of the alkaline phosphatase activity of the cells in the developmental process of the vertebral column. To start with, the sclerotogenous cells show positive alkaline phosphatase reaction. However, this positive reaction gradually fades away to an almost negative degree when the cells reach a stage of protochondrium. The process of losing phosphatase reaction of cells at the protochondrium condition has also been observed previously by MOOG[1] in chick embryos and by LORCH[2] in fish embryos. Reappearance of phosphatase in the cells at a later stage of calcification and marrow formation is a feature of utmost importance. DANIELLI[3] has also emphasised this point and has recorded: 'This

[1] F. MOOG, Biol. Bull. *86*, 51 (1944).
[2] I. J. LORCH, Quart. J. micr. Sci. *90*, 381 (1949).
[3] J. F. DANIELLI, *Cytochemistry – A Critical Approach* (1953), p. 65.

intracellular phosphatase is not associated with bone formation and may largely disappear, to be succeeded by a second wave of formation of phosphatase, a great part of which is found outside the cells'. A point of discrimination is to be made about the nature of phosphatase reactions found in the cells before the disappearance and after the reappearance. Before the disappearance, the alkaline phosphatase localisation is seen to be mostly intracellular, whereas after the reappearance it is extracellular in nature. However, the deep phosphatase localisation of the mesenchymatous cells is throughout an important peculiarity of this study. The plastering cells are mesenchymatous cells and that is why they always show positive phosphatase reaction. The study confirms the view that alkaline phosphatase is associated in the development of the bone element of the vertebral column, and at the time of calcification, there is a phenomenon of reappearance of phosphatase in the cells. This study also supports the view previously expressed by LORCH [2] that, in the absence of extracellular phosphatase, there cannot be formation of bone.

This problem was suggested by Dr. SIVATOSH MOOKERJEE and I am indebted to him for his constant help and encouragement.

ASOKE BOSE

Department of Zoology, Presidency College, Calcutta, June 19, 1959.

Zusammenfassung

Zu Beginn der Entwicklung der Wirbelsäure im Hühnerembryo findet sich die alkalische Phosphatase intrazellulär. Mit der späteren Verkalkung und Entwicklung des Knochenmarks ist die Reaktion extrazellulär nachweisbar.

Protective Action of Irradiated Bone Marrow Cells

The potential of the host's immunogenic tissue surviving following lethal irradiation determines whether a foreign graft will take. If it is feasible to completely suppress the immunogenic system of the host by x-ray, the immunologic potential of the graft determines how long the host will survive. Provided that the remaining host's immunogenic tissue after lethal irradiation is negligible, 'takes' and progressive growth in the new hosts of foreign bone marrow, if still functional, would ensue[1]. Even if the implants were hypo-functional, or afunctional, they would nevertheless modify the irradiation response.

Since it appears undesirable to inject cells fully capable of reacting immunologically against the host, in the present study, bone marrow was subjected to irradiation *in vitro* prior to transplantation. The degree of protection afforded by isologous (DBA_1), homologous (C57BL/6) and heterologous (WR) bone marrow exposed to fractionated doses was assessed from the number of mice (DBA_1) surviving lethally irradiated controls. The effects of all treatments are presented in tabular form (Table).

The observations made indicated that: (1) isologous bone marrow cells exposed *in vitro* up to 950 r exercised some protective effect in lethally irradiated mice, and (2) irradiation of homologous and heterologous bone marrow before injection, (a) did not render them incapable of reaction against the host; (b) were destroyed, or, (c) could not repair the host's radiation-injured cells.

Assuming that radiation is cumulative, the total amount of x-radiation to the animals' bone marrow is obtained simply by adding the doses of lethal body irradiation and the *in vitro* exposure of the isologous bone marrow injected[2].

[1] G. W. SANTOS and L. J. COLE, J. nat. Cancer Inst. *21*, 279 (1958).
[2] W. SHELDON, K. C. ATWOOD, M. L. RANDOLPH, and H. E. LUIPPOLD, Biophys. biochem. Cytol. *4*, 265 (1958).

Experiment	No. of Mice	Treatment of host; X-irradiation*	Treatment of graft; X-irradiation	M S T of mice dying of exposure	No Survival over Total number
I Isologous bone marrow** (DBA/1)	5	509 r	No bone marrow	11	0/5
	5	509 r	None	Permanent	5/5
	5	950 r	475 r	28	1/5
	5	950 r	950 r	14	1/5
	5	950 r	1425 r	10	0/5
II Homologous bone marrow (C57B/6)	5	950 r	No bone marrow	11	0/5
	5	950 r	None	22	2/5
	5	950 r	475 r	15	0/5
	5	950 r	950 r	9	0/5
	5	950 r	1425 r	19	0/5
III Heterologous bone marrow	5	950 r	No bone marrow	11	0/5
	5	950 r	None	12	0/5
	5	950 r	475 r	9	0/5
	5	950 r	950 r	10	0/5
	5	950 r	1425 r	10	0/5

* $LD_{100/11}$ = 950 r; rate 190 r/min. 250 Kvp, 15 ma; 1/2 mm Cu + 1 mm Al filters ** 10^8–10^{12} cells/0·5 ml saline i.v. 24 h post-irradiation