intacts. Dans ce sens, nos observations sont un argument supplémentaire en faveur d'une équivalence de ces structures avec les mitochondries. Sans les identifier avec les mitochondries tissulaires, dont l'organisation est très compliquée, nous désirons rappeler ces analogies en proposant le terme de *chondrioïdes*.

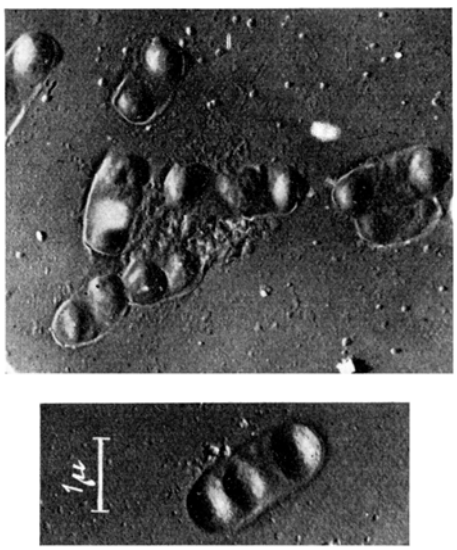


Fig. 5 et 6. Répliques *Escherichia coli* B (en croissance exponentielle): Révélation des chondrioïdes par la méthode de séchage lent.

Les *nucléoïdes*, colorés par la méthode hydrolyse-Giemsa de PIEKARSKI-ROBINOW[1] consécutive à un séchage lent, sont nettement disposés «entre» les chondrioïdes, soit plus près du centre de la cellule; ils ne sont donc nullement superposables aux chondrioïdes.

Nous remercions la «Fondation Fritz Hoffmann-La Roche pour l'expansion en Suisse du travail scientifique exécuté par équipe» pour l'aide généreuse qu'elle nous a apportée.

E. KELLENBERGER et LUCIE HUBER

Institut de physique, Université de Genève, le 6 mai 1953.

Summary

When slowly dried up in their culture medium, bacteria show chondrioids (equivalents of mitochondria) which are entirely different from the nucleoïds (equivalents of nuclei). Observations are made in the electron microscope by means of the replica technique, or directly with the phase contrast microscope. The chondrioids contain the Formazan deposited by vital reduction of Tetrazol.

[1] C. G. ROBINOW, Addendum de DUBOS, *The bacterial Cell* (Harvard University Press, Cambridge, Mass., 1949).

Observations on the Fixation *in vitro* of Uranium to Sections of Bone

Evidence that a great part of the uranium injected into the living animal is readily deposited in bone tissue has been supplied by W. F. NEUMAN *et al.*[1]. The same authors have analysed *in vitro* the interaction between bone minerals and phosphate buffers containing radioactive P; they maintain that fixation of isotopes to bone tissue involves both an exchange reaction between cations of the solid and of the fluid phase and a process of recrystallisation.

The results of an autoradiographic study of ground sections of bone treated *in vitro* with radiocalcium and radiophosphorus have been reported in previous papers[1]. These experiments have consistently shown that a relatively greater amount of labelled Ca and P gets fixed *in vitro* (as well as *in vivo*[2]) to recently laid down, less calcified structures of bone in comparison with more calcified, older structures. The present paper is a preliminary report on the results of an autoradiographic study of the fixation of U to bone sections *in vitro*.

Technique. Ground sections (from 30 to 45 μ in thickness) of compact bone were treated with a $1^0/_{00}$ solution of uranyl nitrate in a 0·025 N buffer of $NaHCO_3$. Whole fresh bone, bone fixed in 95° ethanol or formalin, and bone freed of its organic components were studied. After U treatment, the sections were washed for 2 or 3 days in frequently renewed distilled water, air dried at room temperature and tightly pressed in a photographic printing frame against the emulsion side of C_2 Ilford Nuclear Research plates, 50 μ of emulsion thickness.

A quantitative analysis of the U deposited in the various structures of the same section of bone was attempted by counting the *alpha* tracks in a sufficient number of microscopic fields. In order to compare the *alpha* activity of bone sections which underwent different treatments (see further on), a single large section of uniform thickness was divided into various sectors. Each sector was separately treated. All the sectors were then exposed together for the same length of time on the same autographic plate, and the *alpha* activity of *structurally corresponding regions* in the various sectors compared.

Quantitative historadiography (ENGSTRÖM) was applied to the bone sections prior to treatment with U, to estimate the degree of relative calcification of recently built and old structures[3].

Results. (1) The uptake of U for unit volume of section is from 30% to 100% greater in recently built primary or secondary bone than in old bone tissue. The degree of calcification in recently laid down structures resulted to be from 10% to 25% lower than in the neighbouring old bone tissue.

The *distribution* of U in bone seems, therefore, to be qualitatively identical to that previously described for Ca^{45}. However, the differential uptake in recent as compared to old bone tissue is much greater for radiocalcium than for U (Fig. 1 and 2).

(2) Removal of the organic components from bone ground substance increases the ability of bone tissue to fix U. The amount of U deposited in sections ashed at 600° to 700°C for 3 to 4 h is from 15% to 30% greater than in control sections of whole bone; 4 times greater than in whole bone in sections boiled for 24 h in distilled water[4]; from 6 to 8 times greater in sections treated with boiling glycol/K hydroxide (GABRIEL).

[1] M. W. NEUMAN and W. F. NEUMAN, J. biol. Chem. *175*, 711 (1948). – W. F. NEUMAN, M. W. NEUMAN, and B. J. MULRYAN, J. biol. Chem. *175*, 705 (1948). – W. F. NEUMAN and B. J. MULRYAN, J. biol. Chem. *185*, 705 (1950); *193*, 237 (1951).

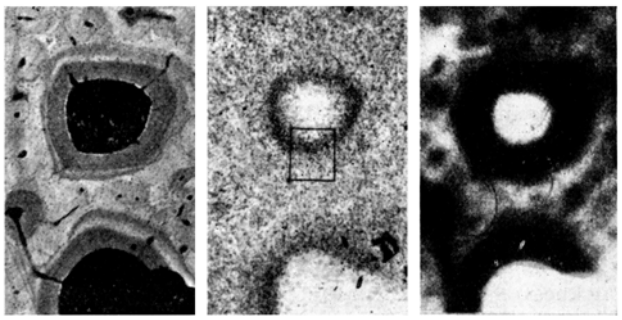
[1] R. AMPRINO, Exper. *8*, 20 and 380 (1952); Z. Zellforsch. *37*, 240 (1952).

[2] For the distribution of P *in vivo*, *cf.* B. ENGFELDT, A. ENGSTRÖM, and R. ZETTERSTRÖM, Bioch. Bioph. Acta *8*, 375 (1952).

[3] Technical details, *cf.* R. AMPRINO and A. ENGSTRÖM, Acta Anat. *15*, 1 (1952). – R. AMPRINO, Z. Zellforsch. *37*, 144 (1952).

[4] According to DALLEMAGNE *et al.* (Arch. intern. Physiol. *57*, 411 [1950]) this treatment removes from bone ground substance nearly all the organic components, a remarkable amount of minerals and a great part of the carbon dioxide.

The *distribution* of U is uniform throughout the sections freed of organic components; thereby showing that in these conditions the same amount of U for unit volume of tissue is fixed both to old and recently built structures.



A *B* *C*

Fig. 1.—Ox, 3 years. The same area of compact bone from the diaphysis of the right metatarsus. *A*, negative historadiograph; *B*, autoradiograph after U treatment; *C*, autoradiograph after Ca^{45}·22:1.

(3) The rate of depositions of U is high within the first hour of treatment of the section, then it decreases. No further fixation of U seems to occur after 10 to 12 h treatment. Comparable results were obtained both with whole bone and bone freed of its organic components.

(4) U replaces the Ca^{45} previously fixed to bone ground substance. The decrease of *beta* activity of Ca^{45} is clearly

Fig. 2.—Higher magnification of the area indicated in Figure 1 *B*. 225:1.

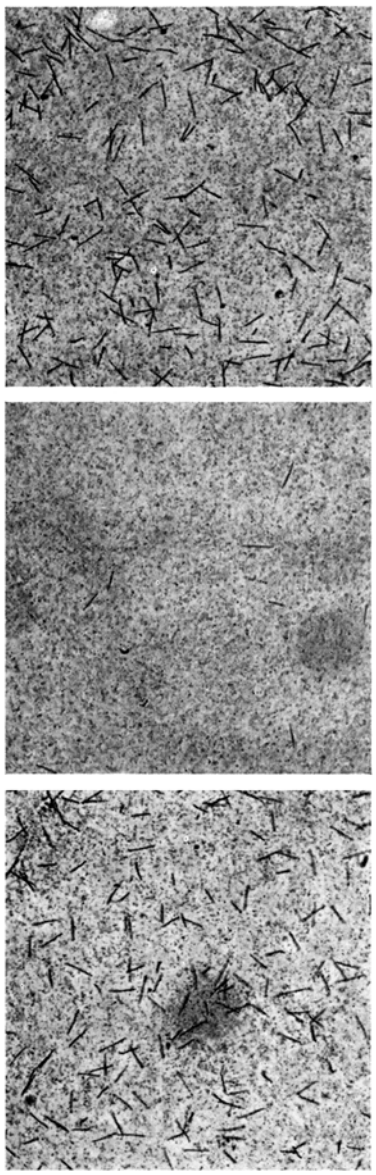
detectable after 1 h treatment with U $1^0/_{00}$ and becomes much more apparent after 6 h treatment. It seems, however, that U cannot replace all the Ca^{45} previously fixed to a section of whole bone, even after a long treatment with frequently renewed solutions of U.

No change in the *relative distribution* of Ca^{45} in the various structures of bone after prolonged treatment with U has ever been detected.

(5) Concurrent fixation of both Ca^{45} and U takes place in sections treated with a solution of the two cations. The uptake of both elements is relatively greater in recently formed structures than in old bone tissue.

Whenever sections of whole bone are treated first with radiocalcium and then with U *and* Ca^{45}, both a further fixation of Ca^{45} and an easily detectable fixation of U are demonstrated.

(6) Sections of whole bone treated with U can still fix a remarkable amount of Ca^{45} (Fig. 1 *C*). The uptake of the latter is relatively greater in recently formed structures. It must be stressed that the greater part of radiocalcium which is deposited in these conditions does not replace the U. In fact, no significant decrease of the number of *alpha* tracks per unit volume of tissue takes place after treatment with $CaCl_2$ (Fig. 3, *A*, *C*).



A

B

C

Fig. 3.—Calf, 10 months. Metacarpus. Autoradiographs of structurally corresponding (e.g. old primary bone) regions of three successive sectors of the same section treated with U. Sectors *B* and *C* kept per 6 h in $NaHCO_3$ and respectively in $CaCl_2$ 0·025 N after U treatment; *A*, control sector.

On the other hand, in bone sections freed of organic components, the treatment with $CaCl_2$ determines a reduction of more than 30% of the previously fixed U.

(7) Nearly 90% of the U deposited in sections of whole bone can be readily removed by treatment with a 0·025 N solution of $NaHCO_3$ (Fig. 3, *A*, *B*) or Na_2CO_3.

No detectable removal of previously fixed radiocalcium occurs after treatment with $NaHCO_3$ or Na_2CO_3.

No removal of U occurs when sections are treated with 0·025 N solutions of sodium chloride or sodium phosphate.

(8) Nearly all (e.g., from 95% to 98%) the U fixed to sections of whole bone can be removed by glycol-ashing or microincineration at 700°C. About 70% of the U fixed to previously glycol-ashed sections can be removed by further glycol-ashing.

(9) The U is removed almost entirely from sections of whole bone by decalcification with HNO_3 1:1,000.

(10) A remarkable amount of U gets fixed to decalcified sections of bone, e.g., from 60% to 80% of the U which is bound to notdecalcified control sections. The distribution of U is uniform in recent and old structures in decalcified bone. The greater part of the U thus fixed can be removed by the use of HNO_3 (1:1,000 × 20′)

Fixation of U also takes place in strips of the fibrous layer of periosteum peeled off from bone compacta. The treatment for 20′ with HNO_3 1:1,000 greatly reduces the *alpha* activity of these pieces.

In *conclusion*, U and Ca^{45} show much the same distribution patterns in sections of bone treated *in vitro* with solutions of these elements. The results obtained with the use of U under some conditions (see experiments reported at 7, 9, 10) are, however, apparently different from the results of similar experiments made with radiocalcium[1]. Such differences seem to indicate that the fixation of the two cations to bone ground substance *in vitro* may not obey the same rules.

R. Amprino

Institute of Anatomy, University of Turin, March 15, 1953.

Résumé

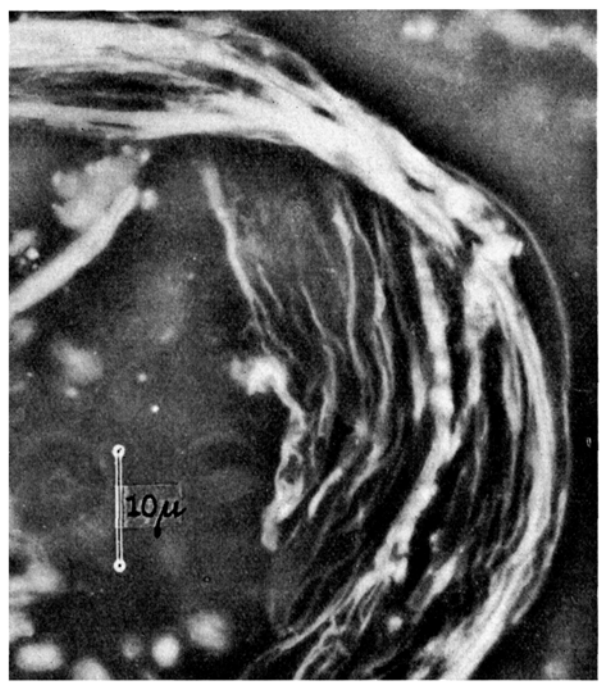
L'auteur a étudié par la méthode autoradiographique la fixation *in vitro* de l'uranium à la substance fondamentale du tissu osseux. Des coupes minces d'os compact total frais ou fixé, d'os minéralisé et d'os décalcifié ont été soumises à l'action de faibles solutions d'uranium. Les résultats de cette recherche sont comparés à ceux que l'auteur avait obtenus précédemment par l'emploi du radiocalcium et du radiophosphore.

La distribution de l'U est qualitativement identique à celle du Ca^{45} et du P^{32}: c'est-à-dire qu'une quantité plus importante d'U se fixe par unité de volume de substance osseuse aux parties moins calcifiées, récemment formées. D'autre part, on a observé des différences assez considérables entre la fixation du Ca^{45} et de l'U; en particulier, ce dernier se fixe *in vitro* non seulement aux cristaux minéraux, mais aussi aux composants organiques de la matrice osseuse.

[1] For the fixation of Ca^{45}, *cf.* R. Amprino, Exper. *8*, 380 (1952).

Membrane Structures in Mucus

In fresh untreated specimens of mucus from the human pharynx and nasal cavity, in saliva, expectorations and gastric contents, and in secretions of the isolated duodenal pouch of a dog, extremely thin membranous structures are regularly seen by the microscope method of the author[1]. Some of these may cover an area of up to several millimeters. Twisted packets of membrane, seemingly identical with Curschmann spirals, are frequently observed in normal expectorations. Sometimes these untwist and unfold into curtain-like formations. Whether the membranes are formed by alteration of the mucus surface or by direct secretion is uncertain. In any case, their influence on the permeability of mucus is obvious.



A typical membrane formation from pharyngeal mucus.

A. Wilska

Institute of Physiology, University of Helsinki, March 15, 1953.

Zusammenfassung

Im Schleim verschiedener Herkunft sind ausserordentlich dünne Membranbildungen mit der lichtmikroskopischen Kontrastmethode des Autors zu beobachten.

[1] A. Wilska, Nature *171*, 353 (1953).

Extra Reproduction of a Chromosome in Yeast

In spite of the lack of unanimity of opinion regarding the time of reproduction of chromosomes, there appears to be justification for the belief that multiple chromonemata are present at various stages of mitosis[1]. It is remarkable that a chromosome which is compound even at the microscopic level "splits" exactly and separates at anaphase into two and only two daughter chromosomes. It may, occasionally, split into three instead of two chromatids as reported by Huskins[2]. What appears to be a similar but rare phenomenon was observed by us in two strains of diploid yeast.

Normal mitosis of our two chromosome control brewery yeast as seen in Feulgen preparations has already been described elsewhere[3]. Figure 1 illustrates an early anaphase showing two pairs of chromosomes. When one

[1] B. P. Kaufmann, Bot. Rev. *14*, 57 (1948).
[2] C. L. Huskins, Amer. Nat. *81*, 401 (1947).
[3] S. Duraiswami and M. K. Subramaniam, Exper. *7*, 422 (1951).