Electron Microscope Laboratory, Anatomical Department, Nara Medical College, Naraken, Japan, and Anatomical Department, Medical School, Osaka University, Osaka, Japan, August 8, 1954.

Zusammenfassung

An den ultradünnen Schnittpräparaten von Erythrozyten der Karausche *Carassius auratus* wurde ihre submikroskopische Struktur mit dem Elektronenmikroskop sowie auch mit dem Lichtmikroskop untersucht. Im Cytoplasma der Erythrocyten kann man immer die undurchsichtigen Granula beobachten, welche durch ein feines Kanälchen in Zusammenhang mit der Zell- und Kernmembran stehen. Es ist festgestellt, dass der Kern aus achromatischen Fäden mit Schraubenwindung, undurchsichtigen Chromatingranula und Kernmembran von Doppelnatur besteht. Die Befunde der Schnitte stimmen mit den schon früher von YASUZUMI und seinen Mitarbeitern nachgewiesenen Resultaten überein.

Etude de la structure des sels osseux et des pseudoapatites à l'aide des radio-isotopes

On sait qu'il existe toute une série de phosphates de calcium, synthétiques et biologiques, dont le rapport Ca/P varie de 1,72 à 2,26 et qui cependant possèdent tous la même structure apatitique. Ces phosphates synthétiques et biologiques – que nous avons appelés pseudoapatites[1] – sont cependant distincts, par leurs propriétés physiques et chimiques, des apatites naturelles des minéralogistes. Nous expliquons que les pseudoapatites puissent avoir un Ca/P inférieur à 2,14 en admettant que des ions calcium font statistiquement défaut dans le réseau cristallin[2]; la pseudoapatite de Ca/P 2,14 correspond à la structure saturée (10 Ca pour 6 P). De plus, il existe des pseudoapatites dont le rapport Ca/P, supérieur à 2,14, peut atteindre 2,26. Il faut alors admettre la présence de calcium supplémentaire, fixé dans la structure, d'une manière encore inconnue mais qui n'est certainement pas une simple rétention par adsorption physique. Ce calcium supplémentaire correspond au maximum à un demi-atome par maille apatitique (10,5 Ca pour 6 P).

Nous avons apporté une première preuve à l'appui de cette conception: on peut compléter la structure de n'importe quelle pseudoapatite séchée à température ordinaire, quel que soit son Ca/P initial, en augmentant son taux de calcium jusqu'à obtenir un rapport Ca/P 2,26[3].

Le composé de Ca/P 2,26 présente un grand intérêt théorique, car il semble être le constituant fondamental de la fraction minérale de l'os: le demi-atome de calcium qui élève le rapport Ca/P de 2,14 à 2,26 apparaît comme une caractéristique de la substance minérale osseuse et de certaines pseudoapatites de synthèse. Il ne trouve d'ailleurs pas sa place dans le réseau apatitique calculé par les cristallographes à partir de la fluorapatite et de l'hydroxyapatite. Ajoutons encore que si l'on porte à 105°C les pseudoapatites lacunaires, elles perdent la possibilité de fixer ce demi-atome de calcium[3].

Nous avons cherché à déterminer si, dans le phosphate qui représente le constituant fondamental de la substance minérale osseuse, ce demi-atome de calcium se comportait comme les autres atomes de calcium: nos premières expériences ont été faites avec le radio-isotope.

L'un de nous a montré que l'attaque de la substance minérale osseuse par l'acide chlorhydrique dilué permet, non seulement de la débarrasser du matériel adsorbé, mais d'en extraire du calcium de façon préférentielle, de telle sorte que le rapport Ca/P de la phase solide décroît en fonction de la quantité d'acide utilisé[1].

Divers échantillons de sels osseux (méthode de GABRIEL) attaqués plus ou moins profondément par HCl et dont les Ca/P s'étagent entre 2,26 et 2,13 sont immergés jusqu'à équilibre dans une solution de Ca^{45} ($CaCl_2$): on détermine ensuite l'activité spécifique de chacun des échantillons. Quelle que soit l'importance de l'attaque chlorhydrique et le degré d'abaissement du rapport Ca/P de la phase solide, le pourcentage d'échange est constant.

Si les sels osseux n'ont pas été préalablement traités par l'acide chlorhydrique, leur activité spécifique est plus grande.

Nous interprétons ces résultats de la façon suivante.

1° La substance minérale osseuse non traitée par HCl renfermant encore son calcium adsorbé, et la quantité de ce dernier étant très faible, pour que l'activité spécifique globale puisse être modifiée, il faut que le pourcentage d'échange du calcium adsorbé soit extrêmement élevé.

2° La substance minérale osseuse traitée par HCl a libéré tout le calcium adsorbé et ne renferme donc plus que les différents atomes de calcium du composé dont le rapport Ca/P était primitivement 2,26. Or, comme l'activité spécifique est la même quel que soit le rapport Ca/P auquel on aboutit, on peut conclure que le comportement de tous les atomes de calcium est le même vis-à-vis du radio-isotope.

Ce travail a été réalisé grâce à l'aide du Centre interuniversitaire belge des Sciences nucléaires et «l'Air Research and Development Command. U.S. Air Force», contrat n° A.F.61(514)-647 C.

CLAUDINE FABRY et M. J. DALLEMAGNE

Institut de thérapeutique expérimentale, Université de Liège, le 11 octobre 1954.

Summary

The main constituent of bone salts, which has a Ca/P ratio of 2,26, contains 10 calcium per 6 phosphorus, plus half an atom of calcium which properties are important to be known. This half atom has the same behaviour as the other calcium atoms in so far as the radioisotope is concerned.

[1] M. J. DALLEMAGNE, thèse d'agrégation (Edition Gordinne, Liège 1943); résultats non publiés.

Distribution of S^{35}-Sodium Sulfate in Early Chick Embryos

Recent researches have shown that S^{35}-sodium sulfate is incorporated in sulfo-mucopolysaccharides of mammalian tissues[1]. Studies carried on by means of contrast

[1] C. FABRY, J. Physiol. (Paris) *46*, 361 (1954).

[2] A. S. POSNER, C. FABRY et M. J. DALLEMAGNE, Biochim. Biophys. Acta *15*, 304 (1954).

[3] C. FABRY, Biochim, Biophys. Acta *1954* (sous presse).

[1] D. D. DZIEWIATKOWSKI, J. biol. Chem. *189*, 187 (1951). – L. L. LAYTON, Cancer *4*, 198 (1951). – H. BOSTRÖM, J. biol. Chem. *196*, 477 (1952). – H. BOSTRÖM and S. GARDELL, Acta Chem. Scand. *7*, 216 (1953).

autoradiography proved the existence of a differential sulfate fixation in various tissues and organs, in adults as well as in fetuses[1]. The main uptake of radiosulfate occurs in cartilage[2]; the sulfate is incorporated in the synthesis of the chondroitinsulfuric acid of the matrix[3]. Relatively smaller amounts of S^{35} get fixed in the wall of arteries, in the fibrous tissue of sclera and cornea, in the mucous cells, in the mast cells, etc.[4]. Variations in the uptake of radiosulfate may occur in different parts of a given organ or tissue; DZIEWIATKOWSKI, DAVIES and YOUNG[5] produced good evidence of a relatively higher incorporation of S^{35}-sulfate in the epiphysial plate of the long bones of newborn rats and in the layer of the cartilaginous epiphyses which surrounds the ossification centre. AMPRINO[6] has reported on the distribution of S^{35}-sulfate during differentiation and growth of the cartilaginous anlagen of bones in chick embryos from 7 to 20 days of incubation; evidence was presented that quantitative and qualitative variations in the S^{35} uptake occur in various parts of a given cartilage according to developmental stages: these variations are related to changes in the amount of cartilage matrix, in its basophilia and in the number and size of chondrocytes.

The following experiments were performed to analyse autoradiographically the distribution of S^{35}-sulfate in chick embryos from 80 h (e.g., 36 to 38 somites) to 7 days of incubation.

Technique. 0·25 or 0·50 ml of a solution of S^{35}-sodium sulfate (corresponding to 5 and 10 μ C respectively) were allowed to fall in drops on the extra-embryonic area, e.g., vitelline or chorioallantoic vessels, of embryos aged 3 to 5 days of incubation. The embryos were fixed 12 to 24 h later in Rossmann fluid in the icebox at 0°C, dehydrated and embedded in paraffin. The serial sections, 10 μ thick, were mounted on flexible glass slides, deparaffinised and thoroughly washed in 70° alcohol; the slides, dried at room temperature, were pressed, sections down, on the 25 μ thick emulsion of C_2 Ilford Nuclear Research plates. Contrast autoradiography was employed; after an exposure of several weeks, the plates were developed and the sections stained.

The radioactivity of the tissues was evaluated by measuring the opacity of selected areas of the autoradiographs photometrically, according to the quantitative technique suggested by ODEBLAD and more recently used by BOSTRÖM and co-workers[7]. The autoradiographic opacity, corresponding to the radioactivity of various tissues and organs, was in general compared with that of the nervous centres which showed consistently the lowest uptake of radiosulfate. Differences

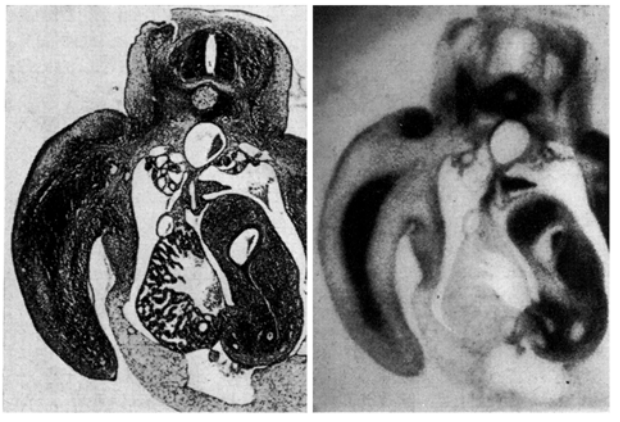

Fig. 1.–Cross section of the trunk of 114 h embryo. 20 ×.

of autoradiographic opacity between two organs, or two distinct regions of the same organ, were taken into account only when they could be found in three consecutive sections at least. The values reported below correspond to the mean of 6 to 10 measurements each.

Results. (1) The distribution of the radiosulfate is more uniform in embryos of 4 than of 5 to 6 days. This behaviour probably depends on two factors: *a*) a decrease in the S^{35} uptake of some tissues during that period of development, *b*) a conspicuous increase in the fixation of S^{35} per unit volume in other tissues, particularly in supporting tissues, during their histological differentiation. In fact, the ratio between the radioactivity of the mesenchyme encircling the notochord and the radioactivity of the spinal cord was found to be 3·1:1 in embryos 85 to 90 h of incubation; the ratio between the activity of the vertebral precartilage and of the spinal cord is 4·8:1 in a 114 h embryo (cf. Fig. 1) and 5·8:1 at 144 h (Fig. 2); in the latter embryo the cartilage matrix is already highly basophilic and the collagen fibres are masked by the chondromucoid.

(2) In 5 days embryos, remarkable differences in the radiosulfate uptake appear not only in different organs but also in the various parts of a given organ. In the autoradiographs of the embryo's head the opacity which corresponds to the diencephalic wall is on the average 30% greater than that of the neighbouring nervous layer of the retina. Differences of the same degree exist at the end of the sixth day in regions of the encephalic wall from which grey and white matter respectively will form (Fig. 2).

(3) Variations in the amount of the radiosulfate uptake are apparent also in the epidermis of various cutaneous districts: in general, the uptake is a little greater in the epidermal thickenings.

(4) From the fifth day on, the greatest differences in the degree of radioactivity occur in tissues and organs of mesodermal or mesenchymal origin. The radioactivity of the mesenchymal blastema which surrounds the intestinal epithelium is about twice the radioactivity of the mesenchyme underlying the epidermis, e.g., presumptive derm and subcutaneous. Less remarkable differences in the S^{35} uptake are found in the various parts of the mesenchymal blastema of the intestinal wall (cf. Fig. 1); they probably depend partly on variations of the number of cells per unit volume of tissue, partly on qualitative differences of the intercellular substance.

(5) Great differences in radioactivity between skeletogenous and non-skeletogenous mesenchyme respectively become evident towards the end of the fifth day

[1] E. ODEBLAD and H. BOSTRÖM, Acta pathol. microb. Scand. *31*, 339 (1952). – H. BOSTRÖM and E. ODEBLAD, Anat. Rec. *115*, 505 (1953). – U. FRIBERG and N. R. RINGERTZ, jr., Exper. *10*, 67 (1954).

[2] D. D. DZIEWIATKOWSKI, E. R. BENESCH, and R. BENESCH, J. biol. Chem. *178*, 931 (1949). – L. L. LAYTON, Cancer *3*, 725 (1950). – D. CAMPBELL and B. H. PERSSON, Exper. *7*, 304 (1951). – H. BOSTRÖM, J. biol. Chem. *196*, 477 (1952). – H. BOSTRÖM, E. ODEBLAD, and U. FRIBERG, Arch. Bioch. Biophys. *38*, 283 (1952).

[3] D. D. DZIEWIATKOWSKI, J. biol. Chem. *189*, 187 (1951). – H. BOSTRÖM and S. AQVIST, Acta chem. Scand. *6*, 1557 (1952). – H. BOSTRÖM and B. MANSSON, J. biol. Chem. *196*, 483 (1952).

[4] E. ODEBLAD and H. BOSTRÖM, Acta pathol. microb. Scand. *31*, 339 (1952). – H. BOSTRÖM and E. ODEBLAD, Anat. Rec. *115*, 505 (1953).

[5] D. D. DZIEWIATKOWSKI, J. Exp. Med. *93*, 451 (1951); *95*, 489 (1952). – D. V. DAVIES and L. YOUNG, J. Anat. *88*, 174 (1954).

[6] AMPRINO, C. r. Ass. Anat., 41 Réun., Gênes 1954 (in press); Rend. Acc. Naz. Lincei S. VIII, *16*, 781 (1954)

[7] E. ODEBLAD, Acta Radiol. Suppl. *93* (1952). – H. BOSTRÖM, E. ODEBLAD, and U. FRIBERG, Arch. Bioch. and Biophys. *38*, 283 (1952).

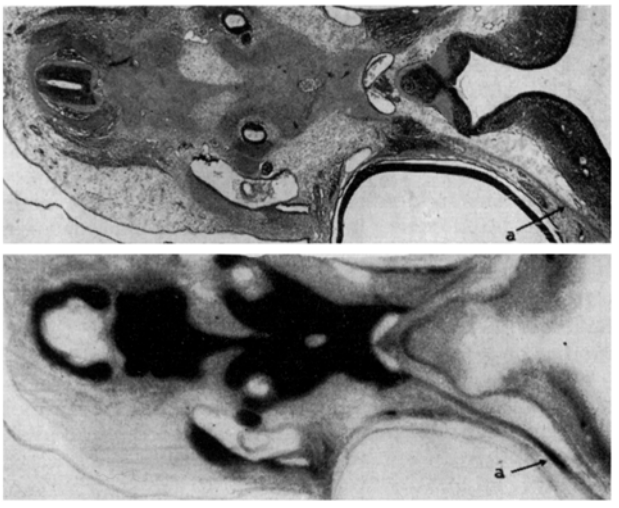

Fig. 2.–Cross section of the base of the head of 144 h embryo. 14·5 ×.

(cf. Fig. 1). In the wing and in the pelvic limb, the ratio between the radioactivity of the regions in which cartilage and derm and subcutanous tissue respectively will differentiate amounts to 3·4:1 ca. The increasing of this ratio runs parallel with the increasing of both the amount and the basophylia of the intercellular matrix in the precartilaginous blastemas (e.g. embryos of 7 to 8 days). Remarkable differences of radioactivity occur also in the various regions of each precartilaginous blastema, depending on the different degree of chondrification: the ratio of radioactivity between procartilage and precartilage ranges between 2·7:1 and 2·3:1. A conspicuous uptake of radiosulfate is apparent in 6 days embryos in the clusters of osteoblasts which mark the sites of intramembranous ossification, before the formation of bone matrix proper (Fig. 2 *a*).

(6) Remarkable is the fixation of radiosulfate in the mesenchymal anlage of the spleen (Fig. 1). At the beginning of the sixth day, the ratio between the radioactivity of the spleen rudiment and that of the spinal cord is 3·4:1 ca. Therefore, the sulfate uptake is nearly as great in the spleen as in precartilage; the ratio of radioactivity between the two is 1:1·8 ca.

(7) The subendothelial mucous tissue of the prevalvular cushions of the heart and big arteries fixes a relatively large amount of radiosulfate. In 87 to 90 h embryos, the radioactivity of this material is nearly as high as that of the mesenchymal blastema which surrounds the notochord and will differentiate in the cartilage of the vertebral body.

(8) A relatively great uptake of radiosulfate occurs from the sixth day on in the periendothelial mesenchyme of the main arteries and not in veins of corresponding size. BOSTRÖM and ODEBLAD (l.c.) have shown that a remarkable fixation of radiosulfate takes place in the arterial wall of 22 day rabbit fetuses and adults; the fact that this process starts in very early developmental stages deserves consideration.

(9) Relatively small, on the contrary, is the uptake of radiosulfate in the intestinal epithelium and in the anlage of the liver (Fig. 1). The ratio between radioactivity of the liver and that of the spinal cord was found to be of the order of 1·1:1 at the end of the fifth day. However, the material which accumulates in the lumen of the intestine, and which is presumably a secretory product, appears highly radioactive even at this stage of development (Fig. 1).

(10) The activity of the mesonephros is a little higher than that of the liver, and the radioactivity of the primordium of the suprarenal cortex is still higher in 5 to 6 days embryos.

This preliminary study leads us to the conclusion that in relatively early stages of embryonic development in the chick, quantitative differences of the uptake of radiosulfate occur in the anlagen of several organs and in the various regions of a given tissue, from which histological constituents displaying different structures and functions will differentiate. Obviously, the technique adopted in the present research does not enable us to establish the chemical form, whether organic or inorganic, in which the radiosulfate is fixed in embryonic tissues.

R. AMPRINO

Institute of Anatomy, University of Turin, June 25, 1954.

Résumé

La distribution du radiosulfate introduit dans les embryons de poulet de 3 jusqu'à 7 jours a été étudiée par l'autoradiographie de contraste. Il existe des différences marquées dans la fixation du S^{35} au niveau des ébauches d'organes différents et même dans les diverses parties de chaque tissu. Ces différences sont particulièrement remarquables entre les divers dérivés du mésoderme et elles s'accentuent pendant la différenciation ultérieure.

The Use of Nuclear Emulsions to Determine Small Amounts of P^{33} Present in Samples of P^{32}

This investigation is an attempt to apply the method of radioautography to the quantitative determination of small amounts of radio-active material. The immediate problem is to determine the amount of P^{33} [1] present in samples of P^{32}.

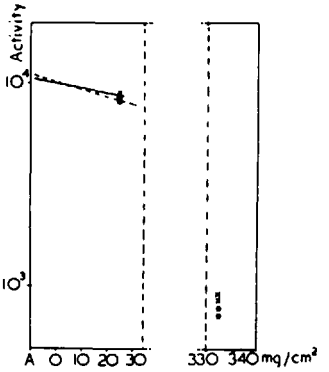

Fig. 1.—A-(by air and window correction)
×× P^{32} absorption curve
○○ mixture absorption curve

The experimental method is based on counting of the numbers of the densely ionised terminal parts of the β-ray tracks in nuclear emulsions. The emulsion thickness used of 200 μ was chosen to include almost the whole length of the tracks from P^{33} [2] while recording only a small proportion of the tracks of P^{32}.

[1] E. N. JENSEN and R. T. NICHOLS, Phys. Rev. *83*, 215 (1951). – R. K. SHELIN, R. B. HOLTZMANN and C. Y. FAN, Phys. Rev. *83*, 215 (1951).

[2] L. E. GLENDENIN, Nucleonics *2*, 16 (1948). – A. BONETTI and G. TOMASINI, Nuovo Cimento *8*, 701 (1952). – L. KATZ and A. S. PANFOLD, Rev. Mod. Phys. *24*, 28 (1952).