

UPTAKE OF S^{35} IN THE INTERVERTEBRAL DISCS AFTER
INJECTION OF S^{35} -SULPHATE.
AN AUTORADIOGRAPHIC STUDY

By

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In autoradiographs from sections through whole mice injected with S^{35} -sulphate, an uptake of S^{35} was noted in the peripheral zone of the nucleus pulposus (fig. 1). The study to be described in this paper, with newborn piglets as experimental animals, was undertaken in order to localize radiosulphur in the intervertebral discs more precisely and to follow the changing autoradiographical distribution patterns over a period of time. The method might be useful when studying the metabolic alterations in different morphological structures appearing with ageing or as the result of degeneration in intervertebral discs.

METHODS

The radiosulphate was produced by neutron irradiation of analytically pure potassium chloride in the uranium reactor of AB Atomenergi, Stockholm, according to the reaction $Cl^{35} \text{ np } S^{35}$. Purification of the radiosulphate formed was carried out according to methods described by *Deshpande* (1959) (3). Radiochemical purity was tested by paper chromatography.

Five piglets were injected intraperitoneally with a dose of 1 mC carrier-free S^{35} -sulphate per kg. bodyweight. The pigs were littermates and when injected they were about 24-hours old with an average weight of 1.9 kg.

After injection they were killed at intervals of 1.5 hours, 6 hours, 24 hours, 4 days, and 16 days. At the end of the experiment the piglets weighed 1.9, 1.8, 1.95, 3, and 7 kg. respectively.

Immediately after the animals were killed a portion of the lumbar

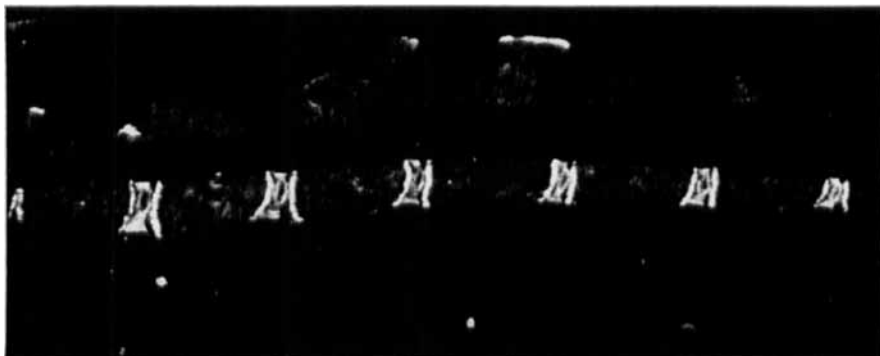


Fig. 1.

Distribution of S^{35} in the vertebral region of an adult mouse 24 hours after the intravenous injection of S^{35} -sulphate.

vertebral column was removed and rapidly frozen by immersion in a solid mixture of carbon dioxide and acetone. Sagittal sections, 20 μ thick, were then prepared in a refrigerated room at -10° C. The sections were freeze-dried and autoradiographical exposure was made by apposition at -10° C against Gevaert Dentus x-ray film. More extensive descriptions of the autoradiographic technique have been given previously (16, 17). The exposure time varied from 5 days for the pig killed 1.5 hours after injection to 60 days for the pig killed after 16 days.

RESULTS

One and a half hours after the injection of S^{35} (fig. 2), radioactivity is apparent throughout the entire specimen. In the vertebral bodies, activity is greatest in the epiphyseal lines and periosteum while in the osseous tissue the trabecular structure is clearly delineated. With the exception of the vascular channels, there is uniform uptake throughout the epiphyseal cartilage and since the activity is somewhat stronger than in the annulus fibrosus, the border between these structures is quite distinct. In the annulus, activity increases slightly towards the inner portion. With its moderate and diffuse activity the nucleus pulposus contrasts with the cartilage plate. Activity in the nucleus is nevertheless somewhat greater towards the periphery.

Six hours after the injection of S^{35} (fig. 3) activity is more distinctly concentrated to the epiphyseal cartilage, the inner portion of the annulus, and the periphery of the nucleus. Activity along the epiphyseal line is no longer so much more evident than in the epiphyseal cartilage.

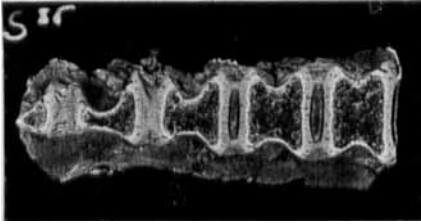


Fig. 2.

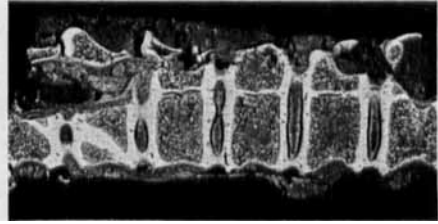


Fig. 3.

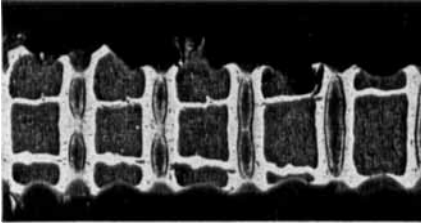


Fig. 4.

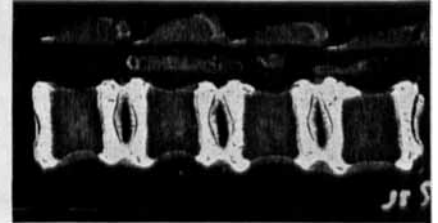


Fig. 5.

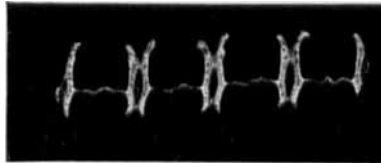


Fig. 6.

Figs. 2-6.

Distribution of S^{35} in the lumbar vertebral column of piglets at the following intervals after intraperitoneal injection of S^{35} -sulphate:
 Fig. 2: 1.5 hours. Fig. 3: 6 hours. Fig. 4: 24 hours. Fig. 5: 4 days. Fig. 6: 16 days.

One day after injection (fig. 4) the appearance has changed radically with nearly complete disappearance of activity from the diaphysis of the vertebral body and concentration in the three sites already mentioned, the epiphyseal cartilage, the inner zone of the annulus, and the periphery of the nucleus.

By four days after injection (fig. 4) radioactivity has largely disappeared from the vertebral body apart from the periosteum and the primary spongiosa adjacent to the epiphyseal line. Activity in the epiphyseal cartilage, the inner zone of the annulus, and the periphery of the nucleus is by now impressive while activity has largely disappeared from the other regions. There is no clear distinction ventrally and

dorsally between the inner portion of the annulus and the periphery of the nucleus. Between the nucleus and the cartilage plate (or more correctly at this stage the epiphyseal cartilage) there is a distinct borderline. Activity appears in thin lines radiating from the periphery of the nucleus in towards its centre.

Despite a much longer exposure time for the autoradiographs made 16 days after injection (fig. 6), the images are quite weak. The most important difference is seen in the nucleus. A distinct concentration of activity is still apparent at the periphery and in addition to this there is obvious although much weaker activity in the central part of the nucleus.

DISCUSSION

Within the nucleus a series of morphological, chemical, and physical changes take place throughout life. The gelatinous, semifluid state of the newborn nucleus becomes successively dehydrated with metamorphosis of this structure, generally fibroid, in some instances chondroid. Systematic morphological studies on dogs have demonstrated that this metamorphosis originates at the periphery of the nucleus and the inner layer of the annulus fibrosus, so intimately associated with the nucleus in this species (11). In this region there is vigorous cellular activity. The most important chemical property of the nucleus is its content of polysaccharides. According to *Malmgren & Sylvén* (1952) (12), these polysaccharides consist of chondroitin sulphate or sulphate isomers. In a series of papers *Gardell* (7-10) has demonstrated that both chondroitin sulphate and keratosulphate are present, a finding which has been confirmed by others (2). *Dzietwatkowski* (1951) (4) and *Boström* (1952) (1) have shown that S³⁵-labelled sulphate is taken up by cartilage mainly as ester sulphate in chondroitin sulphuric acid. Radiosulphur can also be recovered from keratosulphate (*Rodén* 1956) (15).

The manner in which S³⁵ was incorporated in the nucleus pulposus in our experiments with mice and newborn piglets indicates that synthesis of the two sulphomucopolysaccharides of interest takes place in the peripheral zone. This synthesis is apparently preceded by at least a day-long period during which the sulphur is diffusely distributed throughout the disc, cartilage, and bone. *Friberg* (1958) (6) observed that in guinea pigs sulphur is present in inorganic form up to 48 hours after injection. Inorganic sulphur in bone has also been observed by *Engfeldt et al.* (1954) (5).

Activity was low 16 days after the injection of S^{35} , largely because the rapidly-growing piglet "diluted" the injected sulphur. Activity in the central portion of the nucleus at this time can be interpreted in two ways. Either the S^{35} -polysaccharides have already degraded to give inorganic S^{35} or else polysaccharides have migrated from the periphery of the nucleus; the question remains open.

In this experiment on newborn piglets, the incorporation of radio-sulphur illustrates the metabolically most active components in the intervertebral space—the periphery of the nucleus, the inner portion of the annulus, and the epiphysis of the vertebral body. Metabolic activity has its morphological counterpart. In the autoradiograms there is no distinction between the ventral and dorsal parts of the nucleus and the adjacent inner portion of the annulus. In like manner there is intimate union between the inner layer of the annulus and the epiphyseal cartilage of the vertebral body. On the other hand, the border is very distinct between nucleus pulposus and the cartilage plate, the portion of the epiphysis of the vertebral body abutting upon the disc.

Whether one examines a histological section or an autoradiogram, the inference is the same—that the tissue layers which form the periphery of the nucleus and the perinuclear portion of the annulus represent the "growth zone" of the disc. Here is the seat of the fibroid or chondroid metamorphosis of the nucleus pulposus and here, it appears, the synthesis of the mucopolysaccharides takes place. An echo of embryological development can be discerned. Chorda dorsalis forms the centre of the future disc and at an early stage is enclosed by pericardal precartilage. The definitive nucleus is formed by combined development of the chorda and the inner portion of the annulus derived from the perichordal pre-cartilage (13, 14). It is only to be expected, then, that the persisting "growth zone" of the nucleus is situated about its periphery.

SUMMARY

S^{35} injected as sulphate intraperitoneally into newborn piglets is concentrated in the intervertebral space to the epiphyseal cartilage, the inner zone of annulus fibrosus, and the periphery of nucleus pulposus. As a probable expression of synthesis of the sulphomucopolysaccharides, the incorporation of S^{35} at these sites accords closely with inferences deduced from morphological features.

To obtain distinct autoradiograms of S^{35} incorporation at least one day should elapse after injection.

RESUME

S³⁵ en injection intrapéritonéale comme sulfate chez des porcelets nouveaux-nés se concentre dans l'espace intervertébral jusqu'au cartilage épiphysaire, la zone intérieure de l'anneau fibreux et la périphérie du noyau pulpeux. Comme l'expression probable d'une synthèse de sulfomucopolysaccharides, l'incorporation de S³⁵ dans ces endroits concorde parfaitement avec les conclusions tirées des faits morphologiques.

Pour obtenir des autoradiogrammes distincts de l'incorporation de S³⁵, wenn als Sulphat in die Peritonealhöhe von neugeborenen

ZUSAMMENFASSUNG

S³⁵, wenn als Sulphat in die Peritonealhöhle von neugeborenen Ferkeln injiziert, wird im Zwischenwirbelraum des Epiphysenknorpels, der inneren Zone des annulus fibrosus und der Peripherie des nucleus pulposus gespeichert. Als ein möglicher Ausdruck der Synthese von Sulphomucopolysacchariden stimmt die Einverleibung von S³⁵ an diesen Stellen mit Folgerungen, die aus morphologischen Erscheinungen gezogen werden, nahe überein.

Um deutliche Autoradiogramme von S³⁵-Einverleibung zu erhalten, sollte zumindest ein Tag nach der Injektion verstrichen sein.

ACKNOWLEDGEMENTS

We should like to thank AB Atomenergi and especially civilingenjör C. G. Österlund and ingenjör A. Petersen for the neutron irradiation, civilingenjör S. Forberg for valuable help with the purification of the S³⁵-sulphate, and the Knut and Alice Wallenberg foundation for financial support.

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