# Informations - Informationen - Informazioni - Notes

## STUDIORUM PROGRESSUS

### On the Enzymatic Exchange of the Sulphate Group of the Animal Sulpho-Mucopolysaccharides

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#### INTRODUCTION

Among the acid mucopolysaccharides, those esterified with sulphuric acid, the sulpho-mucopolysaccharides, constitute an important group with a wide distribution in the body. Thus, chondroitin sulphuric acid is one of the main constituents of the hyaline cartilage. It is also present in the aortic wall, in tendons, sclera, and the dermal connective tissue. Mucoitin sulphuric acid is claimed to occur in the gastric mucosa[2]. Hyaluronosulphate[3] is also claimed to be present in the cornea. Heparin[4] has been found to be a specific mucopolysaccharide produced by the mast cells.

During the past few years, additional knowledge of the metabolism of the sulpho-mucopolysaccharides has been gained, by means of studies of the *sulphate exchange* in these compounds with the isotope technique. $S^{35}$-labelled sulphate has been used as the tracer substance (DZIEWIATKOWSKI *et al.*[5], LAYTON *et al.*[6], BOSTRÖM *et al.*[7] and others).

In the present paper, a short survey is given of the recent developments in this field with particular reference to the work performed in Scandinavia.

#### IN VIVO STUDIES

##### *Excretion of $S^{35}$*

If a tracer dose of $S^{35}$ as *sodium sulphate* is given to an experimental animal, most of the radioactive sulphur is rapidly excreted[8]. According to DZIEWIATKOWSKI[9], 95 per cent of the $S^{35}$ given to rats is excreted in the urine and faeces during the first 5 days. The concentration of inorganic sulphate in the tissues decreases rapidly. Only a small proportion of the $S^{35}$ excreted occurs as ethereal sulphates[9].

##### *Sulphate fixation*

Numerous investigations have been made to determine the exact localization of that part of the $S^{35}$-labelled sulphate retained in the tissues of the experimental animals. Thus, SINGHER and MARINELLI in 1945[1] reported a higher sulphate fixation in the bone marrow than in the rest of the bone. DZIEWIATKOWSKI *et al.* showed in 1949[2] a high sulphate incorporation and a slow elimination in cartilage, bone and bone marrow; this was in sharp contrast to the low sulphate uptake and rapid elimination in the blood, liver and brain.

The autoradiographic method has proved to be eminently suitable for ascertaining the exact localization of several radioactive tracers, even in relatively small structures. By means of this technique, the sulphate fixed in articular and epiphyseal cartilage of suckling rats was made visible by DZIEWIATKOWSKI[3] and in the cartilage of the nasal septum of adult rats by CAMPBELL and PERSSON[4]. Together with ODEBLAD, the present authors have used the autoradiographic method for a systematic study of the sulphate fixation in a large number of normal tissues and organs in rats and rabbits given injections of $S^{35}$-labelled sulphate. A brief account of the results of these investigations, which have already been published in various journals, will now be given.

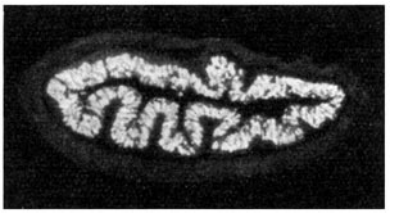

Fig. 1.–Autoradiograph of ileum from an adult rabbit 24 h after an intravenous injection of $S^{35}$-labelled sodium sulphate. × 25.

In the *respiratory tract*, the highest sulphate fixation took place in the tracheal and bronchial cartilage and in the cartilage plates of the lung[5]. The tracheal epithelium and the respiratory epithelium of the lung showed a low or moderate uptake, whereas the $S^{35}$ content of the lymphatic follicles was extremely low.

All sections of the *gastrointestinal tract* investigated[5] (distal part of the oesophagus, fundus ventriculi, duodenum, distal part of the ileum and descending colon) showed the highest degree of sulphate fixation in the epithelial layer (Fig. 1). In other layers of the oesophagus, stomach and intestines and in the *liver* and *pancreas* a diffuse, low or moderate $S^{35}$ uptake was noted.

In the *cardiovascular system*[5], the highest $S^{35}$ incorporation occurred in the aorta and in the heart valves. The *heart muscle* and *the spleen*, on the other hand, showed only a moderate, diffuse uptake.

In the *nervous system*, a locally differentiated but fairly low $S^{35}$ incorporation was demonstrated[6]. In general, a considerably higher uptake was found in the

[1] From the Chemistry Department II, Karolinska Institutet, Stockholm.

[2] P. A. LEVENE, *Hexosamines and Mucoproteins* (Longmans, Green & Co., London, 1925).

[3] K. MEYER and E. CHAFFEE, Amer. J. Ophtalmol. *23*, 1320 (1940).

[4] H. HOLMGREN and O. WILANDER, Z. mikrosk.-anat. Forsch. *42*, 242 (1937). – E. JORPES, H. HOLMGREN, and O. WILANDER, Z. mikrosk.-anat. Forsch. *42*, 279 (1937).

[5] D. D. DZIEWIATKOWSKI, R. E. BENESCH, and R. BENESCH, J. Biol. Chem. *178*, 931 (1949).

[6] L. L. LAYTON *et al.*, Cancer *3*, 725 (1950).

[7] H. BOSTRÖM, Arkiv Kemi *6*, 43 (1953).

[8] H. BORSOOK, G. KEIGHLEY, D. M. YOST, and E. Y. MCMILLAN, Science *86*, 525 (1937). – D. D. DZIEWIATKOWSKI, J. Biol. Chem. *178*, 197 (1949).

[9] D. D. DZIEWIATKOWSKI, J. Biol. Chem. *178*, 389 (1949).

[1] H. O. SINGHER and L. MARINELLI, Science *101*, 414 (1945).

[2] D. D. DZIEWIATKOWSKI, R. E. BENESCH, and R. BENESCH, J. Biol. Chem. *178*, 931 (1949). – D. D. DZIEWIATKOWSKI, J. Biol. Chem. *178*, 197 (1949).

[3] D. D. DZIEWIATKOWSKI, J. Exptl. Med. *93*, 451 (1953).

[4] D. CAMPBELL and H. PERSSON, Exper. *7*, 304 (1951).

[5] E. ODEBLAD and H. BOSTRÖM, Acta Pathol. Microbiol. Scand. *31*, 339 (1952).

[6] H. BOSTRÖM and E. ODEBLAD, Acta Psychiatr. Neurol. Scand. *28*, Fasc. 1 (1953).

grey than in the white matter in all nervous tissues investigated. In the *cerebrum*, a particularly high uptake was noted in a cortical layer consisting of polymorphous and fusiform cells. In the *cerebellar cortex*, the molecular layer and the PURKINJE cells showed a moderate uptake. In the granular layer of the *cerebellum*, on the contrary, a considerable $S^{35}$ uptake was noted. Finally, in the *choroid plexus*, there was a very high $S^{35}$ incorporation.

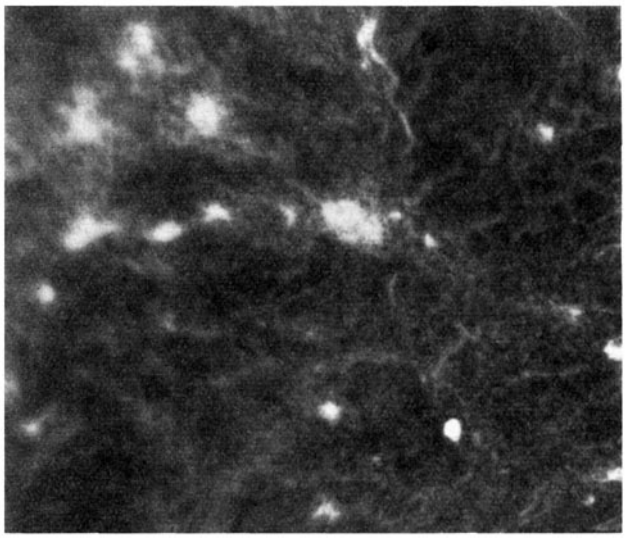

Fig. 2.–Stripping film autoradiograph showing $S^{35}$-incorporation in the mast cells of the subcutis of young rats 5 days after the injection of $S^{35}$-labelled sodium sulphate. × 200.

In the autoradiographs of the *eye*[1], a high $S^{35}$ content appeared, corresponding to the cornea and sclera, while the vitreous body contained no $S^{35}$.

The *skin*[2], finally, showed a low uptake of $S^{35}$ in the cornified layer. In the corium, a smooth distribution of somewhat more $S^{35}$ was observed, interrupted by the pictures of hair follicles and small vessels, which showed a still higher concentration of $S^{35}$. A remarkably high sulphate fixation occurred in the *mast cells*[1] (Fig. 2) of the subcutis, contrasting to the extremely low sulphate fixation in other parts of this layer.

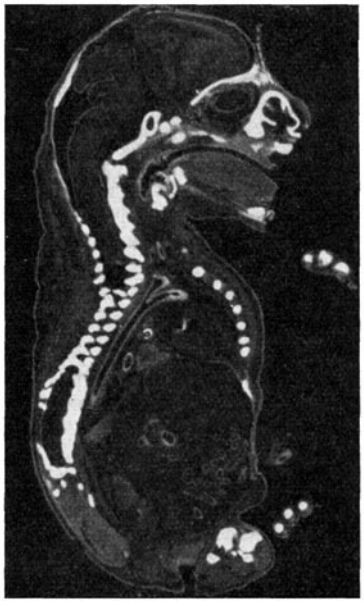

Fig. 3.–Autoradiograph of a three week old rabbit foetus, whose mother was killed 16 h after having received an intravenous injection of $S^{35}$-labelled sodium sulphate.

According to the findings of LAYTON and co-workers[2], maternal sulphate is utilized by mammalian embryos. In order to study the $S^{35}$ distribution in embryonal tissues, rabbit foetuses, about 29 mm in length, of which the mother was sacrified 16 h after having received an injection of $S^{35}$-labelled sodium sulphate, were subjected to autoradiographic examination[3]. Well-differentiated autoradiographs were obtained, indicating that the $S^{35}$ incorporation varied markedly in different tissues (Fig. 3). Thus a very high uptake was noted in all *cartilage structures*. In the *aorta* a considerable fixation of the isotope was noted; and other tissues, e.g. foetal *lungs, intestines, tongue, dental papilla* of a presumptive tooth, *cardiac valves*, also showed a fairly high $S^{35}$ incorporation. On the other hand, no or very little $S^{35}$ was fixed in the liver and in the brain.

### *Mode of sulphate fixation*

DZIEWIATKOWSKI and co-workers[4] assumed that the high $S^{35}$ incorporation, demonstrated by them in 1949, in the cartilage of suckling rats after the intraperitoneal administration of $S^{35}$-labelled sodium sulphate mainly indicated an incorporation of the radioactive sulphur in the sulphate group of condroitin sulphuric acid. In a later investigation, DZIEWIATKOWSKI[5] made an attempt to isolate this labelled chondroitin sulphate from the cartilage, after adding large amounts of non-labelled chondroitin sulphuric acid as a carrier. The aforementioned conception has been verified in our laboratory by the isolation of protein-free labelled chondroitin sulphuric acid from the costal cartilage and skin of adult rats given injections of $S^{35}$-labelled sulphate.

Isolation from the same animals of other sulphur-containing compounds, such as cystine, taurine and methionine, showed, however, only a low or non-ap-

*Table I*

A comparative study of the $S^{35}$-incorporation in chondroitin sulphuric acid prepared from cartilage and in taurine, cystine and methionine prepared from the liver of adult rats after a single injection of $S^{35}$-labelled sodium sulphate.

| Substance | Radioactivity in counts/min/cm² at differ. times after inj. | | | |
|---|---|---|---|---|
| | 2 h | 8 h | 24 h | 48 h |
| Chondroitin sulphuric acid isolated from the costal cartilage | 1392 | 2136 | 2953 | 2933 |
| Taurine isolated from the liver | 32 | 68 | 53 | 35 |
| Methionine isolated from the liver | – | – | 3 | |
| Cystine isolated from the liver | – | – | 4 | – |

[1] E. ODEBLAD and H. BOSTRÖM, Acta Pathol. Microbiol. Scand. *31*, 339 (1952).

[2] H. BOSTRÖM, E. ODEBLAD, and U. FRIBERG, Acta Pathol. Microbiol. Scand. *33*, Fasc. 4 (1953).

[1] E. JORPES, E. ODEBLAD, and H. BOSTRÖM, Acta Haematol. *9*, 273 (1953).

[2] L. L. LAYTON, Arch. Biochem. Biophys. *28*, 142 (1950).

[3] H. BOSTRÖM and E. ODEBLAD, Anat. Record. *115*, 505 (1953).

[4] D. D. DZIEWIATKOWSKI, R. E. BENESCH, and R. BENESCH, J. Biol. Chem. *178*, 931 (1949).

[5] D. D. DZIEWIATKOWSKI, Biol. Chem. *189*, J, 187 (1951).

preciable uptake[1] (Table I). On the other hand, labelled sulphate can be found in ethereal sulphates, as demonstrated by LAIDLOW and YOUNG[2].

The greatest incorporation of $S^{35}$ takes place in those tissues and organs in which sulpho-mucopolysaccharides are present. Thus, on the autoradiograph, chondroitin sulphuric acid can be seen in, for example, various kinds of cartilage, in the aortic wall and in the chondroid substance of the heart valves. On the other hand, the marked density of the mast cells visible on the autoradiograph must be considered to correspond to the $S^{35}$ incorporated in heparin or in its precursors. The high uptake of $S^{35}$ in the cornea and sclera affords evidence of an incorporation in the sulpho-mucopolysaccharides that are known to occur in these membranes. In the vitreous body, on the contrary, which contains the non-esterified hyaluronic acid, no uptake of $S^{35}$ can be noted. The uptake of $S^{35}$ in the gastro-intestinal tract must be ascribed mainly to an incorporation in a possible mucoitin sulphuric acid. Since, however, the intestinal tract is known to be the main extrahepatic source of phenol conjugation[3], the possibility that some of the $S^{35}$ present in the intestinal mucosa occurs as ethereal sulphates cannot be ruled out.

*The renewal of the sulphate group of chondroitin sulphuric acid*

In order to study the rate of the sulphate exchange in the sulpho-mucopolysaccharides, the following experiment was performed[4]:

Adult white rats were given intraperitoneal injections of $S^{35}$-labelled sodium sulphate. 9 groups of animals, each comprising 20 rats, were used. The groups of animals were sacrified at different times after the injection, the first group after 2 h and the last after 16 days. The blood of all the animals in a group was pooled, as were the ribs with the intercostal muscles and the livers. Chondroitin sulphuric acid was prepared from the costal cartilage by applying on a small scale the method of JORPES[5], as modified by STRANDBERG[6]. The different preparations of chondroitin sulphuric acid obtained were hydrolyzed in hydrochloric acid and the sulphate precipitated as $BaSO_4$ for measurements of the radioactivity. The inorganic sulphate of the intercostal muscles was extracted and precipitated as $BaSO_4$ and the total sulphur of the blood was transformed into sulphate by oxidizing with sodium peroxide before the precipitation as $BaSO_4$.

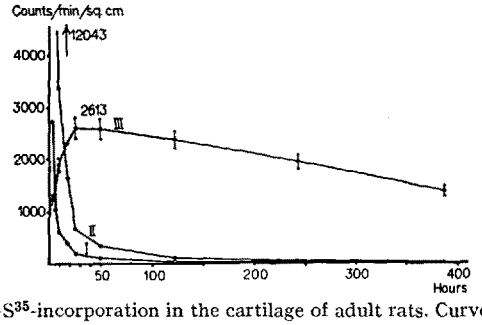


Fig. 4.–$S^{35}$-incorporation in the cartilage of adult rats. Curve I total sulphur in blood. Curve II free sulphates in the costal muscles, Curve III the chondroitin sulphuric acid. The errors in measurements are marked on curve III.

[1] H. BOSTRÖM and S. ÅQVIST, Acta Chem. Scand. *6*, 1557 (1952).
[2] J. C. LAIDLAW and L. YOUNG, Biochem. J. *42*, Proc. 1 (1948).
[3] R. T. WILLIAMS, *Detoxication Mechanisms* (John Wiley, New York, 1947), p. 70.
[4] H. BOSTRÖM, J. Biol. Chem. *196*, 477 (1952).
[5] E. JORPES, Biochem. Z. *204*, 354 (1929).
[6] L. STRANDBERG, Acta Physiol. Scand. *21*, 222 (1950).

The different samples of $BaSO_4$ originating from the sulphate sulphur of chondroitin sulphuric acid of cartilage, the inorganic sulphate of intercostal muscles and the total sulphur of the blood were plated and the radioactivity measured by means of a GEIGER-MÜLLER counter. The results of this experiment are shown in Figure 4. Contrary to the rapid decrease in the radioactivity of the total sulphur fraction and the inorganic sulphur fraction (Curves I and II, respectively), the radioactivity of the ester sulphate group of chondroitin sulphuric acid (Curve III) showed an increase to a maximum value at 24 h after the injection, followed by a decrease during the whole time of observation. From the descending part of this curve the biological half-life-time of the sulphate group of chondroitin sulphuric acid in the cartilage of adult rats could be estimated to be about 16 days. In a similar way the approximate biological half-life-time of the ester sulphate of chondroitin sulphuric acid prepared from the skin of the same rats was estimated to be 8–9 days (Fig. 5, Curve I).

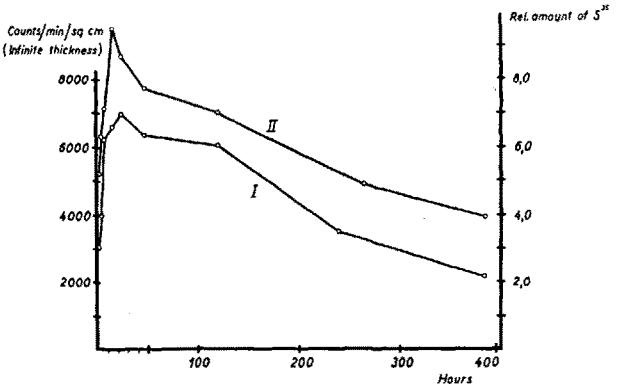


Fig. 5.–Uptake and elimination of $S^{35}$ from the skin of adult rats after a single injection of $S^{35}$-labelled sodium sulphate. Curve I. The radioactivity of the sulphate group of the isolated chondroitin sulphuric acid. Curve II. The radioactivity in the corium as estimated by quantitative autoradiography.

In another experiment[1], four rats from the same litter were given injections of $S^{35}$-labelled sodium sulphate. At different intervals of time a piece of skin was excised from the dorsal area of each rat. All the specimens were subjected to autoradiography, using X-ray film, and the relative amounts of $S^{35}$ fixed in the corium layer of the skin were estimated by means of quantitative autoradiography according to ODEBLAD[2]. When the curve obtained in this way (Fig. 5, Curve II) was compared with the curve for the radioactivity in the ester sulphate group of chondroitinsulphuric acid isolated from the skin, both curves were found to follow each other quite closely (Fig. 5).

*Factors influencing the sulphate exchange in vivo*

The uptake of $S^{35}$ seems to be higher in foetuses and young growing animals than in adult ones. Here it is a question not only of the ordinary sulphate exchange in the sulpho-mucopolysaccharides, but also of an accumulation of newly synthesized material. In accordance herewith, the $S^{35}$ fixation in chondrosarcomatous tissues has been found to be higher than in the cartilage (GOTTSCHALK and ALLEN[3]).

[1] H. BOSTRÖM, E. ODEBLAD, and U. FRIBERG, Acta Pathol. Microbiol. Scand. *33*, Fasc. 4 (1953).
[2] E. ODEBLAD, Acta Radiol. Suppl. 93 (1952).
[3] R. GOTTSCHALK and H. ALLEN, Proc. Soc. Exptl. Biol. Med. *80*, 334 (1952).

According to DZIEWIATKOWSKI[1] the treatment of rats with thyroxin increases the sulphate fixation in the tissues. On the other hand, it has been shown that the administration of cortisone to animals reduces the speed of the sulphate fixation in the tissues, probably reflecting an actual decrease in the rate of renewal of the sulphate groups in the sulpho-mucopolysaccharides[2]. Such a slow sulphate exchange has recently been found by REDDI and NORSTRÖM[3] to occur in scorbutic animals. Certain salicylates also exert a similar retarding effect[4].

## IN VITRO EXPERIMENTS

### *Methods*

LAYTON *et al.*, using a tissue culture technique, made a series of *in vitro* studies of the sulphate fixation in a number of tissues[5]. They found a highly differentiated fixation in embryonic chick tissues and a greater retention of sulphate in granulation tissue and injured muscle tissue from healing wounds than in corresponding normal tissues.

Since we found that slices of cartilage take up free sulphates from the surrounding medium as well, a technique for studying the sulphate exchange *in vitro* was elaborated in our laboratory[6]. The technique was as follows:

Fresh costal cartilage from newly killed calves was cut in thin slices. 5 g portions of the slices were then incubated in a Krebs-Ringer-bicarbonate solution containing $S^{35}$-labelled sodium sulphate at a temperature of 37°C in the presence of a gas mixture containing oxygen and carbon dioxide. After a few hours the reaction was stopped by boiling the samples. Chondroitin sulphuric acid of a high degree of purity was then isolated from the cartilage according to a simplified small-scale method. The contaminating inorganic sulphates were removed by passing the samples through an anion exchange column. After splitting off the ester sulphate by acid hydrolysis and precipitating as $BaSO_4$, the radioactivity of the sample was measured in a GEIGER-MÜLLER counter. The figures for the uptake of radioactive sulphate obtained in parallel experiments agreed fairly well with a standard deviation amounting to 3–4 per cent in a double determination. No corresponding $S^{35}$ incorporation took place in 24 h when sodium sulphate labelled with $S^{35}$ was added to pure chondroitin sulphuric acid alone.

### *The $S^{35}$ incorporation in the slices*

The $S^{35}$ incorporation in chondroitin sulphuric acid increased with the time of incubation. During the first two hours of incubation, the $S^{35}$ incorporation seemed to be more rapid than subsequently. In the interval 2 h–24 h, there seemed to be a linear relationship between the incorporation of $S^{35}$ and the time of incubation (Fig. 6).

The ability of the cartilage to incorporate sulphate ions remained fairly constant during the first 10 h after the slaughter, if the material was kept in an ice-box at a temperature of + 4°C. After that time a slow reduction in activity was noticed. On the other hand, if fresh cartilage was frozen and then thawed before incubation, no $S^{35}$ uptake occurred in the slices; this agrees with the findings of LAYTON and co-workers. Homogenization in a small blender also destroyed the activity, as did heating of the slices to 47°C.

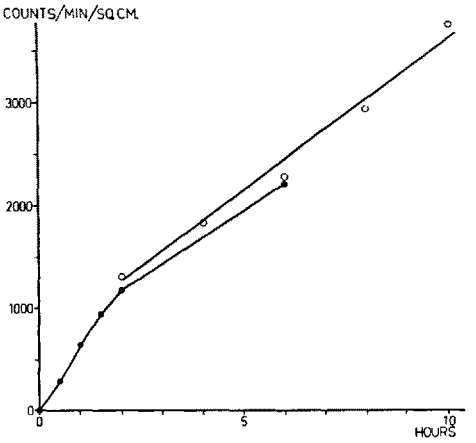


Fig. 6.–The $S^{35}$-uptake in the sulphate group of chondroitin sulphuric acid of the cartilage *in vitro* as a function of the time of incubation.

A considerably higher uptake was noted in slices of medium thickness (0·25–0·50 mm) than in thin and thick slices. There was an increase in the $S^{35}$ incorporation with an increase in temperature until heat inactivation occurred. A considerably slower uptake took place when the bottles were aerated by a nitrogen-carbon dioxide mixture instead of the ordinary oxygen-carbon dioxide mixture.

### *Inhibition of the $S^{35}$ incorporation*

In order to obtain more information on the process leading to incorporation of sulphate in the cartilage, the effect of several more or less "group-specific" enzyme inhibitors was systematically studied in *in vitro* experiments. Most of the reagents employed were dissolved directly in Krebs-Ringer-bicarbonate solution; the acid reagents were brought to pH 7·4 before use. A few water-insoluble reagents were dissolved in ethanol, which in small amounts had no significant effect on the process to be studied. The inhibitors were added to the slices suspended in Krebs-Ringer-bicarbonate solution half an hour before addition of the isotope.

The results of the experiments presented in Table II showed that a moderate degree of inhibition was brought about by many of the substances, *e.g.* hydroxylamine, semicarbazide, potassium cyanide, sodium azide, thiourea, and formalin. Among the inhibitors tested, however, the SH inhibitors such as arsenicals, mercurials, iodosobenzoate, monoiodoacetic acid, copper ions and selenite were found to be the most effective. The inhibitions caused by mercurials and iodobenzoate could to some extent be prevented by glutathione.

### *Effect of cortisone and salicylates*

Cortisone was among the other substances tested. According to LAYTON's findings[1], this substance reduces the fixation of $S^{35}$ in various tissues, an observation which we have confirmed in our experiments[2]. Cortisone in the form of cortisone alcohol was dissolved directly in the Krebs-Ringer-bicarbonate solution. It proved to have a strongly inhibiting effect on the sulphate ex-

[1] D. D. DZIEWIATKOWSKI, J. Biol. Chem. *189*, 717 (1951).
[2] L. L. LAYTON, Proc. Soc. Exptl. Biol. Med. *76*, 596 (1951). – H. BOSTRÖM and E. ODEBLAD, Arkiv Kemi *6*, 39 (1953).
[3] K. REDDI and A. NORSTRÖM, (Unpublished observation).
[4] H. BOSTRÖM and B. MÅNSSON, Unpublished observation.
[5] L. L. LAYTON *et al.*, Cancer *3*, 725 (1950).
[6] H. BOSTRÖM and B. MÅNSSON, Arkiv Kemi *6*, 23 (1953).

[1] L. L. LAYTON, Proc. Soc. Exptl. Biol. Med. *76*, 596 (1951).
[2] H. BOSTRÖM and B. MÅNSSON, Arkiv Kemi *6*, 23 (1953).

change in chondroitin sulphuric acid of the cartilage *in vitro*. With a concentration of $2{\cdot}8 \times 10^{-4}$ M per liter (10 mg per cent) the inhibition amounted to 35 per cent

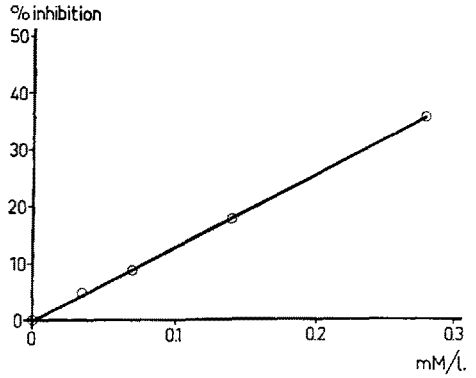


Fig. 7.–Effect of cortisone alcohol on the $S^{35}$-incorporation in chondroitin sulphuric acid in slices of cartilage *in vitro*.

(Fig. 7). Of the substances hitherto tested, only the SH reagents proved to be more powerful inhibitors. A very similar effect *in vitro* was obtained with salicylates.

*Stimulation of the $S^{35}$ incorporation*

It was recently found in our laboratory that the process studied could not only be inhibited by different agents but could also be stimulated in different ways. Thus, the presence of glucose (0·1 %) in the suspension medium gave an $S^{35}$ incorporation in the samples of chondroitin sulphuric acid of cartilage which was 15–20 per cent higher than that in samples incubated according to the standard method, without the addition of glucose to the Krebs-Ringer-solution.

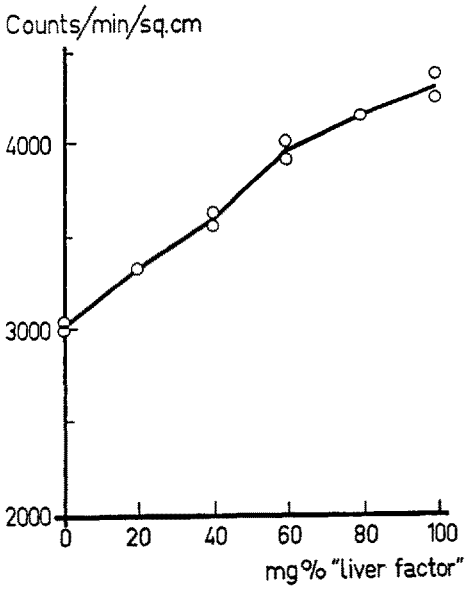


Fig. 8.–Stimulating effect on the $S^{35}$-incorporation in slices of cartilage *in vitro* by a crude preparation of the "liver factor".

A stimulation of quite another order of magnitude was, however, obtained when small amounts of a liver homogenate were added to the slices[1]. In this case, radioactivity exceeding that of the control samples by more than 100 per cent was noted. In further experiments it was shown that the active principle is present in the liver extract. It is thermostable and can be dialyzed through a cellophane bag.

*Table II*

Effect of some enzyme inhibitors on the $S^{35}$ uptake by slices of cartilage *in vitro*.

| Inhibitor | Concentration of inhibitor M/l | Per cent inhibition |
|---|---|---|
| *Metal enzyme inhibitors** | | |
| Potassium cyanide | $8{\cdot}0 \times 10^{-3}$ | 53 |
| Sodium azide | $8{\cdot}0 \times 10^{-3}$ | 72 |
| Thiourea | $8{\cdot}0 \times 10^{-3}$ | 12 |
| 8-hydroxyquinoline | $8{\cdot}0 \times 10^{-4}$ | 75 |
| *Carbonyl group reagents** | | |
| Hydroxylamine | $8{\cdot}0 \times 10^{-3}$ | 95 |
| Semicarbazide | $8{\cdot}0 \times 10^{-3}$ | 42 |
| Sodiumbisulphite | $8{\cdot}0 \times 10^{-4}$ | 39 |
| Phenylhydrazine | $8{\cdot}0 \times 10^{-4}$ | 67 |
| Potassium cyanide | $8{\cdot}0 \times 10^{-3}$ | 53 |
| Dimedon | $8{\cdot}0 \times 10^{-3}$ | 0 |
| *Sulphuric group reagents** | | |
| Iodosobenzoate | $4 \times 10^{-4}$ | 50 |
| Iodoacetate | $6 \times 10^{-5}$ | 50 |
| Phenylmercuric nitrate | $2 \times 10^{-5}$ | 50 |
| p-Chloromercuric benzoate | $4 \times 10^{-5}$ | 50 |
| Lewisite | $8 \times 10^{-6}$ | 50 |
| Diphenylchloroarsine | $8 \times 10^{-6}$ | 50 |
| Sodium arsenite | $8{\cdot}0 \times 10^{-4}$ | 88 |
| Sodium selenite | $8{\cdot}0 \times 10^{-5}$ | 46 |
| Copper chloride | $8{\cdot}0 \times 10^{-4}$ | 79 |
| Chloroacetophenone | $5{\cdot}0 \times 10^{-5}$ | 66 |

* Type of inhibitor.

The result of a typical experiment showing the increase in the sulphate exchange caused by different amounts of the "liver factor" is recorded in Figure 8.

*Zusammenfassung*

Es wird über Versuche zur Bestimmung des enzymatischen Umsatzes der Sulfatgruppe von Mucopolysacchariden mit Hilfe von $S^{35}$-Sulfat berichtet. Letzteres ist in hohen Konzentrationen im Knorpel, in den Schleimhäuten, in der Intima aortae und in den Gewebsmastzellen nachweisbar. Das Ausmass dieser Sulfatfixierung kann entweder auf Grund von Organen isolierter Chondroitinschwefelsäure oder nach der Intensität der Schwärzung des autoradiographischen Bildes bestimmt werden.

$S^{35}$-Sulfat wird von dünnen Knorpelschnitten auch aus einer Lösung aufgenommen, womit das Studium des Umsatzes der Sulfatgruppen *in vitro* möglich ist. Durch Einfrieren, Homogenisieren oder Erhitzen des Knorpels auf 47°C wird seine diesbezügliche fermentative Aktivität zerstört. Der Umsatz der Sulfatgruppen wird besonders stark durch SH-Inhibitoren und auch durch Cortison gehemmt. Zusatz von Leberextrakt bewirkt eine hochgradige Stimulierung der Sulfatveresterung durch einen dialysierbaren und thermostabilen Aktivator.

[1] H. BOSTRÖM and B. MÅNSSON, Acta Chem. Scand. 1953 (in print).