11. Strandberg, L. *Acta Physiol. Scand.* **21** (1950) 222.
12. Stein, W. H., and Moore, S. *Cold Spring Harbor Symposium Quant. Biol.* **14** (1949) 179.
13. Schneider, W. C. *J. Biol. Chem.* **161** (1945) 293.
14. Åqvist, S. E. G. *Acta Chem. Scand.* **5** (1951) 1031.

Received November 5, 1952.

Exchange of the Acetyl Group of Chondroitin Sulphuric Acid in Slices of Cartilage

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In an earlier paper[1], we have reported on the exchange of the sulphate group of chondroitin sulphuric acid in slices of cartilage. The object of the present study was to investigate the exchange of the acetyl group by means of the previously described slicing technique, after addition of sodium acetate labelled with C^{14} in the methyl group. A relatively low but definite uptake of C^{14} was demonstrated for both the methyl and the carboxyl groups of the acetic acid.

Fresh costal cartilage from 1 to 3-day-old calves was freed from muscles, connective tissue and perichondrium, and sliced to a thickness of approximately 0.2 mm. The slices were incubated in portions of 10 g with shaking in an oxygen-carbon dioxide atmosphere (93.5 % O_2 plus 6.5% CO_2) at 37° C in Erlenmeyer flasks, each containing 50 ml of Krebs' solution [3], sodium acetate labelled with C^{14} in the methyl group *, and 50 000 I.U. of penicillin. The slices were taken at different times and boiled for five minutes. Sodium salts of chondroitin sulphuric acid were then prepared from all the samples, according to a method described elsewhere [2]. The yield was 200 to 300 mg from each portion of 10 g. The figures obtained on analyzing the different preparations are given in the table.

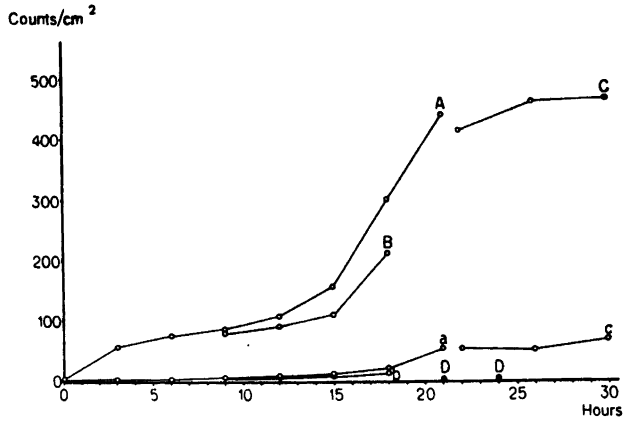
150 mg of the preparations obtained were hydrolyzed under reflux with 10% by volume of sulphuric acid for three hours at 100° C. The acetic acid was then isolated by distillation. 0.05 *N* NaOH was added to pH 7 (glass electrode control), and the solution evaporated to dryness. The sodium acetate was then degraded according to the method of Phares [4]. After plating of the barium carbonate obtained, employing a filtering technique, the radioactivity of each of the carboxyl and methyl carbons was measured in a Geiger-Müller counter with a 2.1 mg per cm^2 mica end-window tube.

Three different experiments, A, B, and C, were carried out in which 1.6 mg, 0.5 mg, and 1.0 mg, respectively, of sodium acetate labelled with C^{14} in the methyl group and with an activity of 2.5×10^6 c.p.m. per cm^2 (measured as $BaCO_3$ at infinite thickness) were added. The rate of uptake of C^{14} in the methyl and carboxyl carbon of the acetyl group of the chondroitin sulphuric acid was determined. The periods of incubation varied from 0 to 30 hours. In two control experiments the incubation was performed with cartilage slices which had been boiled for five minutes prior to incubation with 1.0 mg of labelled acetate.

The results of the experiments are shown in the figure. Curves A, B, and C represent the uptake of C^{14} in the methyl group of the acetyl group in the chondroitin sulphuric acid as a function of time. Curves a, b, and c denote the corresponding uptake in the carboxyl group. The points D mark the retention in the methyl carbon in the acetyl group of the chondroitin sulphuric acid prepared from slices that had been boiled prior to incubation with the isotope.

It is seen from curves A, B, C, and a, b, c that in slices of calves' cartilage some methyl-labelled sodium acetate is built into the acetyl group of the chondroitin sulphuric acid, the labelled carbon being found both in the methyl group and, although to a lesser extent, in the carboxyl group. If the slices were boiled prior to addition of the isotope, there was no uptake (experiment D).

* Obtained from *The Radiochemical Centre Amersham, England.*



The fact that the sodium acetate added was labelled in the methyl group only, but that the carboxyl group of the acetic acid from chondroitin sulphuric acid nevertheless also showed a low but definite uptake, suggests that some change in the precursor has occurred in the system examined. On the other hand, the methyl group showed a rate of uptake that was about ten times as high as that of the carboxyl group. This fact seems to support the supposition that an exchange, including an introduction of acetate, has occurred in the chondroitin sulphuric acid.

The possibility that the observed incorporation of C^{14} in the chondroitin sulphuric acid should be due to contamination with free labelled acetate, is considered to be ruled out on the following grounds. In the two samples in which the cartilage had been boiled prior to incubation with acetate (D), and in the sample that was boiled immediately after addition of the isotope (the time O on curve A), there was no significant uptake. Moreover, the applied method of preparation of chondroitin sulphuric acid includes extraction with NaOH followed by neutralization with glacial acetic acid and dialysis against running distilled water. With this process the precursor is diluted about 2 000 times with inactive acetate. Finally, the chondroitin sulphuric acid preparations obtained were allowed to pass through an anion exchange column (Dowex 2, 20 to 40 mesh, 30 cm × 1 cm) without any observable change in the content of isotope.

The possible contamination with proteins also appears to lack importance as a source of error. Firstly, the low content of nitrogen in the preparations (Table) rules out the presence of any large amounts of proteins. Secondly, the possibility that any C^{14}-labelled complexes would follow the acetate during the isolation and the subsequent degradation is very slight.

It is of some interest to compare the concentration of isotope in the acetyl group of the chondroitin sulphuric acid obtained in this experiment with the in-

Nitrogen % of dry substance		Sulphur % of dry substance		Acetic acid % of dry substance	
Found	Calculated	Found	Calculated	Found	Calculated
2.88 (2.61, 3.22)	2.78	5.69 (5.58, 5.78)	6.37	10.1 (9.6, 11.4)	11.9

corporation in the sulphate group of the chondroitin sulphuric acid obtained, when labelled sodium sulphate is added to slices of cartilage in about equivalent concentrations and under otherwise identical conditions. It was found that in the latter case the content of isotope was many times higher, as has been shown elsewhere [2]. At present, it is impossible to state whether these findings reflect a real difference in the speed of exchange of the acetyl groups and of the sulphate groups or whether the difference in the content of isotope found is merely due to different sizes of the sulphate and acetyl pools.

In addition, the course of the curves reproduced in the figure, indicating a considerably higher rate of uptake at the end of the experiment, differs from that of the curves representing the exchange of sulphate in the chondroitin sulphuric acid, which is almost linear during the first 20 hours. The cause of this discrepancy has not been investigated. The possibility that the increase in the C^{14} incorporation found in the later part of the former curve may be due to bacterial influence cannot be completely ruled out.

The authors wish to express their thanks to Professor Erik Jorpes for valuable discussions and stimulating advice during the work.

This investigation has been aided by grants from the *Rockefeller Foundation*, the *Swedish Medical Research Council*, the *Knut and Alice Wallenberg Foundation*, and the *Vitrum Company*, Stockholm.

1. Boström, H., and Månsson, B. *J. Biol. Chem.* **196** (1952) 483.
2. Boström, H., and Månsson, B. *Arkiv Kemi, in press.*
3. Krebs, H. A., and Henseleit, K. *Z. physiol. Chem.* **210** (1932) 33.
4. Phares, E. F. *Arch. Biochem. and Biophys.* **33** (1951) 173.

Received November 5, 1952.

Studies on Quinones and Hydroquinones. IV *. A Note on the Structure of Tribromophenol Bromide

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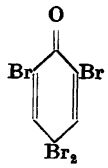
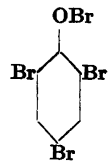
It is possible to propose alternative structures, containing either a carbonyl group or with the hypobromite configuration, for the substances obtained by the bromination of certain quinones, with bromine methanol [1]. A simple substance for comparison is tribromophenol bromide for which two structures, the one (I a) quinonoid and the other (I b) a phenyl hypobromite, have been suggested. Elston, Peters and Rowe [2] investigated analogous polyhalo derivatives and found that the



I a



I b

ultraviolet absorption spectra supported the quinonoid structure. Recently Yasnikov and Shilov [3] studied the reaction between tribromophenol and bromine water containing radioactive Br, and concluded that tribromophenol bromide should have the quinonoid structure (I a). We have now determined the infrared absorption spectrum of tribromophenol bromide (Fig. 1). The two bands at 5.97 μ and 6.31 μ are due to the C = O group and the C = C

* Part III. Acta Chem. Scand. In the press.