A Quantitative Autoradiographic Study on the Uptake of Labelled Sulphate in the Aorta of the Rabbit

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The aorta is an elastic organ which is known to contain chondroitin sulphuric acid [1]. Nothing is as yet known about the rate of renewal of this substance in the aorta. It has, however, been shown by different workers [2-4] using tracer methods that a fairly high sulphate fixation takes place in the aorta, as in other tissues containing sulpho-mucopolysaccharides. This seems to be due to an exchange of the ester sulphate groups in these compounds. From earlier investigations [4-7] we have collected experience on the application of quantitative autoradiography with S^{35} in studies on the sulphate exchange of the mucopolysaccharides. It is fairly difficult to isolate sufficient amounts of mucopolysaccharides from the rabbit or rat aorta for metabolic studies. The autoradiographic technique was therefore chosen for the present investigation, in which an attempt was made to follow the incorporation and elimination of S^{35} in the adult rabbit aorta after a single injection of S^{35}-labelled sulphate.

27 adult female rabbits *, weighing on an average 2.9 kg, were given carrier-free S^{35}-labelled sodium sulphate ** intravenously, in doses of 0.65 mC per kg of body weight. The animals were divided into nine groups with three animals in each, and were sacrificed by air embolism 2, 4, 8, 16, 24, 48, 120, 240 and 384 hours, respectively, after injection of the radiosulphate. Part of the thoracic aorta was excised and fixed in pure methanol for 24 hours. It was then passed through *iso*-propanol (for 3 hours) and xylene (for 3 hours), embedded in paraffin and cut into 10 μ thick sections. These were mounted on metacrylate slides and deparaffinized in xylene and ethanol. They were then pressed against Gevaert Dentus rapid film for 24—27 days in an iron press for exposure [8] (exposure time noted). The films were developed in Kodak DK 20 with 0.2 per cent KBr for 30 minutes at + 18.3° C. The density of the autoradiographic images was then measured with a recording photometer [8]. After correction for the decay of the radioactive sulphur during the exposure time, the relative amounts of S^{35} in the aorta could be calculated.

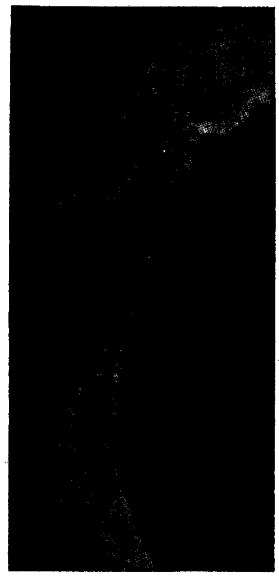

Fig. 1.

The autoradiographs obtained are exemplified in Fig. 1, which shows a fairly even distribution of S^{35} in all the layers of the aortic wall 48 hours after injection. The results of the quantitative estimations of the relative S^{35} content in the media are assembled in Fig. 2. As seen from the curve, a maximal accumulation of S^{35} seems to occur after about 2 days; about two weeks after the injection, half of this value could be noted.

Fixation with methanol results in retention of both inorganic sulphates and orga-

* The same animals as those used in a previous investigation [13].

** Obtained from A.E.R.E., Harwell, England.

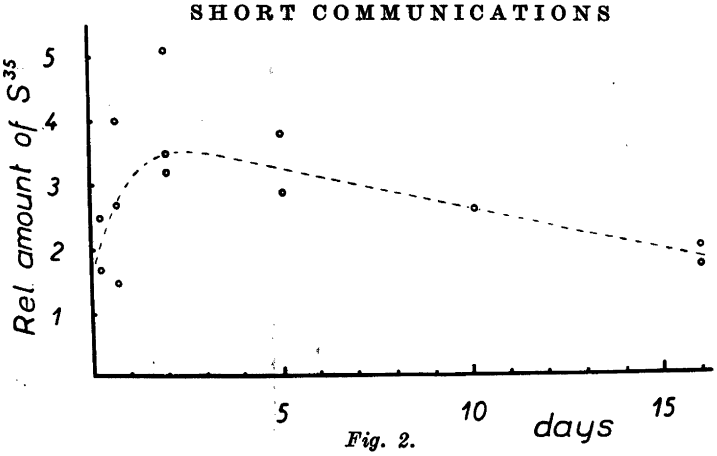

Fig. 2.

nic sulphur-containing compounds in the sections. It is therefore impossible, on the basis of the present investigation alone, to draw any conclusions with regard to the S^{35}-containing compounds in the aortic wall. The following facts have, however, been established by means of earlier investigations. (1) The greater part of the labelled sulphate disappears rapidly from the tissues [4,9]. (2) Sulphate-sulphur is not utilized in the synthesis of sulphur-containing amino acids [10,11]. (3) Good agreement is found if, after the administration of labelled sulphate, the incorporation of S^{35} in ester sulphates in the cartilage [4] and skin [12] of rats as determined on isolated chondroitin sulphuric acid is compared with the increase and decrease of S^{35} in these tissues [5,6], as estimated by quantitative autoradiography.

For these reasons it appears probable that, in the aorta as well, the greater part of the S^{35} present more than 24—48 hours after the injection of sulphate is in the form of ester-bound sulphur in chondroitin sulphuric acid. Thus, the curve in Fig. 1 reflects the exchange of the sulphate group in the chondroitin sulphuric acid of the aorta, the rate of which exchange seems to be moderate.

1. Meyer, K., and Rapport, M. M. *Science* **113** (1951) 596.
2. Dziewiatkowski, D. D. *J. Biol. Chem.* **189** (1951) 187.
3. Layton, L. L. *Cancer* **4** (1951) 198.
4. Boström, H. *J. Biol. Chem.* **196** (1952) 477.
5. Boström, H., Odeblad, E., and Friberg, U. *Arch. Biochem. and Biophys.* **38** (1952) 283.
6. Boström, H., Odeblad, E., and Friberg, U. *Acta pathol. et microbiol. Scand.* In press.
7. Jorpes, E., Odeblad, E., and Boström, H. *Acta Haematologica*, In press.
8. Odeblad, E. *Acta Radiol. Suppl.* **93** (1952).
9. Dziewiatkowski, D. D. *J. Biol. Chem.* **161** (1945) 723.
10. Tarver, H., and Smith, C. L. A. *J. Biol. Chem.* **130** (1939) 67.
11. Boström, H., and Åqvist, S. E. G. *Acta Chem. Scand.* **6** (1953) 1557.
12. Boström, H., and Gardell, S. *Acta Chem. Scand.* **7** (1953) 216.
13. Odeblad, E., and Boström, H. *Acta Radiol.* In press.

Received January 12, 1953.

A Small Cell for Electrolytic Alkoxylation of Furans

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To date 14 different 2,5-dialkoxy-2,5-dihydrofurans have been prepared by electrolytic alkoxylation of furans [1-7]. These alkoxylations were carried out in a cell with an outer and an inner cathode, containing about 300 ml of electrolyte [1].