

1

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**DEXTRAN SULFATE AS ANTICOAGULANT, PROCESS OF PREPARING SAME, AND STERILE SOLUTION THEREOF**

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This invention relates to the manufacture of anticoagulants for use with blood and plasma, and one object is to provide anticoagulants which may be readily prepared on a large scale. A further object of the invention is to provide anticoagulants which are non-toxic.

Anticoagulants, i. e. substances which prevent blood from clotting, have a wide application in medicine and other biological sciences. They may be used to prevent blood from clotting (a) after blood has been shed ("in vitro") or (b) used within the body ("in vivo"). In case (a), the fluid blood may be readily handled for subsequent transfusion and for many forms of haematological testing (e. g. erythrocyte sedimentation rate, haematocrit, etc.). For such work, anticoagulants should not affect the erythrocyte properties, and should not interfere with the usual haematological tests in other ways. In case (b), repeated administration is usually required, as the conditions likely to result in intravascular thrombosis (e. g. the effects of coronary thrombosis) usually take many weeks to disappear. It is therefore essential that an anticoagulant used in this way shall not cause toxic effects even after prolonged and repeated administration. The prophylactic administration of anticoagulants in conditions where past experience shows that thrombosis may occur (e. g. after abdominal operations, etc.) is also frequently desirable.

The only previously described substance which has a sufficiently low toxicity for administration "in vivo" is the naturally occurring substance "heparin." The present market price of heparin is about £5 per gramme, since it has to be prepared from animal carcasses, in which it is present in minute quantities. Heparin is also in demand for use "in vitro," but in view of its expense, sodium citrate has been used extensively as a substitute for heparin. However, sodium citrate is not ideal for use in this connection, and it has been suggested that certain heparin-like compounds might combine the desirable properties of heparin with ease of preparation on a large scale. Such heparin-like compounds might assist the survival of the cellular components of the blood, such as the leucocytes and platelets to a greater extent than does sodium citrate.

Various polysaccharides with sulphate groups have been shown to possess anticoagulant properties similar to those of heparin, but none has been considered suitable for administration "in vivo." Such compounds, especially those derived from bacterial dextran and certain other compounds, can be produced in large quantities and at low cost.

One compound recently proposed for use as an anticoagulant (see Upsala Läkereforenings Förhandlingar, NF 50-51, 144-46) is a sulphuric acid ester of partially-hydrolysed dextran.

According to this invention there is provided an anticoagulant consisting of, or comprising mainly, a water-

2

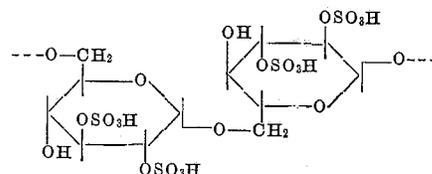
soluble salt of dextran sulphate, the latter being the sulphuric-acid ester of a dextran having an intrinsic viscosity less than 0.12 (measured at 37° C. in a B. S. S. No. 1 Viscometer) and said ester having a sulphur content, calculated on its sodium salt, of not less than 9 per cent.

The said sulphuric ester may have up to three sulphate groups per glucose unit.

The anticoagulant according to this invention may be successfully employed "in vitro" or "in vivo."

The anticoagulant of this invention is non-toxic, and, when employed for blood or plasma, does not interfere with the erythrocyte properties of the blood, or the usual haematological tests in any way. Since the said anticoagulant prevents clotting after the blood has been shed, the fluid treated therewith can be readily handled for subsequent transfusion. Like heparin, the anticoagulant properties of the dextran sulphate produced by the invention may be counteracted "in vivo" or "in vitro" by certain basic substances (e. g. protamine); this is a very desirable property in case of emergency, for example in cases of sudden haemorrhage.

The following is the graphic formula of a dextran sulphate from which the anticoagulant of this invention is prepared:



The following shows the difference between the molecular weights, as deduced from their intrinsic viscosities, of the dextrans employed in the preparation of the said known sulphuric-acid ester of partially hydrolysed dextran and of the dextrans employed in the preparation of the dextran sulphates salts according to this invention.

1. Known dextran sulphates: Molecular weight of dextran employed, 22,000-200,000; dextrans of such molecular weights have an intrinsic viscosity of at least 0.12, which increases with increasing molecular weight.

2. Dextran sulphate compounds according to this invention:

In the case of three compounds produced, the dextrans employed each had a molecular weight of less than 22,000 and an intrinsic viscosity of less than 0.12.

The following is an example of the preparation of a non-toxic anticoagulant dextran sulphate salt according to the invention:

(1) *Degradation of dextran of high molecular weight*

100 ml. N sulphuric acid are added to 1 litre of 6% dextran and the solution is boiled under reflux for 4 hours. The pH before and after heating is 1.4. The solution is neutralised with about 100 ml. N sodium hydroxide. 1,158 ml. of solution of degraded dextran is obtained.

In order to ascertain the approximate extent of degradation resulting from the above operation, a sample of the resultant solution may be diluted to 3% dextran and the relative viscosity of such diluted sample determined in an Ostwald Viscometer. (In experiments, values in the range 1.11 to 1.16 have been obtained for this relative viscosity).

(2) *Fractionation*

985 ml. acetone are added to 1,145 ml. of the solution of degraded dextran (86 ml. acetone per 100 ml. solution) slowly and with good stirring at 20° C. The syrup formed is allowed to settle and the supernatant