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Dextran and its Use as a Plasmasubstitute *

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As is well known, we possess in blood and plasma therapeutic agents for the treatment of shock, *e. g.* after severe losses of blood, contusions and extensive burns. The use of blood and plasma for the purpose of infusion is, however, accompanied by certain disadvantages. In order to obtain sufficient blood and plasma there is needed, among other things, a large and complicated organization with Blood Donation Centres. It is troublesome to keep blood or to separate out plasma. The cost of blood and plasma is therefore high. In the event of war or other catastrophies it is quite impossible to meet the demand for blood and plasma. Blood and plasma are very delicate means for infusion; they do not bear sterilization by heating. Therefore there always exists a certain risk that one will transport certain infectious diseases with the infusion. Both blood- and plasma-infusions, at times, give rise to undesirable reactions. With blood-infusions one must pay attention to the patient's bloodgroup.

It is therefore natural that physiologists and chemists have tried to find solutions which would be able to replace the expensive and delicate blood or plasma for transfusions.

Even during the First World War Bayliss tried to use solutions of gum-arabic for the purpose of infusion. Later, yet other substances such as gelatin, polyvinylalcohol, pectin, polyvinylpyrrolidon etc. have been tested for this purpose. The infusion of these colloids has however been accompanied by certain inconveniances. Some of the substances tested have given undesirable reactions, some have antigenic properties, and some could not be broken down by the organism, hence they are accumulated, especially in the liver.

Before I pass on to give an account of the attempts of Grönwall and myself to obtain a plasmasubstitute from dextran [1—5], I think it is necessary for

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me to state some of the conditions a colloid should fulfill, in order that it should be applicable as a plasmasubstitute.

For shock after not only bleeding but also contusions and burns, it is necessary by means of infusion of liquids to increase the volume of the circulating blood, so that the blood pressure is kept up to the normal value. This can not be done in a satisfactory way with crystalloid solutions, since substances of low molecular weight quickly leave the bloodstream. There is required instead infusion solutions containing colloids, which produce the same colloid-osmotic-pressure as the plasma proteins. An assumption, that the colloids should produce this pressure and keep the water in the blood vessels, is that they should have such a large molecular size that they do not pass out through the walls of the blood vessels but remain in the blood. The molecular weight of the colloid should consequently not be too small. The molecular weight however should not be too large since the colloid-osmotic-pressure then becomes too small. The molecular weight should be of the order of magnitude of 100,000. It is not necessary for the substance to bee monodisperse. However, it can be thought to be desirable that the distribution of the molecular weight should have a convenient appearance.

The colloid must be able to be injected intravenously in large quantities and on repeated occasions without being accompanied by any undesirable reactions. It must consequently be quite untoxic and not have antigenic properties. It should of course be able to be given to all, independent of blood-group and such like.

The solution must not be too viscous. Preferably the viscosity should be of the same order of magnitude as for plasma.

The substance should be of such a nature that the body is able to get rid of it at a suitable speed, so that it does not remain in the blood too long or accumulate in the organs.

The colloid solution should be able to be sterilized by means of heating at 120° C so that one can be absolutely sure that neither bacteria nor virus infection is transferred. The sterilized solutions should be very stable so that the flasks containing the prepared solutions can be kept for a long time at room-temperature without special precautions.

Finally the substance should be able to be prepared on a large scale and the price should be lower than that for dried plasma.

It is not so difficult to find colloids which only fulfill some of the conditions set forth. But if one shall find a colloid which fulfills all the conditions set forth at the same time, one must surely have a little luck.

Fig. 1. Electron-microscope photograph of dextran molecules. 30,000 ×.

It has now appeared that by means of partial hydrolysis of the polysaccharide dextran we can prepare a substance which fulfills the requirements indicated above for a plasma substitute.

Dextran was discovered during the last century by the German sugar-chemist Scheibler. He found that in sugar-refining there sometimes appeared a mucous substance which caused a great deal of trouble, among other things that thereby the sugar juices filtered slowly. Already during the last century it was shown that dextran is a polysaccharide which on hydrolysis gives glucose. It was also shown that dextran is formed in sugar juices which are infected with the bacterium *Leuconostoc mesenteroides*.

It was also during work with sugar juice that we came into contact with dextran for the first time. Since 1941 in Tiselius' laboratories we have carried out researches on the colloids of sugar beet in close collaboration with the Swedish sugar-industry. During these studies we happened to get a sugar beet juice infected with *Leuconostoc mesenteroides*. We obtained some dextran, on which we carried out some researches. Among other things we showed that the molecular weight of dextran was unusually high, of the order of magnitude of several millions. An exact value could not be given however, since the

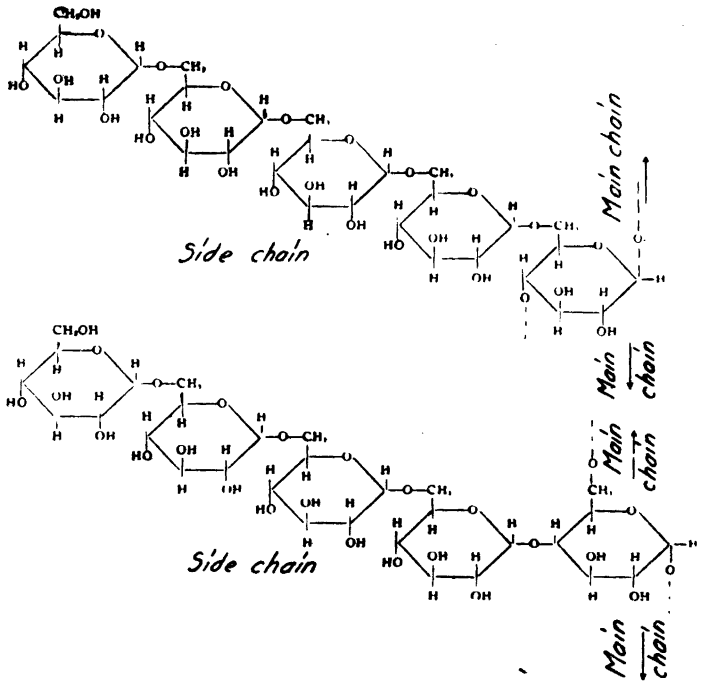

Fig. 2. Formula of dextran.

dextran did not have a single molecular weight. Later however, in collaboration with Halling and Kinell the dextran has been fractionated into more distinct fractions for which molecular weight determinations etc. are being carried out [6]. Quite early we could show that dextran has a long molecule. This appears even from electron microscope pictures of dextran molecules which I took together with Kai Siegbahn during 1943 [7,8] (Fig. 1). Magnification of the image is 30,000 ×. The thickness of the threads at the narrowest place is 30—100 Å units. 50 Å units is equivalent to about 10 glucose units. Here and there one can see knots on the threads at regular distances.

A molecular breadth of about 50 Å units can be explained with the formula for dextran which Levi, Hawkins and Hibbert suggested on the basis of methylation experiments [9] (Fig. 2).

They consider that dextran consists of long main chains with short side chains. Mainly the bindings are 1—6 but 1—4 bindings also occur. What in the electron microscope is visible as a thread should consequently be a main chain with short side chains.

Dextran is not broken down by the usual amylases.

Among the foreign works on dextran which are carried out during later years, the researches on the enzymatic synthesis of dextran from saccharose are quite the most interesting. Hehre could show that one could obtain from cultures of *Leuconostoc mesenteroides* a bacteria-free filtrate which contained an enzyme which could convert saccharose to dextran according to the formula:

$$n \text{ saccharose} = \underset{\text{dextran}}{(\text{glucose} - H_2O)_n} + n \text{ fructose}$$

Hehre has shown that, in distinction from the synthesis of starch, glucose-1-phosphate is not an intermediate product here [10].

After this very short survey of the chemistry of dextran we return to the problem of producing a plasma substitute from dextran. This work was started with Grönwall in January 1943 at the Institutes of Biochemistry and Physical Chemistry in Uppsala.

It was very quickly shown by experiments with animals that the viscous aqueous solutions which are obtained from the natural dextran could not be injected intravenously. But when the dextran was partially hydrolyzed to a molecular weight of the same order of magnitude as that of the proteins of blood, we could inject the test animals with large quantities without any injurious results. The hydrolysis of dextran is carried out by the aid of hydrochloric acid and is stopped when a product of suitable molecular weight has been obtained. The dextran can be precipitated from the water solution by the addition of alcohol. It must of course be purified very carefully. The solution must be so pure that one can, for example, after severe burns, when large losses of plasma take place, inject up to ten liters intravenously during some days into one and the same patient.

The viscosity and the colloid-osmotic-pressure for the 6 % solutions used are of the same order of magnitude as for plasma. Besides the partially hydrolyzed dextran the solutions also contain 0.9 % of sodium chloride. The solutions can be heated at 120° C and the sterilized solutions are very stable.

If a normal infusion-dose of dextran be injected intravenously, for example, into a dog or a man, the concentration of dextran in the blood sinks to 0 in about 5 days. The time that the dextran stays in the blood seems to be ideal. A small part of the dextran, namely the fraction which has the smallest molecular size, is filtered out through the kidneys into the urine. Even after repeated large infusions no accumulations in the organs can be detected. Animals had for a long time been injected with large quantities of dextran. Some time after the last injection the animals were killed and examined histologically by Gellerstedt, without any sign of injury or accumulation

being observable. We were neither able to detect any dextran by means of polarimetric determinations nor by means of Hint and Thorsén's recently produced dextran determination method, which admit the detection of small quantities of dextran even in the presence of glycogen. Since the quantities of the dextran injected had been very great, one must form the conclusion that dextran is slowly broken down in the body. A support for this presumption is also given by some hormone investigations which I have performed. If one gives dextran-injected rabbits thyroid gland preparation, the dextran disappears out of the blood more quickly than normally. The most probable interpretation of this result is that when the metabolism is increased by means of the thyroid gland hormone, then the dextran also breaks down more quickly [11].

After dextran infusion the sedimentation-rate of the erythrocytes is increased. The original dextran produces a greater sedimentation than the partially hydrolyzed dextran. The sedimentation produced is less and less the more one breaks down the dextran. Thorsén and Hint have confirmed this by accurate tests with dextran fractions of different molecular size. This proved that the change of the sedimentation-rate of the erythrocytes was primarily caused by the largest dextran molecules in the therapeutically used dextran solution.

The therapeutic effect was studied by our animal tests for shocks caused by bleeding, histamine and contusion which we produced in rabbits and cats in narcosis. A good effect on blood pressure, heart activity and breathing could always be recorded (Fig. 3). The figure shows a blood pressure curve from such a test with a rabbit. Along the y-axis is the animal's blood pressure, the x-axis is a time-axis. Before the test the rabbit's blood pressure was 100 mm Hg. In deep narcosis the animal's hind legs were crushed. (Repeated crushings at I, II and III.) The blood pressure dropped by this means to about 30 mm and stayed at this value. After some minutes 20 ml of dextran solution were injected intravenously (at IV) after which the blood pressure quickly rose to the normal value (V).

Since the animal tests passed over favourably, we dared to begin clinical tests firstly on a very limited scale. Since these tests gave promising results, an extensive clinical test was begun under Bohmansson's guidance. In these tests, Thorsén and Wilander and, as burn-specialist, Rosenqvist took part [12]. Besides the work already published Thorsén is now compiling extensive clinical material.

It is obvious that it was necessary that the clinical tests should be carried out with the greatest care and therefore the tests took a large time. Now, however, about 2,000 l of dextran solution have been injected into about a thousand patients, hence the conclusions which were drawn from the tests could be regarded as well founded.

Fig. 3. Blood pressure curve from a test with a rabbit.

Since this is a meeting of chemists and not a congress of physicians and since the time is limited I cannot now, unfortunately, give an account of all the interesting details in these clinical tests. I shall restrict myself to say that the result of shock-treatment by dextran is good and that when required one can inject several liters of dextran solution without injurious secondary reactions. The dextran concentration in plasma can rise to several per cent, in which case the concentration of protein can be appreciably lower than normal. The concentration of the colloids seems to adjust itself so that the colloid-osmotic pressure of the blood becomes normal. A part of the blood-protein can consequently be temporarily replaced by dextran without inconvenience. Afterwards plasma protein is newly formed at the same time as the dextran disappears.

In his thesis on the treatment of extensive burns Rosenqvist judges the effect of dextran as of the same value as that of plasma [13]. At the Scandinavian Surgical Congress in June 1947 the clinical results were presented by Thorsén [14] and Bohmansson [15], after which dextran could be released for commerce.

Thorsén maintained that the shock prophylaxis which is often used in operations is not fully satisfactory. After operations latent shock is present in so many cases that colloidal and not crystalloidal solutions should be infused. This implies that dextran can be calculated to get a use not only for accidents and extensive burns or operations where shock has already entered but also in the daily routine of surgical departments to prevent shock in operations.

Bohmansson summed up the result of the clinical tests with these words: »Dextran is a safe and very good plasma substitute for the treatment of shock and for the prevention of shock.»

Raw dextran is now prepared by *Svenska Sockerfabriks Aktiebolaget* and hydrolyzation, purification and preparation of the infusion solutions are carried out by *A.B. Pharmacia* in Stockholm.

Finally I should also desire to state that it would not have been possible to carry through this work without the fine collaboration between the scientific institutions in Uppsala, the industries concerned — *i. e. A.B. Pharmacia* and *Sockerbolaget* — and the doctors who performed the clinical tests.

SUMMARY

A brief survey is given of some of the conditions which a colloid should fulfill, in order that it shuld be applicable as a plasma substitute. Some properties of the polysaccharide dextran are described. By means of partial hydrolysis of dextran it is possible to prepare a substance which fulfills the requirements indicated for a plasma substitute.

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