turned to advantage over other forms of radiotherapy in the treatment of malignant disease in patients.

Another use of the cyclotron lies in its ability to produce certain radioactive isotopes which cannot be produced in the atomic pile. The particular importance of this cyclotron lies in its ability to produce in a hospital certain isotopes which, on account of their very short life, must be used immediately they are produced. Facilities have been provided adjacent to the machine for this purpose.

[1] *Nature*, 171, 297 (1953).
[2] Wood and Boag, M.R.C. Special Report Series No. 267, "Researches on the Radiotherapy of Oral Cancer" (London: H.M.S.O.).

# DEXTRAN AND ITS APPLICATIONS

ON the evening of December 3 the Microbiology Group of the Society of Chemical Industry held a meeting in the hall of the Medical Society of London, 11 Chandos Street, London, W.1, presided over by Dr. L. A. Allen, at which various applications of dextran were discussed.

Prof. M. Stacey introduced the subject with a paper on "Polyglucose Structures with Special Reference to the Dextran Group". He commented on early work in Sweden and in Birmingham, saying that the dextran polysaccharides have achieved an important medical use as blood plasma substitutes or blood volume expanders, and their derivatives are possible anticoagulants. Since they can readily be produced from cane or beet sugar by fermentation methods on an industrial scale, other uses, such as colloids of precise size and shape, will doubtless be found for them.

Chemical and physical methods of carbohydrate chemistry are now so well developed and of such diversity that we can now determine with great accuracy the homogeneity, size, shape and molecular structure of most polysaccharides. Homogeneity can be determined by physical techniques such as measurements of optical rotation, electrophoresis, ultracentrifugal properties, diffusion, scattering of light, and immunochemical studies, all of which also give information on molecular size and shapes. Constituent units can be determined by hydrolysis with acids followed by paper and column chromatography, ionophoresis, etc., while acetolysis, methylation, periodic oxidation, infra-red studies, ionophoresis, etc., pick out the nature of ends of chains, repeating units, types of linkage and structures generally. Number average and weight average can all be determined by classical methods for dealing with macromolecules; for example, the physical methods already mentioned, viscosity measurements, and methods involving reaction at reducing-chain ends such as copper number measurements and the addition of labelled hydrogen cyanide.

Almost every type of polyglucose structure is now known where the glucose units are in the pyranose form. In starch the amylose component is a chain of at least two hundred glucose units joined by (1 → 4)-α-linkages, while the ramified amylopectin has short chains of twenty glucose units joined as in amylose but cross-linked by (1 → 6) linkages. Glycogen consists of shorter chains with (1 → 4)-α-linkages and (1 → 6) cross-linkages. Cellulose is entirely linear with very long chains of probably a thousand glucose units linked in the (1 → 4)-β manner. The polyglucose made by the crown gall micro-organism possesses mainly (1 → 2) linkages, while the yeast 'glucan' has mainly (1 → 3) linkages. Nigeran from *Aspergillus niger* has alternating (1 → 4) and (1 → 3)-α-linkages, and luteose from *Penicillium zukal* has a long glucosan chain with (1 → 6)-β-linkages.

The predominating linkage in the dextrans is the (1 → 6)-α-linkage, but a variety of types is known varying from completely linear structures to highly ramified types with additional (1 → 4)-α- and (1 → 3)-α-cross-linkages and combinations thereof. All are macromolecules with molecular weights of the order of millions. Great interest arises in methods for degrading the dextrans and in separating and selecting homogeneous fractions for infusion purposes. Breakdown can be effected by acidic, alkaline, enzymic and oxidative hydrolysis, or by ultrasonic or thermal treatment. Fractionation precipitation techniques, using mainly organic solvents and aqueous solutions, not only separate large and small molecules but will also pick out linear from branched chains—the infra-red technique being useful for rapid control of fractionation.

Recently, it has become possible to control the enzymic synthesis of polyglucose molecules and indeed to grow them to size. Great advances have been made in the enzymic synthesis of starch and glycogen. For these, phosphorylase acts upon glucose-1-phosphate to give the straight-chain amylose. This may then be acted upon by the branching '*Q*-enzyme', which effects chain-shortening by breaking about one in every twenty (1 → 4)-α-linkages and which then re-forms the molecule by creating (1 → 6) cross-linkages giving the highly laminated structures of glycogen and amylopectin. This 'transglycosylase action' is responsible for synthesizing the highly branched dextrans, for at least two enzymes seem to be involved: a (1 → 6)-α-linked straight-chain former and a transglycosylase which forms (1 → 4) and/or (1 → 3) linkages.

For polysaccharide synthesis generally three factors are needed: enzyme, specific substrate and receptor molecule. For dextran synthesis by *Leuconostoc* species, sucrose is a highly specific substrate, and preformed dextran forms the receptor molecule or 'primer'. When a small number of large dextran molecules is used to prime the reaction, macromolecules of very large size result. When a relatively large number of small dextran molecules is used to provide receptor groups for synthesis from non-reducing chain ends, they are all lengthened to the same extent, and relatively small dextran molecules result. Thus, it is now possible actually to grow dextran 'to size'—for example, to molecular weights of seventy thousand for infusion purposes. The method uses the living cell, whereas American workers are studying similar polymerizations with isolated enzyme systems.

Mr. A. E. James then presented a paper on "The Technology of Dextran Production", showing that the production of dextran for use as a plasma volume expander may be divided into five main stages: fermentation, degradation, fractionation, purification and final testing after bottling. Dextran is produced as a large macromolecule during the fermentation of sucrose by a selected strain of *Leuconostoc mesenteroides*, although the use of the dextran 'sucrase' enzyme has been reported in the United States. The dextrans produced by different organisms vary from almost linear to highly branched molecules. The

reduction of the molecular size of the native dextran to approximately that of the blood proteins may be achieved by several degradative steps such as acid or alkaline hydrolysis, thermal or pyrolytic breakdown, irradiation with ultrasonic vibrations or enzymic breakdown. Recent work has indicated the possibility of direct synthesis of the required molecular weight, thus avoiding degradation.

The material from degradation is very polydisperse, and the objectionable larger molecules and the useless small ones have to be removed by selective precipitation. Acetone and the lower alcohols have been used as precipitating agents. This process does not yield a homogeneous dextran with respect to molecular weight, and the commercially available transfusion solutions contain a range of molecular sizes dictated partly by medical requirements and partly by economic considerations. Ultrasonic degradation appears to be unique, as it yields a product requiring virtually no fractionation to yield a satisfactory medical preparation.

The desired fraction of dextran is freed from precipitants, pyrogen, ionic impurities and colour by selective adsorption and ion-exchange, and may then be filled directly as a solution in water, sodium chloride solution or glucose solution. Alternatively, it may be spray-dried for bulk testing and distribution. After bottling, rigorous chemical bacteriological and pharmacological tests are applied.

Dr. F. Fletcher followed with a discussion of the "Properties of Dextran from the Physiological Aspect". He pointed out that the maintenance of a normal blood volume depends on the hydrostatic pressure of the capillary blood and the colloidal osmotic pressure gradient between the plasma and tissue protein. Plasma contains albumin, globulin and fibrinogen ; but it is the albumin fraction (55 per cent of total protein) of molecular weight about seventy thousand which mainly contributes to the colloidal osmotic pressure.

It is essential that any colloid intended as a replacement for plasma protein be retained in the blood vessels and not rapidly lost through the capillaries to the tissues or urine. The retained colloid must exert a colloidal osmotic pressure similar to that of the normal plasma protein. Dextran can be prepared to meet these criteria and is a satisfactory substitute for plasma protein.

Dextran fractions of various ranges of molecular weight have been studied, and the distribution and excretion-rates have been compared. The molecular-weight distribution of such fractions can be determined by ultracentrifuge, scattering of light, or techniques of the measurement of osmotic pressure, but it is more usual for measurements of intrinsic viscosity to be made. The relationship between intrinsic viscosity and molecular weight has been published by various workers.

The distribution of dextran in the body is influenced by its molecular-weight distribution. Relatively small molecules below a weight-average molecular weight of sixty thousand are excreted in the urine within a few hours after administration. Molecules slightly larger can also pass readily through the capillary walls into the tissue fluid, thus tending to reduce the effective osmotic pressure gradient. Therefore, on the basis of these physiological considerations, the higher molecular range of dextran used in Britain appears to be preferable for clinical use compared with dextran fractions of lower average molecular weight.

Prolonged storage of a non-physiological colloid in the body is undesirable. That dextran is metabolized in the body has been suggested by the finding of radioactive carbon dioxide in the expired air of animals receiving injections of dextran labelled with carbon-14, obtained by the *Leuconostoc* fermentation of radioactive sucrose. Structural differences exist between dextrans produced by different strains of *Leuconostoc mesenteroides*, and immunological studies on structurally different types of dextran might influence the type of material selected for clinical use.

Mr. A. W. Wilkinson continued the physiological discussion with a paper on "The Clinical Applications of Dextran". After paying a tribute to Prof. Stacey and other pioneer workers on dextran, he commented on its importance in blood studies, and went on to say that the cells of the living body are surrounded by an environment of extracellular fluid ; at any moment this extracellular fluid may be arbitrarily divided into one quarter, which is within the blood vessels in the form of plasma, and three quarters, the interstitial fluid, which is outside the blood vessels in the immediate neighbourhood of the cells. This separation depends on a balance between the hydrostatic forces in the blood vessels and the interstitial space, and the osmotic pressure of the plasma proteins. In normal tissues flow in the capillaries is intermittent and depends chiefly on requirements related to tissue activity. After injury there is a very marked increase in capillary permeability in the injured tissues and the volume and protein content of interstitial fluid increases, this increase in volume being due to a greater turnover of fluid rather than to simple local accumulation, because lymph formation is also increased up to eight times. If the injury is large, so much fluid may be lost from the blood vessels that the volume of blood in active circulation may be reduced enough to cause reduction of the peripheral blood pressure and a state of 'shock'. Shock may also be caused by loss of whole blood. It is now recognized that, in shock, capillary permeability is increased only in the injured tissues.

The most satisfactory treatment of shock is by the early, rapid and complete replacement of lost blood or plasma by the transfusion of blood, plasma or a substitute for plasma, such as dextran. To be successful as such a replacement, the dextran must be fractionated so that as little as possible is lost in the urine or through the unusually permeable capillaries of the injured tissues and as much as possible remains within the vessels.

The effects of injecting a litre of a 6 per cent solution of dextran in 0·9 per cent saline have been studied in normal volunteers. There is an immediate reduction in packed cell volume and of total plasma protein and plasma albumin concentration. These changes afterwards slowly pass off, and the plasma dextran concentration, initially 1–1·5 gm./100 ml., falls slowly over 8–10 days to less than 0·1 gm./100 ml., but even at this time the packed cell volume remains below the original value. Detailed study of the changes during the first 24 hours after the infusion has shown that during the 2 hours or so following the initial increase in plasma volume, which varied from 0·3 to 1·3 litres, there is a reduction in plasma volume almost to the initial quantity. During this short period more than 40 per cent of the total quantity of plasma albumin in solution and nearly 40 per cent of the injected dextran leave the blood vessels. During the next 8–12 hours there is a gradual increase in plasma volume, and

the quantity of plasma albumin in circulation increases to that found immediately after the infusion stopped; but the increase in total circulating dextran is much less. After 24 hours only 8 per cent of the injected dextran has been recovered in the urine and 65–75 per cent remains in circulation within the vessels, so that about 20 per cent is untraced. The explanation of these changes seems to be that the acute overloading of the circulation produced by the rapid injection of this dextran solution is relieved by the loss of intravascular fluid into the interstitial space, with subsequent slow return over the next 8–12 hours. Similar results have been obtained in shocked patients, and two examples of the rapid and lasting improvement in blood pressure and clinical state were described.

All these volunteers exhibited 'reaction' which could be resolved into two phases, urticarial and vasomotor, that were separated in time. Whereas the urticarial phase may be due to the peculiar characteristics of the dextran used, the vasomotor phase seems to be related to the rapid shift of fluid from vessels to interstitial space.

Finally, Mr. A. R. Lockwood briefly reviewed "The Utilization of Dextran and some of its Derivatives". The current utilization of dextran in non-clinical applications depends largely upon the economic separation of the crude polysaccharide from the crude fermenter broths. Solvent precipitation is the only practical means so far employed, but is costly and must usually be followed by spray or roller drying. Some attention has been directed to using the whole fermenter broth—for example, in oil-well drilling muds and edible syrups. Sugar syrups made by the addition of sucrose to *Leuconostoc* pure cultures form an effective way of conveying dextran into a variety of food products without the problem of prior separation.

Derivatives of current interest include the sulphuric polyesters, which have a heparin-like blood anticoagulant action; some of them have been shown recently to be suitable for clinical use. The carboxymethyl ethers are likely to have superior stabilizing powers compared with the neutral polysaccharides. Other ethers and mixed ether/esters have been described, of which the most interesting is the polymerizable allyl derivative. Attention has been directed to the importance of microbial polysaccharides in soil structure; but the exploitation of dextran in this field may have to follow the development of large-scale, low-cost crude dextrans, possibly from the direct fermentation of natural sugar juices.

The discussion at the meeting was opened by Dr. C. R. Ricketts, who said that he thought dextran production might be regarded as the dextran industry now that there are six or eight factories engaged in it. He thought that the present phase of 'tailoring' the molecules of dextran after its initial preparation would be followed by direct production, by fermentation, of the molecular size and form required. The work on the effect of addition of short-chain dextrans and of sugars to the fermentation mixture in modifying the dextran produced provides a fruitful field for simplification in manufacture of the kind suggested and might have an important effect on making preparations for clinical use.

In reply to a question by Mr. D. Armstrong why dextran of a molecular weight of sixty thousand is excreted by the kidney whereas albumin of similar molecular weight presumably is not, Dr. Fletcher said that molecular shape is also important in excretion and that albumin can be re-absorbed back from urine by the kidney whereas dextran cannot. Mr. A. L. Bacharach suggested that in practice this matter is approached empirically and is not based on purely physiological concepts. In commenting that Prof. Stacey's paper showed the advantages of ultrasonics in achieving uniformity of molecular size, Mr. H. F. Frost asked if this method is used commercially, and Mr. Lockwood replied that there has been difficulty in getting large ultrasonic units of suitable types but that they are now being employed. Dr. D. H. F. Clayson asked if the breakdown of dextran in the body is due to attack of the $(1 \rightarrow 6)$ linkages and, if so, could it be controlled by adding inhibitors such as amylase to the blood. Prof. Stacey replied that there is no knowledge yet on either of these points, for no enzymes which could break such linkages have been isolated from body fluids. An inquiry was made by Mr. H. J. Bunker as to the raw materials for the large-scale development of dextran to which Mr. Lockwood referred, and Prof. Stacey replied that any sugar material can be used, including dextrins, and that recent restrictions in production in the Jamaican sugar industry because of over-production suggest that even raw cane sugar might be available for the purpose. Cane sugar undoubtedly must be one of the most important raw materials of the future. Mr. J. Green asked Mr. Wilkinson what was his evidence for saying that the dextran should not have molecular weight above three hundred thousand for clinical use, and the latter said that above this value aggregation of red cells can be demonstrated and this is said to be undesirable; he thought that this tendency might well not be of great importance, since turbulence in the capillaries could be expected to overcome it.

## THE SHIRLEY INSTITUTE

### ANNUAL GENERAL MEETING

THE thirty-fourth annual general meeting of the British Cotton Industry Research Association was held at the Shirley Institute, Manchester, on December 10, 1953, under the presidency of the chairman of the Association, Mr. N. G. McCulloch. In his speech Mr. McCulloch referred particularly to the statutory levy that came into operation during 1953, as a result of which all cotton processors in Great Britain now contribute to the Institute and are eligible to take advantage of the services which the Institute offers. He hoped that all new members would take full advantage of this opportunity. Mr. McCulloch also emphasized that firms who process rayon and synthetic fibres are encouraged to continue in membership or become new members, since so much of the Institute's work is concerned with, or applicable to, these fibres as well as to cotton. The honorary treasurer of the Association, Mr. J. Lindley, also referred to the levy, and paid special tribute to the generous way in which the industry had responded to the appeal for funds which it had been necessary to make in order to tide the Institute over the period before the first collection of the levy.

Dr. F. C. Toy, director of research, presented his annual report and directed attention to the more important of the year's activities. He mentioned especially two essays in the educational field: the travelling exhibition of the Institute's work on sizing