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MOLECULAR INHOMOGENEITY AS A SOURCE OF ERROR IN INULIN CLEARANCE STUDIES

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In recent years there has been disagreement among workers in different parts of the world as to whether the straight-line relationship between plasma level and inulin excretion holds, whatever the concentration of the polysaccharide in the blood. The published results, e.g. those of Ferguson, Olbrich, Robson & Stewart (1950) and of Kennedy & Kleh (1953) are not always strictly comparable, on account of differences in the technique employed. Barnard, Bassir & Hough (1955) observed in a number of individuals a marked fall in the renal clearance of inulin with the passage of time, especially 24–48 hr after a single intravenous injection. After considering a few other possibilities, these authors postulated molecular inhomogeneity of the inulin they used as a feasible explanation of their results. In the present paper evidence is presented on some of the physical properties of six different brands of inulin, as well as the fractional excretion of one of these after a single intravenous injection.

EXPERIMENTAL PROCEDURE

Paper electrophoresis

Analysis of original solutions. Aqueous solutions of intravenous inulin were obtained from Israel (1), Beverley (2), Messrs Thomas Kerfoot and Co. (3 and 4, unpurified and recrystallized respectively), the United States (5) and Edinburgh (6). The numbers in brackets correspond to the code numbers in Figs. 1 and 2. After dilution to 1% (w/v), each was subjected to paper electrophoresis in barbitone buffer at pH 8·6, and stained with a mixture of resorcinol and thiourea in an acid medium: 0·1 ml. of each solution was applied to strips of Whatman No. 31 filter-paper. Each run lasted 20 hr at 130 V, the current being approximately 0·5 mA/cm width of paper. After the electrophoresis, the paper strips were dried at 120° C in a hot-air oven. The dry paper was then sprayed with the resorcinol-thiourea reagent. This consists of two solutions A and B, which are mixed in the proportion of 8:2 (v/v) just before spraying. Solution A consists of approximately 8·2 N-hydrochloric acid; and solution B of thiourea 0·25 g, resorcinol 0·1 g, glacial acetic acid 100 ml. After spraying the strips are suspended in a hot-air oven at 120° C for exactly 5 min, and then left to dry at room temperature, preferably in darkness. The coloured electrophoretograms were scanned by the device of Bassir (1954) with a blue filter.

Analysis of urine inulin. After a single intravenous injection of inulin (supplied as ready-made

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solution in phials by Messrs Kerfoot and Co.) into a subject, urine was voided into a series of separate containers over a period of 24 hr. A blank specimen of urine taken before the injection, and each of the other samples, were submitted to the paper electrophoresis technique described above.

Differential precipitation with ethanol

In the first experiment 2 ml. aliquots of 10% (w/v) solutions of the different brands of inulin were treated with successive 2 ml. portions of 20, 40, 60, 80 and 100 (v/v)% aqueous ethanol. After each addition of alcohol the mixture was shaken for 1 hr and centrifuged. The precipitate was collected and drained after each extraction, and the next portion of alcohol added to 2 ml. of the supernatant. Each precipitate was taken up in hot distilled water, and 2 ml. of this added to 7 ml. of approximately 8·2 N-HCl + 1 ml. resorcinol-thiourea reagent (Roe, Epstein & Goldstein, 1949). After heating for 5 min in boiling water to develop the colour, the inulin content was estimated in a spectrophotometer at 520 mμ.

RESULTS

Fig. 1 shows the diagrams obtained from scanning the electrophoretograms of the original solutions. It can be seen that more than one fraction is present

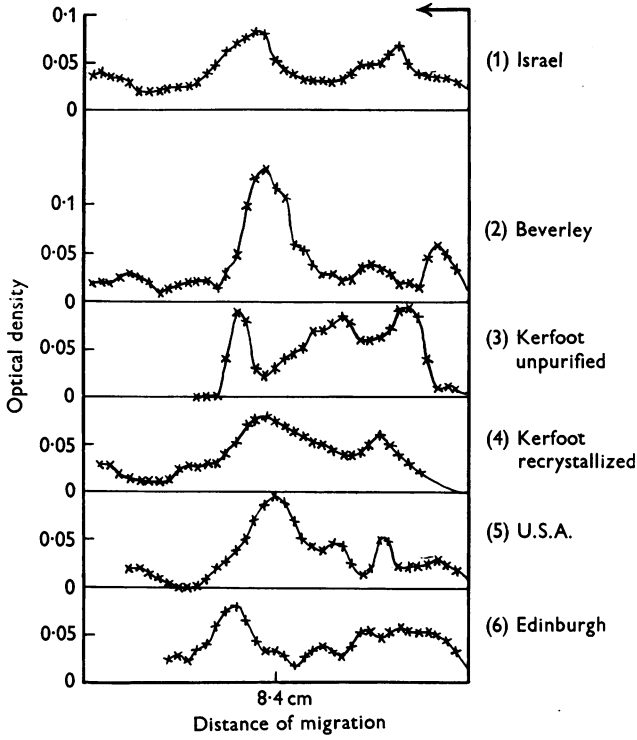

Fig. 1. Paper electrophoresis of intravenous inulin solutions. The graphs are diagrammatic representations of the distribution of inulin on filter-paper after electrophoresis of the samples from Israel, Beverley, Kerfoot (unpurified and recrystallized), United States and Edinburgh, respectively. The optical density readings represent the intensity of the colour given in the resorcinol-thiourea reaction by which the inulin fractions on the test paper are visualized.

in each brand of inulin, and the mobilities of the fractions are different. The Israel and unpurified Kerfoot samples appear similar in having a slowly moving fraction which forms an appreciable fraction of the whole. The Edinburgh and recrystallized Kerfoot samples do not show any pronounced peaks, while the Beverley and U.S.A. specimens appear to consist mainly of a fast-moving fraction sharply delimited from a few minor components.

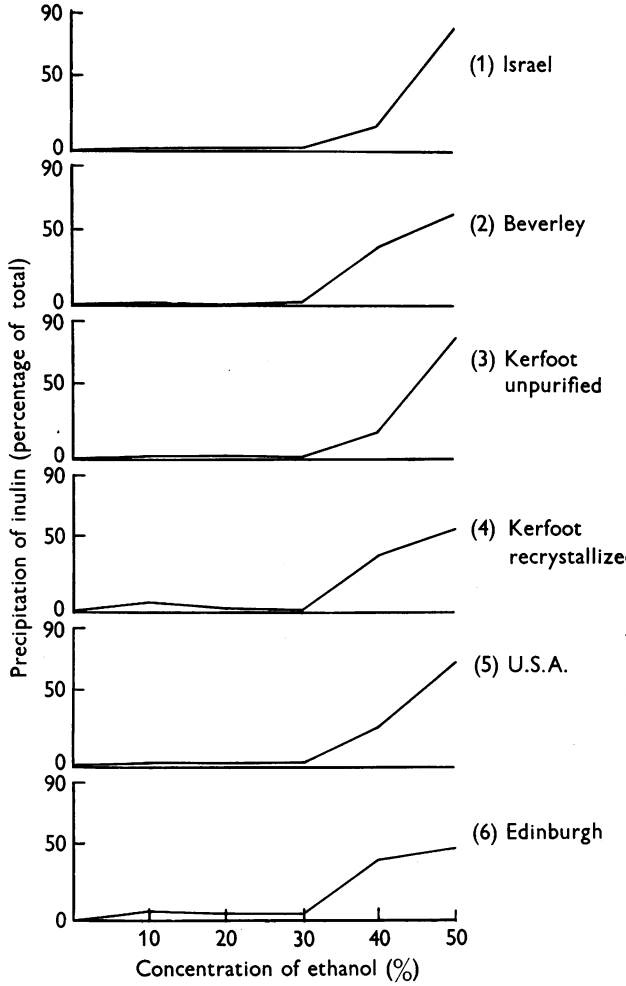

Fig. 2. Precipitation of inulin with various concentrations of ethanol.

Fig. 2 illustrates the results of the differential precipitation of inulin, with ethanol, from aqueous solutions. In this experiment, the pattern of recovery of the polysaccharide is approximately the same for the recrystallized Kerfoot and Edinburgh brands on the one hand, and the Israel and unpurified Kerfoot

variety on the other hand. The Beverley and U.S.A. specimens show intermediate characteristics. The result of a second precipitation experiment was almost identical with the first. At the most favourable concentration of alcohol, the percentages of the total inulin precipitated were approximately as follows: 65 for the Edinburgh and recrystallized Kerfoot specimens; 50 for the Beverley and U.S.A. samples; and 40 for the unpurified Kerfoot and Israel brands.

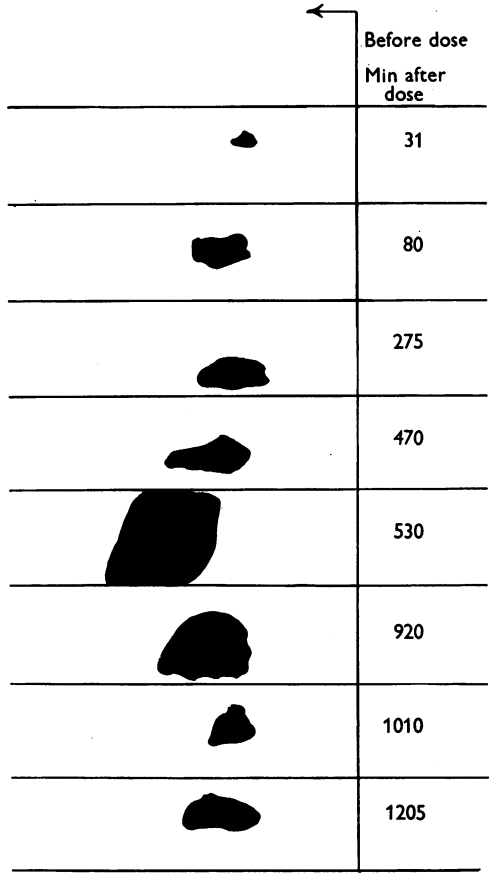

Fig. 3. Map showing migration of main component during electrophoresis of urinary inulin on paper.

Fig. 3 is a diagrammatic representation of the migration of the main component of inulin (Kerfoot) excreted in the various samples of urine, after a single injection into a female subject. It suggests that a continuous increase in the proportion of fast-moving fraction of inulin excreted occurs up to 9 hr after the dose, and a decrease thereafter. Experiments on three other subjects have yielded similar results.

DISCUSSION

Both the electrophoretic analysis of pure intravenous inulin solutions and the physico-chemical characterization by precipitation with ethanol indicate that (*a*) the molecular size (and perhaps structure) of the dissolved particles is heterogeneous; and (*b*) the relative amounts of the components differed considerably in the brands of inulin studied.

Beattie (1954, unpublished) found a decrease in the clearance of inulin from two different species of Dahlia, especially 15 or more hours after injection. Artichoke inulin gave a value different from either of these. A similar decrease in clearance rate was observed in the dog by Beattie & Bell (1953), after the intravenous injection of fructosans which had been shown, by ethanol precipitation and other physico-chemical means, to exhibit molecular inhomogeneity. If the inulin injected were heterogeneous with respect to molecular size, and assuming simple glomerular filtration, we should expect the different particle sizes to be excreted in the urine at different rates. This is shown in Fig. 3 to be borne out in practice.

In a previous study, Hough, Barnard & Bassir (1955) found that the pattern of urinary excretion of inulin after a single injection shows hysteresis, and that the two parts of the excretion curve may be formulated mathematically as

$$x = x_1 (1 - e^{-\lambda_1 t}) + x_2 (1 - e^{-\lambda_2 t}),$$

where x_1, x_2, λ_1, and λ_2 are constants which it is possible to evaluate from an analysis of the curve. It seems possible therefore that there are two separate excretory processes, one of which predominates over the other in the period up to about 10 hr after injection.

In any case, it is clear that because of the molecular inhomogeneity of the different types of inulin and the uneven manner in which the fractions are excreted in the urine, clearance tests conducted with different kinds of inulin may give irregular or misleading results, unless the times of sampling are rigidly standardized (preferably in the middle of the early part of the excretory process), or comparison is made simultaneously on a clinically normal subject. It also follows that if it is desirable to obtain good agreement between the results of different workers in different countries, not only the same technique but also samples of the same brands of inulin must be employed.

SUMMARY

1. By means of paper electrophoresis and differential precipitation with ethanol, commercial samples of inulin solution from various parts of the world have been shown to have different physio-chemical properties.

2. This heterogeneity of particle structure appears to be related to the pattern of excretion of the inulin after a single intravenous injection; and it

may, therefore, give rise to errors if clearance tests are performed with inulin from different sources.

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