operated. On an industrial scale, filter cloths with enzyme action can be mounted in a plate-and-frame filter press; in the laboratory scale, small pieces of active paper can be used by mounting them in a filter holder (Gelman Instrument Co., Michigan). In this way, good flow rates can be obtained without plugging or channelling, and the bed size can readily be altered by adding or removing sheets. By using several sheets with different enzymes attached, it is possible to build a "multi-enzyme" reactor.

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De-esterification using Ion Exchangers

ION exchangers, as the name implies, are used principally for the removal of unwanted ions, but they have also been used for ionic catalysis. In particular, cation exchangers in the H^+ form have been employed to catalyse reactions requiring H^+ in which the product is required free from acid.

The hydrolysis of esters can be catalysed by acids or bases, but the latter are more effective. Esters of different types of alcohol (primary, secondary, hemiacetal) have different stability to base-catalysed hydrolysis. This property was employed in the selective hydrolysis of peracetylated sugars with acetonic potassium hydroxide to give compounds which are esterified at the primary hydroxyl groups only[1-3]. The sugar products of these reactions were accompanied by potassium acetate from which they had to be separated.

This communication describes the use of anion exchange columns to accomplish two tasks: first, to catalyse the hydrolysis of esters and, second, to absorb the acid so formed. Peracetylated sugars and sugar alcohols were used as starting materials. Preliminary experiments indicated that weak anion exchangers such as 'Dowex 3' or 'Duolite *A*6' were not effective and so 'Dowex 1' was used. A column of this material effectively hydrolyses β-glucose pentacetate or β-galactose pentacetate to the free sugar, or sorbitol hexacetate to the alditol at room temperature. If, however, the temperature is below 5° C, then the deacetylation is not completed. Thus 'Dowex 1' × 1 50–100 mesh (400 g) was equilibrated with normal sodium hydroxide, washed three times with water and twice with actone : water 1 : 1 and placed in a cooled Liebig condenser measuring 200 × 2 cm and washed with acetone–water until the *p*H of the eluant was below 8. Ten grams of β-glucose pentacetate or β-galactose pentacetate in 20 ml. acetone was then applied to the column, which was maintained below 5° C. The column was eluted with acetone–water. The first 650 ml. of eluate contained the products, which were a mixture of several acetates of the sugars, with 6-*O*-acetyl glucose or 6-*O*-acetyl galactose, respectively, predominating. These were crystallized and identified. When sorbitol hexacetate was applied, thin layer chromatography of the eluate (silica-gel *D*5 from Camag developed with chloroform : methanol, 88 : 12, and made visible with iodine), showed the product to be a mixture of acetates. Of these, the 1,6-di-*O*-acetyl sorbitol predominated. Also present were monoacetyl sorbitol, esterified at a primary alcoholic group, and triacetyl sorbitol (esterified at C1, C6 and C2 or possibly C5) and some tetraacetyl sorbitol.

At both room temperature and in the cold, the column absorbed the acetate that was formed. Attempts to use the column for a third task, namely, that of separating the sugar–ester products from each other, were unsuccessful.

Preliminary experiments indicate that the method of ion exchange de-esterification would be widely applied, not only for sugar esters but also for lipids. Thus glyceryl tripropionate was hydrolysed to a mixture of glycerol and monoglycerate. A paper chromatogram of the latter reacted slowly with alkaline permanganate–periodate spray[4] (for glycols), and weakly with the alternate periodate–*p*-anisidine spray[5] but strongly with alkaline hydroxylamine–acidic ferric chloride spray[6] (for esters). Quantitative periodate oxidation confirmed that it was a mixture of α- and β-monopropionin. The mixture could have arisen as a result of acyl migration[7].

Polyacetylated oligosaccharides can also be deacetylated in this fashion, for the 'Dowex 1' column does not affect most glycosidic linkages. Even the relatively labile β-fructofuranoside linkage is not hydrolysed, and, on deacetylation, sucrose octacetate gave sucrose admixed with sucrose monoacetate. On the other hand, in suitable conditions, 'Dowex 50' and other cation exchangers hydrolysed glycoside bonds but not acetyl bonds.

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RADIOBIOLOGY

Attenuation in the Chest Wall of 20 keV X-rays from an Inhaled Radioactive Aerosol

EXTERNAL counting of the low intensity *L* uranium X-rays (energies 13·6, 16·9 and 20·2 keV) is a promising technique for the estimation of insoluble compounds of plutonium in the human lung. These low energy radiations are, however, very easily absorbed in the tissues of the chest wall; the half-value thickness (HVT) is about 0·7 cm of soft tissue and only 0·03 cm of bone. The effects of variations in body build are clearly important and difficult to assess, and calibration of the counting equipment is a severe problem. One approach has been the use of a phantom[1] or cadaver[2]. In neither of these cases is there any certainty that the radiation from plutonium inserted into the chest cavity will undergo the same attenuation by surrounding tissues as *in vivo*.

There are now techniques for the preparation of monodisperse aerosols of polystyrene particles, labelled with radio-nuclides, and suitable for inhalation by volunteers[3]. This has made it possible to study the plutonium calibration problem *in vivo*, using radio-nuclides which decay by

orbital electron capture, and so emit characteristic X-rays. As the first radio-nuclide for investigation we chose 17 day palladium-103, which emits the characteristic K X-rays of rhodium (K_α at 20·2 keV, K_β at 22·8 keV) and is thus very suitable as a component of "mock plutonium"[4].

Irradiated palladium was purified and converted into the bis-diethyldithiocarbamate, which was dissolved in xylene containing 0·2 per cent of polystyrene. Because the palladium-103 emits no significant gamma-radiation we used 27·9 day chromium-51 to provide a "label" (0·323 MeV gamma-rays). This was added to the polystyrene solution as the acetylacetonate, to give a mixture of approximately equal activities at the beginning of the experiments. A monodisperse aerosol of polystyrene particles 5 μm in diameter was generated by the spinning disk technique[3].

Seven healthy adult men volunteered to inhale the radioactive aerosol; the weights and heights of the subjects covered a wide range as shown in Table 1. The lung contents of chromium-51 were determined by gamma-ray spectrometry using two collimated crystals of NaI(Tl), 15 cm diameter by 8·9 cm thick, placed above and below the chest of the supine subject as described elsewhere[3]. From the estimated contents of chromium-51 (Table 1) the contents of palladium-103 were calculated from the known initial activity ratio and the half-lives.

The X-rays from the palladium-103 were measured inside a lead shield with a gas-flow (90 per cent Ar, 10 per cent CH_4) proportional counter, approximately 30 cm square, placed about 1 cm above the highest point of the chest of the supine subject. This counter, which will be described in detail elsewhere, uses the multi-wire method of internal anti-coincidence for background reduction[5]. The main counting volume of 9 l. is defined on four sides by 103 wires which also form the cathodes for thirty-nine anti-coincidence counters; these reduce the background response from about 725 c.p.m. to 13 c.p.m. above a threshold of 8 keV. A further reduction to 4 c.p.m. results from the use of pulse rise time discrimination[6], which also reduces the contribution at low energies from the chromium-51 gamma-rays, but without affecting significantly the response to 20 keV X-rays. For these experiments, an energy band of 14–29 keV was used and the background in this band was 2·3 c.p.m.

The counting rate per microcurie of palladium-103 was determined at various times after the inhalation, and in Fig. 1 the results are plotted as a function of the ratio of weight to height (a measure of the average cross-section area of the body) for the seven subjects. Two sets of results are shown—those obtained from the first measurement after inhalation and the average of those obtained after 48 h and up to 14 days, when the early phase of elimination from the lung was complete. At 48 h the average retention was 69 per cent (range 62–76 per cent), and in all subjects the retention was decreasing only slowly.

There is a remarkable degree of correlation between the counting efficiency and the crude measure of body thickness represented by the ratio of weight to height. A very similar correlation was observed when the results were plotted against the chest circumference. There was only a small change in the counting efficiency as a result of the removal of some 30 per cent of the activity, presumably from the ciliated region of the lung. There was no significant systematic change after 2 days; when we ignore effects of statistical fluctuations, the range of all the values for any one subject corresponds to a change in average absorption of only 5 mm of soft tissue (about half one HVT at 20·2 keV).

Table 1

Subject	Age	Weight (kg)	Height (cm)	Chest circumference (cm)	Radioactivity inhaled, μCi ^{51}Cr	^{103}Pd
D.V.B.	46	61·5	184	88·6	0·304	0·226
D.N.	30	64·5	170	93·5	0·098	0·081
R.P.R.	40	70·5	178	95	0·278	0·172
J.E.M.J.	56	79·5	180	96·3	0·260	0·171
J.R.	42	80·6	178	99·7	0·367	0·273
N.G.S.	51	88·0	183	105	0·311	0·205
J.W.C.J.	47	107·0	194	114	0·315	0·195

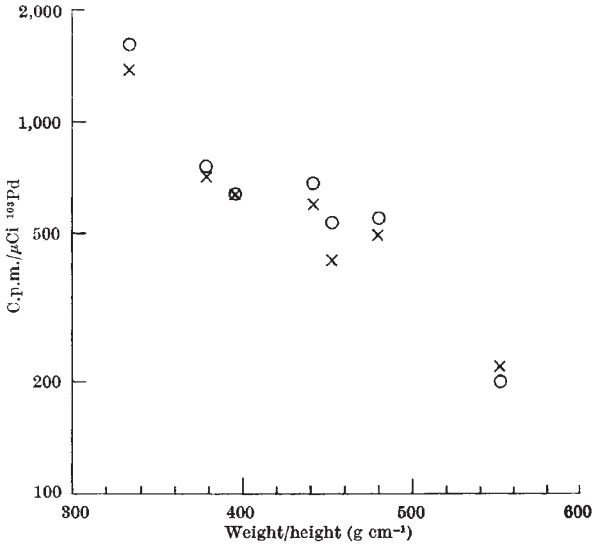

Fig. 1. Variation of counting efficiency with the ratio weight/height. ○, Measurements at about 30 min after inhalation; ×, average of measurements made between 2 days and 14 days after inhalation.

The factor of eight between the efficiencies for the subjects at the extremes of build corresponds to a difference in absorption of about 3 cm of soft tissue, and this is in very good agreement with the results of investigations using ultrasonics for subjects of similar builds[7]. In that method, however, only the soft tissue thickness external to the rib cage was measured, whereas our measurements give results which include the effects of the ribs, and of differences in the size of the lungs which affect the geometrical efficiency.

Because the uranium L X-rays emitted by plutonium-239 have energies between 13·6 and 20·2 keV, the variations in absorption of that radiation among subjects will be even bigger than observed by us. It should also be pointed out that the fraction of counts attributable to K_β radiation (22·8 keV) from palladium-103 in the lung is higher than that from a bare source, so that this method probably under-estimates the effects of absorption at 20·2 keV. We propose to carry out further experiments using other radio-nuclides which emit X-rays of lower energies and with more efficient counters being developed commercially from our design.

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