inhibition caused by phenol or aminophenol. Inhibition in the presence of phenyl sulphate was considerably greater than that produced by similar concentrations of phenol. The effect could therefore not be attributed to hydrolysis of phenyl sulphate during incubation. Addition of carrier sulphate to the medium did not overcome inhibition of $^{35}SO_4^{2-}$ uptake by phenyl sulphate. It is possible that phenyl sulphate may be inhibitory by means of a competitive action on enzymes involved in sulphate metabolism. Of other sulphur-containing substances investigated, potassium methanesulphonate inhibited, whereas potassium ethyl sulphate failed to do so. The further elucidation of this problem and that of the mode of action of other inhibitors must await the availability of a satisfactory cell-free system.

The sulphation of phenol by granulation tissue was a somewhat surprising finding, since Segal (1955) found no such activity in a variety of other tissues. The only reported association of chondrosulphatase and aryl sulphatase activities has been found in the large periwinkle (*Littorea littorina*) by Dodgson & Spencer (1954). Our reasons for investigating the sulphation of phenol were, first, to study the specificity of the sulphating system and, secondly, to have a readily available substrate for future experiments on the problem of sulphation in granulation tissue. Hilz & Lipmann (1955) have shown the presence of a sulphate-activating and a transferring system in liver. It seems likely that a similar activating system occurs in granulation tissue and that a transfer to either carbohydrate or phenol may then take place.

SUMMARY

1. The Q_{O_2} and SO_4^{2-} uptake of guinea-pig granulation-tissue slices decreased with the maturation of the tissue after tendonectomy.

2. The SO_4^{2-} uptake by granulation tissue was stimulated by addition of glucose (2×10^{-2}M) or pyruvate (2×10^{-2}M), and was completely inhibited, together with Q_{O_2}, by fluoroacetate (2×10^{-2}M).

3. Sulphate uptake by the tissue was also inhibited by gentisate and phenol.

4. Phenol acted as an alternative substrate and was shown by chromatography to be converted into phenyl sulphate, which itself was an inhibitor of $^{35}SO_4^{2-}$ uptake by granulation tissue.

The authors thank Professor H. A. Krebs for his nterest.

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The Metabolism of Sorbitol in the Human Subject

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Sorbitol has been used as a sweetening agent for diabetics since it was shown that moderate amounts of sorbitol taken by mouth by normal or diabetic subjects cause only a small and insignificant increase in the blood-sugar concentration (Thannhauser & Meyer, 1929). While this small increase was considered to be due partly to a slow rate of absorption from the intestine, it was also widely believed that, in man, orally ingested sorbitol is metabolized by a pathway not involving glucose (Payne, Lawrence & McCance, 1933).

Animal experiments have shown that as much as 64% of injected sorbitol may be excreted unchanged in the urine (Todd, Meyers & West, 1939; Smith, Finkelstein & Smith, 1940; Ellis & Krantz, 1941, 1943; Stetten & Stetten, 1951), whereas only about 3% of orally ingested material is lost in this way. Recently Stetten & Stetten (1951), Wick, Almen & Joseph (1951) and Wick, Morita & Barnet (1955) have injected uniformly labelled D-[^{14}C]sorbitol (D-[U-^{14}C]sorbitol) into normal and alloxan diabetic rats and recovered most of the radioactivity in the respiratory carbon dioxide. Hepatectomized rats were unable to metabolize sorbitol in this way. Embden & Griesbach (1914) and later Anschel (1930) suggested that sorbitol was metabolized via fruc-

tose, and sorbitol dehydrogenase, the enzyme effecting this conversion, was found in liver (Breusch, 1942). The properties of this enzyme have been further investigated by Blakley (1951). On the other hand, Wick *et al.* (1955) have shown that in alloxan-diabetic rats fed on a high fructose diet less fructose than sorbitol was oxidized to carbon dioxide, and suggest that at least two pathways exist for the oxidation of these two compounds.

There seemed need therefore for a re-investigation of the metabolism of sorbitol in man. Our initial experiments were with unlabelled sorbitol, and showed that after an oral dose of 35 g. of sorbitol given to either normal or diabetic subjects, sorbitol did not appear in the blood in concentrations greater than 2 or 3 mg./100 ml. Less than 2 % of the administered dose appeared in the urine, and less than 10 % in the faeces, even after the bacterial flora of the gut had been suppressed by aureomycin or terramycin. These last observations suggested that sorbitol was not significantly degraded in the intestine by bacteria but was metabolized by the human subject. The extent of this metabolism has therefore been investigated with D-[U-^{14}C]sorbitol. The activity of the expired carbon dioxide and that of the blood glucose have been measured after the ingestion of the labelled sorbitol as well as after a similar dose of uniformly labelled D-[^{14}C]glucose (D-[U-^{14}C]glucose). The results show conclusively that more than 70 % of orally administered sorbitol is metabolized to carbon dioxide by both normal and diabetic human subjects and that at least part of the sorbitol is converted into glucose. A brief report of this work has already been published (Adcock & Gray, 1956).

EXPERIMENTAL

Clinical material and methods

Experiments with unlabelled sorbitol. The subjects were six normal and eight diabetic adults. There were equal numbers of men and women in normal and diabetic groups. The diabetic subjects were all ambulatory patients attending the Diabetic Clinic of King's College Hospital; their diabetes was well controlled without insulin by diet alone. After the subject had fasted overnight, the bladder was emptied and capillary blood was collected from the lobe of the ear for glucose and sorbitol estimations. Sorbitol (35 g.) dissolved in 300 ml. of water was taken by mouth. Blood for analysis was collected at half-hourly intervals for 2·5 hr. In some cases urine was collected for 24 hr., and faeces for 3 days, after beginning the experiment.

Experiments with ^{14}C-labelled sorbitol. Labelled sorbitol was given by mouth to four subjects: *A* was a normal male, 28 years old; *B* and *C* were female and male diabetic subjects respectively, 75 and 70 years old respectively, neither of whom was receiving insulin; subject *D* was a female diabetic of 39 years, well controlled on 6 units/day of Lente insulin which, however, was not given on the day of the investigation.

Each subject fasted overnight, and then 35 g. of D-[U-^{14}C]sorbitol (total activity 30 μc) dissolved in 250–300 ml. of water was drunk over a period of 3 min. Normal diet was allowed 3 hr. after the sorbitol had been taken. At intervals during the following 24 hr. samples of expired air were collected into a Douglas bag for 5 min. periods, while the subject was sitting comfortably relaxed in a chair. Urine was collected at convenient intervals during the 24 hr. after the beginning of the experiment; faeces were collected in 24 hr. batches for 3 days. Three 30–40 ml. samples of blood for isolation and estimation of the radioactivity of the blood glucose were collected from subjects *C* and *D* by venepuncture from the antecubital vein at convenient times during the first 9 hr. after the sorbitol was given.

The investigations were repeated on subjects *C* and *D* with D-[U-^{14}C]glucose instead of the labelled sorbitol.

Methods

Estimation of sorbitol. Sorbitol in blood, urine and faeces was estimated by the method of Adcock (in preparation).

Estimation of blood sugar. Blood sugar (i.e. total reducing substances after removal of proteins) was estimated by the method of Nelson (1944) after precipitation of the protein with zinc sulphate and barium hydroxide (Somogyi, 1945).

Estimation of fructose. Fructose was estimated in blood plasma after protein precipitation by the Somogyi method, by the method of S. W. Cole (cited by Bacon & Bell, 1948).

Treatment of breath samples. No samples of expired air were collected from subject *B*. Small but unmeasured portions of each 5 min. collection from subject *C* and subject *B* after sorbitol were passed through an absorption tower containing CO_2-free N-NaOH. In the experiment on subject *C* after glucose, and in the two experiments on subject *D*, a measured portion of each 5 min. collection was drawn off over mercury from the delivery tube to the Douglas bag into a sample tube before passing through the absorption tower. In each case the total volume of expired air was measured by means of a gas meter.

Isolation of glucosazone from blood samples. The blood samples were collected with fluoride and oxalate as anticoagulants and treated with the Somogyi protein-precipitating reagents. After centrifuging the protein and after acidifying with acetic acid to prevent alkaline decomposition of the glucose, the supernatant was evaporated to dryness. The residue was extracted with ethanol, the filtered extract evaporated to dryness, and the residue taken up in 2 ml. of water. This solution was heated at 100° for 30 min. with 0·3 g. of crystalline sodium acetate and 0·2 g. of phenylhydrazine hydrochloride. Glucosazone readily crystallized and after cooling to room temperature was separated by centrifuging, washed by suspension in ethanol, filtered and rewashed with ethanol on a sintered-glass microfilter funnel. The m.p. was 205°, undepressed by admixture with authentic glucosazone. Weighed quantities of glucosazone were oxidized by wet combustion to CO_2 as described below.

Treatment of urine and faeces for radioactivity measurements. Portions of urine (2 ml. each) were evaporated to dryness after the addition of 0·1 ml. of NH_3 soln. (sp.gr. 0·88) and oxidized as described below to CO_2 for absorption in CO_2-free NaOH.

The complete faecal collections were weighed and homogenized in an Atomix blender after the addition of suitable known volumes of water. Small weighed amounts were

Table 1. *Total radioactivity in urine, faeces and breath after* 35 *g.* (30 μC) *of* D-[U-^{14}C]*sorbitol*

	Urine (0–24 hr. after sorbitol)		Faeces (0–3 days after sorbitol)		Breath (0–24 hr. after sorbitol)		Total radioactivity recovered	
	μC	% of dose	μC	% of dose	μC	% of dose	μC	% of dose
Subject *A*. Normal	0·45	1·5	3·94	13·1	24	80	28·39	95
Subject *B*. Mild diabetic	0·71	2·4	1·88	6·3	—	—	—	—
Subject *C*. Mild diabetic	0·8	2·7	0·28	0·9	26	87	27·08	90
Subject *D*. Severe diabetic	2·71	9·0	1·46	4·9	19·6	65	26·77*	90

* Diarrhoea after 2 hr.: loss estimated as 3 μC.

dried on a water bath before combustion to CO_2. When samples had been delayed before delivery to the laboratory the samples were dried after the addition of small quantities of alkali to avoid possible loss of volatile materials.

Combustion procedure. The various samples were oxidized with a chromic–sulphuric acid mixture (Van Slyke, 1954), the carbon dioxide being isolated as barium carbonate by the method of Berlin, Tolbert & Lawrence (1951) with minor modifications (Adcock, in preparation).

Radioactive materials. D-[U-^{14}C]Glucose and D-[U-^{14}C]-sorbitol were purchased from the Radiochemical Centre, Amersham.

Radioactivity measurements. All measurements of radioactivity were on barium carbonate mounted at infinite thickness on aluminium planchets of 15 mm. diam., with an end-window Geiger–Müller counter. The counts were corrected for background and were calibrated with a standard barium carbonate sample of high and known activity prepared by combustion of a small quantity of the labelled sorbitol solution given to the patients.

RESULTS

Experiments with unlabelled sorbitol. Of the six normal subjects, four were investigated in the fasting state and the other two at least 3 hr. after food. In neither group of patients did sorbitol given by mouth have a significant effect on the blood sugar. In all eight diabetic subjects, however, the oral administration of 35 g. of sorbitol was followed by a significant increase in the blood-sugar concentration ranging from 9 to 49 mg./100 ml. In neither normal nor diabetic groups did the concentration of sorbitol attain measurable levels in the blood; the method used was capable of estimating sorbitol in blood in concentrations greater than 2–3 mg./100 ml. In one normal subject who received 50 g. of sorbitol, sorbitol was present in a concentration of 9 mg./100 ml. after 1 hr.

The excretion of sorbitol in the urine during the 24 hr. after the sorbitol was given varied between 0·07 and 0·91 g., so that the loss in the urine amounted to less than 3 % of the dose administered. The majority of the excreted sorbitol was eliminated during the first 5 hr.; none could be detected after 24 hr. No unchanged sorbitol could be detected in the faeces of three subjects and only 10 % or less of the dose could be found in the faeces of those other patients whose gastro-intestinal tract had been sterilized by the adequate administration of antibiotics.

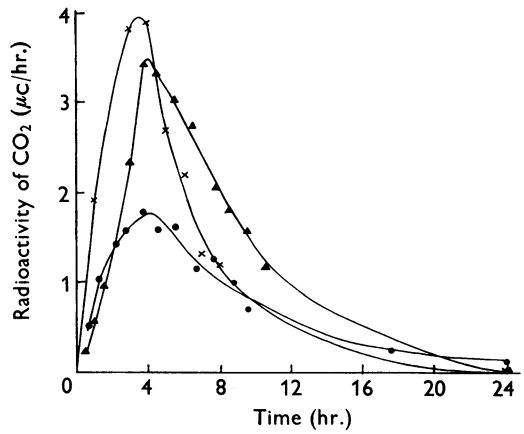

Fig. 1. Radioactivity of expelled CO_2 after 35 g. (30 μC) of D-[U-^{14}C]sorbitol. The specific activity of the CO_2/hr. has been calculated on the assumption that the output of CO_2 was constant at 4 g./5 min. (see text). ×, Subject *A*, normal; ▲, subject *C*, mild diabetic; ●, subject *D*, diabetic requiring insulin.

Table 2. *Total blood sugar and specific activity of blood glucose after* 35 *g. of* D-[U-^{14}C]*sorbitol of activity* 2·17 μC/*g. of carbon*

	Time after sorbitol (hr.)	Blood sugar (mg./100 ml.)	Specific activity of blood glucose (μC/g. of carbon)
Subject *C*	2·75	100–160	0·35
	4·25		0·22
	7·5		0·094
Subject *D*	1·0	228	0·49
	3·25	238	0·38
	9·25	279	0·058

Experiments with D-[U-^{14}C]*sorbitol.* Table 1 shows the total radioactivity in the urine, faeces and breath after ingestion of the labelled sorbitol. The total radioactivity of the urine from the normal subject and of the two mild diabetics was less than 3 % of the dose given, thus confirming the experiments

Table 3. *Total radioactivity in urine, faeces and breath after* 35 *g.* (30 μC) *of labelled glucose*

	Urine (0–24 hr. after glucose)		Faeces (0–3 days after glucose)		Breath (0–24 hr. after glucose)		Total radioactivity recovered	
	μC	% of dose	μC	% of dose	μC	% of dose	μC	% of dose
Subject *C*	1·63	5·4	2·08	6·9	22·7	76	26·41	88
Subject *D*	12·26	40·9	0·80	2·7	13·2	44	26·26	88

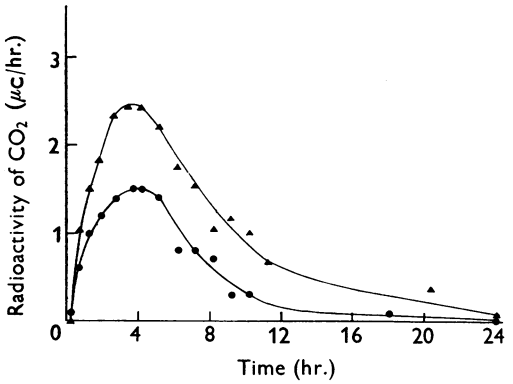

Fig. 2. Radioactivity of expelled CO_2 after 35 g. (30 μC) of D-[U-^{14}C]glucose. The specific activity of the CO_2/hr. has been calculated on the assumption that the output of CO_2 was constant at 4 g./5 min. (see text). ▲, Subject *C*, mild diabetic; ●, subject *D*, diabetic requiring insulin.

Table 4. *Total blood sugar and specific activity of blood glucose after* 35 *g. of* D-[U-^{14}C]*glucose of activity* 2·17 μC/*g. of carbon*

	Time after glucose (hr.)	Blood sugar (mg./100 ml.)	Specific activity of blood glucose (μC/g. of carbon)
Subject *C*	1·0	233	1·0
	4·0	156	0·64
	7·5	157	0·18
Subject *D*	1·0	396	0·96
	4·5	307	0·28
	7·75	315	0·14

with unlabelled material. Neither of these diabetic patients was excreting significant amounts of glucose in the urine. The radioactivity of the urine from the severe diabetic amounted to more than 9% of the dose given, presumably owing to the presence of labelled glucose. In the four subjects the radioactivity of the faeces passed during the 3 days accounted for between 1 and 13% of the labelled sorbitol given. The radioactivity of the faeces after 3 days was negligible.

In the normal subject and subject *C* (the mild diabetic from whom breath samples were collected) 80% of the administered radioactivity could be accounted for in the breath. In subject *D*, the diabetic who required insulin, only 65% could be accounted for in the breath, but the increased loss in the urine made up for this so that in all subjects investigated only 10% or less of the radioactivity could not be accounted for.

Fig. 1 shows that the radioactivity of the expired carbon dioxide was maximal between 3 and 4 hr. after the sorbitol feeding. In the normal subject the activity had declined to little more than 25% of the maximum value by 8 hr.; in the mild diabetic, the maximum activity was nearly equal to that observed in the normal subject and there was a decrease to 25% of that maximum within about 12 hr. The maximum radioactivity in the breath of the more severe diabetic was only about 50% of that observed in the other two subjects. In no subject was radioactivity detected in the breath after about 24 hr. Curves of the cumulative excretion of radioactivity in the breath did not show significant differences between the three subjects.

The specific activity of the blood sugar was determined at convenient intervals by isolation of glucosazone from venous blood in subjects *C* and *D* (Table 2). In the two experiments taken together, the activity appeared maximal within 1 hr. of the sorbitol ingestion and within 7–9 hr. declined rapidly to reach a value of 20% of the maximum observed. The sugar content of the specimens from subject *C* was inadvertently not estimated but this subject's blood sugar was known to be well maintained between 100 and 160 mg./100 ml.

Experiments with D-[U-^{14}C]*glucose.* Table 3 shows the total radioactivity in the urine, faeces and breath after ingestion of the labelled glucose. The total radioactivity of the urine from subject *C* was only 5% of the dose given. The radioactivity of the urine from subject *D*, the more severe diabetic, amounted to 41% of that given. The radioactivity of the faeces passed during the 3 days after the feeding of labelled glucose amounted to 6·9 and 2·7% of the dose given.

In the mild diabetic 76% of the administered radioactivity could be accounted for in the breath. In the more severe diabetic only 44% could be accounted for in the breath, but the increased loss in the urine made up for this so that in the two subjects only 12% of the radioactivity could not be accounted for.

Fig. 2 shows that whereas the radioactivity of the breath in the more severe diabetic followed a curve

almost the same as was found after sorbitol, in the mild diabetic the maximum activity attained was not so high as in the experiment with sorbitol. The peak of radioactivity in the severe diabetic, however, was flattened, presumably because of the excretion of labelled glucose, so that the total activity accounted for in the breath was of the same order after glucose as that found after sorbitol.

Table 4 shows that when D-[U-^{14}C]glucose was fed to subjects *C* and *D*, the specific activity of the blood glucose reached a higher level after 1 hr. than was found after the labelled sorbitol feeding. In both cases the activity at 7–8 hr. was only about 15% of that found at 1 hr.

The amount of fructose in blood samples obtained after sorbitol had been fed to subject *D* was found to be below the limit of sensitivity of the method, nor were significant amounts of fructose found in the blood of two other mild diabetic subjects who had taken unlabelled sorbitol. The method used would have determined concentrations of fructose above 2–3 mg./100 ml.

DISCUSSION

Absorption of sorbitol. The experiments with unlabelled sorbitol confirmed the absence of a significant increase in the blood-sugar concentration after a moderate dose (35 g.) of sorbitol, and that even in a diabetic subject the increase in blood-sugar concentration was less than 50 mg./100 ml., whereas increases above this were found in diabetic patients after a similar quantity of glucose. The absence of detectable sorbitol in the faeces suggested the possibility that bacterial degradation of sorbitol in the gut might be significant, but the experiments on subjects in whom antibiotic therapy had suppressed bacterial growth in the gut excluded this. The small, but significant, radioactivity in the faeces after the labelled material suggested that intestinal bacteria had in fact assimilated, and perhaps metabolized, a small proportion of both glucose and sorbitol. It therefore seemed likely that sorbitol was absorbed and that the failure of detectable amounts to appear in the blood was due to rapid metabolism, excretion, or storage in the body. Sorbitol is stated to be excreted by glomerular filtration (Smith *et al.* 1940) and a comparison of blood and urine concentrations is in agreement with this. The urine analyses, however, show that only a small quantity of orally ingested sorbitol was lost by renal excretion.

Metabolism of sorbitol to carbon dioxide. The measurements of breath $^{14}CO_2$ were discontinuous, and although the carbon dioxide content was reasonably constant at about 4% (v/v) the volume of expired air/5 min. varied according to the activity of the subject throughout the experiment. The expired ^{14}C has therefore been calculated on the assumption that the output of carbon dioxide was constant at 4 g./5 min. This corresponds well with the average daily carbon dioxide production for a sedentary worker calculated from the calorie requirement/hr., an R.Q. of 0·82 and a calorific value for oxygen of 4·825 kcal./l. (Hawk, Oser & Summerson, 1947). This was also in agreement with the known composition of the diet of the diabetic patients. The total radioactivity excreted in the breath has therefore been obtained by measuring the area from 0 to 24 hr. below the interpolated curves shown in Figs. 1 and 2. The results with labelled sorbitol clearly show that in the normal and in the mildly diabetic subject at least 75% of orally given sorbitol is rapidly metabolized to carbon dioxide. In the more severely diabetic subject the recovery of labelled carbon in the respiratory carbon dioxide amounted to only 65% and this lower figure was clearly due to the increased proportion of labelled material lost in the urine. The number of experiments is too small to assess the significance of the delay in the elimination of labelled carbon dioxide in subject *C*, the mild diabetic.

Possible pathway of metabolism of sorbitol. Blakley (1951) has shown that liver slices are capable of converting sorbitol into fructose and that the pathway for its metabolism is probably as follows:

Sorbitol $\rightleftharpoons$ fructose $\rightarrow$ fructose 6-phosphate
$\rightleftharpoons$ glucose 6-phosphate $\rightarrow$ glucose.

Sorbitol may be metabolized in the liver, may be stored as glycogen, or secreted into the blood as fructose or glucose and utilized elsewhere. The high specific activity of the glucosazones prepared from the blood filtrate must be attributed to either labelled fructose or labelled glucose or both in the blood. The highest observed activities of the glucosazones corresponded to 0·35 and 0·49 μc/g. of carbon, whereas the specific activity of the administered sorbitol was 2·17 μc/g. of carbon. Had the activity of the glucosazone been due to blood fructose formed directly from the labelled sorbitol, and therefore with the same specific activity as the sorbitol, between 16 and 23% of the total blood sugar would have been present as fructose. This would have amounted to 16–23 mg. of fructose/100 ml. in subject *C*, whose blood sugar was between 100 and 160 mg./100 ml., and 52 mg. of fructose/100 ml. in subject *D*. If there was isotopic dilution with unlabelled endogenous intermediaries, the blood fructose would have needed to be even higher. The demonstration that blood fructose cannot have exceeded 2 mg./100 ml. showed clearly that the radioactivity of the blood sugar was mostly, if not entirely, due to that of the glucose. Oral sorbitol is therefore presumably converted in the liver of the

human subject into fructose, a proportion of which is converted into glucose, which may in part be temporarily stored as glycogen and ultimately passed into the blood stream as glucose. The proportion of fructose not converted into glucose may be directly metabolized by the glycolytic pathway via fructose 6-phosphate.

The height of the peaks of activity of the respiratory carbon dioxide appeared to be inversely related to the blood-sugar concentrations. This would be expected because the higher the blood sugar the greater the blood-sugar pool with which the isotope was diluted, and also because loss of glucose in the urine occurred when the blood-sugar concentration was above the renal threshold, i.e. 180 mg./100 ml.

The relative specific activity of blood glucose and of respiratory carbon dioxide at corresponding times are compared in Table 5. As expected, the specific activity of the blood glucose after D-[U-^{14}C]sorbitol was lower than that after D-[U-^{14}C]glucose. After sorbitol, there was isotope dilution with pools of intermediaries in its conversion into glucose, as well as with the pools of glucose itself. After the labelled glucose, however, dilution occurred with the glucose pools only.

The peak of labelling of the carbon dioxide was lower and later than that of the blood glucose, which suggests that the labelled carbon dioxide was derived from the labelled glucose in the blood. Although the plasma bicarbonate was not measured in the subjects, and it may safely be assumed to have been not significantly altered, the system is very complex with its pools of bicarbonate, glucose and non-glucose precursors of carbon dioxide. For these reasons the fact that the maximum labelling of the breath $^{14}CO_2$ after sorbitol was greater than that after glucose cannot by itself be taken as an indication that sorbitol was in part oxidized without first being converted into blood glucose. But comparison of the ratios (peak ^{14}C in breath carbon dioxide)/(peak ^{14}C in blood glucose) after sorbitol and after glucose respectively should give information on this question, provided that the respective peaks come at about the same time in any pair of experiments. It is difficult to fix the blood-glucose peak from the three values available in each experiment, but owing to the speed of absorption one can be certain that the peak of labelling occurs within the first hour. Whatever curves are drawn, peaks of blood-sugar ^{14}C are found at about 0·5–1 hr. Table 6 shows the ratio (peak ^{14}C in carbon dioxide)/(peak ^{14}C in blood glucose) obtained by assuming that the 1 hr. values for the latter are maximal and, for subject *C* after sorbitol, by interpolating the results at 1 hr. These ratios are much higher after sorbitol than after glucose in both subjects *C* and *D* and show a significantly lower dilution of $^{14}CO_2$ relative to the blood-sugar ^{14}C from labelled sorbitol than from labelled glucose and provide definite evidence of an alternative pathway of metabolism of sorbitol to carbon dioxide.

Table 5. *Specific activity of blood glucose and expired* CO_2 *after* D-[U-^{14}C]*sorbitol or glucose, each of activity* 2·17 μc/*g. of carbon*

The specific activity of blood glucose was determined on glucosazones prepared from blood samples at the times stated. The specific activity of the CO_2 was determined from the curves presented in Figs. 2 and 3.

	Mild diabetic (*C*)			Severe diabetic (*D*)		
	Time (hr.)	μc/g. of carbon		Time (hr.)	μc/g. of carbon	
		Glucose	CO_2		Glucose	CO_2
Sorbitol experiment	2·75	0·345	0·145	1	0·49	0·073
	4·25	0·22	0·27	3·25	0·38	0·126
	7·5	0·094	0·168	9·25	0·058	0·055
Glucose experiment	1	1·0	0·099	1	0·96	0·065
	4	0·64	0·185	4·5	0·28	0·116
	7·5	0·18	0·115	7·75	0·14	0·053

Table 6. *Comparison of peak activities of* ^{14}C *in breath* CO_2 *and blood glucose*

	After glucose			After sorbitol		
	Peak ^{14}C (μc/g. of carbon)			Peak ^{14}C (μc/g. of carbon)		
	Breath CO_2 (*a*)	Blood glucose (*b*)	Approx. ratio (*a*/*b*)	Breath CO_2 (*a*)	Blood glucose (*b*)	Approx. ratio (*a*/*b*)
Subject *C*	0·19	1·0	0·2	0·28	0·6*	0·5
Subject *D*	0·12	0·96	0·1	0·15	0·49	0·3

* Interpolated value.

SUMMARY

1. Human diabetic and non-diabetic subjects excreted in the urine less than 3 % of an oral dose of 35 g. of sorbitol. No sorbitol could be detected in the faeces.

2. After 35 g. of oral sorbitol, there was no significant increase in blood sugar in normal subjects, but in diabetic subjects the blood sugar increased by a much smaller amount than after glucose. The concentrations of sorbitol in the blood were immeasurably small.

3. Small and insignificant amounts of sorbitol were present in the faeces after sorbitol was fed to patients on antibiotic therapy.

4. Experiments with uniformly labelled D-[^{14}C]-sorbitol and D-[^{14}C]glucose showed that in the normal and in the mildly diabetic subject at least 75 % of orally given sorbitol is rapidly metabolized to carbon dioxide. In a diabetic subject requiring insulin the recovery of labelled carbon in the respiratory carbon dioxide amounted to only 65 % and this lower figure was clearly due to the increased proportion of labelled material lost in the urine.

5. It was shown that a substantial proportion of oral sorbitol is rapidly converted into blood sugar and the pathway of this conversion is discussed.

We thank Mrs F. Sharples for her advice on the radioactivity estimations, Dr R. D. Lawrence and the staff of the Diabetic Clinic at King's College Hospital for their help with the investigations on their patients, our three patients and one normal subject for their willing co-operation, and Dr J. J. Scott for much helpful discussion and advice. We are also indebted to Howards of Ilford Ltd. and Frank Cooper Ltd. for financial support of this investigation and to the former for seconding one of us (L.H.A.) to the Department of Chemical Pathology for the whole period of this work. Equipment purchased by the Joint Research Committee of King's College Hospital and Medical School was used for the radioactivity measurements

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The Use of Radioactive Isotopes in Immunological Investigations

10. THE FATE OF SOME INTRAVENOUSLY INJECTED NATIVE PROTEINS IN NORMAL AND IMMUNE RABBITS*

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Several workers have studied the elimination from the blood stream of antigenic proteins injected intravenously into normal or specifically immunized animals. The subsequent fate of these antigens after elimination from the blood has also been the subject of a number of investigations. However, none of these studies has yielded any detailed information about the rates of elimination of antigens from the blood of immune animals during the first hour or so after injection, or of the immediate fate of these eliminated antigens, though it is during this period that any reaction with circulating antibody would be expected to occur. Such studies might therefore be expected to throw some light on the behaviour of antibodies *in vivo*, and to yield information on their role in the defence mechanisms of the body.

The proteins that have been used in most previous studies on their fate in animals have been labelled in different ways by the introduction of comparatively

* Part 9: Francis, Mulligan & Wormall (1955*a*).