tartrate was shown by paper chromatography in a solvent system of butan-1-ol:acetic acid:water (12:3:5, v/v) to be at least 99% radiochemically pure. Non-radioactive monosodium L(+)-tartrate was provided by Fides, Union Fiduciaire, Switzerland.

Methods. Adult CFY rats (b.wt 200–250 g), a strain of Sprague-Dawley origin were obtained from Anglia Laboratory Animals, Huntingdon, England, and were allowed a pellet diet and water ad libitum. The rats were dosed by oral intubation or by injection into a tail vein with monosodium ^{14}C-L(+)-tartrate at a dose level of 400 mg/kg in aqueous solution. The rats were kept singly in glass metabolism cages, which enabled urine, faeces and expired air to be separately collected, the urine into receivers cooled in solid CO_2, and the expired air ($^{14}CO_2$) into traps containing ethanolamine: 2-ethoxyethanol (1:4, v/v). The rats were sacrificed after 2 days. Radioactivity was measured using procedures previously described[9].

Results. An oral dose of monosodium ^{14}C-L(+)-tartrate was rapidly absorbed and excreted by rats. Excretion of radioactivity in the urine was almost completed within 12 h and in the expired air within 24 h (figure). At 48 h after the oral dose to rats (3♂ + 3♀), 70.1 ± 4.1%, 13.6 ± 7.3% and 15.6 ± 2.7% had been excreted in the urine, faeces and expired air respectively (± SD). After the i.v. dose, 81.8 ± 4.9%, 0.9 ± 1.1% and 7.5 ± 0.5% had been excreted by these routes respectively (± SD). Rates of excretion of radioactivity by male and female animals were similar.

Discussion. The urinary excretion data obtained in these studies are in agreement with an earlier investigation which reported that rats excreted in the urine a mean of 68% of an oral dose of 400 mg/kg of tartrate given as Rochelle salt[6]. However, the earlier work[2] suggested that tartaric acid was only metabolized by the gut flora and not by the tissues. The results obtained in these studies contradict this view since significant amounts of $^{14}CO_2$ were excreted after parenteral administration of ^{14}C-L(+)-tartrate showing that systemic metabolism of tartaric acid occurred. Comparison of results obtained after oral or i.v. doses indicates that an oral dose of L(+)-tartrate was extensively absorbed and that a part was completely metabolized to $^{14}CO_2$ after oral or parenteral administration.

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On a relation of low phosphoglucomutase activity to starch accumulation in spiked sandal

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Summary. A considerable decline in the activity of phosphoglucomutase appeared to be a cause for the starch accumulation in the leaves of sandal affected by spike disease.

In the sandal (Santalum album L.) affected by spike disease, mycoplasmal in nature, the leaves show stunted growth, chlorosis and accumulation[1] of large amounts of starch and sugars, and there is necrosis of phloem elements in the diseased state. The sugar accumulation in the chlorotic spiked leaves, presumably occurring as a result of impaired translocation due to the necrosis of phloem elements, naturally leads to increased starch formation to prevent abnormal rise in the osmotic pressure of the tissue. However, the enzymes related to starch breakdown also determine starch-balance in the tissue. Examination of the diastatic activity of the diseased sandal leaves, showed it, contrary to expectation, to be at a high level[2], thus apparently showing no correlation to the high starch content therein. It is now known that in the plant the breakdown of starch in tissues other than storage tissues and germinating seeds[3–5] is largely brought about by phosphorylase[6–8]. Glucose-1-phosphate (G-1-P) formed during the phosphorolysis, is converted to G-6-P by phosphoglucomutase for entry into glycolysis. Therefore, these 2 enzymes, of relevance to starch balance in the tissue, were studied in the healthy and spiked sandal leaves to examine their relation to the starch accumulation in the diseased state.

Material and methods. Samples of young and mature leaves from healthy and spiked sandal trees were taken during July and September respectively, selecting 6 trees in each case. As the spiked trees remain vegetative throughout, the healthy leaf samples also were taken from trees in vegetative stage for proper comparison.

Phosphorylase and phosphoglucomutase activities and starch content in healthy and spiked sandal leaves

	Healthy Leaves		Spiked Leaves	
	Young	Mature	Young	Mature
Phosphorylase activity* (µg Pi liberated/100 mg tissue/30 min)	4.15 (0.36)	5.20 (0.49)	18.30 (0.56)	18.70 (0.61)
Phosphoglucomutase activity* (µg Pi converted/150 mg tissue/30 min)	51.7 (2.7)	78.0 (4.4)	45.4 (3.5)	10.3 (1.7)
Starch content* (mg/100 g dry leaf)	105.7 (11.8)	73.3 (12.1)	179.2 (16.5)	461.0 (48.5)

*Average of 6 replications. Figures in parenthesis represent SD.

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The samples were taken between 08.00 and 09.00 h and used up for analysis without delay. Starch was determined on ovendry (80 °C) samples according to the method of Sensabaugh and Rush[9].

Phosphorylase activity was estimated following the procedure described by Madhav Singh et al.[10]. A 10% (w/v) leaf homogenate in neutralised cysteine solution (0.015 M) formed the enzyme source. Other things remaining the same, the assay system comprised 0.4 ml citrate buffer (0.1 M, pH 6.2), 0.3 ml 1% soluble starch, 0.15 ml NaF (0.3 M), 0.5 ml homogenate and 0.15 ml G-1-P (0.05 M, pH 6.2). Pi liberated was estimated by chlorostannous acid reagent. The enzyme activity was expressed as μg Pi liberated/100 mg tissue/30 min under the conditions of the experiment.

With respect to phosphoglucomutase, the enzyme extract (10% leaf homogenate) was prepared following the procedure described by Cardini[11], and the activity was estimated according to the method of Ramasarma et al.[12]. The reaction mixture (1.5 ml enzyme preparation + 0.5 ml veronal buffer (pH 7.5) containing 8.25 μmoles G-1-P and 0.07 μmoles G-1,6-di P and 1.7 μmoles $MgSO_4$) was incubated for 30 min at 36 °C, after which the reaction was stopped by adding 1 ml 5N H_2SO_4 and the incubation tubes were kept in boiling water bath for 10 min to hydrolyse residual G-1-P. The tubes were then centrifuged and the supernatant was separated and made up to 25 ml. Pi was estimated in an aliquot of this. In the control, the H_2SO_4 was added to the reaction mixture before the start of incubation. The enzyme activity was expressed as μg Pi converted/150 mg tissue/30 min under the conditions of the experiment.

Results and discussion. The results obtained are presented in the table. It can be seen that in the diseased leaves, compared to the healthy, while phosphorylase activity increased, phosphoglucomutase activity decreased, more so at mature stage. Diseased leaves showed high starch content particularly at mature stage.

In the healthy leaves, phosphorylase activity remaining stationary, the increase in phosphoglucomutase activity and decrease in starch content from young to mature stage, seemed to have a correlation. In the diseased leaves, increased phosphorylase activity and decreased phosphoglucomutase activity could cause G-1-P to accumulate. Though, in the plant, G-1-P undergoes conversion to sucrose for translocation, this conversion seemed not to be freely occurring in the spiked sandal due to restricted phloem translocation. On the other hand, much of the G-1-P in these leaves appeared to be undergoing reconversion to starch for the reason that, of the substrate (adenosine diphosphoglucose/uridine diphosphoglucose) and the acceptor (oligosaccharide/maltose) needed for starch synthesis[4,13,14], the former can arise from the accumulating G-1-P, and the high diastatic activity in the starch-loaded diseased leaves could make the latter readily available. Thus, a considerable decline in the activity of phosphoglucomutase appeared to be a contributing factor for the starch accumulation in the spiked sandal leaves.

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Immunological studies on the key enzyme of arginine biosynthesis in Pseudomonas aeruginosa

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Summary. A method to manufacture specific antisera with a minute amount of pure enzyme is presented. The influence of antibodies on activity and inhibition of an allosterically regulated enzyme was studied.

N-acetylglutamate 5-phosphotransferase (E2, EC 2.7.2.8) catalyzes the second step in the biosynthesis of the amino acid arginine (arg.). In Pseudomonas aeruginosa this enzyme possesses the key position of the whole anabolic sequence. It is inhibited allosterically by the endproduct arg.

It was of interest to investigate the influence of specific antibodies on the enzymic activity and the allosteric properties of the enzyme in vitro. Immunochemical techniques are considered valuable tools in all sectors of biochemical research. Therefore, it was hoped to add to the methodology in metabolic regulation. The arg biosynthesis was chosen as a model pathway because much work has been devoted to the knowledge of the respective enzymes at this institute (e.g.[3-7]).

Pseudomonas aeruginosa PAO-1, a wildtype strain, was cultivated by the method of Haas and Leisinger[3] with slight modifications. The assay procedure for E2 activity was described[3] as: The reaction catalyzed by the enzyme yields N-acetylglutamyl 5-phosphate and ADP. Hydroxylamine present in the assay mixture traps the product forming N-acetylglutamyl hydroxamate which in turn is complexed with Fe^{3+} to produce a brownish colour measurable at 540 nm.

The purification procedure[3] was followed, except for the early heat denaturation step. E2 in the crude extract was observed to be more heat-stable in the presence of N-

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