Regional and temporal variations in the composition of air make it impossible to obtain a constant correction factor for this effect. Thus, analysis of the composition of 3 samples of air[6] taken at different sites in the City of Leeds on 12/6/67 gave values for the SO_2 content of 66, 56 and 74 $\mu g/m^3$. The occurrence of temporal variations was demonstrated by titration; thus determinations of the rate of uptake of acidic substances in 3 ml deionized-distilled water on 7 consecutive days gave equivalent values of 0.730, 0.750, 0.690, 0.852, 0.647, 0.577 and 0.703 μmoles NaOH/h.

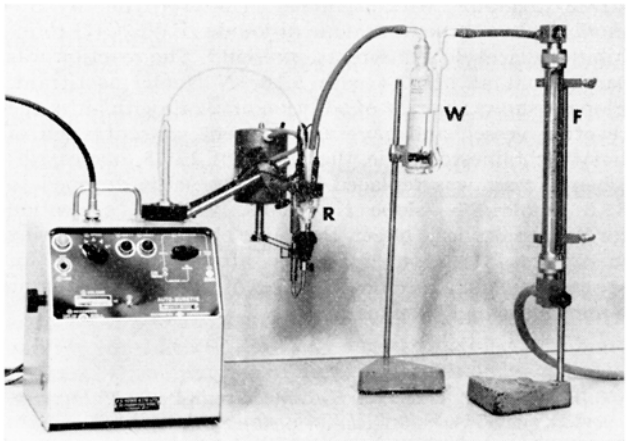

Fig. 4. Equipment suitable for producing an inert atmosphere in a reaction vessel (R) during potentiometric titrations. F, flowmeter (0–25 ml/min); W, wash bottle containing CO_2-free distilled water.

It is clear that the most convenient way to eliminate the error is to carry out titrations in an inert atmosphere, using an arrangement like that shown in Figure 4. Pure N_2 from a cylinder is led through a pressure reducing valve and then to a flowmeter (Figure 4, F) of suitable calibration (0–25 ml/min). It is advisable to pass the N_2 through CO_2-free distilled water in a wash bottle (Figure 4, B) before it reaches the reaction vessel, particularly when titrations are prolonged, to prevent undue evaporation of the vessel contents[7].

Résumé. Les estimations des enzymes dans de petits échantillons de tissu, ou dans des tissus de faible activité, par des méthodes potentiométriques pH-stat avec un alcali comme titrant sont sous la dépendance d'erreurs causées par l'absorption des substances acides de l'air. On peut éviter ces erreurs par l'usage en éprouvette d'une atmosphère pure et inactive.

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The Relationship of Phosphate and Lipids to Xanthine Dehydrogenase

Phosphate is required for the reduction of one electron acceptor by cream xanthine dehydrogenase[1] (XD) and seems to have a stabilizing action on this enzyme[2]. On the other hand, there is evidence that both the milk[3] and the mammalian enzymes[4,5] are associated with lipid components. The postulate that lipid or phospholipide material is responsible for binding together various oxidative enzyme systems was the main support for our investigation on the phosphate and lipid participation in the XD activity of the mammalian enzyme.

For the purification of the enzyme, a homogenate (1:5 w/v) obtained from rat liver was heated at 65 °C for 15 min. After centrifugation at 1000 *g* the aqueous phase was siphoned off and submitted to the following steps performed at 0 °C.

Preparation A1. To each 100 ml of the homogenate were added 47 ml of saturated ammonium sulphate. After 20 min most of the protein which had been salted out was separated by centrifugation and discarded. To each 100 ml of the clear aqueous solution were added 47 ml of saturated ammonium sulphate. After 45–60 min the brown protein was collected by centrifugation and dissolved in twice-distilled water.

Preparation A2. Obtained by dialysis of preparation A1 for 18 h against twice-distilled water at 5 °C. The solution was centrifuged (1000 *g*) for 15 min and a supernatant free of turbidity with a deep red-brown colour was obtained. The E_{280}/E_{450} for this preparation was usually 13.5–14.7.

Preparation B1. Obtained by solubilization of the enzyme by treatment with butanol as suggested by MORTON[3]. While stirring, 1 vol of aqueous butanol was slowly added to the homogenate. After centrifugation (1000 *g* for 15 min) the transparent yellowish aqueous phase was siphoned off, fractionated with ammonium sulphate, and dialysed as A2. This preparation presented an E_{280}/E_{450} equal to 11.3.

XD activity was determined by the method previously described[5]. Phosphorus was determined by the method described by GÖMÖRI[6].

The activity of the enzyme preparations measured by the production of formazan/mg dry weight are shown in Figure 1. Results on aged preparations showed that B1 and A2 were inactivated differently. When incubated at 37 °C, B1 preparation lost activity more rapidly than A2 and both preparations presented 100% inhibition after 24 h of incubation (Figure 2).

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Analytical data on various enzyme preparations

	Preparations[a]						
	A1	A2	A2	A2	B1	B1	B1
Dry weight (mg/ml)	118.8	18.4	14.3	15.8	5.1	4.7	3.6
XD activity (μg formazan/mg dry weight/h)	0.526	3.96	8.36	5.26	14.49	18.14	20.00
Protein (mg/ml)	23.19	13.16	–	–	–	–	2.27
Specific activity (μg formazan/mg protein)	2.61	5.5	–	–	–	–	33.3
Total P (% dry weight)	0.26	0.57	0.91	0.60	1.72	1.85	1.98
Lipid P (% total P)	63.1	60.0	59.3	69.3	56.8	61.3	58.8
Total acid soluble P (% total P)	12.7	–	19.2	19.2	–	14.2	11.6
Total inorganic P (% total P)	5.1	0	–	–	0	–	–
E_{280}/E_{450}	–	–	13.5	14.7	–	11.3	

[a] Different A2 and B1 preparations refer to independent enzyme solutions prepared as follows: A1 without dialysis, A2 with dialysis, B1 treated with butanol.

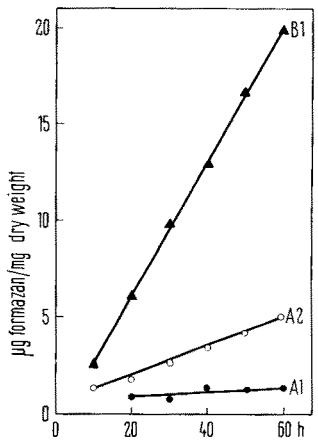

Fig. 1. Xanthine dehydrogenase activity of liver enzyme preparations: A1 without dialysis, A2 with dialysis, and B1 treated with butanol. Each Thunberg tube contained 0.1 ml of a 0.05 *M* hypoxanthine solution, 0.3 ml of a 0.1% triphenyl tetrazolium chloride solution and 1–2 ml of the enzyme preparation. All tubes contained phosphate buffer pH 7.4 to a total of 3.4 ml. Abscissa: incubation time.

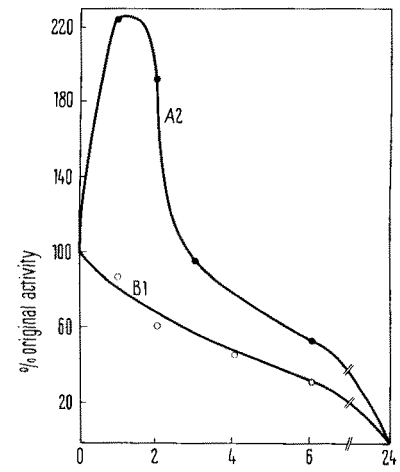

Fig. 2 Xanthine dehydrogenase activity of enzyme preparations incubated at 37 °C. Determinations were carried out at different times after incubation of 1–2 ml of enzyme solution: A2 non-treated and B1 treated with butanol.

The analysis of the preparations for their P content showed the results given in the Table. The total P content in all preparations was surprisingly high. The P present in soluble form in lipid solvents would indicate that a large percentage of the enzyme preparations was composed of a lipid material. A uniformity from preparation to preparation for the data of the total P (chiefly lipid) was obtained.

The lipid material attached to the enzyme molecule seems to exert a protection against inhibitory effects of physical or chemical agents. The incubation (37 °C) of the A2 preparation (not extracted) breaks these lipid bonds and is sufficient to cause a sharp increase in the enzyme activity, an increase not observed with B1 preparation yet dissociated as soluble enzyme.

The earliest hypothesis of MAHLER[7] on the mechanism of action of the enzyme concerning a functional coupling of the metal, which in this case would be molybdenum, to flavin, remains tenable. The participation of Mo on the electron-transport pathway of the enzyme and the possibility that P is essential to the enzyme activity led us to suppose the existence of a phosphomolybdate complex in the enzyme. $FADH_2$ would be the specific reductor for a phosphomolybdate complex in the enzyme, as in known chemical reactions in which specific reagents reduce phosphomolybdate complexes but not free molybdate, such as suggested by KINSKY and MCELROY[8] for Neurospora nitrate reductase[9].

Résumé. La xanthine déshydrogénase, enzyme du foie de rat, a été obtenue par 2 procédés différents: (a) avec et (b) sans traitement par le Butanol. La teneur totale en phosphore des 2 préparations a été etonnament élevée. Soixante %, à peu près, du phosphore total s'est montré soluble dans des solvents de lipides. Certaines hypothèses sur la participation des phosphates et des lipides sur l'activité de l'enzyme sont discutées.

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