Table 3. EFFECT OF DELAY IN APPLYING INFRA-RED AFTER RED ON THE PERCENTAGE OF GERMINATION

	Red alone	Infra-red applied t minutes after red				
		t	0	30	45	60
Per cent of germination	71		15	19	24	31

supporting the mechanism previously suggested[3] according to which it is the system formed by the action of the red light which is sensitive to the effect of the infra-red.

The full details of these and other related experiments will be published elsewhere.

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Direct Deamination of Adenosine Diphosphate by Washed Myofibrils

THE deamination of adenine nucleotides by homogenates and extracts of skeletal muscle is usually ascribed to the action of an enzyme, originally described by Schmidt[1], which specifically deaminates adenosine monophosphate. Direct deamination of adenosine triphosphate has not been demonstrated, though the work of Banga and Josepovits[2] suggested a simultaneous production of inorganic phosphate and ammonia from adenosine diphosphate. This effect required the presence of myosin plus a 'protin' preparation, and an isomer of adenosine diphosphate was postulated as an intermediate compound. This claim has been criticized adversely by Bailey[3].

Evidence supporting the possibility of a direct deamination of adenosine diphosphate has now been obtained. Muscular tissue (psoas, longissimus dorsi and thigh muscles) from the rabbit was used in these investigations. It was homogenized with a high-speed homogenizer to the myofibril-level, centrifuged, washed and recentrifuged repeatedly. For this procedure, 0·16 M potassium chloride, acetate buffer (0·2 M, pH 5·5) or borate buffer (0·1 M, pH 8·6) were all found to be suitable. The fibrils were finally suspended in acetate or veronal buffer (pH 5·5–6·0) and stored at 0° C.

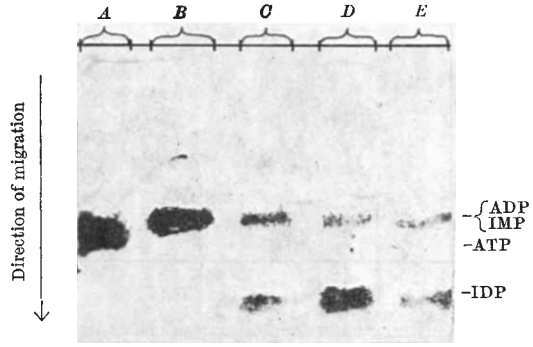
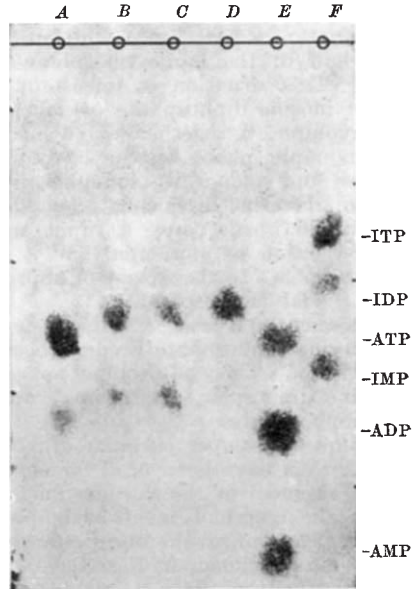


Fig. 2. (*a*) Ionophoretic analysis of reaction products (refer to Fig. 1,*a*). Potential gradient approx. 16 V./cm. for 2–3 hr., citrate buffer (0·1 M, pH 3·0), Whatman paper 3 MM. *A*, *B*, *C*, *D*, reaction times 0, 5, 30, 60 min. respectively. *E*, inosine mono- and diphosphate markers.



(*b*) Chromatographic analysis of reaction products (refer to Fig. 1,*a*). *A*, time 0 min.; *B*, time 60 min.; *C*, time 60 min. + inosine monophosphate; *D*, time 60 min. + inosine diphosphate; *E*, adenosine mono-, di- and triphosphate markers; *F*, inosine tri-, di- and monophosphate markers (propanol/ammonium hydroxide/water, 60/30/10, as solvent)

Fig. 1 shows the effect of adding either (*a*) adenosine triphosphate, or (*b*) adenosine diphosphate to such a fibril suspension. With adenosine triphosphate, there is a rapid hydrolysis of the terminal phosphate group, followed by a slow dismutation of adenosine diphosphate due to residual myokinase acting at a pH well below its optimum. The production of ammonia

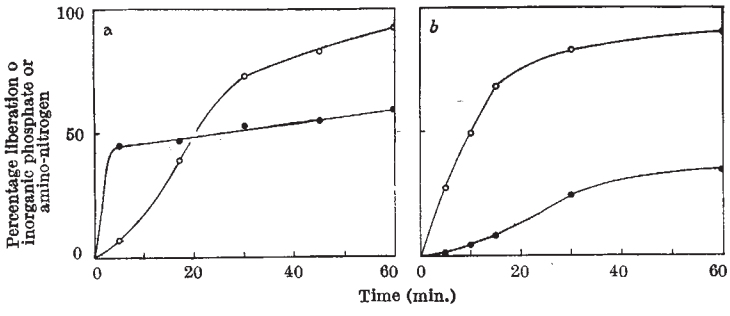


Fig. 1. Washed myofibrils (equivalent to 0·8 gm. original muscle), acetate buffer (0·05 M, pH 5·5), cysteine (0·01 M), room temperature.

(*a*) Adenosine triphosphate added (13·9 μM). (*b*) Adenosine diphosphate added (7·4 μM). ○, Amino-nitrogen liberated. ●, Inorganic phosphate liberated

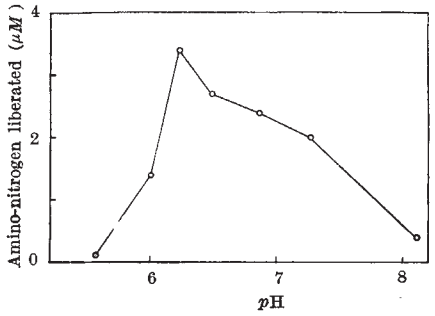


Fig. 3. *p*H activity curve for deamination of adenosine diphosphate in presence of versene (0·01 *M*). Muscle equivalent 0·63 gm., veronal buffer (0·03 *M*), adenosine diphosphate (7·4 μM), 30 min. incubation at room temperature. (Liberated inorganic phosphorus found to be zero at all *p*H values)

begins slowly but rapidly increases as adenosine diphosphate is formed. At the end of the reaction period deamination is practically complete; but little more than half of the labile phosphate has been hydrolysed. This situation is consistent with the formation of inosine diphosphate (which is not dismuted by myokinase[4]), together with a small amount of inosine monophosphate by the combined action of myokinase and adenosine monophosphate-deaminase. When adenosine diphosphate is added originally, the washed fibrils cause a rapid and nearly complete production of ammonia, and a slow production of phosphate to the extent of approximately one-third of the labile phosphate.

In both these cases, it is suggested, direct deamination of adenosine diphosphate occurs, together with some dismutation. The end-product appears to be mainly inosine diphosphate, with small amounts of inosine monophosphate.

The techniques of paper chromatography and of paper ionophoresis have been used for checking the presence (or absence) of the various nucleotides at all stages of the reactions mentioned above. The results (see Fig. 2) confirm the interpretation already given of the curves shown in Fig. 1.

A fuller investigation of the direct deamination of adenosine diphosphate disclosed by these experiments is complicated by its dismutation caused by myokinase. The accompanying table shows the effect of *p*H on the relation between dismutation and deamination of adenosine diphosphate. Increase of *p*H from 5 to 7 progressively favours dismutation, and inosine monophosphate ultimately replaces the diphosphate as the main end-product. It was, however, found that 'versene' (ethylene diamine tetra-acetate), at a concentration of 0·01 *M*, prevented dismutation at all values of *p*H, without affecting deamination. In the presence of this reagent, the *p*H-optimum for the activity of adenosine diphosphate deaminase was found to be approximately 6·2 (see Fig. 3).

Effect of *p*H on Relation between Deamination and Dismutation of Adenosine Diphosphate by Washed Myofibrils. Muscle equivalent 0·6 gm., veronal buffer (0·03 *M*), adenosine diphosphate (10·3 μM), room temperature. Reaction allowed to go to completion

*p*H	ΔP	ΔN	IMP formed (ΔP)	IDP formed (ΔN—ΔP)	Ratio $\frac{\text{IDP}}{\text{IMP}}$
5·1	2·5	9·1	2·5	6·6	2·6
5·4	3·6	9·3	3·6	5·7	1·6
5·9	6·7	9·3	6·7	2·6	0·4
6·2	7·6	9·6	7·6	2·0	0·3
7·0	10·3	9·8	10·3	−0·5	—

Expressed in μM

It has proved impossible so far to measure the effect of calcium and magnesium ions on the deamination of adenosine diphosphate, because these ions, particularly magnesium, progressively stimulate dismutation as their concentration is increased up to 0·005 *M*. Cysteine (0·01 *M*) is found to activate deamination strongly, iodoacetate (0·01 *M*) has little effect, while fluoride and selenite (0·01 *M*) halve its activity.

Homogenized, washed rabbit heart muscle does not exhibit adenosine diphosphate-deaminase activity, neither do preparations of actin or myosin.

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June 12.

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Influence of Cysteinamine, Methylamine and Cortisone on the Toxicity and Activity of Nitrogen Mustard

According to Bacq and Herve[1,2], cysteinamine (2-mercaptoethylamine) and methylamine protect mice against a lethal dose of X-irradiation. The amines prevent death, but do not inhibit X-ray damage to the testes. It was tempting, therefore, to see whether they reduce the toxicity of a 'radiomimetic' drug[3] without at the same time reducing its specific activity. Colter and Quastel[4] have already shown that, *in vitro*, methylamine protects choline oxidase against nitrogen mustard.

On the first day of the experiment, female rats of 51 ± 5·8 gm. mean body-weight received a subcutaneous transplant of Walker carcinosarcoma. Treatment was started on the second day with various doses, per 100 gm. body-weight, of methyl-*bis*-(2-chloroethyl)amine hydrochloride (*HN*2) given intraperitoneally in 0·4 ml. of distilled water. In each experiment approximately half of the animals were given previous injections of cysteinamine, methylamine or cortisone acetate. The cysteinamine and methylamine were given intraperitoneally in 0·2 or 0·3 ml. of distilled water per rat, the solution being made up immediately before use. A suspension of 0·125 gm. of cortisone acetate (cortone acetate) was injected subcutaneously twice a day with an interval of six hours between injections. The nitrogen mustard was given half-way between the two injections. All rats received ten daily injections of *HN*2, intervals being allowed on Sundays, or, in imitation of clinical timing, four daily injections with a day's interval after the third injection. On the fourteenth day, the tumours and other tissues were dissected. The mean weight ratios between untreated and treated tumours were used as a measure of activity of the nitrogen mustard.

The results are shown in the table. Rats injected with *LD*100 of *HN*2 died even when they had received cysteinamine three hours previously. When, however, the interval between the injection of cysteinamine and mustard was reduced to half an hour, 50 per cent protection was afforded.

*HN*2 is more toxic to tumour-bearing than to normal rats[5]. When injected, for example, with a daily dose