Utilization of Metaphosphate for Phosphorylation by Cell-free Extracts of *Mycobacterium smegmatis*

In a recent communication in *Nature*[1], we pointed out that it has been suggested by several authors that metaphosphate may be a source of 'high-energy' phosphate in the cells of a number of lower organisms, but that this had not been proved[2]. The question has since been investigated in this laboratory.

Table 1. Utilization of Labile Phosphate by Cell-free Extracts of *M. smegmatis*

Mixtures incubated	7-min. labile phosphate			Stable phosphate			Orthophosphate		
	Start	Finish	Change	Start	Finish	Change	Start	Finish	Change
(1) Extract + glycerol + metaphosphate	101·6	87·8	−13·8	64·1	66·8	+2·7	59·5	70·8	+11·3
(2) Extract + glycerol + adenosine triphosphate	112·2	15·8	−96·4	115·6	193·1	+77·5	66·8	86·6	+19·8
(3) Extract + glycerol + adenosine triphosphate + metaphosphate	195·6	59·8	−135·8	123·0	230·4	+107·4	69·1	99·4	+30·3

All figures represent μgm. phosphorus per ml. of mixture

The experimental method was based on that used by Hunter[3] in showing the phosphorylation of glycerol by cell-free extracts of mycobacteria. Four-day old surface growth of *M. smegmatis* was harvested, washed, and about 5 gm. (wet-weight) ground by hand with about 10 gm. acid-washed Merck alumina. It was then made into a slurry with 25 ml. of *M*/10 phosphate buffer, *p*H 7·40, and ground in a ball-mill for 10 min. Whole cells and alumina were removed by centrifuging three times at 2,700 *g*, each time for 10 min. All these operations were performed at as low a temperature as possible. The *p*H was re-adjusted to 7·40. The extract was dialysed against three changes of tap water at about 4° C. After dialysis the *p*H was readjusted to 7·40.

The other solutions used in the experiment were: (i) 2 per cent glycerol; (ii) 1·5 per cent solution of the barium salt of adenosine triphosphate (Schwartz Laboratories) dissolved in *N*/20 hydrochloric acid, the barium precipitated by adding potassium sulphate, the solution brought to *p*H 7·40, and centrifuged; (iii) 2 per cent sodium metaphosphate (Hopkin and Williams) was suspended in 1 per cent potassium sulphate, the *p*H adjusted to 7·40, the suspension kept overnight, its *p*H readjusted, the suspension centrifuged, and the supernatant diluted 1 : 1 with water.

5 ml. of the cell-free extract and 1 ml. each of the required solutions were pipetted into 25-ml. conical flasks; water was added where necessary to give a final volume of 9 ml., and the flasks then thoroughly evacuated and incubated at 37° C. for 4 hr. Two samples, taken at both the beginning and end of this period, were pipetted into an equal volume of ice-cold 10 per cent trichloracetic acid and stirred. One sample was centrifuged and orthophosphate determined on the supernatant. The other was heated at 90° C. for 15 min. to extract the phosphorus compounds, centrifuged, and the total phosphorus and 7-min. labile phosphate in the supernatant determined. The results of one such experiment are shown in Table 1.

Under the conditions of these experiments, therefore, metaphosphate was hydrolysed to a slight extent to orthophosphate by the dialysed extract. In the absence of adenosine triphosphate, no further change took place. When adenosine triphosphate was added as the sole 'high-energy' phosphate source, more than 50 per cent of its labile phosphate was converted to glycerophosphoric acid. On the addition of metaphosphate in addition to adenosine triphosphate, a considerably greater conversion of labile phosphate to glycerophosphoric was found. These results show that the cell-free extract was capable of utilizing metaphosphate for the phosphorylation of glycerol in the presence of adenosine triphosphate, presumably by phosphorylation of adenosine diphosphate to adenosine triphosphate. As the experiments were done under anaerobic conditions, metaphosphate must have provided the energy for the phosphorylation.

As would be expected, adenosine diphosphate was capable of replacing adenosine triphosphate in the system, but adenosine monophosphate was not.

The enzyme system is being further investigated and detailed findings will be published elsewhere.

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[1] Winder, Frank, and Denneny, Joan M., *Nature*, 174, 353 (1954).
[2] *Added in proof.*—See, however, Hoffmann-Ostenhof *et al.*, *Biochim. et Biophys. Acta*, 14, 285 (1954).
[3] Hunter, G. J. E., *Biochem. J.*, 55, 320 (1953).

Tumour-initiating Action of Urethane and its Inhibition by Purine Precursors

Urethane injected, given by mouth, or applied to the skin, induces the formation of multiple lung adenomata in mice and rats. It also inhibits the growth of some transplanted animal tumours, and induces remissions in human leukæmia. Its many other biological effects include the induction of chromosome abnormalities and mutations.

It was shown recently[1] that urethane has the power of initiating the carcinogenic change in mouse skin without being itself carcinogenic for this tissue, or producing any other detectable change in it. This was demonstrated by a technique based on that used by Berenblum and Shubik[2] and by others for the investigation of the stages of chemical carcinogenesis. Urethane was applied to the skin of the back and followed, after an interval, by weekly applications of croton oil. Many papillomata and some carcinomata developed. No skin tumours appeared on mice treated repeatedly with urethane alone, and only a very few on control mice treated with croton oil alone.

Little is known of the chemical processes underlying the various biological effects of urethane. Todd (quoted by Haddow and Sexton[3]) suggested that urethane may interfere with nucleotide synthesis.