

Table 1. EFFECT OF CHLORPROMAZINE AND 2:4 DINITROPHENOL ON SYSTEMS DEFICIENT IN ADENOSINE DIPHOSPHATE AND PHOSPHATE

Additions	Chlorpromazine ( $2 \times 10^{-4} M$ )	Oxygen uptake ( $\mu$ atm.)	
		Deficient in adenosine diphosphate	Deficient in phosphate
Nil	—	4.5	1.1
Nil	+	3.6	2.1
2:4 Dinitrophenol ( $3.3 \times 10^{-4} M$ )	—	8.1	6.0
2:4 Dinitrophenol ( $3.3 \times 10^{-6} M$ )	+	3.1	3.6

System: ATP, 0.0017 M; KCl, 0.025 M;  $MgSO_4$ , 0.0067 M; cytochrome *c*,  $2 \times 10^{-5} M$ ; L-glutamate, 0.01 M; inorganic orthophosphate, pH 7.2, 0.01 M in adenosine diphosphate-deficient system and 0.0017 M in phosphate-deficient system. Tris buffer, pH 7.2 at 0.067 M. Mitochondria in 0.25 M sucrose, 1.0 mgm. nitrogen. Final volume, 3.0 ml. Temperature, 38° C. Gas phase, air. Incubation time, 10 min.

Table 2. EFFECT OF CHLORPROMAZINE ON THE EXCHANGE REACTION PHOSPHORUS-32-ADENOSINE TRIPHOSPHATE

	Counts/min. incorporated into adenosine triphosphate	Inhibition (per cent)
Control	3,150	Nil
Chlorpromazine ( $2 \times 10^{-4} M$ )	1,460	54

System: ATP, 0.0017 M; inorganic orthophosphate buffer, pH 7.2, 0.0033 M;  $MgSO_4$ , 0.0067 M; KCl, 0.025 M; tris buffer, pH 7.2, 0.03 M; versene,  $10^{-3} M$ ; phosphorus-32 equivalent to 15,000 counts/min. added. Time of incubation, 15 min. Temperature, 20° C. Specific activity of phosphorus-32, 1,000 counts/min./ $\mu$ mole.

phosphorylation at a point prior to that which is sensitive to 2:4 dinitrophenol. We suggest, for example, a modification of the scheme of Chance and Williams<sup>2</sup>, as follows:

- (1)  $B + iP \rightleftharpoons B.P$
- (2)  $AH_2 + B.P \rightleftharpoons A + BH_2 \sim P$
- (3)  $BH_2P + X \rightleftharpoons X \sim P + BH_2$
- (4)  $X \sim P + ADP \rightleftharpoons ATP + X$
- (5)  $BH_2 \sim P \rightarrow BH_2 + iP$
- (6)  $X \sim P \rightarrow X + iP$

In this scheme,  $AH_2$  would represent reduced diphosphopyridine nucleotide and  $B$  oxidized flavo-protein.  $X$  is the unknown intermediate postulated by Chance. In this scheme chlorpromazine would interfere with reaction (3) and 2:4 dinitrophenol with  $X \sim P$  of reaction (4). Reaction (5) would be relatively slow, reaction (6) relatively rapid.

These results are being published in full elsewhere<sup>3</sup>.

M. J. R. DAWKINS  
J. D. JUDAH  
K. R. REES

Department of Morbid Anatomy,  
University College Hospital Medical School,  
and Department of Biochemistry,  
University College,  
London, W.C.1.

<sup>1</sup> Boyer, P. D., Falcone, A. B., and Harrison, W. H., *Nature*, **174**, 401 (1954).

<sup>2</sup> Chance, B., and Williams, G. R., "Adv. in Enzymol.", **17**, 65 (1956).

<sup>3</sup> Dawkins, M. J. R., Judah, J. D., and Rees, K. R., *Biochem. J.* (in the press).

### Separation of Glucose-1-Phosphate and Glucose-6-Phosphate by Paper Chromatography

GLUCOSE-1-PHOSPHATE and glucose-6-phosphate are not readily separated by the solvent systems normally used for the analysis of the naturally occurring phosphoric esters on paper chromatograms (for example, propanol-ammonia-water, *t*-butanol-picric acid—

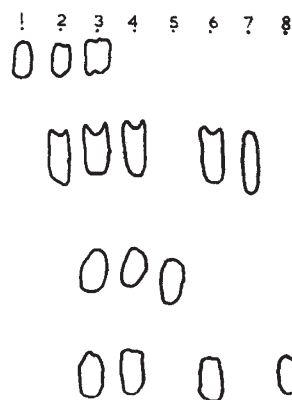


Fig. 1. Chromatogram of phosphoric esters and orthophosphate: (1) fructose-1,6-diphosphate; (2) fructose-1,6-diphosphate + glucose-6-phosphate; (3) fructose-1,6-diphosphate, glucose-6-phosphate, free orthophosphate and glucose-1-phosphate; (4) glucose-6-phosphate, free orthophosphate and glucose-1-phosphate; (5) free orthophosphate; (6) glucose-6-phosphate + glucose-1-phosphate; (7) glucose-6-phosphate; (8) glucose-1-phosphate. Temperature, 20° C.; running time of chromatogram, 48 hr. The paper was equilibrated overnight before the addition of solvent and had been previously washed with acetic acid, distilled water and ammonia (ref. 4).

water, etc.<sup>1</sup>). Though very similar in structure, the esters differ in that glucose-6-phosphate has two *cis*-hydroxyl groups available for substitution and the addition of boric acid to some of the solvent systems previously described enables glucose-6-phosphate to be differentiated clearly from glucose-1-phosphate<sup>2</sup>.

The addition of boric acid to saturate methyl cellosolve-methyl ethyl ketone-3*N* ammonium hydroxide (7:2:3 v/v)<sup>3</sup> gives a sharp separation of glucose-6-phosphate from glucose-1-phosphate and from fructose-1,6-diphosphate and inorganic orthophosphate (Fig. 1).

This work was carried out with Dr. F. A. Isherwood at the Low Temperature Research Station, Cambridge.

F. E. G. HARRAP

Levington Research Station,  
Ipswich, Suffolk.  
July 28.

<sup>1</sup> Hanes, C. S., and Isherwood, F. A., *Nature*, **164**, 1107 (1949).

<sup>2</sup> Isherwood, F. A., *British Medical Bulletin*, **10** (3), 202 (1954).

<sup>3</sup> Mortimer, D. C., *Can. J. Chem.*, **30**, 653 (1952).

<sup>4</sup> Isherwood, F. A., and Hanes, C. S., *Biochem. J.*, **55**, 284 (1953).

### Spatial Summation for Heat-Pain

THE report of an experimental study by Greene<sup>1</sup> on this topic reached me too late for reference to be included in my recent communication<sup>2</sup>.

Greene used three methods of stimulation. Two of these—needle-scratch and cold immersion—produced no significant amount of spatial summation. In his third method, of thermal radiation, he used a bank of six 500-W. quartz infra-red lamps so arranged as to produce an even field of radiation over a large area, and found a mean temperature difference in pricking-pain sensation thresholds of 1.1° C. as between an area of 7.8 cm.<sup>2</sup> and one of 2.5 cm.<sup>2</sup>.

This represented a statistically significant degree of spatial summation, but less than that which I reported for the water-heating method, the difference being probably due to two main variations in the