

## A New Polywater

SINCE the report of an anomalous form of water produced in silica capillaries (which we call silica polywater)<sup>1</sup>, similar polywater has been reported by workers using silica and 'Pyrex' capillaries<sup>2-5</sup>. Its existence has, however, been disputed<sup>2,6</sup>. Attempts to find ways of making silica polywater, other than by the methods of Deryagin, have failed<sup>3</sup>.

We have made a form of polywater (which we shall call fluorite polywater) with an infrared spectrum similar to that observed by Lippincott *et al.*<sup>4</sup> but with the frequencies of the peaks somewhat displaced. Two calcium fluoride optical flats were spaced 25  $\mu\text{m}$  apart using two thin parallel strips of platinum separated by 2 cm. The flats were clamped in such a way that water vapour had free access to the gap between them. 2-3 days over 10 per cent aqueous  $\text{K}_2\text{SO}_4$  solution in an evacuated vessel at room temperature produced enough condensate for an infrared spectrum. A broad (about 100  $\text{cm}^{-1}$  wide) peak occurred at 1,550  $\text{cm}^{-1}$ , and a smaller, sharper peak at 1,450  $\text{cm}^{-1}$ . No absorption was observed in the region 3,000-4,000  $\text{cm}^{-1}$ . No such spectrum could be obtained by soaking the flats in distilled water.

Silica polywater was produced in 'Pyrex' capillaries using Deryagin's method<sup>7</sup> and blown on to the same calcium fluoride optical flats. This gave a peak at 1,400  $\text{cm}^{-1}$  and a broad peak in the region 1,600-1,650  $\text{cm}^{-1}$ . There was also a broad peak at 3,500  $\text{cm}^{-1}$ . These results are in agreement with those of Lippincott *et al.*<sup>4</sup> and are what is to be expected from a mixture of ordinary water and silica polywater.

We conclude that it is possible to produce a form of polywater without the presence of a surface containing -Si-O groups.

Most of the structures proposed for silica polywater have involved either an extended hydrogen-bonded network<sup>4,5</sup> or "clusters" containing various numbers of hydrogen-bonded water molecules<sup>8-10</sup>. The spacing of the oxygen atoms at the surface of silica and 'Pyrex' is somewhat uncertain, but the majority of theories try to fit 0.28 nm. The spacing of the fluoride ions in calcium fluoride, however, is 0.386 nm<sup>11</sup>. The models proposed by Erlander<sup>8</sup> and Donohue<sup>10</sup> may be made compatible with this spacing. The network of hexagonal units proposed by Lippincott *et al.*, however, is a good fit to 0.28 nm spaced silica surface in one direction and, with only minor bond bending, fits a 0.386 nm spacing along another axis.

Some of the properties of polywater formed in quartz capillaries (such as viscosity) may be the result of dissolved or suspended silicate. The infrared evidence for the two forms of polywater is, however, conclusive. There seems no reason why a family of similar polywaters should not exist.

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<sup>1</sup> Deryagin, B. V., and Fedyakin, N. N., *Dokl. Akad. Nauk. SSSR*, **147**, 408 (1962).

<sup>2</sup> Willis, E., Rennie, G. K., Smart, C., and Pethica, B. A., *Nature*, **222**, 159 (1969).

<sup>3</sup> Peel, D. H., and Martin, F. S., *Chem. Ind.*, 1046 (1969).

<sup>4</sup> Lippincott, E. R., Stromberg, R. R., Grant, W. H., and Cessac, G. L., *Science*, **164**, 1482 (1969).

<sup>5</sup> Bellamy, L. J., Osborne, A. R., Lippincott, E. R., and Bandy, A. R., *Chem. Ind.*, 686 (1969).

<sup>6</sup> Cherkin, A., *Nature*, **224**, 1298 (1969).

<sup>7</sup> Anisomova, B. I., Deryagin, B. V., Ershova, I. G., Litsnikov, D. S., Rabinovich, Ya. I., Simonova, V. K., and Churaev, N. V., *Zh. fiz. Khim.*, **41**, 2377 (1967).

<sup>8</sup> Erlander, S. R., *Phys. Rev. Lett.*, **22**, 177 (1969).

<sup>9</sup> Bolander, R. W., Kassner, jun., J. L., and Zung, J. T., *Nature*, **221**, 1233 (1969).

<sup>10</sup> Donohue, J., *Science*, **166**, 1000 (1969).

<sup>11</sup> Swanson, H. E., and Tatge, E., *US Nat. Bur. Stand. Circ.* **539**, 1, 69 (1953).

## BIOLOGICAL SCIENCES

### Recognition Sites around the Catalytic Centre of DNA-dependent RNA Polymerase

WE have been studying the structure of enzymes of nucleic acid metabolism by amino-acid sequence analysis and affinity labelling techniques and hope in this way to discover specific enzyme inhibitors. We report here the inhibition of purified DNA-dependent RNA polymerase from *Escherichia coli* by the nucleoside analogues,  $\beta$ -D-ribose-6-methyl-thiopurine (MMPR, methylthioinosine) and its periodate oxidation product (MMPR-OP), and by  $\beta$ -D-arabino-6-mercaptopurine (Ara-6MP). We also report a lack of inhibition by the arabinosides of cytosine (Ara-C) and adenine (Ara-A). The results lead us to propose a tentative stochastic model for the catalytic centre of DNA-dependent RNA polymerase from *E. coli* B.

Table 1. EFFECTS OF VARIOUS MOLARITIES OF MMPR AND MMPR-OP ON RNA POLYMERASE

Inhibitor	nmoles 2- <sup>14</sup> C-UTP incorporated	Percentage inhibition
None	9.40	—
MMPR-OP		
$3.1 \times 10^{-5}$ M	8.60	9
$6.3 \times 10^{-5}$ M	7.90	16
$1.3 \times 10^{-4}$ M	7.50	21
$2.5 \times 10^{-4}$ M	6.65	29
$5.0 \times 10^{-4}$ M	5.65	40
$1.0 \times 10^{-3}$ M	3.80	60
MMPR		
$5.0 \times 10^{-4}$ M	6.8	28
$1.0 \times 10^{-3}$ M	5.9	37

The reaction mixture contained in 0.5 ml.: 5 units of RNA polymerase; 0.4  $\mu\text{moles}$  each of ATP, GTP and CTP; 50  $\mu\text{moles}$  of Tris-HCl buffer (pH 7.8); 2.0  $\mu\text{moles}$   $\text{MnCl}_2$ ; 50  $\mu\text{g}$  calf thymus DNA; 1.0  $\mu\text{moles}$  spermidine; and 0.2  $\mu\text{moles}$  2-<sup>14</sup>C-UTP. Inhibitors or water were preincubated with enzyme in 50  $\mu\text{moles}$  of Tris-HCl buffer and 2  $\mu\text{moles}$   $\text{MnCl}_2$  for 15 min at 37° C before the addition of the template and substrates and incubations were continued for 20 min.

DNA-dependent RNA polymerase was extracted and purified by the methods of Furth *et al.*<sup>1</sup> and Maitra and Hurwitz<sup>2</sup> as modified by Persico<sup>3</sup>, which yield the purified polymerase containing the  $\sigma$  subunit. Enzymatic activity was assayed by the incorporation of radio-labelled UTP into an acid-insoluble product. The purified enzyme (2-5 units) was preincubated at 37° C for 15 min with 50  $\mu\text{moles}$  of Tris-HCl buffer (pH 7.8), 2  $\mu\text{moles}$  of  $\text{MnCl}_2$ , and various molarities of nucleoside analogue inhibitors or deionized, glass distilled water. After preincubation, the incubation mixtures were cooled to 3° C and to these were added 50  $\mu\text{g}$  of calf thymus DNA (Sigma); 0.4  $\mu\text{moles}$  each of CTP, GTP and ATP (Sigma); 1.0  $\mu\text{mole}$  of spermidine (Sigma); and 0.2  $\mu\text{moles}$  of 2-<sup>14</sup>C-UTP (Schwarz BioResearch, 250 c.p.m. per nmole) or 5-<sup>3</sup>H-UTP (Schwarz BioResearch, 3,800 c.p.m. per nmole). The complete systems were incubated at 37° C for various times, and the reactions were terminated by cooling the mixtures to 3° C, adding 0.5 mg of egg albumin as carrier, and 2.0 ml. of ice-cold 5 per cent trichloroacetic acid. The acid-insoluble precipitates were each washed three times with ice-cold 5 per cent trichloroacetic acid, partially dried, and solubilized in 'Soluene' (Packard). Radioactivity was measured with a liquid scintillation spectrophotometer (Packard, model 3380).