Table 1. Comparison of crystal data of IDU and BrDU.

Physical property	IDU	BrDU
Crystal system	Triclinic	Triclinic
a(Å)	4.98	4.87
b(Å)	6.83	6.72
c(Å)	9.60	9.56
α	101°40′	100°10′
β	109°18′	107°24′
γ	98°20′	98°31′
Volume of cell (Å^3)	292.9	285.1
Measured density (g cm^{-3})	2.014	
Molecules per cell	1	1
Calculated density (g cm^{-3})	2.008	1.789
Space group	$P1$	$P1$

iodine to oxygen distance being even shorter than that reported here.

On the basis of this information it does not seem unreasonable to suggest that the strong intermolecular attraction may be the explanation for the role played by IDU in the treatment of *herpes simplex* keratitis. The ability of the iodine to form intermolecular charge-transfer bonds with atoms having lone pairs of electrons, such as oxygen or nitrogen, may cause the increased interchain attraction which is observed when IDU is substituted for thymidine in DNA, with subsequent retardation of the reproductive ability of the DNA. In the case of IDU incorporation by *herpes simplex*, this could prevent the synthesis of viral DNA or retard it to the extent that the body could combat the virus successfully.

There are a number of oxygen and nitrogen atoms on the complementary purine base (adenine), and on the complementary chain skeleton, with which the iodine might be able to form charge-transfer bonds; such a bond need not be with carbonyl oxygen as is the case in the IDU crystal. Hence a large number of possibilities exist as to the actual intermolecular bonding scheme which could be effected by the electron-accepting property of the iodine, some possibly involving disruption of the normal hydrogen-bonding system, and some not. Whether such intermolecular attraction would actually take place in addition to the normal hydrogen-bonding scheme between base pairs, or would replace this scheme with an alternate one, is a matter of speculation. More direct information would require structural investigation of the aberrant DNA.

The other short intermolecular distances in the IDU crystal correspond to normal hydrogen bonds. Crystals of thymidine are orthorhombic and the cell dimensions bear no resemblance to those of IDU and BrDU, so that it has a different crystal structure.

N. CAMERMAN
J. TROTTER
Department of Chemistry, University of British Columbia, Vancouver

References

1. H. E. Kaufman, A. B. Nesburn, E. D. Maloney, *Arch. Ophthalmol.* **67**, 583 (1962).
2. I. Tamm and H. J. Eggers, *Science* **142**, 24 (1963).
3. W. Szybalski, in *Molecular Basis of Neoplasia* (Univ. of Texas Press, Austin, 1961), p. 147.
4. S. Kit, *ibid.*, p. 133.
5. Details in preparation.
6. O. Hassel and C. Rømming, *Quart. Rev. London* **16**, 1 (1962).

11 March 1964

Nucleotides: Separation from an Alkaline Hydrolysate of RNA by Thin-Layer Electrophoresis

Abstract. *Mixtures of the 2′, 3′ nucleoside monophosphates obtained from an alkaline hydrolysis of RNA can be resolved into the four main RNA mononucleotides by electrophoresis on thin layers of cellulose. The separated nucleotides are recovered by removing the cellulose layer from the supporting glass plate and suspending the cellulose layer in* tris *hydrochloride buffer. The extracted cellulose is removed by low-speed centrifugation.*

The separation of low molecular weight derivatives of nucleic acid by thin-layer chromatography on cellulose or on ion-exchange cellulose layers has been reported (*1–4*). The systems developed by Randerath (*1, 2*), are most suitable for the separation of components of nucleotide mixtures of differing phosphate content, such as monophosphates and diphosphates. The separation of 10^{-3} μmole amounts of the four 5′ mononucleotides of RNA has been described by Randerath (*5*) for polyethylenimine-cellulose layers with a stepwise elution technique; but this method does not give good resolution of the 2′, 3′ phosphates obtained from an alkaline hydrolysis of RNA.

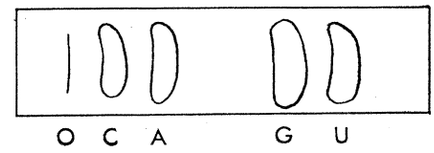

Fig. 1. The separation obtained with sodium formate buffer, *p*H 3.4, ionic strength 0.1, with a 75-minute run. *O*, origin; *C*, cytidylic acid; *A*, adenylic acid; *G*, guanylic acid; and *U*, uridylic acid.

This lack of resolution increases very markedly when the sample applied to the thin layer is in the 10^{-1}-μmole range, which is the amount of material necessary for adequate identification by spectroscopy in the ultraviolet region. The isobutyric acid, ammonia (specific gravity 0.90), and water solvent used by Coffey and Newburgh (*2*) to separate the four RNA mononucleotides works well with a 10^{-1}-μmole sample, but 5 hours or more are necessary for development and separation. The propanol, ammonia (specific gravity 0.88), and water solvent used by Dyer (*3*) on DEAE-cellulose (diethylaminoethyl) layers to separate guanylic acid from the remaining three mononucleotides operates at such a high *p*H that the ion-exchange process is not possible. This method produced a great deal of "tailing" when 10^{-1} μmole samples were applied to DEAE-cellulose layers. These difficulties indicated that thin-layer electrophoresis rather than chromatography might be a more suitable method for the separation of the four RNA mononucleotides with samples in the micromole range.

The ribonucleotides tested were obtained from Nutritional Biochemicals Corporation and from an RNA sample, purified by phenol extraction of HeLa cells, which was degraded with 0.3*N* NaOH at 35°C for 18 hours. The hydrolysate was neutralized by shaking with a small quantity of Dowex 50 in the H form, and then dialyzed against distilled water for 1 hour at 25°C.

MN-cellulose powder 300 (*6*) was washed by centrifugation in 1*N* NaOH, water, 0.1*N* HCl, water, ethanol, and water. Forty grams of the washed powder was brought to 300 ml with distilled water and then stored.

Plates were prepared by vigorously shaking the suspended cellulose powder and pouring 100 ml into a spreader (*7*) which was then passed over 20 (200 × 50 mm) plates set in position on the Desaga mounting board.

The plates were dried at 105°C for 15 minutes.

Samples were streaked in 10-μl quantities so that a narrow band about 30 mm wide formed at 25 mm from one edge of the plate.

Sodium formate buffer, *p*H 3.4, ionic strength 0.1, was used to separate the four mononucleotides. The glass plate was dipped into the buffer except for the region to which the nucleotide band had been applied. Two paper wicks were placed at the ends of the wet plate and held in place by a second glass placed over the first. The buffer was applied to the wicks and flowed by capillary action over the region of the plate containing the nucleotide band. The two plates and wicks were placed in the electrophoresis chamber with the wicks extending into the buffer which was covered with Varsol to cool the plates during electrophoresis.

The four compounds were resolved in 75 minutes when a potential difference of 450 volts was applied across the plate (Fig. 1). With a formate buffer at *p*H 3.1, guanylic acid and uridylic acid were separated from each other and the other nucleotides in 50 minutes. These times are comparable to those obtained with high-voltage (3000 volts) paper electrophoresis, which requires an expensive power supply. Complete resolution of these compounds with a potential difference of 450 volts takes 10 to 20 hours with ordinary paper electrophoresis (*7*, *8*). Another advantage of the thin-layer method is that the thickness of the layer can be continuously varied to suit the amount of the sample.

After drying the layers, the bands were located with a Mineralight UV lamp and removed from the plate with a razor blade. The thin roll of cellulose obtained was placed in a small conical centrifuge tube to which 2.0 ml of 0.05*M* *tris*-HCl buffer, *p*H 7.2 was added. The centrifuge tube was placed in a bath at 50°C and shaken vigorously several times. After 10 minutes the cellulose was removed by centrifugation and the supernatant was filtered through Schleicher and Schuell No. 589 Blue Ribbon filter paper. The bands were then identified by their ultraviolet spectrum.

FRANK M. DEFILIPPES
Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

References and Notes

1. K. Randerath, *Biochem. Biophys. Res. Commun.* **6**, 452 (1962); ———, *Angew. Chem. Intern. Ed. Engl.* **1**, 435 (1962).
2. R. G. Coffey and R. W. Newburgh, *J. Chromatog.* **11**, 376 (1963).
3. T. A. Dyer, *ibid.* **11**, 414 (1963).
4. G. Weimann and K. Randerath, *Experientia* **19**, 49 (1963).
5. K. Randerath, *Thin-Layer Chromatography* (Verlag Chemie, Weinheim, 1963), pp. 195–199.
6. From Machery, Nagel and Co., Düren, Germany.
7. Purchased from DeSaga, Brinkmann Corp.
8. J. M. Davidson and R. M. S. Smellie, *Biochem. J.* **52**, 594 (1952); R. Markham and J. D. Smith, *ibid.* **52**, 552 (1952).

10 April 1964

Development of Mouse Eggs in Diffusion Chambers

Abstract. *Mouse eggs in intraperitoneal diffusion chambers continued normal development up to the stage of implantation, and shedding of the zona pellucida occurred. At the time corresponding to implantation within the uterus, eggs attached to the Millipore surface, where both embryonic and trophoblastic cell populations developed. There was migration of cells, especially of trophoblastic giant cells, away from the point of egg attachment. Egg development was not influenced by the endocrine state of the host.*

Studies in vivo on the development of early mouse eggs in extrauterine locations have met with only limited success (*1*, *2*). In vitro, mouse eggs have never been cultured beyond the blastocyst stage. Since the advantages of techniques used in vivo and in vitro can be combined in the diffusion chamber (*3*), the idea that the chamber might allow even further development of mouse eggs was tested.

Usually one, but up to eight eggs were placed in 0.1-ml Lucite diffusion chambers of drum shape, bounded by cell-impermeable Millipore filters (pore size, 0.45 μ). The sealed chambers were placed in the peritoneal cavity of male mice, and pregnant and nonpregnant female mice. Peritoneal fluid soon displaced the original chamber medium, which consisted of equal volumes of calf serum and 0.9 percent NaCl solution. After 1 to 27 days, studies were made of the eggs and the cellular derivatives of eggs attached to the filters (see Figs. 1 and 2), the contents of the chamber fluid, and the contents of the fibrin clot (Fig. 3) formed after 1 day in the chamber cavity. The clot formed after 1 day in control chambers was also studied, and was uniformly acellular.

At least one egg continued development in nine out of 19 chambers into which eggs were placed at the 2-cell stage (1½ days after fertilization) and in all 30 chambers that received eggs at the 8- to 16-cell and early blastocyst stages (2½ days and 3½ days). The older stages also develop better in vitro (*4*). However, among eggs that did develop in the chambers, there was no systematic difference in development according to whether the eggs had been transferred at an early or late stage. The interpretation is that the 2-cell eggs which did develop became normal blastocysts in the chamber cavity, and thus exhibited the same subsequent behavior as those eggs introduced directly at the blastocyst stage.

When the developing egg reached the late blastocyst stage, it attached to the filter surface. This attachment is considered analogous to egg implantation within the uterus, because of both the chronology and the morphology of the attachment. Implantation in the mouse occurs on the 5th day of pregnancy (*5*), and in chambers containing either originally 2-cell eggs or later stages, attached eggs were on the Millipore surface on the 5th day after fertilization, but never before this time. Unlike the uterus, the Millipore filter provides an impenetrable barrier to vertical invasion by eggs, and thus implantation must be transcribed into two dimensions on the filter surface. As in the uterus, the peripheral cells of the attached egg differentiated into trophoblastic giant cells which migrated away from the

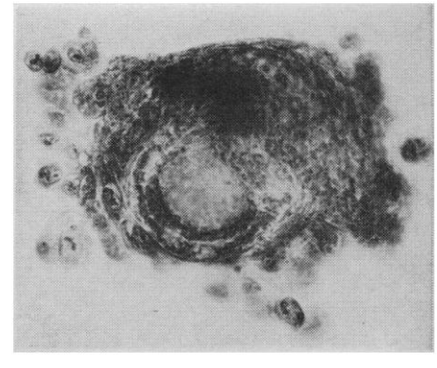

Fig. 1. Stained blastocyst attached to filter surface after 4 days in chamber. The zona pellucida no longer surrounds the egg. The inner cell mass has escaped and spread to the top and to the right. Trophoblastic giant cells surround the other side of the egg, where the inner cell mass has differentiated (gastrulated) into primitive ectoderm and endoderm. (× 150)