

noted to be infected by *Physoderma* in Texas, although corn becomes severely infected with *P. maydis*, indicates that barnyard grass is not a collateral host for *P. maydis* and that the *Physoderma* observed in India is a separate species. None of the graminicolous species of *Physoderma* given by Karling (1) are morphologically similar to the one under study. The fungus is presented as a new species with the name *P. echinochloae*.

*Physoderma echinochloae* sp. nov.<sup>1</sup> Rhizomycelium endobioticum, tenue, delicatulum, ramosum; sporangia perdurantia endobiotica, cellulas mesophylli fasciculos vasculares circumdantes implentia, luteo-brunnea, hemisphaerica, in latere uno applanata et operculum circumscissilem reteguntia, 18–26 × 10–16 μ; episporium leve; germinatio non visa. Hab. in foliis *Echinochloae crusgalli*, Patna, Bihar, India.

Rhizomycelium endobiotic, tenuous, delicate, ramose. Resting sporangia endobiotic, filling the mesophyll cells surrounding vascular bundles, yellowish-brown, hemispherical, flattened on one side and revealing the circumscissile lid, 18–26 × 10–16 μ. Episporium smooth. Germination not observed. Habitat on leaves of *Echinochloa crusgalli* Beauv., Patna, Bihar, India, 12-7-1952, leg. M. J. Thirumalachar.

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## Separation of the Purines and Pyrimidines by Ionophoresis on Filter Paper

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The technique of ionophoresis on filter paper is being extensively employed in separations of various types of mixtures, as reviewed by some authors (1–3). Nucleotides, the constituents of nucleic acid, have also been ionophoretically separated (4, 5). In this communication successful separations by ionophoresis on paper of the purine and pyrimidine bases, adenine, guanine, cytosine, thymine, and uracil, the building units of nucleic acids, are described.

The equipment used for this purpose was essentially the same as described by Durrum (6) with slight modifications and simplifications. A jar (used as a dome for candles) is inverted over a wooden base coated with paraffin. The opening at the top of the jar is ordinarily kept closed by a cork. One or more paper strips (each 1 cm wide and 57 cm long) of Whatman 1 filter paper are supported over a horizontal glass rod within the jar, the ends of the papers dipping into the buffer or any other conducting solution, contained in two different containers. The level

of the liquid in the two containers is made the same by temporarily connecting them through a siphon tube. Voltage is applied from the mains (220 v) through platinum electrodes. A milliammeter is connected in a series to record the current passing through the strips. After rinsing the paper strips with the conducting solution and adjusting the level of the liquid, the mixture to be separated is applied in state of solution from a micropipet at the apex of the supported strip (apex height about 28 cm) by temporarily opening the cork at the top. Usually 0.01 ml of the solution containing 5–15 μg of each of the purine and pyrimidine bases was found to be sufficient for the purpose. For advantages of detection, the constituents of the mixture usually were run individually side by side with the mixture on separate strips of paper. After passage of current for the requisite number of hours, the papers were taken out and held before the Chromatolite lamp (an ultraviolet lamp emitting 2537 Å radiation and specially designed for chromatographic purposes). Purines and pyrimidines appear as blue-black spots on a fluorescent background due to quenching of the fluorescence of paper in those regions (7).

Since purines and pyrimidines are mainly basic in character, buffers of acidic pH were tried as conducting medium for ionophoretic separations. After trials, citric acid-phosphate buffer of pH 2 was found to be suitable for the purpose. An average current of 0.15 ma flowed through each strip when 220 v were applied. Guanine, adenine, and cytosine were found to move toward the cathode whereas thymine and uracil remained practically stationary at the original starting line. Thus, separation of thymine and uracil was not possible under such conditions; fortunately, however, they do not occur in the same nucleic acid. They can be separated from each other using other conducting medium. Of the remaining three, guanine was slowest and cytosine the fastest, with adenine rather close behind cytosine. Though 2 hr were quite sufficient for separation of either guanine and adenine or guanine and cytosine, 6–8 hr were required for separation of adenine and cytosine. An 8-hr-run of a mixture of uracil (or thymine), guanine, adenine, and cytosine was found to be quite sufficient for their neat separations. The distances traveled by the components are found to vary slightly from experiment to experiment. Average distances are: uracil and thymine, 0 cm; guanine, 11–12 cm; adenine, 15–16 cm; and cytosine, 17–18 cm.

Thymine and uracil were separated from each other by using alkali solution as the conducting medium. The bases were applied as sodium salts by dissolving them in alkali. Using 0.1 N alkali, the two separate in about 8 hr, uracil being a bit ahead of thymine (uracil, 10–11 cm; thymine 9–10 cm). Runs for longer hours did not improve the results, but instead created disadvantages due to considerable electrolysis. Using stronger alkali (0.2 N), separation can be effected in a shorter period (4 hr), uracil being a bit ahead of thymine. (uracil, 4–5 cm; thymine, 3–4 cm). In this

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case too, runs for longer periods failed to effect better separation.

Application of this technique in the identification of yeast nucleic acid has already been reported (8).

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## Erythrocyte Mosaicism in a Pair of Sheep Twins

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Almost a decade ago Owen (1) reported in *SCIENCE* his discovery of the phenomenon of compound blood types associated with multiple births in cattle. Subsequently, this condition became known as *erythrocyte mosaicism* (2). Individuals with red cell mosaics are believed to have hematopoietic tissues derived in part from their own embryonal cells and in part from embryonal cells of a co-twin, or co-triplets, etc. Fusion of blood vessels between developing embryos provides the channels for the intermingling of embryonal cells with subsequent establishment of these cells in the hematopoietic beds of each individual so joined. When the autograft produces cells of a serological type different from that of the cells produced by the homo-graft, erythrocyte mosaicism ensues.

TABLE 1

SHEEP TWINS N777 AND N778, AND THEIR PARENTS H454 AND 2955. REACTIONS OF UNTREATED CELLS (N777, N778) AND CELLS (N777/S3 AND N778/S3) RECOVERED FOLLOWING DIFFERENTIAL HEMOLYSIS WITH S3 ANTIBODIES\*

Cells	Antibodies—Readings at 3 hr														
	R	O	S2	S3	S4	S5	S6	S7	S8	S10	S11	S12	S15		
H454	4	0	4	4	4	4	0	4	4	0	4	3	4		
2955	0	4	4	4	4	4	0	4	4	0	4	3	4		
N777 }	0	4	3	1	4	4	0	1	1	0	4	2	4		
N778 }	0	4	3	1	4	4	0	1	1	0	4	3	4		
N777/S3 }	0	4	3	0	4	4	0	0	0	0	4	3	4		
N778/S3 }	0	4	3	0	4	4	0	0	0	0	4	3	4		

\* Readings 0, 1, 2, and others represent degrees of hemolysis ranging from 0 (no hemolysis) to 4 (complete hemolysis). Guinea pig complement was used in these tests.

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Although the detection of these mosaics is commonplace in dizygotic twins and higher zygotic multiples in cattle, there have been no reports of this phenomenon in species other than cattle. It is of interest, therefore, to report an example of this condition in a pair of sheep twins.

In another study (3) concerned with the development of blood-typing antibodies for use primarily in separating dizygotic from potential monozygotic ovine twins, we encountered a pair of twin lambs (N777 and N778) whose corpuscles reacted with certain antisera in a manner suggesting mosaicism. That is, only a fraction of their erythrocytes was hemolyzed by the action of antibodies found in three different ovine isoimmune antisera coded S3, S7, and S8 (Table 1), whereas those of their parents (H454 and 2955, Table 1) were completely hemolyzed. Unaffected erythrocytes (N777/S3 and N778/S3, Table 1) recovered following hemolysis with S3 antibodies were nonreactive in tests with S3, S7, and S8 but were hemolyzed by the other antisera (Table 1) which acted on the untreated cells. The ratio, approximately 40:60, of hemolyzed to nonhemolyzed corpuscles in tests with S3 was the same for each twin. There were, however, no antisera among our limited battery which would lyse only those corpuscles not lysed by S3 or S7 or S8. Although similar examples are encountered in cattle, in view of the nonreciprocal character of the evidence for mosaicism, it seemed advisable to strengthen these results by further tests. To this end absorptions were made on the S3 antiserum with the bloods of each of the twins to make certain that the hemolysis of their corpuscles with these antibodies was not nonspecific. Both bloods readily absorbed the antibodies in this serum. Although the male of this pair died a few weeks after these initial tests, the results of the differential hemolytic tests shown in Table 1 were repeatedly confirmed on samples of blood drawn from N778 at intervals of several months. There was no doubt as to the permanence of the mosaic. It was concluded that the twins N777 and N778 had exchanged hematopoietic transplants through communal chorio-vascular channels.

In view of the general impression that vascular anastomosis between ovine embryos occurs rarely (if at all) our results came somewhat as a surprise since we had tested only 26 pairs of twins. The idea that vascular anastomosis between sheep embryos must be very rare probably traces to the writings of Lillie (4). He apparently based his conclusion on the absence of any reports of ovine freemartins rather than on his own study of four pairs of fetal twins. Since the time of Lillie's paper, there have been at least two reports of ovine freemartins (5, 6). We have also located another report, Rotermund's doctoral dissertation (7), on the subject of choriovascular arrangements between ovine fetuses. Rotermund noted fusions of blood vessels in one pair of heterosexual ovine twin fetuses out of a total of 11 pairs studied, but he did not mention whether the female appeared to be a freemartin.