contained the same amounts of organic bases as did the nucleic acids of the nonirradiated culture. These results are summarized in tables 2 and 3. Of course the synthesized ribonucleic and deoxyribonucleic acids might be abnormal without showing this in their chemical composition. For deoxyribonucleic acid this may be examined with bacteria showing transformation. Preliminary experiments with *Haemophilus influenzae*, carried out in the Biology Department of Brandeis University, Waltham, Massachusetts, showed that postirradiation-formed deoxyribonucleic acid is functionally normal. A detailed report on this will be published shortly.

SIMPLE METHOD FOR SEPARATING LEPTOSPIRAE FROM CONTAMINATING MICROORGANISMS

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Previous methods of purifying contaminated leptospiral cultures have proved tedious and inconsistent. The present work was in progress when Pokorny and Havlik (Geskoslov. epidemiol., mikrobiol., immunol., **6,** 204, 1957; abstr. in Bull. Hyg., **32,** 1197, 1957) reported successful use of membrane filters, shrunk by boiling, to free leptospiral cultures from various bacteria, yeasts, and fungi.

Characteristically turbid, 5-day cultures of the following leptospiral serotypes in Schüffner's medium were employed: *Leptospira autumnalis*, *L. ballum*, *L. bataviae*, *L. biflexa*, *L. canicola*, *L. grippotyphosa*, *L. hyos*, *L. icterohaemorrhagiae*, *L. javanica*, and *L. pomona*. Dense growth in Schüffner's medium of *Escherichia coli* and of *Staphylococcus aureus*, and a turbid suspension in Schüffner's medium of *Haemophilus influenzae* were employed singly as contaminants. Sterile, ½-in Millipore filters (Millipore Filter Corp.) of type HA (pore size 0.45 ± 0.02 μ[3]) were employed in the Millipore Swinny Hypodermic Adapter (Millipore Filter Corp. no. XX 30 012 00).

Equal volumes of leptospiral culture and a suspension of a contaminant were mixed and 1 ml was drawn into a 2-ml syringe through a needle. Two drops were inoculated into a medium suitable for growth of the contaminant (Schüffner's medium for *E. coli* and *S. aureus*; chocolate agar for *H. influenzae*). The needle was removed and a sterile Millipore adapter containing an HA filter was then attached to the syringe and 0.4 ml of the material was filtered into a tube containing 3 ml of Schüffner's medium. Filtrates from mixtures containing *H. influenzae* were also inoculated onto chocolate agar. To favor growth of contaminants, all media were incubated at 37 C for 3 days after inoculation; then portions of all Schüffner's media inoculated with filtrates were subcultured for bacterial growth onto appropriate agar slants. Incubation of the filtrate-inoculated tubes was continued for 4 weeks at

TABLE 1

Results of filtrate cultures after passage through millipore filters

Serotype	Contaminant*		
	Escherichia coli	*Staphylococcus aureus*	*Haemophilus influenzae*
Leptospira autumnalis	+	+	+
L. ballum	+†	+†	−
L. bataviae	+	+	+
L. biflexa	+†	+†	+†
L. canicola	+	+	+
L. grippotyphosa	+	+	+
L. hyos	+	+†	−
L. icterohaemorrhagiae	+	+	+
L. javanica	C	+	+
L. pomona	+	+	+

* + = Growth of leptospirae in filtrate culture; − = No growth of leptospirae in filtrate culture; C = Contaminant isolated from filtrate culture.

† Leptospiral growth occurred with initial incubation at 30 C.

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[3] Manufacturer's specifications.

30 C. Darkfield examination and Gram stain were employed to verify the presence of microorganisms.

Contaminants were isolated from every unfiltered leptospira-contaminant mixture. In addition, *E. coli* was isolated from a single filtered leptospira-*E. coli* mixture. In this instance it is felt that mechanical rupture of the delicate membrane occurred during assembly since visible turbidity developed within the first hour of incubation. As seen in table 1, no contamination of any other of the 30 filtrate cultures resulted and leptospiral growth occurred in all but 3. In most cases, macroscopic growth of the spirochete was evident within 10 days.

Attempts to use a filter of smaller porosity (Millipore type PH, pore size $0.3 \pm 0.02\ \mu^3$) resulted in a decreased number of positive leptospiral cultures. In addition to molecular membrane filtration, trials were carried out with Becton-Dickinson Swinny Filter Adaptors (no. 423 FA) equipped with Seitz EK filter pads (Republic Seitz Filter Corp.; effective pore size $0.1\ \mu^3$). With the latter equipment, filtrates were occasionally contaminated until a modification of the filter pad holder was carried out.

The Millipore HA filter, used in conjunction with the Millipore Swinny Hypodermic Adapter, offers a simple and rapid means of removing other microorganisms from fluids containing leptospirae. The high percentage of positive results, the small size and low cost of equipment, the small amount of culture required, and the simplicity of operation compared with vacuum or pressure equipment and large filters or with animal inoculation, all combine to make advantageous the use of the procedures described in this report.

ERRATUM

ISOLATION OF *LEPTOTRICHIA BUCCALIS*

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An error appeared in the above article which was recently published (J. Bacteriol., **76**, 330–331, 1958). In the legend of Figure 2, the magnification should read "×450" instead of "×900."