and, consequently, the cell wall *per se* of *P. aeruginosa*.

The results of our experiments reported herein, moreover, indicate that the treatment of cell walls of *P. aeruginosa* by EDTA cannot be used as a tool in the isolation of chemically pure lipopolysaccharide as suggested by Leive(11) for *E. coli* since we have shown that lipopolysaccharide thus treated was altered in its biological activity.

Summary. Subcellular fractions and extracted lipopolysaccharide of *P. aeruginosa* were assayed for toxic activity by the mouse lethality technique. Toxic activity was demonstrated to be associated with the cell wall fraction. The isolated lipopolysaccharide was shown to be altered by treatment with lysozyme and with EDTA as evidenced by its loss of lethality for mice.

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A Whole Blood Microculture Technique for Cytological Examination Of Neonatal Rabbits.* (31274)

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Several techniques describing methods for culturing small quantities of human, cattle, sheep, deermouse, and mink whole blood for the subsequent study of chromosomes have been developed(1-4). These procedures (primarily for human whole blood) have not always been directly applicable to laboratory animals. Although the quantitative values associated with these techniques vary, the literature indicates that whole blood cultures are in general composed of chemically defined culture media, blood serum, phytohemagglutinin, heparinized whole blood, and colchicine or its synthetic analog, colcemide. A variety of methods for the hypotonic treatment of the cultured cell, cell fixation, slide preparation, and staining also exists and alterations in these steps are apparently based on individual preference.

Since our study of spontaneous and induced malformations in newborn rabbits would be aided by the cytological examination of such malformed neonates, it was necessary that a repeatable technique be found which would insure good chromosome spreads with limited effort. The whole blood microculture seemed most applicable, since leucocyte separation procedures(5,6) and the bone marrow biopsy technique(7) would be impractical because of the neonatal rabbit's size. Extension of a working microtechnique to older rabbits would also be advantageous because of the small quantities of blood required. The following technique has been developed for efficient chromosomal assay of the neonatal rabbit.

Materials and methods. Erlenmeyer flasks (30 ml) and rubber serum caps were prepared for sterilization by soaking and washing in

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a hot water-detergent solution, rinsing in tap water, and twice in distilled water. The flasks were rinsed again with distilled water (20-30 sec) using a Manning Model 23 Vacuum-Type Ampoule Washer. This last rinse utilized a small stream of distilled water directed at the inner surfaces of the glassware to insure that the culture flask walls were free of all traces of protein and detergent residue. Subsequently, each flask was inverted until thoroughly dried, fitted with a serum cap, vented by means of a cotton-plugged 26-gauge hypodermic needle, and autoclaved at 270°C. After sterilization (30 min), the venting needles were removed while the flasks were hot. When cool, the flasks were filled with the culturing constituents which were introduced aseptically through the serum cap by a hypodermic syringe and needle.

Each culture contained 5.0 cc of Waymouth's tissue culture medium MD705/1(8), 0.2 cc phytohemagglutinin M (code 0528 Difco Laboratories), and 0.5 cc of pooled fetal calf serum (Grand Island Biological Laboratory). The phytohemagglutinin-M *must* be passed through a Seitz (or similar) filter and directly into the flask to insure bacteria-free cultures, as occasional lots were contaminated. The ingredients were mixed before adding the blood by gently swirling each flask. Whole blood was aseptically drawn into a syringe containing 100 U.S.P. units of *preservative-free heparin* per 0.1 cc of blood. The thoroughly combined blood and heparin was inoculated into each flask at the rate of 0.2-0.4 cc whole blood per culture. The flasks were again gently swirled and then incubated, undisturbed, at 38°C for approximately 96 hours. Three to four hours prior to culture termination, 1 cc colcemide (CIBA) solution (2 μg/cc) was gently combined into each culture. Subsequently the serum caps were removed, and 15 ml deionized or distilled water, pH 7.0 (38°C), was added directly to each culture flask. The hypotonic treatment was maintained for 10 min at 38° C, during which time serum clots, if present, were removed (a Pasteur pipette works well).

The hypotonic treated cultures were then transferred to centrifuge tubes and centrifuged in a clinical centrifuge for 6 minutes at 600 rpm. The supernatant liquid was carefully removed using flame-drawn glass tubing connected to a faucet aspirator, care being taken not to disturb the packed cell pellet. To the resulting cell pellet was added 1.5 ml cold fixative (5°C), 1 part glacial acetic acid, and 3 parts of 100% methanol (modification of Clarke's fixative)(9). The packed cell pellet was undisturbed for 15 minutes and then suspended in the fixative. After an additional 15 minutes, it was recentrifuged, the supernatant discarded and fresh cold fixative added. (The cell suspension should be centrifuged and the fixative replaced several times before refrigerating or preparing slides.)

The final fixative volume, changed immediately before slide preparation, was adjusted to roughly three times the packed cell volume. The actual dilution was determined by the cell population preferred.

Prior to slide preparation, precleaned glass slides were further cleaned for 4 hours in a 1:1 solution of 70% ethanol and anesthetic ether, rinsed with and stored in distilled water at 5°C until immediately before using. One to two drops of prepared cell suspension were dropped onto a cold, drained (but still wet), slide, using a Pasteur pipette held 2-3 cm above the slide. The cell suspension was ignited immediately and air-dried for 12 hours before staining. Batch staining was done in a 1:20 solution of Giemsa stain fluid and slightly basic distilled water (pH 7.1-7.4) for 5-10 min. Excess stain was gently washed from the slides with cold tap water (pH 7.0) (acidity causes destaining). After drying, cover slips were permanently mounted to the slide with diaphane.

Results and discussion. Preservative-free heparin contributed greatly to the success of this procedure, as confirmed by Razavi(3). Heparin with preservatives was found to inhibit development up to 100%. In addition to this problem, sporadic contamination affected many of the preliminary cultures. Study of cultures containing either filtered or unfiltered PHA-M resulted in no observable contamination in filtered vials from 13 separate bottles of PHA-M after 96 hours at 37°C and 100% contamination in unfiltered vials from 8 separate bottles of PHA-M after 24 hours at 37°C. The reconstituting fluids

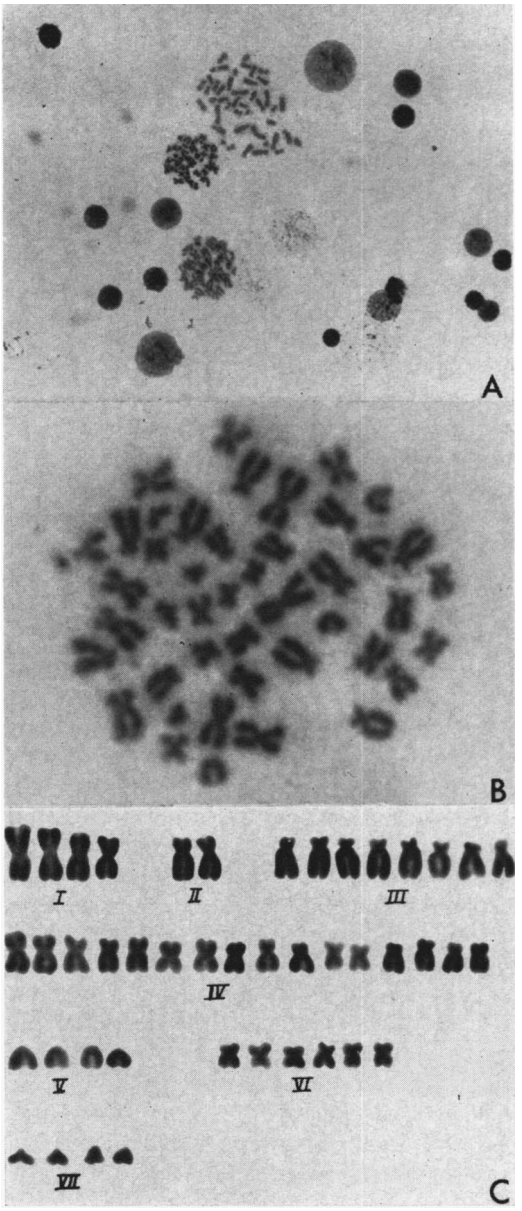

FIG. 1.

were also checked with no observable contamination. Bacteriological examination of the culture ingredients showed that one lot of supposedly sterile PHA-M was contaminated with *Bacillus cervi* and subsequently all PHA-M has been Seitz-filtered, thus eliminating further contamination. Blood was obtained by cardiac puncture in the newborn and from the heart, ear vein, or ear artery of the adult rabbit. If Vacutainers (Becton, Dickinson & Co.) are used for collection, blood can be transferred aseptically from the collection tube to the culture media with a syringe containing the required amount of heparin. Unhemolyzed erythrocytes in the packed cell pellet should be kept to a minimum by adjusting the hypotonic volume, since they contribute a cluttered background to the prepared slide.

A culture developed according to this procedure should produce about 5-8 slides, each containing a high number of metaphase chromosomes. In Plate 1, Fig. A and B show approximate cell density and a sample metaphase, respectively, and Fig. C shows a karyotype in the manner of Myers *et al*(10).

Summary. A technique for preparing neonatal rabbit blood for chromosome studies is described. It uses whole blood, Waymouth's tissue culture media, phytohemagglutinin M, and fetal calf serum. The advantages are the small quantity of blood required, the simplicity of equipment and facilities needed, and the efficiency of the combination of culture ingredients and procedures in the production of usable metaphases.

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