fers on control media, passages through normal mice and storage at 4°C partially or completely restored the bacitracin sensitivity to all resistant variants. 4. Mouse virulence was decreased in 7 of 9 strains. It was restored in 6 of the 7 strains by subsequent passages in normal mice. 5. Group specificity was lost in 3 of 7 group B strains after induced resistance. 6. Growth on sub-inhibitory concentrations of bacitracin stimulated transient conversion from beta to alpha hemolysis in most strains studied. 7. The streptolysin "S" titre was decreased in 2 of 3 resistant variants. Ribo- and desoxyribonuclease activity and the proteinase-streptokinase ratios remained unchanged after acquired resistance.

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The Cultivation of Coxsackie Virus.*† (17955)

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Recent laboratory studies(1-6) have established a newly discovered(1) group of viruses, the Coxsackie or "C" group, as the etiological agents responsible for human disease diagnosed clinically as nonparalytic poliomyelitis (1-4,7), "summer grippe"(5), aseptic meningitis(3) and epidemic myalgia(4). The viruses have been recovered from feces and throat swabs of patients and from sewage and flies(3). This group of viruses is characterized by its pathogenicity for newborn mice and hamsters with resultant myositis, paralysis and death.

In the experiments reported herein evidence is presented for the successful propagation *in vitro* in the presence of living tissue cells of a member of the Coxsackie group of viruses, type 4, Minnesota 1 strain.§

Materials and Methods. Virus. The strain of virus was isolated from a fecal specimen which had been obtained on about the 10th day following the onset of the illness of a patient whose diagnosis, both on admission and discharge from the University of Minnesota Hospitals, was nonparalytic poliomyelitis. The feces were made up in 10% suspension, homogenized for 5 minutes in a Waring blender and centrifuged at 10,000 r.p.m. for 20 minutes. The supernatant fluid was removed, filtered through a Seitz-EK sterilizing filter pad and the filtrate was employed for injection intraperitoneally, 0.05 ml, into each member of a litter of Rockland Swiss albino mice within 24-36 hours of their birth. When these infant mice were ill or dead, they were frozen, triturated in a mortar in 0.85% saline to yield a 10% suspension, filtered as described above, and the filtrate, 0.03 ml, was used as the inoculum for passage to infant mice. Following 10 mouse passages in series, 0.03 ml of filtrate similarly prepared was used to initiate each tissue culture passage series.

Tissue Cells. Infant mice killed within about 48 hours before or after birth provided the tissues which were employed to

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1. Dalldorf, G., and Sickles, G. M., *Science,* 1948, v108, 61.

2. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.,* 1949, v89, 567.

3. Melnick, J. L., Shaw, E. W., Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 344.

4. Curnen, E. C., Shaw, E. W., and Melnick, J. L., *J.A.M.A.,* 1949, v141, 894.

5. Melnick, J. L., Ledinko, N., Kaplan, A. S., and Kraft, L. M., *J. Exp. Med.,* 1950, v91, 185.

6. Howitt, B. F., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 443.

7. Unpublished data.

§ The authors are indebted to Dr. G. Dalldorf for allocating Minnesota strain 1, Coxsackie group, as type 4, subgroup A, Coxsackie group of viruses.

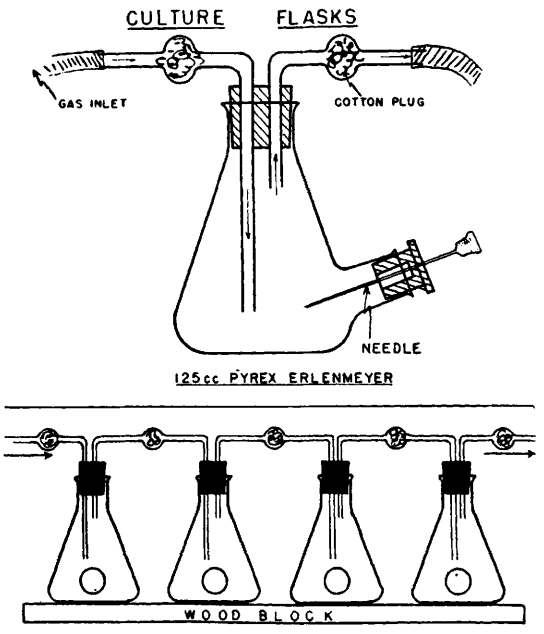

FIG. 1.
Tissue culture flask and flask assembly.

prepare 3 tissue pools made up of cells from (a) intestines, (b) brains, and (c) muscle (a mixture of hepatic, splenic and muscle tissues were used for the first 5 passages). Each tissue-pool was finely minced in Simm's UF/3X7 fluid with sharp iris scissors to a particle size that entered a 10 ml serological pipette. One drop of this tissue pulp and 0.03 ml of virus suspension were added to 5 ml of Simm's UF/3X7 contained in a newly designed tissue culture 125 ml flask (Fig. 1). The utilization of this flask and of Mohr clamps for closure made it possible to pass daily a gaseous mixture of 5% carbon dioxide, 21% oxygen, and 74% nitrogen over the surface of the content, and to maintain an identical atmosphere within each battery of flasks. After incubation had been carried out at 35.5°C for 72 hours, the supernatant fluid was withdrawn from each flask, pooled, and 0.03 ml was employed as the inoculum for each flask in the successive passage serial transfer.

Results. Three passage series, A, intestinal tissue cells; B, brain tissue cells; C, a mixture of muscle, hepatic and splenic cells for 5 passages and, thereafter, muscle alone, had been carried through 24 passage transfers when this report was written. The virus content of the supernatant fluids, as successive tenfold decrements, was determined for each dilution at intervals by the injection intraperitoneally, 0.03 ml, into each of a litter of mice from 1 to 5 days old. The results for 24 passages are summarized in Fig. 2.

It can be seen that the virus through the 12th passage was almost uniformly lethal. The titer after 10 passages was 10^6. When virus representative of the 16th passage was tested, virus was present in a concentration of 10^6 as evidenced by paralysis of the test mice. However, it became apparent that a change in relative pathogenicity had occurred, since many mice survived at all dilutions to give an over-all mortality of 42%. The surviving mice showed marked tremors, spasticity, paralysis and residual stunting. Since the dilution factor after the 24th passage was more than $(166)^{24}$, unequivocal evidence for multiplication of the virus had been effected.

Summary. A Coxsackie virus, Minnesota 1 strain, following its isolation from a fecal sample of a patient with a clinical diagnosis of nonparalytic poliomyelitis, was maintained successfully for 24 successive passages in a tissue medium made up of minced embryonic mouse cells and Simm's UF/3X7 fluid.

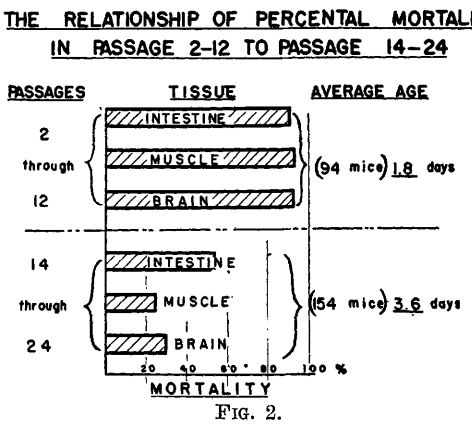

FIG. 2.
The relationship of percental mortality of the newborn mice employed for testing virus after from 2 to 12 passages in tissue culture and of the mice similarly employed for testing virus after from 14 to 24 passages.

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