and RNA synthesis.

1. Karnofsky, D. A., Ridgway, L. P., and Patterson, P. A., *Endocrinology,* 1951, v48, 696.
2. Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.,* 1945, v161, 83.
3. Wells, B. B., and Kendall, E. C., *Proc. Staff Meet. Mayo Clin.,* 1940, v15, 324.
4. Cavallero, C., Bertazzoli, C., Rossi, L., and Sala, G., *Lo Sperimentale,* 1951, v101, 210.
5. Baker, B. L., and Ingle, D. J., *Am. J. Anat.,* 1948, v82, 75.
6. Lowe, C. U., Williams, W. L., and Thomas, L., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 818.
7. Kass, E. H., and Kendrick, M. I., *Fed. Proc.,* 1952, v11, 472.

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Poliomyelitis and Coxsackie Viruses Isolated from Normal Infants in Egypt.* (19964)

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In Egypt, poliomyelitis is most unusual in native adults and has been rarely reported at any age. Yet, as Paul(1,2) has pointed out, the endemic paralytic attack rate for this disease in Egyptian infants is appreciable. Furthermore, a serological survey carried out on "normal" sera collected in Egypt in 1950, revealed that neutralizing antibodies to all three types of poliomyelitis virus are also acquired at an early age among native Egyptians. For type 2, more than 50% of infants have antibodies before they are 2 years of age, and for all 3 types 75 per cent or more before they are 4 years old. Complement fixing antibodies, which are transient in poliomyelitis, were also measured in this population(3), and the results supported the view that in Egypt infection with poliomyelitis virus must be widespread in the early years of life.

To extend these studies, 36 rectal swabs were collected in Egypt‡ from normal babies between 6 and 12 months of age, *i.e.,* at a time in life when the studies referred to above indicated maximum prevalence of infection. The purpose here was to determine how frequently one might detect poliomyelitis virus (and other viruses) in the intestinal tract of normal infants. The samples were collected by Dr. Robert Ward, while in Egypt carrying out a study of the occurrence of poliomyelitis virus in flies(4), and were generously supplied to us. The swabs were tested not only for poliomyelitis virus, but for Coxsackie (C) viruses as well, and both viruses were found.

Collection of specimens. Specimens were collected only from well children between 6 and 12 months of age, who were inhabitants of villages in the neighborhood of Cairo, representing an area under study by the International Health Division of the Rockefeller Foundation. It was in this same district that specimens for the serological studies mentioned above(2,3) had been collected. The swabs were placed in 1 ml of sterile water and frozen on dry ice. They were transported in the frozen state to New Haven where the laboratory studies were carried out. Sera from the children were also obtained, frozen soon after collection, and also transported on dry ice.

Methods. After thawing, the fluid contained in each swab was expressed by placing the swab in the barrel of a 1 ml syringe and forcefully squeezing it with the piston. The expressed fluid was added to the fluid in which the swab had been soaked, a total of about

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TABLE I. Isolation of Viruses from Egyptian Infants.

Pool	Poliomyelitis virus	Coxsackie virus	Tissue culture agents*
A	—	—	—
B	—	+	+†
C	—	+	—
D	+	+	—
E	—	—	—
F	—	+	+*
G	—	+	—
H	—	+	—
I	—	—	—
J	—	+	—
Total	1/10	7/10‡	2/10

* On passage in tissue culture this agent became markedly cytopathogenic for monkey testicular fibroblasts. It remained nonpathogenic *in vivo* for mice (infants and adults), hamsters, and monkeys, and has not yet been identified.

† This strain produced mild fibroblastic degeneration in monkey testicular cultures. Tissue culture fluids produced paralysis with myositis in infant mice typical of Coxsackie viruses. The strain was subsequently identified as belonging to the Boston type(8).

‡ The 7 positive pools contained 26 individual samples. When these were run separately, 9 yielded positive tests of C virus, 5 gave incomplete tests, and 12 were negative.

1 ml being available from each specimen. The fluids were centrifuged for 15 minutes at 18,000 rpm in the PR-1 refrigerated International centrifuge and the supernate saved. The 36 rectal specimens were made into pools, each containing material from 3 to 4 swabs. Before inoculation, a mixture of penicillin and streptomycin was added so that the inoculum contained 1000 units of each per ml. The pools were tested intracerebrally in monkeys for poliomyelitis virus and subcutaneously in 1-day-old mice for C viruses. In addition the specimens were tested in roller tube cultures of monkey testicular tissue according to the methods previously described(5). Sera from the same 36 infants were each tested, in infant mice for the presence of C viruses, and in both infant and young adult mice for the presence of encephalitis viruses. The latter tests were run in view of the previous isolation of 3 strains of West Nile virus from "normal" sera collected in this area in 1950(6).

Results. As shown in Table I, one strain of poliomyelitis virus, 9 of Coxsackie virus, and an unknown agent were isolated from these normal children.

Poliomyelitis virus as detected by monkey inoculation. Following intracerebral inoculation in monkeys, the 10 pools yielded one, D, which was positive for poliomyelitis virus. When tested for cytopathogenicity in roller tube cultures of monkey testicular tissue, this sample was negative.

Coxsackie viruses as detected by mouse inoculation. The 7 pools which were positive for C viruses produced paralysis in infant mice 3 to 9 days after inoculation. With several of the samples, all inoculated infant mice succumbed. Lesions were typical(7) and limited to the skeletal muscle. The individual components of the 10 pools were then rethawed and run separately. Some of the pools contained more than one positive specimen; the 7 positive pools were made up of a total of 26 samples, and of these, 9 samples were positive, 5 gave incomplete tests (all mice died without signs of paralysis having been observed), and 12 were negative. Seven of the Coxsackie strains were typed(8): 2 belonged to Israel-7 type, 2 to Texas-1, 1 to Texas-15, 1 to Boston, and 1 to Type 3.

Tissue culture studies. Of the 10 pools, two, B and F, produced cytopathic changes (fibroblastic degeneration) such as have been previously described for Coxsackie and poliomyelitis viruses, respectively, in monkey testicular tube cultures(5). The agents were passed serially through 5 tissue transfers including 15 changes of the fluid medium. Pools B and F had yielded positive tests for Coxsackie virus on direct inoculation into infant mice, and negative tests for poliomyelitis virus in monkeys. The members of the 2 pools were then run separately.

In monkeys, the 4 components of pool B and the 3 components of pool F each gave negative tests for poliomyelitis virus. In mice each pool yielded one sample positive for C virus.

The two agents isolated in tissue culture from pools B and F were tested in infant mice after 3 *in vitro* passages. The F agent was negative in 32 mice. On the other hand, the B agent produced paralysis in 33 mice, 18 of which had been inoculated intracerebrally and 15 subcutaneously. Mice developed signs of disease on the 5th or 6th day, and histological sections of 25 animals revealed typical

Coxsackie lesions in the muscles, with none present in the brain, spinal cord, or fat.§ After 2 passages in mice, the virus was titrated and yielded a LD_{50} of $10^{-6.0}$. It was identified as belonging to the Boston type of Coxsackie virus both by neutralization and complement fixation tests(8).

In tissue culture, the agent isolated from pool F produced definite fibroblastic degeneration by the 5th day which progressed to marked degeneration by the 9th day. It readily passed through an EK Seitz filter. Fluids harvested on the 5th and 9th days after inoculation of the culture tubes failed to produce disease or CNS lesions in 2 cynomolgus and 3 rhesus monkeys inoculated intracerebrally. The agent remained nonpathogenic for mice, both newborn and adult, and also for hamsters. Neutralization tests were carried out in tissue culture(5) using potent antisera to Types 1, 2, and 3 poliomyelitis virus, and none of the sera were able to inhibit the cytopathogenic effect of the agent. Obviously this filterable agent requires more study.

The sera of all 36 children failed to produce disease when tested individually by intracerebral inoculation into 4-week-old Swiss mice (8 mice for each serum) and by intracerebral and subcutaneous inoculation into newborn mice (8 to 16 mice for each serum), indicating that they were free of lethal agents such as West Nile and Coxsackie viruses.

Comment. These findings give additional support to the concept that poliomyelitis is an endemic disease of the tropics, and they supplement earlier studies(2,3) indicating that infection with poliomyelitis virus may occur at a relatively early age in Egypt. In addition to the previous serological evidence, we can now record the isolation, during a non-epidemic time, of poliomyelitis virus from the intestinal tract of a supposedly normal infant in the area, representing one of the 36 infants tested. This observation is similar to that of Gear *et al.*(9) who found 4 of 16 native infants in the Johannesburg area excreting virus at some period during the first year of life. As only one rectal swab was collected from each child in the present study, this single positive among the 10 pools is of significance. Further evidence for the widespread prevalence of poliomyelitis virus in the Cairo area is indicated by the ease with which Ward has been able to detect virus in flies trapped there (4).

Within the last year or so, C viruses have been isolated in the Middle East from patients (10), from Cairo sewage(11), and from flies (4). A variety of types have been identified from these sources(8). The present data indicate how common infection with these viruses may be in this area, which is similar to the experience of Measroch *et al.* who found that 5 of 9 native South African babies tested were excreting C viruses in their feces(12). Only one of their babies had an illness at the time of the collection of the specimens from which virus was isolated.

One of the C viruses described in the present study (pool B) is characterized by its cytopathogenic property in tissue culture. As few C viruses have this property, it is noteworthy that this one is antigenically related to the Boston type (Wieder strain) which has been found to produce cytopathic changes in roller tube cultures of human(13) and monkey tissues(5).

The isolation of a filterable agent, as yet unidentified, depended upon the use of the new tissue culture technic(13). Like similar agents recently isolated in the United States (5), the Egyptian agent is characterized by its cytopathogenicity for fibroblasts in monkey testicular cultures, and its avirulence for monkeys, mice, and hamsters. Antisera to the three poliomyelitis virus types had no inhibitory influence on it. The role of this agent in human disease is completely unknown. Somewhat similar agents cytopathogenic for human tissue cultures have also been isolated in Boston(13). The relationship of the Egyptian agent to American ones with pathogenicity similarly limited to tissue culture remains for further study.

Summary. Thirty-six rectal swabbings from normal Egyptian infants, tested singly or in pools representing material from 3 to 4 infants yielded 1 poliomyelitis virus, 9 Coxsackie (C) viruses, and 1 unidentified filterable agent.

§ We are indebted to Dr. E. Manuelides for preparing and examining these sections.

The unidentified agent was characterized by its cytopathogenicity for cultures of monkey testicular tissue and by its failure to produce disease in monkeys, mice, and hamsters.

1. Paul, J. R., Havens, W. P., and Van Rooyen, C. E., *Brit. Med. J.*, 1944, v1, 841.

2. Paul, J. R., Melnick, J. L., Barnett, V. H., and Goldblum, N., *Am. J. Hygiene*, 1952, v55, 402.

3. Goldblum, N., and Melnick, J. L., *J. Exp. Med.*, 1952, v96, 175.

4. Ward, R., *Fed. Proc.*, 1952, v11, 486.

5. Riordan, J. T., Ledinko, N., and Melnick, J. L., *Am. J. Hyg.*, 1952, v55, 339.

6. Melnick, J. L., Paul, J. R., Riordan, J. T., Goldblum, N., and Zabin, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 661.

7. Literature summarized by Melnick, J. L., and Curnen, E. C., in Viral and Rickettsial Diseases of Man, edited by T. M. Rivers, 2nd Edition, 1952. Lippincott, Philadelphia.

8. Contreras, G., Barnett, V. H., and Melnick, J. L., *J. Immunol.*, 1952, v69, 395.

9. Gear, J., Measroch, V., and Bradley, J., *So. African Med. J.*, 1951, v25, 297.

10. Bernkopf, H., Levine, S., and Olejnik, E., personal communication.

11. Mount, R., personal communication.

12. Measroch, V., Gear, J., and Faerber, G. I., *So. African Med. J.*, 1951, v25, 421.

13. Robbins, F. C., Enders, J. F., Weller, T. H., and Florentino, G. L., *Am. J. Hygiene*, 1951, v54, 286; also personal communication.

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Epidemic Influenza B and C in Navy Recruits during Winter of 1951-52. (19965)

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Recognition has recently been given to a strain of virus classified as "Influenza C" (1,2) but multiple isolations of strains of this type from a population during a single winter has not been previously reported.† Fourteen viruses, serologically related to earlier strains of influenza C, were isolated from Navy recruits between January and April of 1952. Evidence that these viruses were epidemic should be of interest to others concerned with the etiology and epidemiology of influenza during the past winter. Epidemic influenza B occurred simultaneously in this population. Eleven influenza B viruses were isolated.

Materials and methods. Observations on influenza have been made among recruits at the Naval Training Center, Great Lakes, during each winter since 1948. Continuing these observations during November, 1951, and June, 1952, throat swabs for virus isolations and acute and convalescent sera were routinely obtained from Navy recruits hospitalized because of acute respiratory infections including suspected cases of influenza and cases of pneumonia. Paired sera also were available from serial bleedings done at various intervals on 13 companies of recruits undergoing training between January and May. Acute and convalescent sera from patients experiencing

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† A few were identified at the University of Michigan during March 1952(5).