tus and *Streptococcus* Gr. A. (Difco) were found to be inactive in terms of fever, toxicity, and protection against infection. Fourth, numerous control experiments have demonstrated none of the endotoxic effects, indicating contamination by the diluent, syringes, needles, or glassware was highly improbable. Altogether, these observations strongly suggest that contamination by Gram-negative endotoxins can be excluded.

Several findings of other workers are pertinent to our work. Fruhman has reported mobilization of neutrophils in the peritoneal fluid of the rat and mouse following intraperitoneal injection of a *Staphylococcus* A lipopolysaccharide(11). The directed migration of polymorphonuclear leucocytes and mononuclear phagocytes has been found to reside in a polysaccharide fraction of staphylococci as well as other Gram-positive and Gram-negative organisms(12). A strain of *Alcaligenes* has produced precipitation in agar gel with staphylococcal rabbit antiserum and the erythrocyte sensitizing antigen common to *S. aureus* is apparently present in at least one *Pseudomonas* strain(13). Cross reactivity, then, may be a factor, as well as a peritoneal neutrophilia somewhat akin to the dual mechanism proposed by Rowley for stimulation of nonspecific immunity provoked by endotoxins(14).

Further studies are in progress to implement these preliminary findings and to clarify some of the mechanisms involved.

Summary. Pretreatment of mice by the intraperitoneal route with staphylococcal extracts significantly protects the host against a subsequent lethal challenge of *E. coli* and prolongs survival time of mice infected with *S. typhimurium.* The same extracts were also found to produce a biphasic fever and a leucopenic response in rabbits to which refractoriness was rapidly developed.

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Immunologic Identification of Coxsackie A21 Virus with Coe Virus.* (26534)

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By means of reciprocal neutralization tests, the recently described Coxsackie group A, type 21 virus prototype strain was found to be immunologically identical to the Coe virus, recovered from individuals with mild respiratory illness.

The Coe virus, described by Lennette *et al.* (1), was isolated from the throat washings of 4 military recruits with mild respiratory illness. Subsequently, isolations of the Coe virus were reported from patients with respiratory illness in Great Britain(2), the Nether-

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lands(3), and the Washington, D.C. area(4), as well as in a recent outbreak of respiratory disease in the same military installation from which the virus was originally isolated(5). The agent has been further implicated in the etiology of respiratory disease by the recent demonstration of Parsons *et al.*(6) that human volunteers inoculated with the virus developed illnesses resembling the common cold.

Pereira and Pereira(2) found the Coe virus to be ether resistant, and their studies, as well as recent investigations in this laboratory(7), have indicated that the size of the Coe virus approximates that of the enteroviruses. The cardinal characteristic of the agent which distinguishes it from most of the other members of the enterovirus group is its inability to produce a cytopathic effect in monkey kidney cell cultures; however, the agent produces degeneration similar to that of the enteroviruses in a variety of mammalian cells in continuous passage as well as primary cultures of human tissues(1,2).

Attempts were made in this laboratory to propagate all 4 of the originally described Coe virus strains, as well as a recently isolated strain, in suckling mice, and although the inoculated animals occasionally showed transitory illness, it was impossible to establish the virus in infant mice even through multiple, blind passages of brain and muscle material (1). This was also the experience of Pereira and Pereira(2) employing a strain of the virus isolated in Great Britain; not only was it impossible to propagate the virus strain in suckling mice, but these workers were unable to demonstrate virus in the tissues of inoculated mice which had displayed evidence of illness.

The prototype strain (Kuykendall strain) of Coxsackie group A, type 21 virus was also originally isolated in this laboratory. The isolation was made in tissue cultures of human embryonic skin and muscle, the virus being recovered from the stool of an individual with paralytic poliomyelitis whose stools also yielded a type 1 poliovirus. The fourth tissue culture passage material was inoculated into 12 suckling mice, 2 of which became moribund at 10 days. Brain and muscle tissue from these ill mice was passaged into additional suckling mice and, while the brain tissue produced no evidence of illness, 7 of the 12 mice inoculated with the muscle tissue suspension became ill at 8-9 days and the muscles showed gross lesions. Muscle tissue from these mice failed to produce symptoms in 2 litters of mice, but 2 additional attempts were made to infect mice with the same material, and both were successful. At the fourth passage level of infected mouse muscle, most of the inoculated mice became paralyzed at 4-6 days, and the strain was well established in suckling mice at the 5th passage, producing paralysis at 5 days after inoculation. The 7th mouse passage material was sent to the laboratory of Dr. Gilbert Dalldorf where the virus strain was designated as the prototype of Coxsackie group A, type 21 on the basis that in newborn mice it induced flaccid paralysis with marked myositis, but no lesions in the central nervous system(8). Interestingly, it was also found by Dr. Dalldorf's laboratory that the strain occasionally caused paralysis in 10-12 g mice, but could not be propagated in these older mice. The virus was shown to be non-cytopathogenic in monkey kidney cell cultures, but cytopathogenic in HeLa cell cultures and human amnion and uterine tissue cultures(8).

When the Coe virus was recently examined in neutralization tests against immune sera to the newly-recognized group A Coxsackie virus types 20-24, it was found that the virus was neutralized by immune serum to Coxsackie A21 virus. The results of cross-neutralization tests with the viruses and hamster immune sera to Coe and Coxsackie A21 (Kuykendall) strains are presented in Table I. In monolayer and colorimetric neutralization tests(1) both immune sera displayed essentially the same endpoints against both viruses, indicating that immunologically the 2 strains are very closely related, if not identical.

The demonstration of the immunologic identity of these 2 viruses further illustrates that the pathogeniciy of an enterovirus strain for suckling mice is not a valid criterion for placing the immunotype in the Coxsackie group. It is now well known that certain strains of ECHO virus types 9 and 10 (reo-

TABLE I. Results of Reciprocal Neutralization Tests with Coe and Coxsackie Group A, Type 21, Viruses and Immune Sera.

Test system	No. viral TCD_{50} in test	Neutralization titers of serum: Coe immune serum *vs* Coe virus	Coe immune serum *vs* Coxsackie A21 virus	Coxsackie A21 immune serum *vs* Coe virus	Coxsackie A21 immune serum *vs* Coxsackie A21 virus
Monolayer cell cultures in tubes	1000	1024*	—	128	—
	300	1024	512	128	128
	100	1024	1024	256	256
	30	1024	1024	512	512
	10	—	2048	—	512
Metabolic inhibition (colorimetric)	560	2048	—	256	—
	180	1024	2048	512	512
	56	4096	2048	512	1024
	18	≥4096	≥4096	512	1024

* Reciprocal of highest dilution of serum neutralizing test dose of virus.

virus) are pathogenic for infant mice, while other immunologically identical strains do not propagate in infant mice(9), and strains of Coxsackie viruses, particularly A9, have been recognized which are not pathogenic for suckling mice(10,11). In the case of Coe and Coxsackie A21 viruses, it would appear that the majority of strains of the immunotype thus far described lack pathogenicity for infant mice, and the Kuykendall strain may conceivably represent an aberrant strain of the Coe virus. (The pathogenicity of the Coxsackie A21 virus in young adult mice is much the same as that of Coe virus in suckling mice in that illness is occasionally induced, but the strain cannot be propagated.) Reclassification of the enteroviruses as numerical types on the basis of immunologic distinctness rather than biological properties (pathogenicity) should prevent further confusion in classification arising from differences in biological properties of various strains of an immunotype.

Of particular interest is the fact that an enteric virus immunotype, as represented by a strain possessing certain "Coxsackie" characteristics, has been implicated as the etiologic agent of respiratory illness with cold-like symptoms. This further extends the spectrum of clinical manifestations attributable to viral strains immunologically identical to "Coxsackie" viruses.

Summary. Cross neutralization tests in HeLa cell cultures, using both the monolayer and metabolic-inhibition technics, show the Coe virus, etiologically associated with respiratory disease, to be immunologically indistinguishable from the recently-described Coxsackie group A, type 21, virus.

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