TABLE I. Plasma Glucose (mg/100 ml)* After Treatment

	1 hr	3 hr	6 hr
(1) Untreated	170 ± 8	167 ± 10†	161 ± 8
(2) Propranolol	175 ± 10	200 ± 14†	185 ± 8
(3) Insulin (0.5) unit	60 ± 4	—	115 ± 11
(4) Propranolol + insulin (0.5) unit	46 ± 9	—	78 ± 24
(5) Insulin (1) unit	—	56 ± 12	115 ± 4
(6) Propranolol + insulin (1) unit	—	8 ± 2	all dead

* Values are means ± standard error of mean.
† Only 5 of the 10 rats were bled at this time interval.

glucose sank to 8 mg/100 ml at the 3-hour interval and shortly thereafter all animals exhibited typical hypoglycemic convulsions and died. These results are probably due to the inhibition by propranolol of the usual epinephrine-induced glycolytic reaction following insulin administration(4,5).

Summary. "Inderal" (Ayerst 64043, Imperial Chemical Industries 45,520, propranolol) an adrenergic beta receptor antagonist, acts synergistically with insulin in the rat to induce a hypoglycemia much more severe than that resulting from insulin alone. This synergism may possibly be of clinical significance.

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Exacerbation and Transformation of Allergic Encephalomyelitis by Pertussis Vaccine.* (31067)

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Injection of central nervous system (CNS) tissue in animals usually elicits a single attack of experimental allergic encephalomyelitis (EAE), but relapses occur spontaneously on occasion or can be induced by reinjection of CNS antigen(1,2). Demyelinating diseases like multiple sclerosis are characterized by relapses. To simulate more closely such a clinical course, exacerbations and relapses were induced in rats with EAE by injection of pertussis vaccine. For the first time relapses have been induced at will without repeating the primary immunization against neural antigen. Of especial interest was the observation that the primary attack and the exacerbation differed in histologic character and could be differentiated under the microscope.

Methods. EAE was induced in 75 female rats of the highly susceptible Lewis strain (Microbiological Associates, Inc.) by injection of an aqueous homogenate of 200 mg guinea pig spinal cord (without any adjuvant) into 5 pads on the sole of the right hind foot(3). All injections were given under light ether anesthesia. Thirty-seven of the rats developed clinical signs of EAE after 8-13 days (limp tail, weakness). Within 2 days after the onset of symptoms they were treated by one of the following: injection of pertussis vaccine concentrate (either unheated or heated to 80°C for 30 minutes), typhoid vaccine concentrate, or no treatment, according to the schedule and dose listed in

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TABLE I. Exacerbation of Signs of EAE After Pertussis Vaccine, with Conversion to Hyperacute Form.

Rat	Day of onset (limp tail, weakness)	Day of pertussis in dorsum of foot	Day of exacerbation (paralysis)	Histological type of EAE
1	9*	11	15	Hyperacute
2	9	11	14	"
3	10	11	14	"
4	12	14	19	"
5	12	14	24	Ordinary
6	—	20	24	Hyperacute
7	—	20	27	Ordinary
8	—	20	32	"
9	—	20	—	"
10	—	20	—	"

* Day of injection of guinea pig spinal cord in right foot pads counted as day 0.

Table II. The injections were made intradermally into the dorsum of the right (previously injected) foot or the left (previously uninjected) foot, or intravenously. Twenty days after the original injection of guinea pig cord homogenate, 38 rats had not yet developed EAE signs. At that time, they, also, were given one of the treatments in rotation. Random samples of 3 rats from the group with clinical signs and 5 rats from the group without signs were sacrificed at the times of the vaccine injections; all of these revealed the typical EAE lesions, perivascular mononuclear inflammatory infiltrates. Based on the clinical signs of EAE in 37 rats, and the uniform presence of histologic lesions in the samples of rats with or without signs, we assumed that all rats had EAE at the time of the provocative treatment. The animals were examined daily and were sacrificed when they became paralyzed. CNS was fixed in Bouin's solution. Histologic evaluation was based on paraffin sections of entire spinal cord and hind brain stained by hematoxylin-eosin and by phosphotungstic acid-hematoxylin (for fibrin).

Results. The most important result of this experiment was the development of an exacerbation of clinical signs within 2-4 days, fully developed within another 1-2 days, in 4 rats that received pertussis vaccine in the right foot shortly after onset of EAE signs, and in 1 rat without signs that received the same treatment (Table I, rats 1, 2, 3, 4, 6). In the 4 symptomatic animals, clinical disease which was characterized originally by a limp tail progressed rapidly to hind limb weakness and then to paralysis of hind limbs or all extremities. Two of these rats died within a day of development of paralysis. Such rapid progression did not occur in the untreated controls or in the other treated groups. Histologic examination of CNS revealed extremely severe and widespread perivascular inflammation. Unlike the ordinary form of EAE found in controls, the inflammatory exudate was characterized by an abundance of polymorphonuclear leukocytes and fibrin. These histologic features, and the rapid development of clinical signs with frequently lethal outcome, are typical of the hyperacute form of EAE(4). The special character of hyperacute EAE lesions made it possible to associate particular lesions with the exacerbation as opposed to the pre-existing ordinary EAE. In most cases, the two types of lesions were intermixed, but in 1 of these rats, the hyperacute exacerbation was limited to the cerebellar white matter while the spinal cord had ordinary EAE lesions.

Exacerbations or relapses of a different character followed injection of pertussis vaccine in the right foot in 3 additional rats of the group just described (Table I, rats 5, 7, 8). These instances did not start until 5 to 8 days after pertussis injection; they progressed more slowly, and, most important, histologic examination revealed only lesions of ordinary EAE, with none of hyperacute character. Therefore, the evaluation of these events depended on clinical criteria only. Furthermore, their provocation was not specific since simi-

TABLE II. Exacerbation and Conversion of EAE to Hyperacute Form.

Vaccine	Dose*	Route	Conversion†
Pertussis	1×10^{10}	R. foot	5/10‡
Heated pert.	1×10^{10}	" "	0/9
Typhoid	1×10^{10}	" "	0/9
Pertussis	1×10^{10}	L. foot	0/9
"	4×10^{10}	" "	1/10
"	4×10^{10}	I.V.	1/10
None	—	—	0/10

* No. of organisms injected after onset of signs of ordinary EAE or on 20th day.

† Numerator, No. of exacerbations and relapses, histologically of hyperacute type; denominator, total No. of rats.

‡ Results in individual rats of this group are detailed in Table I.

lar occurrences were noted in 1 rat after heated pertussis vaccine, in 4 rats after typhoid vaccine, in 2 rats after pertussis vaccine in left foot, in 5 rats after large doses of pertussis vaccine in left foot, and in 3 rats after intravenous pertussis vaccine.

Among these additional groups, actual transformation to hyperacute EAE did occur, but it was rare and was restricted to the groups that received unheated pertussis vaccine. Relapse with transformation to hyperacute EAE occurred in only 1 rat 15 days after a large dose of pertussis vaccine in left foot, and in only 1 rat 12 days after intravenous pertussis vaccine, and in none of the other groups (Table II).

Discussion. Hyperacute EAE has been produced previously by the injection of CNS antigen mixed with pertussis vaccine as adjuvant(4). This is the first time that hyperacute EAE has been produced by conversion from clinically established ordinary EAE. It is of particular interest that the transformation was accomplished by pertussis vaccine alone, without additional CNS antigen. However, conversions occurred in higher incidence, with shorter incubation period and required less pertussis vaccine when the provocative injection was given in the limb originally injected with guinea pig cord than if it was given elsewhere. These results may be understood if conversion is brought about by utilization of residual CNS antigen in response to the injection of pertussis vaccine. Or, the pertussis vaccine may induce a release of lymphoid cells(5), previously immunized, from the originally injected limb. These results are not explicable as a direct effect of pertussis vaccine on the CNS.

The induction of exacerbations that differ in histologic character from the original attack appears to be unique. It permits the association of particular lesions with the exacerbation as opposed to pre-existing ordinary EAE from the primary attack. An analogous situation occurs occasionally spontaneously, both in EAE in monkeys(2), and in human disease when lesions of acute necrotizing hemorrhagic encephalopathy (analogous to hyperacute EAE) coexist with those of acute disseminated encephalomyelitis (analogous to ordinary EAE)(6). The presence of lesions of varying age in different parts of the nervous system is also a feature of human multiple sclerosis.

This report demonstrates an increasing ability to control the character and course of experimental autoimmune disease of the nervous system. It offers hope that laboratory models can be developed to simulate not only the morphology but also the natural history of human demyelinating disease. Further investigation of the mechanism of pertussis-induced exacerbation and transformation will contribute to the understanding of spontaneous relapses in EAE and in human disease.

Summary. Relapses and exacerbations were induced in the course of allergic encephalomyelitis (EAE) by injection of pertussis vaccine without repeating the primary immunization against neural antigen. Lesions of the relapse differed from those of the primary attack in histologic character and could be identified microscopically.

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Purification and Biophysical Properties of Rhinoviruses.* (31068)

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Rhinoviruses have been established recently as a subgroup of the picornaviruses(1,2). New serotypes continue to be recognized(3). Rhinoviruses contain RNA and protein and display cubic symmetry of the icosahedral pattern(4). They are approximately 18-23 mμ in diameter, ether stable and acid labile. Their low titer, lability, and specialized growth requirements(2,5) have not encouraged extensive study by routine biophysical procedures. This report describes methods that we have developed for obtaining partially purified preparations of human rhinoviruses without significant losses in titer and presents preliminary biophysical data obtained with these agents.

Materials and methods. Virus. Two rhinovirus strains, B 632 and Baylor 1, were used in this study. Strain B 632, one of the original Salisbury strains, was isolated from a person with a common cold(6). The virus was passed twice in rhesus monkey kidney cells and 6 times in KB cells before use. The Baylor 1 type, strain Tippett, was isolated in human aorta cells from a patient with a common cold in Houston, Texas(7). The virus was passed 4 times in aorta cells and 10 times in human lung fibroblast cells (WI-38) before use.

Cell lines, virus growth and assay. The WI-38 cells were cultivated in roller tubes, the KB cells both in roller tubes and spinner culture. Both cell lines were grown in Eagle's medium containing 10% calf serum and 0.075 g% sodium bicarbonate. The cultures were maintained in Eagle's medium containing 2% fetal bovine serum and 0.113 g% sodium bicarbonate.

Virus was grown primarily in WI-38 roller tubes at 33°C in maintenance medium. The titer of the B 632 strain was boosted from 10^5 $TCID_{50}$/ml to $10^{7.5}$/ml by 3 passages in KB cells. An increase in titer was not achieved by similar treatment of the Baylor 1 strain which maintained an average titer of $10^{4.5}$/ml. Viruses were harvested by a single cycle of freezing and thawing when cytopathic effects (CPE) were judged to be at a maximum, usually within 48 hours after inoculation.

Titrations were carried out in roller tubes at 33°C. KB cells were used for the B 632 strain, WI-38 for the Baylor 1 strain. Fifty per cent endpoints were determined.

Purification and concentration. Crude virus harvests were clarified by low speed centrifugation in an International clinical centrifuge for 5 to 10 minutes. After extraction with an equal volume of fluorocarbon (Genetron 113) the preparations were concentrated by dialysis against carbowax (polyethyleneglycol compound, 20 M) or by sedimentation in the Spinco model L ultracentrifuge for 3 hours at 105,000 × *g*. The pellets produced by ultracentrifugation were resuspended either by adding 1 ml of physiological saline and freezing and thawing (Method I, Table I), or by soaking overnight at 4°C in a 1 ml volume of physiological saline (Method III, Table I). Both methods were followed subsequently by mechanical agitation. The suspensions were layered over preformed gradients, prepared by layering 6 CsCl solutions varying in density from 1.46 to 1.25 g/cm^3, and

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