Human Rabies Immune Globulin

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APPROXIMATELY 8,000 persons in the United States receive antirabies serum of equine origin (ARS) each year. This passive immunization provides rabies antibody immediately as well as apparent protection from infection for 12 to 14 days. After that, antibody from active immunization is usually present.

Unfortunately, approximately 16 percent of the persons who receive the equine-origin antirabies serum develop serum sickness; for persons over 15 years of age, this incidence increases to 46 percent (*1*). Most authorities have agreed that to prevent these reactions a human-origin rabies immune globulin (HRIG) was needed. This globulin should be not only safer but also as potent as the ARS now available.

The Rabies Unit at the National Communicable Disease Center, Public Health Service, has coordinated efforts to develop such a globulin for experimental use and to determine whether it is feasible to produce and use such a product in the field. This report summarizes the progress in four areas of this program.

1. Collection and fractionation of plasma
2. Potency and animal protection tests
3. Testing in man
4. Anticipating problems that might arise in subsequent development of the globulin.

Collection and Fractionation of Plasma

Donors, mostly veterinarians, had previously received rabies vaccine. Before donating either a single or double unit of blood, each person received a booster injection of the duck embryo origin rabies vaccine (DEV). The American National Red Cross collected and stored the plasma until it was fractionated; the Medical Laboratory Section, National Communicable Disease Center, conducted the fractionation procedures.

A sample of serum from each donor was tested for rabies serum neutralizing (SN) antibody. For the first three lots of experimental globulin, the plasma of donors was accepted if their SN antibody titers were 1:100 or greater. In the fourth—the most recent—lot, the titer of each donor's serum had to be at least 1:400 to be included for fractionation by the cold ethanol technique (*2*, *3*). The final globulin was reconstituted to 16 percent in 0.3 molar glycine. A titer increase of approximately twentyfold has occurred as a result of fractionating each 250 ml. quantity of plasma to its concentrated 5 ml. of gamma globulin (see box).

A total of 400 ml. of human-origin rabies immune globulin was prepared for the first series of studies to determine its potency and protective value as measured in animals. An additional 2,500 ml. lot of HRIG is now available for studies in human beings.

Potency and Animal Protection Tests

The first lots of human-origin rabies immune globulin used in animal protection tests contained between 33 and 62 international units per ml. The lot prepared during the last 6 months

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This paper was presented at the 96th annual meeting of the American Public Health Association, Detroit, Mich., November 13, 1968.

for use in human beings contained 165 international units per ml. The current potency requirement for antirabies serum is that it contain at least 100 units per ml. The potency values of four lots of HRIG prepared at the National Communicable Disease Center and two lots of commercial antirabies serum are presented in table 1.

The ability of the human-origin immune globulin to protect rabies-challenged animals was tested in comparison with antirabies serum in mice, guinea pigs, and dogs. Although these first lots of HRIG contained 0.2 to 0.5 times as many units as the ARS, there was no significant difference in their capacity to protect the 103 challenged animals (table 2).

These first results therefore indicated that HRIG, which was tested as a heterologous globulin, was as effective in preventing rabies in various species of animals as the antirabies serum. When homologous and heterologous antirabies serums were compared in guinea pigs,

Table 1. Potency value of human-origin rabies immune globulin, equine-origin antirabies serum, and National Institutes of Health reference serum

Test material	Plasma or serum pool		Globulin or serum concentrate		Concentration factor
	Titer [1]	Antibody units per ml.	Titer [1]	Antibody units per ml.	
Human-origin rabies immune globulin:					
Lot 1	1:625	4.5	1:5,900	42	10
Lot 2	1:800	5.7	1:8,700	62	11
Lot 3	1:350	2.5	1:4,700	33	16
Lot 4	1:1,400	5.8	1:29,000	166	21
Equine-origin antirabies serum:					
Lot 1			1:19,000	136	
Lot 2			1:23,400	167	
Reference serum:					
Lot 1B	1:280	2.0			
Lot 2	1:350	2.0			

[1] Dilution of material protecting 50 percent of the mice.

Table 2. Rabies mortality in three species receiving human or equine antibody treatment after challenge

Species	Dosage volume (cc.) [1]	Human-origin rabies immune globulin			Antirabies horse serum concentrate			Deaths of controls
		Lot No.	Antibody units per dose	Deaths	Lot No.	Antibody units per dose	Deaths	
Mice	0.5	1, 2	25	3 of 50	1	67.9	0 of 43	24 of 25.[2]
Guinea pigs	2.0	1, 2	100	1 of 33	1	271.6	1 of 40	20 of 20.[3]
Dogs	[4] 5	3	[4] 16.3	3 of 20	2	[4] 83.6	1 of 20	10 or 14.[5]
Total				7 of 103			2 of 103	54 of 59.

[1] Inoculated in left rear leg. Mice and guinea pigs, 1 hour or 24 hours after challenge; dogs, 24 hours after challenge.

[2] 630 mouse intracerebral LD_{50} (MIC LD_{50}) or 10 mouse intramuscular LD_{50} (MIM LD_{50}) in the right rear leg, 21-day observation. The 50 percent effective dose (ED_{50}) = the amount of brain tissue that will protect 50 percent of the mice against a subsequent challenge with rabies virus.

[3] 10,000 MIC LD_{50} or 63 guinea pig intramuscular LD_{50} (GPIM LD_{50}) in the right rear leg, 21-day observation.

[4] Per kg.

[5] 200,000 MIC LD_{50} in the right rear leg, 180-day observation.

Gamma Globulin Yield, Human-Origin Rabies Immune Globulin, Using Cohn Fractionation Technique

4 units of blood yield 1,000 ml. plasma = 750.0 ml. serum

750.0 ml. serum contain 0.6 percent gamma globulin = 4.5 gms. gamma globulin

4.5 gms. gamma globulin estimated 75 percent recovery = 3.4 gms. gamma globulin

3.4 gms. gamma globulin reconstituted to 16 percent = 21.0 ml. of final product or approximately 5 ml. gamma globulin per unit of blood

there was a strong suggestion that fewer units of homologous than of heterologous antiserum would protect guinea pigs challenged with rabies virus (table 3).

Similarly, homologous antirabies serum protected dogs and mice after challenge at least as effectively as the heterologous serum (*4*). However, in dogs a major interference problem developed between passive and active immunization when the dose of human antiserum was used in conjunction with duck embryo origin rabies vaccine. This problem pointed up the need for more studies in other animals to determine degrees of interference that develop when various doses of antiserum, both homologous and heterologous, were used.

Various regimens were tested to see which might overcome the interference. Results of these studies showed three conditions.

1. Heterologous antibody titers decreased by 50 percent every 1.5 days while homologous antibody titers decreased by 50 percent every 7 days, lasting five times longer.

2. When homologous antiserum was followed by DEV, response to the vaccine was suppressed longer than when heterologous antiserum and vaccine were administered. The response was delayed until the antibody titer decreased to 1:20 or lower.

3. Interference was greatly diminished or overcome when the dose of antiserum was reduced from 100 units to 25 units or when a more potent vaccine was used. When the suckling

Table 3. Rabies mortality in challenged guinea pigs, by type and dosage of antirabies serum

Type of serum	Antibody units per dose	Number tested	Number developing rabies
Experiment 1—2.0 ml. dose:[1]			
Controls		[2] 9	8
Antirabies guinea pig serum	30.8	9	0
	3.08	9	0
	.308	9	4
	$ED_{50} \leq .308$ units		
Antirabies burro serum	322.0	9	1
	32.2	9	3
	3.22	8	3
	$ED_{50} = 5.8$ units		
Antirabies horse serum concentrate lot 1	272.0	9	3
	27.2	9	6
	2.72	9	5
	$ED_{50} = 39.4$ units		
Experiment 2—0.2 ml. dose:[1]			
Controls		[3] 9	7
Antirabies guinea pig serum	3.08	9	0
	.308	9	3
	$ED_{50} = \leq .308$ units		
Antirabies burro serum	32.2	9	5
	3.22	9	3
	$ED_{50} = 6.42$ units		

[1] 0.2 ml. or 2.0 ml. of the appropriate dose of serum or serum concentrate in the left rear leg 24 hours after challenge.

[2] 8,300 MIC LD_{50} in the right rear leg, 30-day observation.

[3] 8,300 MIC LD_{50} in the right rear leg, 40-day observation.

mouse brain vaccine was used, the response to vaccine was apparent in the presence of a passive titer of 1:120.

Testing in Man

The studies described marked the completion of the previously planned animal protection studies with human-origin rabies immune globulin and homologous or heterologous antiserum. The next step was to test the HRIG in man.

Although no human beings had received HRIG, the Rabies Control Unit had done much of the work leading to the testing in man. The major task was to prepare enough globulin. The 2,500 ml. of HRIG that had been collected, fractionated, and tested in the laboratory was of excellent quality and sufficient quantity to complete the two phases of studies planned in human volunteers in the United States during 1969.

In the meantime, the Rabies Control Unit registered an application for investigation of new drugs with the Division of Biologics Standards, National Institutes of Health, Public Health Service. Further, the NCDC Epidemiology Program assigned two physicians to direct testing of HRIG in human beings.

The first study, initiated in June 1969, was to determine the antibody decay rate of the HRIG. Twenty persons from the Atlanta, Ga., area received the globulin without any unusual reactions, and serum samples from these persons are now being tested for rabies antibody. The protocol for this study was developed and reviewed by several physicians and other rabies experts to allow intelligent progress to the next phase of human testing.

The second phase of testing will encompass a similar group of volunteers who will receive both the HRIG and rabies vaccine to determine whether interference between passive and active immunization is observed. There is every reason to believe that HRIG will provide as high initial antibody levels as that produced by the equine-origin antirabies serum. However, this high antibody titer is expected to last longer, and it might produce interference with active immunization. Only when a regimen has been developed which will satisfactorily overcome interference will work on development of HRIG be considered finished.

Anticipated Problems

Collecting, fractionating, and testing lots of human-origin rabies immune globulin have thus far required considerable time and effort. However, much has been learned that can be applied in the future, and the feasibility of producing HRIG for practical use has been demonstrated.

HRIG is expected to be available on a limited basis from a few commercial laboratories in the reasonably near future. Two major problems remain to be overcome.

1. A supply of donors whose antibody titer is at least 1:400 must be obtained.
2. The cost of providing such a specialized globulin to people who are exposed is very high.

The following analysis was done to determine the number of donors required to supply sufficient HRIG for the 8,000 people in the United States expected to need antirabies serum each year.

A total of approximately 240,000 ml. of globulin would be required if each ml. contained 100 units and if the average weight of persons treated was 120 pounds. Each person would require 1,000 units per 40 pounds body weight. Since 10 ml. of globulin could be obtained by plasmapheresis from each donor at least every 2 weeks, one donor could provide 260 ml. of globulin per year. Thus 920 donors would be required to provide the amount used in the United States. If the HRIG contained as high a titer as that developed at the National Communicable Disease Center, only 552 donors would be needed.

Approximately 90 percent of the plasma used in the HRIG development program was donated by veterinarians in single or double units of blood and collected without charge by the American National Red Cross. The remaining 10 percent was obtained from various persons in the El Paso, Tex., and Atlanta, Ga., areas. Each person yielded 2 pints of blood per bleeding by the plasmapheresis method; each was paid $25 per double unit.

If HRIG is to be produced by independent laboratories which use paid donors, the cost of sufficient plasma to be fractionated for treating one man weighing 160 pounds would be $100. It is possible, however, that plasma can be obtained at a much cheaper rate, but the cost of fractionating and testing to assure its quality

will undoubtedly increase the cost of a human antirabies treatment to at least 10 times the present amount.

Summary

A total of 2,500 ml. of rabies immune globulin of human origin (HRIG) has been produced by the Rabies Unit of the National Communicable Disease Center, Public Health Service, and is now being tested in human beings. This globulin has passed all safety tests, and it contains 165 international units per ml., which is equal to the potency of antirabies serum of equine origin (ARS) now prescribed in the United States for persons exposed to rabid animals.

Human rabies immune globulin gave animals challenged with rabies virus as much protection as ARS. The next step is to develop for human use a satisfactory regimen of HRIG in conjunction with rabies vaccine. Being a homologous globulin, this HRIG should preclude serum sickness in exposed persons who are sensitive to equine serum.

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Education Notes

Doctoral Study in Social Work and Social Science. The University of Michigan offers an interdepartmental program which combines social work with economics, political science, psychology, or sociology and leads to a doctor of philosophy degree. Social psychology may be chosen as a field of concentration within psychology or sociology.

The program is designed to prepare students for careers in research, teaching, policy development, and administrative positions in the social welfare field. Candidates for a master's degree in social work in 1969–70, experienced social workers, holders of a master's degree in a social science, or persons with a bachelor's degree only may apply.

Support for this advanced study is available from several sources, including the Children's Bureau and the Public Health Service. Stipends range from $1,600 to $3,400 plus tuition and dependency allowances.

Fellowship applications and applications for admission only will be received up to January 13, 1970.

For detailed information and application forms write to Doctoral Program in Social Work and Social Science, University of Michigan School of Social Work, 1065 Frieze Building, Ann Arbor, Mich. 48104.

Hospital and Health Care Administration. Saint Louis (Mo.) University has redesigned its graduate program in hospital administration and broadened its name. The new title, hospital and health care administration, effective September 1, reflects the increased scope of the program's academic content.

In addition, the academic-residency sequence has been altered to consist of two academic semesters, followed by an 8-month residency, and concluding with a third academic semester.

Courses in health care economics and community health and medical care have been added. The new curriculum will also give additional emphasis to hospital and health care planning, systems analysis and design in health care, and health care research.

Miniature Pigs Bred for Research

A strain of miniature pigs for use in research has been bred at the University of Minnesota under the sponsorship of the Hormel Foundation. Compared with an average pig, weighing from 500 to 1,000 pounds, the adult "mini pig" weighs 100 to 200 pounds.

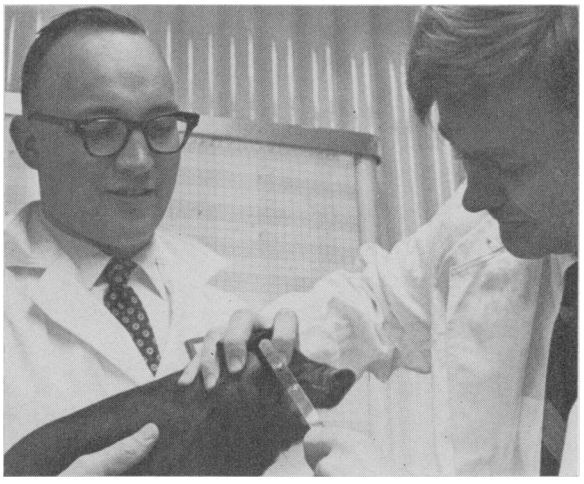
The pig's cardiovascular system, skin, blood vessels of the retina, gastrointestional tract, and dental structure are similar to man's. Like human beings, pigs suffer from atherosclerosis, gastric ulcers, rheumatoid arthritis, nutritional deficiences, influenza, and trichinosis. However, the pig has not been an ideal laboratory animal because of his ungainly bulk and pigly habits.

In addition to size, another drawback of pigs has been lack of clinical data. Pigs have been known to live from 15 to 16 years on a farm, but their lifespan in a controlled environment is not known.

The clinical and other data on the pigs is being sought in a research project at the University of Missouri School of Veterinary Medicine. In the project, a study group of 200 pigs has been divided into three groups. Individual animals in one group will be sacrificed periodically and necropsied. A second group will provide blood, fecal, and urine samples throughout their lifespan. The third group will live out their full lives with as little stress as possible. The animals are housed at the university's Sinclair Comparative Research Farm for the Study of Chronic Diseases and Aging in Columbia, Mo.

Records are kept on each animal, and he is ear notched and tattooed for identification. From physiological, anatomical, radiographic, microbiological, and parasitological observations made at regular intervals upon meaningful numbers of animals, biological profiles from birth through old age are being developed.

A team of researchers is working on this project. Dr. Richard B. Wescott, associate professor of veterinary microbiology and of medical microbiology, is the principal investigator and project coordinator. Wescott, Dr. Myron Tumbleson, and Dr. Charles Middleton have established and will maintain the mini-pig population. Taking care of physiological aspects are Tumbleson, Dr. Margaret Flynn, and Dr. Saul Larks. Electrocardiograms of all the miniature swine, including fetal electrocardiograms of pregnant sows and newborn pigs, are being recorded. All growth changes, blood, fecal, and urine analyses are also being noted.



Two pathologists, Middleton and Dr. Lawrence Morehouse, are determining the normal gross and microscopic anatomy of the pigs. For more detailed study, tissues are being carefully preserved with formaldehyde in plastic bags for future research aims.

The normal skeletal development of the pigs is being determined by Dr. E. Allen Corley through periodic radiographic examinations. Wescott and Dr. A. Roland Dommert are studying the microbiology and parasitology of the animals' gastrointestinal tract.

The project, now in its second year, is aided by a 5-year grant from the Public Health Service. The yearly allotment is $102,000. To achieve the full research goals of the project will require about 15 years, depending on the actual lifespan of the miniature pig.