MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

JANUARY 23, 1969

Clinical Usage of Immune Serum Globulin (Gamma Globulin)

TABLE I. General Features of the Immunoglobulins

	IgG	IgA	IgM	IgD	IgE
Physicochemical Characteristics					
Molecular weight	150,000	150,000+	900,000	150,000	? 200,000
Ultracentrifugation	6.65	6.6 (10, 13,17)	18 (24,32)	7	8.0
Electrophoretic mobil.	γ	Slow β	Between γ and β	Between γ and β	Between γ and β
Normal serum level ($\bar{x}$, mg/ml)	12.4	3.9	1.2	0.03	?
Serum half-life (days)	23	6	5	2.5	?
Distribution in serum (%)	44	40	70	73	?
Immunological Characteristics					
First detectable antibody	+	-	-	-	-
Major part of 2° response	+	-	-	-	-
Binds complement	+	-	+	-	-
Arthus reaction	+	-	+	-	-
Agglutinating activity	"1"	Variable	"10-1000"	-	-
Opsonization	"500-1000"	-	"1"	-	-
Toxin neutralization	"100"	-	"1"	-	-
Reaginic activity	-	-	-	-	+

IgM is the first serum antibody to appear in response to a given antigen. In most systems studied its production is transient and gives way within days or weeks to γG antibody. Experimental studies and observations on patients with selective immunological deficiency states suggest that γG antibody is principally responsible for the control of infection and protection against reinfection (Table II). Secretory γA has been demonstrated in the epithelium of the respiratory tract, gut and urinary tract and is known to be a first line of defense against certain respiratory infections in normal people; it is noteworthy that people with congenital absence of γA have a γG response to similar antigenic stimuli and do not suffer increased duration or frequency of infection.

No biological function has been ascribed to IgD as yet. It now seems clear that γE is the reaginic antibody.

TABLE II. The Relation of Immunoglobulin Deficiency Patterns to Susceptibility to Infection and Ability to Produce Antibody

	γG	γA	γM	Infection Rate	Response to Antigenic Stimuli
1	↓	↓	↓	↑	0
2	N	↓	N	Nl	+
3	↓	↓	N,↑	↑	+
4	↓	N	N	↑	+
5	N	↓	↓	↑	0

Pattern 1 is expected in congenital and Swiss types and is rarely seen in "acquired, adult onset" forms. Pattern 2 is seen in otherwise normal people and is of interest from the genetic standpoint only. Patterns 3 and 4 account for most of the acquired forms, with 5 being rare. It is noteworthy that patients with normal γM and decreased γG are susceptible to infection although they form macroglobulin antibody; γG antibody seems to be required for protection.

Physicochemical Characteristics of Immune Serum Globulin (Gamma Globulin, Normal Gamma Globulin, Normal Human Immunoglobulin)

Immune serum globulin is prepared from large pools of plasma (1000 or more donors) collected from placental and on venous blood. Separation by the cold alcohol fractionation of Cohn is deemed most desirable, although ether fractionation and ammonium sulfate precipitation have been employed. The finished product contains 16.5 gm.% protein which is principally IgG; other immunoglobulins are virtually absent. Antibody content is checked against two bacterial and two viral antigens and each lot must demonstrate at least a 10-fold increase in antibody content over the pool from which it was produced (9).

Quantitative utilization of the product is shown in Figure 1.

Use of Gamma Globulin in Viral Hepatitis

Magnitude of the Problem: (See Figure 2)

TABLE III. Reported Cases of Hepatitis in 3 Epidemiologic Years

	1965-66		1966-67		1967-68	
	Cases	Rate cs/100 T Pop.	Cases	Rate	Cases	Rate
United States	33,076	17.0	37,256	18.9	44,261	22.3
Texas	1,828	17.2	2,459	22.8	2,467	22.6

FIGURE 1

IMMUNE SERUM GLOBULINS (HUMAN) – UNITED STATES, 1965-1966
(net distribution by month)

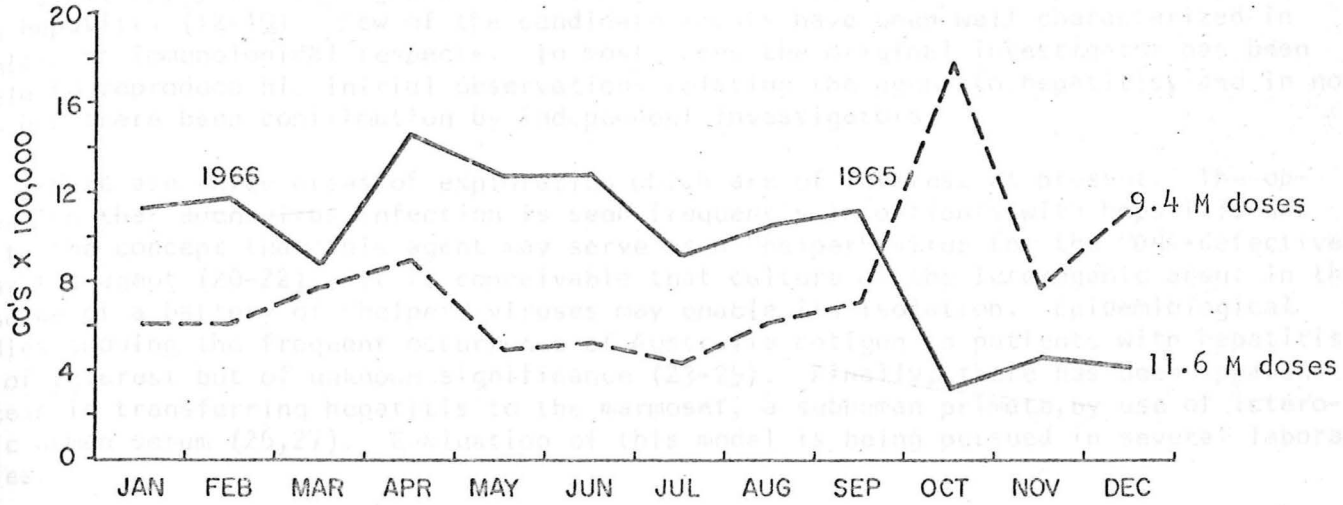
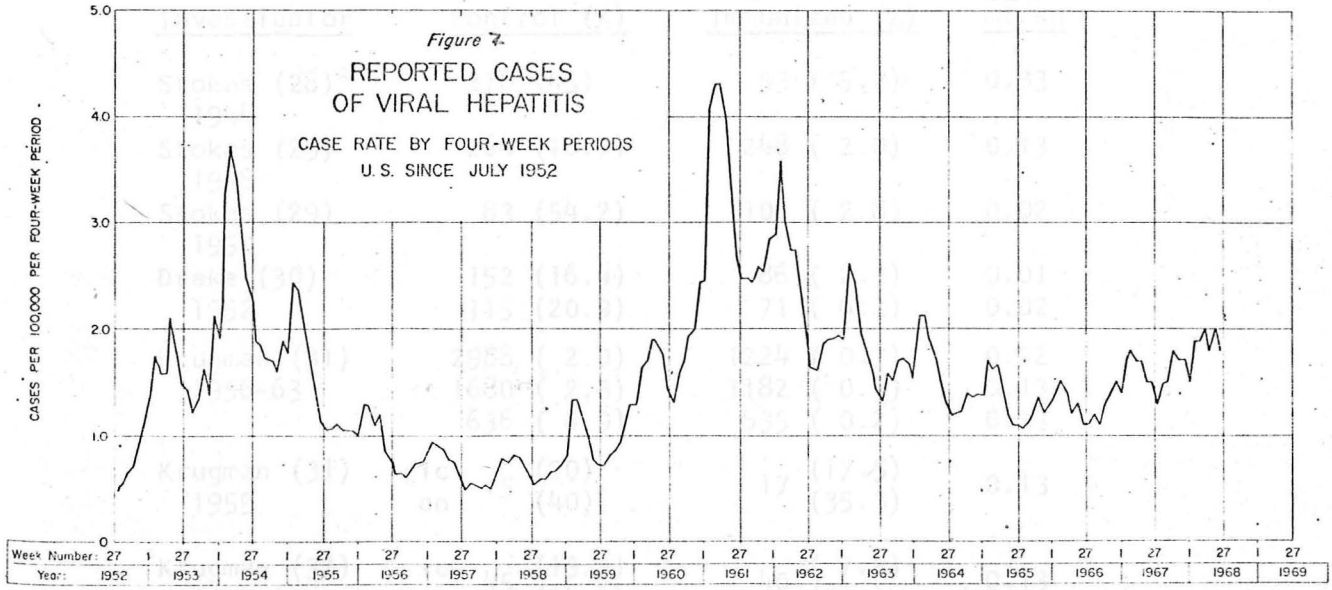


Figure 7.

REPORTED CASES
OF VIRAL HEPATITIS

CASE RATE BY FOUR-WEEK PERIODS
U.S. SINCE JULY 1952



Due to reluctance to report cases to health authorities and failure to recognize anicteric cases, these figures serve only as an index of relative frequency of the disease. True incidence cannot reliably be assessed and the morbidity is incalculable.

Status of the Vaccine

Since 1956, several agents have been isolated from serum or excreta of patients with hepatitis (12-19). Few of the candidate agents have been well characterized in chemical or immunological respects. In most cases the original investigator has been unable to reproduce his initial observations relating the agent to hepatitis, and in no case has there been confirmation by independent investigators.

There are three areas of exploration which are of interest at present. The observation that adenovirus infection is seen frequently in patients with hepatitis has led to the concept that this agent may serve as a "helper" virus for the "DNA-defective" hepatitis agent (20-22). It is conceivable that culture of the icterogenic agent in the presence of a battery of "helper" viruses may enable its isolation. Epidemiological studies showing the frequent occurrence of Australia antigen in patients with hepatitis are of interest but of unknown significance (23-25). Finally, there has been apparent success in transferring hepatitis to the marmoset, a subhuman primate, by use of icterogenic human serum (26,27). Evaluation of this model is being pursued in several laboratories.

Efficacy of Immune Serum Globulin in Infectious Hepatitis

TABLE IV. Efficacy in Prophylaxis Against Hepatitis in Institutional Situation

Investigator	Attack Rate, No. Control (%)	Attack Rate, No. Immunized (%)	Dose ml/kg
Stokes (28)* 1944	278 (45)	53 (5.7)	0.33
Stokes (29) 1948	264 (16.7)	248 (2.0)	0.13
Stokes (29) 1950	83 (54.2)	106 (2.8)	0.02
Drake (30) 1952	152 (16.4) 115 (20.9)	86 (2.3) 71 (4.2)	0.01 0.02
Krugman (31) 1956-63	2988 (2.0) 1680 (2.3) 636 (4.9)	1224 (0.7) 1182 (0.3) 635 (0.2)	0.02 0.13 0.13
Krugman (31) 1959	ic 15 (20) an (40)	17 (17.5) (35.3)	0.13
Krugman (32) 1959-60	ic 45 (13.3) an (24.4)	40 (7.5) (30)	0.13

* Water-borne outbreak

TABLE V. Efficacy in Prophylaxis Against Hepatitis in Household Contacts

Investigator	Attack Rate				Dose GG ml/kg
	Control		Immunized		
	No.	%	No.	%	
Ashley (33) (1952-53)	708	15.8	268	2.2	0.02
Gelperin (34) (1952-53)	475	7.8	837	1.1	Not given
Clark (35) (1955-56)	2109	15.2	2118	2.3	Not given
Mosley (36) (1960-61)	133	31.6	1577	0.8	0.02
Mosley (37) (1966)	250	6.4	236*	3.4	0.02
			238‡	0.8	

* Globulin Lot A
‡ Globulin Lot B

TABLE VI. Comparison of 2 Immune Globulins for Prophylaxis of Infectious Hepatitis (37)

Viral Antigen	Globulin A	Globulin B	Ratio B/A
Poliovirus type 1	370	4,200	11.4
Poliovirus type 2	490	6,400	13.1
Poliovirus type 3	290	4,100	14.2
Coxsackie B3 virus	< 20	69	> 3.4
Measles virus	71	260	3.7
Herpesvirus hominis	470	750	1.6

Transfusion-Associated Hepatitis

Defined as hepatitis developing within 15-180 days after transfusion of blood or blood products. In general, disease developing after 60 days' incubation is presumed to be due to serum hepatitis (SH) virus and that developing with a shorter incubation period to be due to infectious hepatitis (IH) virus. On the basis of those criteria, the IH virus accounts for 40 to 50% of transfusion-associated hepatitis (40-42).

Magnitude of the Problem

Estimates of the incidence of post-transfusion hepatitis vary a hundredfold, ranging from 0.6 cases per 1000 units to around 60 cases per 1000 units.

TABLE VII. Incidence of Post-Transfusion Hepatitis

Investigator	No. Units	Attack Rate Cs/1000 Units
Allen (42)	6,440	7.3
Cohen (43)	2,056	7.8
Mirick (44)	2,950	9.0
Grady (45)	303,351	0.6
Kunin (46)	1.3 M	$\bar{x}$ = 2.1
Collected series US 1943-57		
Shimizu (47)	1,907	60.0*
Hampers (48)	115	87‡

* 88% cases were anicteric

‡ All cases anicteric

Other than the variation in diagnostic criteria, the factors known to influence attack rate are donor source, the epidemicity of hepatitis in the community at large, and the amount of blood received by each patient.

TABLE VIII. Frequency of Transfusion in Three Series of Transfusion-Associated Cases (41)

Units Received	Allen (42) %	Grady (45) %	Surveillance Data (30 States) %
1	15.6	10.5	16.9
2	19.5	17.4	24.4
3-5	31.2	30.2	30.7
6-10	20.8*	26.7	13.7
11 or more	13.0‡	15.1	14.3

* 6 to 9 units

‡ 10 or more units

It is noteworthy that the single transfusion contributes heavily to all collected series of transfusion-associated hepatitis. This problem is not limited to non-university hospitals (41).

It has been demonstrated that certain blood products carry a much higher risk of inducing hepatitis than does whole blood. The risk of developing hepatitis following fibrinogen infusion has been reported as 6 to 40% (41, 49-52). Pooled plasma which has

been frozen and irradiated according to current recommendations has been shown to carry a hepatitis-inducing risk of 10% (53). Conversely, there are preliminary data which suggest the incidence of post-transfusion hepatitis can be sharply reduced by the use of packed cells rather than whole blood (54).

TABLE IX. Influence of Immune Serum Globulin on Transfusion-Associated Hepatitis. Evidence for Effectiveness

Author	Patients	Treatment (# Pts.)		Rate
Grossman (55)	Battle casualties	10 ml x 2	(384)	1.3%
		Controls	(384)	8.9%
Csapo (56)	Premature infants	8 ml/kg	(182)	0.5%
		Controls	(205)	7.3%
Dawson (57)	Hospital patients	10 ml x 2,3,4	(400)	0.0%
		Control (review)	(400)	4.0%
Mirick (44)	Tuberculosis patients	10 ml x 2,3	(656)	1.1%
		Controls	(655)	3.9%

TABLE X. Evidence Against Effectiveness

Author	Patients	Treatment (# Pts.)		Rate
Duncan (58)	Battle casualties	10 ml	(2406)	1.2%
		Control	(2374)	0.9%
Stokes (59)	Volunteers	Virus	(14)	64.3%
		Virus + hyperimmune globulin	(14)	71.4%
Holland (60)	Heart surgery	10 ml x 2	(84)	13.1%
		Control	(83)	7.2%
Mirick (44)	TBc patients	*10 ml x 2	(121)	4.1%
		Control	(161)	4.2%
‡Mirick (44)	TBc patients	10 ml x 2,3	(687)	6.2%
		Control	(733)	6.3%

* First dose given during week before surgery

‡ Anicteric hepatitis

Thus, the efficacy of large doses of gamma globulin in prevention of transfusion associated hepatitis has not yet been established. Implementation of a policy of use of GG for prophylaxis in the high risk (> 40 years), transfused patient would quickly deplete existing supplies (62). Reduction of numbers of transfusions, careful donor selection, preferential use of packed red blood cells where feasible, avoidance of fibrinogen and pooled plasma, are justifiable efforts to reduce the risk of transfusion-associated hepatitis.

Increased Risk Groups

Hemodialysis Centers

There have been several reports of outbreaks of hepatitis in hemodialysis centers in this country and abroad (63-68). To assess the magnitude of the problem in the U.S., the NCDC Hepatitis Unit undertook a survey of 112 dialysis units (69). Their data are summarized in the following table:

Hepatitis in Chronic Hemodialysis Units

Status of 96 units as of October 1, 1966	Number
Patients on chronic hemodialysis	581
Staff working on units	640
Hepatitis cases in patients prior to October 1, 1966 (46 patient cases occurred in 23 units with 236 patients)	46
Hepatitis cases in staff prior to October 1, 1966 (22 staff cases occurred in 11 units with 132 staff members)	22

Status of 108 units as of October 1, 1967	
Patients on chronic hemodialysis	915
Staff working on units	883
Hepatitis cases in patients between October 1, 1966, and October 1, 1967 (48 patient cases occurred in 26 units with 345 patients)	48
Hepatitis cases in staff between October 1, 1966, and October 1, 1967 (25 staff cases occurred in 14 units with 147 staff members)	25

Percentage of hemodialysis patients with hepatitis: 5.2% (48/915)

Percentage of staff with hepatitis: 2.8% (25/883)

Dialysis-Associated Hepatitis in Staff

	Prior to October 1, 1966		Between October 1, 1966 and October 1, 1967	
	Number	Per Cent	Number	Per Cent
Units with cases	11		14	
Staff working on units	132		147	
Hepatitis cases	22	(16.6% of staff)	25	(17.0% of staff)
Nurses	9	40.9	15	60.0
Technicians	7	31.8	9	36.0
Physicians	6	27.3	1	4.0
Icteric	21	95.4	20	80.0
Deaths*	0	0.0	0	0.0
Immune globulin within 6 mos.				
Yes‡	3	13.6	7	28.0
No	14	63.6	18	72.0
Unknown	5	22.7	0	0.0

* The investigators are aware of one hepatitis death in a staff member since October 1967.

‡ Time intervals between globulin administration and onset of hepatitis:
3 cases prior to Oct. 1, 1966: 1 month, 1-1/2 months, unknown.
7 cases between Oct. 1, 1966, and Oct. 1, 1967: 1 week, 1 month, 1-1/2 months, 2-4 months (2 cases), 5 months (2 cases)

Although these figures do not indicate true attack rates, it appears the risk to staff members is considerable, about half that of chronically dialyzed and transfused patients. Epidemiologic data are not sufficient to determine the relative proportion of IH and SH. It is well established that the latter can be transmitted by contamination of unimpressive skin abrasions (70). The efficacy of immune serum globulin in protecting the staff has not been assessed.

Handlers of Subhuman Primates

Since 1961 over 150 cases of infectious hepatitis have been reported in veterinarians and other handlers of subhuman primates (71-73). Maintenance doses of immune serum globulin are recommended for persons with that occupational exposure.

Recommendations of the Public Health Service Advisory Committee on Immunization Practices (MMWR 17:31, 1968)

Immune Serum Globulin for Prevention of Viral Hepatitis

TABLE XI. Guidelines for ISG Prophylaxis of Infectious Hepatitis for General Use

Person's Weight (lbs.)	ISG Dose (ml)*
up to 50	0.5
50-100	1.0
over 100	2.0

* Within limits, larger doses of ISG provide longer-lasting but not necessarily more protection. Higher doses are, therefore, used under certain circumstances.

Categories of Risk and Suggested Use of ISG

Household Contacts - ISG to all contacts.
School Contacts - Not indicated for teacher or students unless epidemiologic study demonstrates that contact is responsible for continued transmission of disease.
Institutional Contacts - ISG to inmates and staff in face of outbreak. Not recommended against endemic disease.

Hospital Contacts - ISG not recommended for routine contacts. Should be used in those accidentally inoculated with blood or serum from patients with IH.
Office and Factory Contacts - Not recommended.
Common Source Exposure - ISG to all persons exposed to source.
Transfusion-Associated Hepatitis - ISG prophylaxis not recommended.

Travelers

TABLE XII. Guidelines for ISG Prophylaxis of Infectious Hepatitis for U.S. Residents Traveling or Living in Foreign Countries

Area	Person's Weight (lbs.)	Short-Term Travel (1-2 months) ISG Dose (ml.)	Extended Travel or Residence (3-6 months)* ISG Dose (ml)
Africa Asia North America Central America Mexico (rural) Pacific Region Philippine Islands South Pacific Islands South America	up to 50 50-100 over 100	0.5 1.0 2.0	1.0 2.5 5.0
Europe North America Canada Caribbean Islands Mexico (urban) Pacific Region Australia Japan New Zealand	Routine ISG prophylaxis is not indicated		

* Repeat every 6 months of travel or residence

Rubella

Magnitude of the Problem

Rubella is a benign disease except when occurring in the first trimester of pregnancy. The incidence of fetal deaths and/or defective child resulting from first trimester rubella has been estimated as being from 10% to 90% (77-82). The wide variation is due in large part to the fact that laboratory means of establishing a precise diagnosis have only recently become available and most studies have been based on clinical diagnosis. It is well known that non-teratogenic enteroviruses can produce disease

indistinguishable from rubella (83-85). Conversely, rubella can exist without rash in 10-20% of patients and there is considerable evidence that subclinical cases in first trimester pregnancy can result in fetal morbidity (86-88). Further, it has been recognized that many features of the congenital rubella syndrome (deafness, mental deficiency, etc.) are not apparent during the neonatal stage and long-term follow-up is required to assess the true incidence of defects (89).

Observations during a small but well done study with 5-year follow-up are shown in Table XIII:

TABLE XIII (81)

Gestational Week of Maternal Rubella	Spontaneous Fetal Loss (%)	Congenital Abnormalities (%)
1-4	22	11
5-8	38	31
9-13	15	38
14-34	6	1

Recently, Melnick reported observations on 13 women having laboratory-documented rubella during the first trimester. Eleven were aborted and the fetus was infected in 10 instances. Two declined abortion and gave birth to defective children (90, 91).

Efficacy of Immune Serum Globulin

Studies have demonstrated the ability of gamma globulin to suppress or modify the features of rubella if given in large doses before exposure or early in the incubation of the disease (92). Other studies, however, have shown that viremia and virus shedding occur even though clinical features have been suppressed by the globulin (93-95). There have been no well constructed studies convincingly showing efficacy of gamma globulin in preventing teratogenic effects of first trimester rubella (78, 82, 96). In view of the demonstrated failure to prevent viremia, this lack of efficacy would not be surprising.

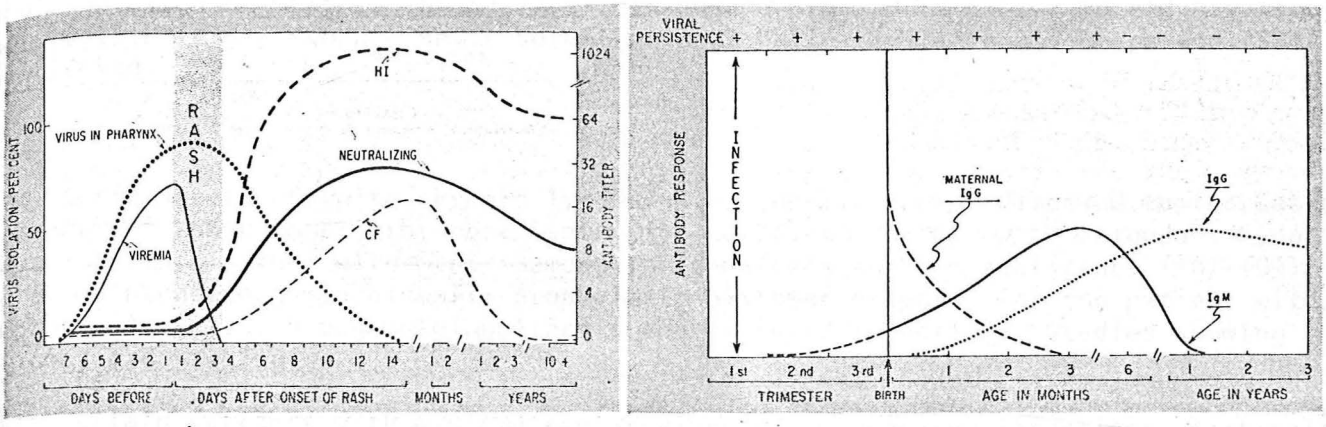
Different globulin preparations have been shown to vary widely in anti-rubella antibody (97, 98). The extent to which the results of the above studies would have been altered by use of standardized preparations is speculative.

Status of the Vaccine

Since isolation of the rubella virus in 1962, several laboratories have been involved in attempts to produce a vaccine. At present, there are several products which are at a desirable level of attenuation, i.e, capable of eliciting seroconversion in tested individuals but incapable of spreading to uninoculated controls. Mild disease and nasopharyngeal shedding of virus for varying periods of time are common consequences of inoculation. Viremia has not been detected with the strains currently being field-tested (98-101).

Management of the Pregnant Patient Exposed to Rubella During the First Trimester

Proper management requires the laboratory confirmation of rubella infection. Improved and simplified serologic tests are becoming increasingly available (90, 102-104).



1) To document infection by serological tests a significant change in titer of one or more antibody types must be demonstrated.

2) An acute serum should be obtained as early as possible, preferably when exposure is recognized or alternatively at the time of onset of the rash.

3) A convalescent serum should be obtained at 10-14 days after the onset of the rash.

4) If the exposed patient does not develop clinical illness, the acute serum should be collected as early as possible and the convalescent serum 28 days after date of exposure.

5) If the acute serum is obtained later than 10 days after the onset of rash it will probably not be possible to establish a diagnosis because of the leveling of the antibody response.

Therapeutic abortion should be strongly considered in a patient having serologically proven rubella in the first trimester of pregnancy (91). Present data would not support the use of immune serum globulin.

Usage in Other Viral Disease

Measles: After the advent of the effective vaccines, there is rarely an indication for use of immune serum globulin in prophylaxis against this disease. A vast experience has shown 0.2 ml/kg to afford protection and 0.04 ml/kg to permit acquisition of attenuated disease.

Varicella: Although no study has shown immune serum globulin capable of preventing varicella, Ross clearly showed attenuation of the disease by use of doses to 0.3 ml/lb. body weight (105). Its use is advocated in exposed susceptible children with debilitating disease and/or immunosuppressive therapy.

Use of Immune Serum Globulin in the Altered Host

Hypogammaglobulinemia

Replacement therapy with immune serum globulin has been shown capable of reducing the frequency of infections in persons with congenital or acquired hypogammaglobulinemia (106). Repeated injections of 0.6 ml/kg should be used at the outset to raise the serum level to a minimum of $\geq$ 300 mg.% (usually 3-4 doses). Maintenance doses of 0.6 ml/kg should be given at monthly intervals. This dosage program is arbitrary and may require modification in a given patient. Serum immunoglobulin levels should be periodically quantitated.

Leukemia, Myeloma

Certain patients with chronic lymphocytic leukemia are predisposed to increased frequency of infections. This predisposition correlates better with attendant hypogammaglobulinemia than with other demonstrable defects in host resistance (107-109). Use of maintenance gamma globulin prophylaxis has been proposed for the patient with lymphatic leukemia, hypogammaglobulinemia and repeated infection. Studies showing efficacy are lacking.

Certain patients with myeloma are predisposed to repeated infections, particularly with the pneumococcus. This defect correlates well with inability to produce serum antibody (109-110). Although use of gamma globulin prophylaxis is often proposed for these patients, the only reasonably well controlled study failed to demonstrate efficacy (111).

Despite multiple testimonial accounts, there is no study showing efficacy of gamma globulin in management of infection in acute leukemia (112).

Modified Gamma Globulin

Gamma globulin is relatively well tolerated intramuscularly but intravenous administration is frequently attended by anaphylactoid reactions (113-115). This reaction seems particularly apt to occur in people with antibody deficiency states. The adverse reaction is thought to be due to presence of molecular aggregates (116-120) which are known to form in commercial gamma globulin preparations (113).

Several investigators have attempted modification of the globulin molecule in manners which would reduce aggregation and complement-binding capabilities without diminishing antibody activity. Early products, prepared principally by pepsin digestion of the globulin molecule, had the obvious disadvantage of rapid elimination and anaphylactoid reactions have been described with its use (121). More recently, plasmin-digested globulin has been shown to have a serum half-life approximating that of native gamma globulin (122). A satisfactory preparation would have obvious advantages in persons requiring large quantities of gamma globulin.

Special Uses of Hyperimmune Human Serum Globulin

Suppression of the Primary Rh Immune Response

It is estimated that some 400,000 women in the U.S. are at risk to Rh immunization annually because of exposure to the antigen during pregnancy. Of the population at risk some 10-25% actually become immunized, thereby posing a threat to subsequent pregnancies. The administration of gamma globulin having high anti-Rh content within 72 hours after delivery has been shown efficacious in preventing immunization of mothers at risk (Rh negative mother, Rh positive baby) (123-125).

Vaccinia

Clinical experience suggests that vaccinia-immune globulin is of value in treatment of generalized vaccinia and vaccinia gangrenosum, but not in post-vaccinal encephalitis (126-129). Reference 129 gives sources of VIG.

Mumps

Hyperimmune serum globulin prepared from convalescent patients has been shown capable of reducing the attack rate of mumps orchitis in males from 28% to 8% (130). The hyperimmune serum commercially available has not been well tested in controlled studies. The advent of an effective vaccine has undoubtedly weakened the tenuous position of hyperimmune mumps globulin.

Tetanus:

Favorable prophylactic efficacy of immune tetanus immune globulin (human) would be anticipated on the basis of the serum antitoxin levels in response to modest doses (132-133). Clinical observations have thus far shown favorable results in prophylaxis. Superiority of this agent in treatment of clinical tetanus has not been established (134).

BIBLIOGRAPHY

The Immunoglobulins

1. Fudenberg, H. The immune globulins. Ann. Rev. Microbiol. 19:301, 1965. (Review, 326 references)

2. Fahey, J. L. Antibodies and immunoglobulins. I. Structure and function. JAMA 194:71, 1965.

3. Bellanti, J. A. Role of local gamma-A-immunoglobulins in immunity. A review. Am. J. Dis. Child. 115:239, 1968.

4. Rowe, D. S., and Fahey, J. L. A new class of human immunoglobulins. I. A unique myeloma protein. J. Exp. Med. 121:171, 1965.

5. Rogentine, G. N., et al. Metabolism of human immunoglobulin D (IgD). J. Clin. Invest. 45:9, 1966.

6. Ishizaka, K., and Ishizaka, T. Human reaginic antibodies and immunoglobulin E. (A review.) J. Allergy 42:330, 1968.

7. Robbins, J. B., et al. The isolation and biological activities of γM and γG anti-Salmonella typhimurium antibodies. J. Exp. Med. 122:385, 1965.

8. Smith, J. W., et al. Comparison of the opsonic activity of γG and γM antiproteus globulins. J. Immunol. 98:336, 1968.

9. WHO Technical Report #327. The use of human immunoglobulin. Report of WHO Expert Committee.

Therapeutic Uses of Immune Serum Globulin

Infectious Hepatitis

10. Janeway, C. A., and Rosen, F. The gamma globulins. IV. Therapeutic uses of gamma globulin. (General review.) New Eng. J. Med. 275:826, 1966.

11. Koff, R. S., and Isselbacher, K. J. Changing concepts in the epidemiology of viral hepatitis. New Eng. J. Med. 278:1371, 1968. (An excellent review of general epidemiological factors)

12. Rightsel, W. A., et al. Tissue culture cultivation of cytopathogenic agents from patients with clinical hepatitis. Science 124:226, 1956.

13. Taylor, T. A., et al. Tissue culture of hepatitis virus. Am. J. Med. 32:679, 1962.

14. O'Malley, J. P., et al. Antibody in hepatitis patients against a newly isolated virus. Proc. Soc. Exp. Biol. & Med. 108:200, 1961.

15. O'Malley, J. P., et al. Identification of A-1 agent as Mycoplasma gallisepticum. Proc. Natl. Acad. Sci. 56:895, 1966.

16. Dunnebacke, T. H., and Williams, R. C. Reinterpretation of nature of "lipovirus" cytopathogenicity. Proc. Natl. Acad. Sci. 57:1363, 1967.

17. Bolin, V. S., et al. Studies on serum and infectious hepatitis viruses of man. I. Preliminary report on isolation of serum and infectious hepatitis viruses from man in tissue culture. Transfusion 1:360, 1961.

18. Liebhaber, H., et al. Recovery of cytopathic agents from patients with infectious hepatitis: Isolation and propagation in cultures of human diploid fibroblasts. Virology 24:109, 1964.

19. Liebhaber, H., et al. Studies of myxovirus recovered from patients with infectious hepatitis: I. Isolation and characterization. J. Exp. Med. 122:1135, 1965.

20. Davis, E. V. Isolation of viruses from children with infectious hepatitis. Science 133:2059, 1961.

21. Communicable Disease Center, Hepatitis Surveillance Report #22, Atlanta, Ga., USPHS, March 31, 1965.

22. Smith, K. D., and Gehle, W. D. Replication of adeno-associated virus in canine and human cells with infectious canine hepatitis as helper. J. Virology 1:648, 1968.

23. Sutnick, A. I., et al. Anicteric hepatitis associated with Australia antigen. Occurrence in patients with Downs syndrome. JAMA 205:80, 1968.

24. Blumberg, B. S., et al. A serum antigen (Australia antigen) in Downs syndrome, leukemia and hepatitis. Ann. Int. Med. 66:924, 1967.

25. Sutnick, A. I., et al. Australia antigen, Downs syndrome and hepatitis. J. Clin. Invest. 46:1122, 1967.

26. Deinhardt, F., et al. Studies on transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passage and description of liver lesions. J. Exp. Med. 125:673, 1967.

27. Holmes, A. W., et al. Experimental hepatitis in marmosets. J. Clin. Invest. 46:1072, 1967.

28. Stokes, J., Jr., and Neefe, J. R. The prevention and attenuation of infectious hepatitis by gamma globulin. JAMA 127:144, 1955.

29. Stokes, J., Jr., et al. Infectious hepatitis: Length of protection by immune serum globulin (gamma globulin) during epidemics. JAMA 147:714, 1951.

30. Drake, M. E., and Ming, C. Gamma globulin in epidemic hepatitis: Comparative value of two dosage levels, apparently near minimal effective level. JAMA 155: 1302, 1954.

31. Krugman, S., et al. Infectious hepatitis: Studies on effect of gamma globulin and on incidence of inapparent infection. JAMA 174:823, 1960.

32. Krugman, S., and Ward, R. Infectious hepatitis: Current status of prevention with gamma globulin. Yale J. Biol. & Med. 34, Dec.-Feb. 1961/2.

33. Ashley, A. Gamma globulin: Effect on secondary attack rates in infectious hepatitis. New Eng. J. Med. 250:412, 1954.

34. Gelperin, A., and Hampton, W. The ecology of infectious hepatitis. Am. J. Pub. Health 45:1327, 1955.

35. Clark, W. H., et al. A special study of infectious hepatitis in the general population of three counties in California. Am. J. Trop. Med. 9:639, 1960.

36. Mosley, W., et al. Epidemiologic studies of a large urban outbreak of infectious hepatitis. Am. J. Public Health 53:1603, 1963.

37. Mosley, W., et al. Comparison of two lots of immune serum globulin for prophylaxis of infectious hepatitis. Am. J. Epidemiol. 87:539, 1968.

Assessment of British Gamma Globulin in Preventing Infectious Hepatitis

38. Report to the Director of the Public Health Laboratory Service. Brit. Med. J. 3:451, 1968.

39. Ward, R., and Krugman, S. Etiology, epidemiology and prevention of viral hepatitis. Progress in Medical Virology, Ed. E. Berger and J. L. Melnick, Vol. 4, p. 88, Karger, Basel and New York.

Transfusion-Associated Hepatitis

40. Koff, R. S., et al. Viral hepatitis in a group of Boston hospitals. III. Importance of exposure to shellfish in a non-epidemic period. New Eng. J. Med. 276:703, 1967.

41. Mosley, J. W. Surveillance of transfusion-associated viral hepatitis. JAMA 193:1007, 1965.

42. Allen, J. G., and Sayman, W. A. Serum hepatitis from transfusion of blood: Epidemiologic study. JAMA 180:1079, 1962.

43. Cohen, S. N., and Dougherty, W. J. Hepatitis among recipients of blood from narcotic addicts. Special report presented at Twelfth Annual Conference of Epidemic Intelligence Service, CDC, USPHS, Atlanta, Ga. April 23-26, 1963. (Quoted by Grady, Ref. 45)

44. Mirick, G. S., et al. Modification of post-transfusion hepatitis by gamma globulin. New Eng. J. Med. 273:59, 1965.

45. Grady, G. F., et al. Risk of post-transfusion hepatitis. New Eng. J. Med. 271:337, 1964.

46. Kunin, C. M. Serum hepatitis from whole blood: Incidence and relation to source of blood. Am. J. Med. Sci. 237:293, 1959.

47. Shimizu, Y., and Kitamoto, O. Incidence of viral hepatitis after blood transfusions. Gastroenterology 44:740, 1963.

48. Hampers, C. L., et al. Post-transfusion anicteric hepatitis. New Eng. J. Med. 271:747, 1964.

49. Mainwaring, R. L., and Brueckner, G. G. Fibrinogen-transmitted hepatitis. A controlled study. JAMA 195:437, 1966.

50. Zanio, E. C. Homologous serum hepatitis following the administration of fibrinogen. Obst. Gynec. 15:404, 1960.

51. Cronberg, S., etal. Fibrnogen transmitted hepatitis. Lancet 1:967, 1963.

52. Skinner, J. S. Serum hepatitis: Occurrence following the use of human fibrinogen. Missouri Med. 54:740, 1957.

53. Redeker, A. G., et al. A controlled study of the safety of pooled plasma stored in the liquid state at 30-32°C for 6 months. Transfusion 8:60, 1968 (Mar.-Apr.).

54. Kliman, A. Low risk of hepatitis after packed red cells. Letter to the Editor. New Eng. J. Med. 277:1320, 1967.

55. Grossman, E. B., et al. Post-transfusion hepatitis in battle casualties and study of its prophylaxis by means of human serum immune globulin. JAMA 129: 991, 1945.

56. Csapo, J., et al. Prevention of transfusion hepatitis. Acta paediat. Acad. Sci. Hungary 4:195, 1963.

57. Dawson, D. A clinical study on the use of immune serum globulin prophylaxis in patients receiving blood and/or plasma transfusions. Current Therap. Research 7:184, 1965.

58. Duncan, G. G., et al. Evaluation of immune serum globulin as prophylactic agent against homologous serum hepatitis. Am. J. Med. Sci. 213:53, 1947.

59. Stokes, J., et al. Methods of protection against homologous serum hepatitis. I. Studies on protective value of gamma globulin in homologous serum hepatitis (SH) virus. JAMA 138:336, 1948.

60. Holland, P. V., et al. γ globulin in prophylaxis of post-transfusion hepatitis. JAMA 196:471, 1966.

61. Creutzfeldt, W., et al. Use of gamma-globulin in prophylaxis of transfusion hepatitis. German M. Monthly 12:101, 1967.

62. Chalmers, T. C. Prevention of serum hepatitis. Letter to the Editor. New Eng. J. Med. 269:870, 1963.

Increased Risk Groups

63. Friedman, E. A., and Thompson, G. E. Hepatitis complicating chronic hemodialysis. Lancet 2:675, 1966.

64. Jones, P. O., et al. Viral hepatitis: staff hazard in dialysis units. Lancet 1: 835, 1967.

65. Hepatitis and renal dialysis. Leading Article. Brit. Med. J. 2:1199, 1965.

66. Reingertz, O., and Melen, B. Hepatitis and artificial kidney. Lancet 1:151, 1966.

67. Eastwood, J. B., et al. Hepatitis in a hemodialysis unit. Ann. Int. Med. 69: 59, 1968.

68. Pendras, J. P., and Erickson, R. V. Hemodialysis: A successful therapy for chronic uremia. Ann. Int. Med. 64:293, 1966.

69. NCDC Hepatitis Surveillance Report #28, Jan. 31, 1968.

70. Ringertz, O., and Zetterberg, B. Serum hepatitis among Swedish track finders: Epidemiologic study. New Eng. J. Med. 276:540, 1967.

71. Hillis, W. D. Outbreak of infectious hepatitis among chimpanzee handlers at United States Air Force Base. Am. J. Hyg. 73:316, 1961.

72. NCDC Hepatitis Surveillance Report #27, Sept. 30, 1967.

73. NCDC Hepatitis Surveillance Report, Sept. 30, 1968.

74. Cline, A. L., et al. Viral hepatitis among American missionaries abroad. A preliminary study. JAMA 199:551, 1967.

75. Frame, J. D. Hepatitis among missionaries in Ethiopia and Sudan. Susceptibles at high risk. JAMA 203:819, 1968.

76. Byrne, E. B. Viral hepatitis: Occupational hazard of medical personnel. JAMA 195:362, 1966.

Rubella

77. Gordon, J. E., and Ingalls, T. H. Rubella: Epidemiology, virology and immunology. Am. J. Med. Sci. 253:349, 1967.

78. Heggie, A. D. Rubella: Current concepts in epidemiology and teratology. Ped. Clin. North America 13:251, 1966.

79. Siegel, M., et al. Fetal mortality in maternal rubella: Results of a prospective study from 1957-1964. Am. J. Obst. Gyn. 96:247, 1966.

80. Lundstrom, R. Rubella during pregnancy. Acta Paediat. Scand. 54 (Suppl. 133): 1-110, 1962.
(Estimated frequency in relation to gestational age - review of series.)

81. Grayston, J. T. Congenital abnormalities following gestational rubella in Chinese. Report of a prospective study including five-year follow-up examinations after the 1957-1958 rubella epidemic on Taiwan (Formosa). JAMA 202:95, 1967.

82. Sever, J. L., et al. Rubella epidemic 1964: Effect on 6000 pregnancies. Am. J. Dis. Child. 110:395, 1965.

83. Gutekunst, R. R., and Heggie, A. D. Viremia and viruria in adenovirus detection in patients with rubella or rubelliform illness. New Eng. J. Med. 264:374, 1961.

84. Sanford, J. P., and Sulkin, S. E. Clinical spectrum of ECHO-virus infection. New Eng. J. Med. 261:1113, 1959.

85. Kibrick, S. Rubella and rubelliform rash. Bact. Rev. 28:452, 1967.

86. Rudolph, A. J., et al. Transplacental rubella infection in newly born infants. JAMA 191:843, 1965.

87. Karmody, C. S. Subclinical maternal rubella and congenital deafness. New Eng. J. Med. 278:809, 1968.

88. Siegle, M., et al. Rubella in mother and congenital cataracts in child. JAMA 203:632, 1968.

89. Lock, Frank R., et al. Difficulties in the diagnosis of congenital abnormalities. Experience in a study of the effect of rubella on pregnancy. JAMA 178:711, 1961.

90. Rawls, W. E., et al. Serologic diagnosis and fetal involvement in maternal rubella. Criteria for abortion. JAMA 203:111, 1968.

91. Rawls, W. E., and Melnick, J. L. Letter to the Editor. JAMA 204:151, 1968.

92. Brody, J. A., et al. Prevention of rubella by gamma globulin during an epidemic in Barrow, Alaska, in 1964. New Eng. J. Med. 272:127, 1965.

93. Green, R. H., et al. Studies on experimental transmission, clinical course, epidemiology and prevention of rubella. Trans. Assn. Amer. Physicians 77:118, 1964.

94. Green, R. H., et al. Studies of the natural history and prevention of rubella. Am. J. Dis. Child. 110:348, 1965.

95. Doege, T. C. Studies of rubella and its prevention with immune globulin. JAMA 200:584, 1967.

96. Lock, F. R., et al. Incidence of anomalous development following maternal rubella. Effect of clinical infection or exposure and treatment with gamma globulin. Am. J. Obst. Gyn. 81:451, 1961.

97. Schiff, G. M., et al. Rubella virus: Neutralizing antibody in commercial gamma globulin. Science 142:58, 1963.

98. Buynak, E. B., et al. Live attenuated rubella virus vaccines prepared in duck embryo cell culture. I. Development and clinical testing. JAMA 204:195, 1968.

99. Parkman, P. D., et al. Attenuated rubella virus: I. Development and laboratory characteristics. New Eng. J. Med. 275:569, 1966.

100. Meyer, H. M., et al. Attenuated rubella virus. II. Production of an experimental live virus vaccine and field trial. New Eng. J. Med. 275:575, 1966.

101. Plotkin, S. A., et al. Rubella vaccine strains attenuated in non-simian cultures. Antimicrobial Agents & Chemotherapy, 1968 (in press).

102. Plotkin, S. A. Virologic assistance in the management of German measles in pregnancy. JAMA 190:266, 1964.

103. Stewart, G. L., et al. Rubella-virus hemagglutination-inhibition test. New Eng. J. Med. 276:554, 1967.

104. Cooper, L. Z., et al. Experience with a modified rubella hemagglutination inhibition antibody test. JAMA 207:91, 1969.

Varicella

105. Ross, A. H. Modification of chickenpox in family contacts by administration of gamma globulin. New Eng. J. Med. 267:369, 1962.

The Altered Host

106. Rosen, F. S., and Janeway, C. A. Gamma globulins. III. Antibody deficiency syndromes. New Eng. J. Med. 275:709, 1966.

107. Miller, D. G., and Karnofsky, D. A. Immunologic factors and resistance to infection in chronic lymphatic leukemia. Am. J. Med. 31:748, 1961.

108. Miller, D. G., et al. A clinical and pathological study of resistance to infection in chronic lymphatic leukemia. Cancer 15:307, 1962.

109. Cone, L., and Uhr, J. Immunological deficiency disorders associated with chronic lymphocytic leukemia and multiple myeloma. J. Clin. Invest. 43:2241, 1964.

110. Zinneman, H. H., and Hall, W. H. Recurrent pneumonia in multiple myeloma and some observations on immunologic response. Ann. Int. Med. 41:1152, 1954.

111. Salmon, S. F., et al. Role of gamma globulin for immunoprophylaxis in multiple myeloma. New Eng. J. Med. 277:1336, 1967.

112. Bodey, G. P., et al. Use of gamma globulin in infection in acute leukemia patients. JAMA 190:1099, 1964.

113. Barandun, S., et al. Intravenous administration of human γ globulin. Vox Sang. 7:157, 1962.

114. Moore, C. E., et al. Experimental and clinical adventures with large doses of gamma globulin and other globulins as anticancer agents. Surgery 41:972, 1957.

115. Coon, W. W. Experiences with large infusions of γ globulin. Am. J. Surg. 102: 548, 1961.

116. Christian, C. L. Studies of aggregated gamma globulin. J. Immunol. 84:112, 1959.

117. Frommhagen, L. H., and Fudenberg, H. The role of aggregated gamma globulins in the anticomplementary activity of human and animal sera. J. Immunol. 89:336, 1962.

118. Richardson, H. B., and Seebohm, P. M. Anaphylactoid reaction to human gamma globulin. Report of a case and inquiry into mechanism. Arch. Int. Med. 117: 568, 1966.

119. Stiehm, E. R., and Fudenberg, H. H. Antibodies to gamma-globulin in infants and children exposed to isologous gamma globulin. Pediatrics 35:229, 1965.

120. Henney, C. S., and Ellis, E. F. Antibody production to aggregated human γG in acquired hypogammaglobulinemia. New Eng. J. Med. 278:1144, 1968.

121. Jager, R. V. Intravenous administration of modified gamma globulin. Several studies on a patient with agammaglobulinemia. Arch. Int. Med. 119:60, 1967.

122. Janeway, C. A., et al. Intravenous gamma globulin. Metabolism of gamma globulin fragments in normal and agammaglobulinemic persons. New Eng. J. Med. 278:919, 1968.

Special Uses of Hyperimmune Human Gamma Globulin

123. Fuda, V. J., et al. Suppression of the primary Rh immune response with passive Rh IgG immunoglobulin. New Eng. J. Med. 277:1022, 1967.

124. Ascari, W. Q., et al. $Rh_o(D)$ immune globulin (human). Evaluation of women at risk of Rh immunization. JAMA 205:1, 1968.

125. Hughes-Jones, N. C., and Stevenson, M. The anti-D content of IgG preparations for use in the prevention of Rh hemolytic disease. Vox Sang. 14:401, 1968.

Vaccinia

126. Kempe, C. H., et al. Hyperimmune vaccinal gamma globulin: Source, evaluation, and use in prophylaxis and therapy. Pediatrics 18:177, 1956.

127. Kozinn, P. J., et al. Progressive vaccinia associatedwith agammaglobulinemia and defects in immune mechanisms. Pediatrics 16:600, 1955.

128. Lundström, R. Complications of smallpox vaccination and their treatment with vaccinia immune globulin. J. Ped. 49:129, 1956.

129. Recommendation of the USPHS Advisory Committee on Immunization Practices. Ann. Int. Med. 66:358, 1967.

Mumps

130. Gulis, S. S., et al. Studies on prevention of mumps orchitis by gamma globulin. Am. J. Med. Sci. 210:661, 1945.

Tetanus

131. McComb, J. A., and Dwyer, R. C. Passive-active immunization with tetanus immune globulin (human. New Eng. J. Med. 268:857, 1963.

132. McComb, J. A. Prophylactic dose of homologous tetanus antitoxin. New Eng. J. Med. 270:175, 1964.

133. Levine, L., et al. Active-passive tetanus immunization. Choice of toxoid, dose of immune globulin and timing of injection. New Eng. J. Med. 274:186, 1967.

134. Young, L. S., et al. Efficacy of serological and antimicrobial therapy in treatment of US tetanus cases. Antimicrobial Agents & Chemotherapy, 1968 (in press).