STUDIES ON LEUKOCYTE ANTIBODIES, WITH SPECLAL REFERENCE TO THE PRODUCTION OF ANTIBODIES SUBSEQUENT TO BLOOD TRANSFUSION

KAZUMASA YAMADA, MORIJI MIURA, MASATO ITO AND KOJI MUNAKATA

1st Department of Internal Medicine, Nagoya University School of Medicine
(Director: Prof. Susumu Hibino)

The importance of blood transfusion in stimulating the production of most leuko-agglutinins is established beyond all doubt. Among 60 leuko-agglutinating sera studied by Dausset, 90 per cent were from patients who had received multiple (average of 22) transfusions. Brittingham, Andre *et al.* and Payne also noted a high degree of correlation. Other than leuko-agglutinins, various types of homologus leukocyte antibodies have been found in human sera, but have been much less studied to date than the agglutinins.

The present report mainly shows the occurance of such antibodies, as leuko-agglutinins, complement-fixing leukocyte antibodies, incomplete anti-leukocyte antibodies by means of the antihuman-globulin consumption test leukocyte phagocytosis inhibiting factor and leuko-precipitins subsequent to transfusion. Transfusion reactions associated with those antibodies were also investigated.

Experimental studies on the antigenic differences and similarities between different morphologic parts of the leukocyte with special reference with the antigenicity of neutrophilic granules, and also immunoagranulocytosis.

I. OCCURANCE OF VARIOUS TYPES OF HOMOLOGOUS LEUKOCYTE ANTIBODIES SUBSEQUENT TO BLOOD TRANSFUSION AND THEIR SIGNIFICANCE IN TRANSFUSION REACTIONS

A) Leukoagglutinins

a) Materials and methods

The materials examined consisted of sera obtained from 417 cases with histories of blood transfusion for various blood diseases, malignant tumors, tuberculosis and others. As controls, sera of 238 healthy individuals with no histories of blood transfusion were examined.

The suspension of leukocyte was prepared by the method shown in Fig. 1, while was conducted by the method indicated in Fig. 2.

For the agglutinin test, leukocytes obtained from more than two healthy individuals were used. In cases with positive results, tests were repeated with leukocytes of other healthy individuals and leukocytes obtained from chronic myelogenous leukemia.

Received for publication,. July 12, 1963.

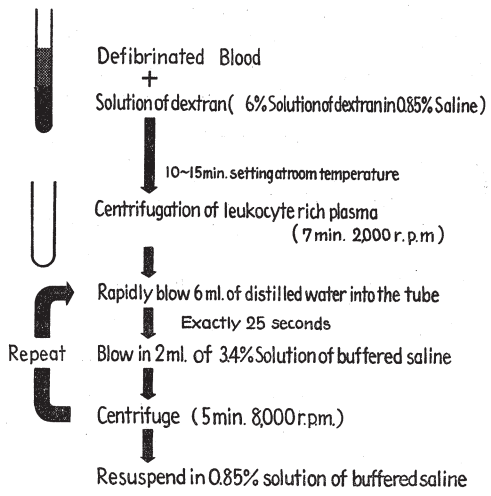

FIG. 1. Preparation of leukocyte suspension (Walford's method)

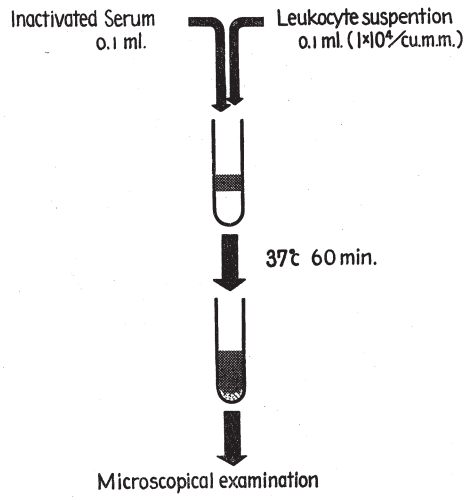

FIG. 2. The leuko-agglutinin test

b) Results

1) Relation between frequency of transfusion and total volume of transfusion.

Of the 238 healthy individuals with no histories of blood transfusion there was found a single case where leukocyte agglutinins were present in the serum.

In Table 1 is shown the relationship between numbers of positive agglutinins and frequency of transfusion in the 417 cases with histories of previous transfusion.

In the group with 1 to 3 previous transfusions there was seen 1 positive reaction among the 194 (rate of 0.51%) in those with 4 to 10 transfusion the rate was 1.28 (2 out of 156 cases) and in those with 11 to 20 transfusions the positive rate rose to 10% (4 out of 40), indicating a rise in positive reaction with increase in frequency of transfusion. The rate was 25% (3 out of 12) in those with 21 to 30 transfusions, 54.6% in those with 31 to 50 transfusions. Thus the positive rate rose proportionately to frequency of transfusion. Fig. 3 shows the relationship between frequency of transfusion and total volume of transfusion in cases with positive leuko-agglutinins.

It was found that even when the total volume exceeded 4000 ml., no positive cases were found when the frequency of transfusion was less than 20 times. But in those with more than 21 transfusion there were 11 out of 25 that were posistive. These findings suggest that production of leukocyte antibodies has little relation to total volume of blood transfused, but is merely influenced by the frequency of transfusion as reported elsewhere.

2) Frequency of cases with positive leuko-agglutinins in various diseases.

In Fig. 4 are shown the results of observations made on positive agglu-

TABLE 1. Leukocyte Iso-antibody Subsequent to Blood Transfusion

Frequency of transfusion	Number of patients		Leuko-agglutinins	
	Cases	Sub total	Cases	Positive rate
1	83		0	
2	54	194	0	0.51
3	57		0	
4	33		0	
5	32		1	
6	27		1	
7	29	156	0	1.28
8	17		0	
9	9		0	
10	9		0	
11	7		0	
12	7		1	
13	3		0	
14	4		1	
15	5		0	
16	5	40	0	10.0
17	5		2	
18	2		0	
19	1		0	
20	1		0	
21~30	12	12	3	25.0
31~40	7	11	2	54.6
41~50	4		4	
51~100	3	3	2	66.6
101~	1	1	1	100.0
Total	417		19	4.5
Non transfused	238		1	0.4

tinins in various diseases and according to frequency of transfusion. It will be seen that positive cases were 10 out of 50 (20%) in blood diseases, 3 out of 40 (7.5%) in digestive tract diseases, and 4 out of 88 (4.5%) in malignant tumor cases, the rate being highest in blood diseases.

Further, of the 19 positive cases 16 were in those with histories of more than 10 transfusions, a finding that deserve attention.

3) Relation between type of transfusion reaction and leukocyte antibodies.

In Table 2 and Figs. 5 and 6 are shown the results of investigation on the rates of appearance of the transfusion reaction, times of its appearance in relation to the frequency of transfusion. Urticaria was observed in 34 out of 43 cases (79.1%) in those with less than 4 transfusion, and only 4 cases (9.3%) in those with after 9th transfusion.

Febrile reactions occured in 19 out of 29 cases (65.5%) in those with more

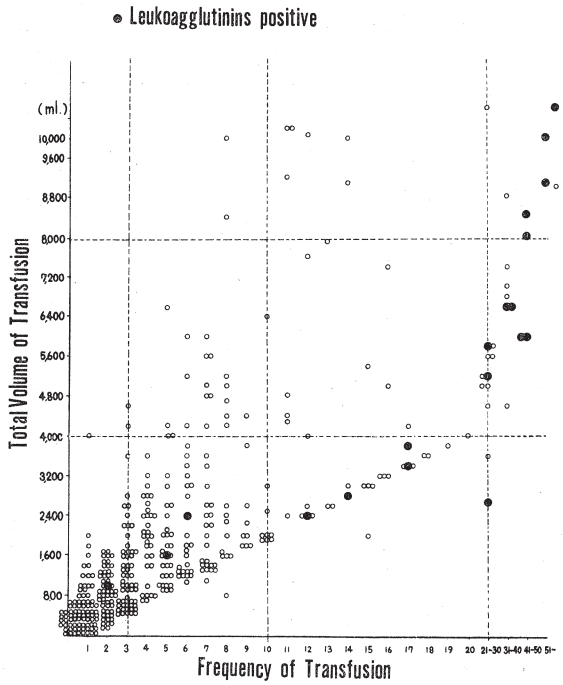

FIG. 3. Leuko-agglutinins in relation to frequency and volume of transfnsion

TABLE 2. Leuko-agglutinin in Group with and without Transfusion Reaction

Leuko-agglutinin	Group with positive transfusion reaction			Group with negative transfusion reaction		
	Cases	Number of transfusion	Total volume of transfusion	Cases	Number of transfusion	Total volume of transfusion
Positive	9	35.3	5900	10	47.7	4570
Negative	72	7.4	2680	326	4.9	2710
Total	81	10.5	3040	336	6.2	2780
Positive rate	11.1% (9/81)			2.9% (10/336)		

than 5 transfusions. These findings indicate that urticaria tends to occure in cases of less than 4 transfusions and fever in those with more than 5 transfusions.

Table 3 shows, the relation between transfusion reaction and leuko-agglutinin. The cases are devided into two groups with positive and negative transfusions reaction. Agglutinins positive cases were 9 out of 81 (11.1%) in those with positive transfusion reactions, while the figures were 10 out of 336 (2.9%) in those with negative transfusion reactions, indicating clearly a higher positive rate in the former group. The positive group is further devided into the febrile, urticarial, febrile-urticarial and others, and the frequencies of the leuko-

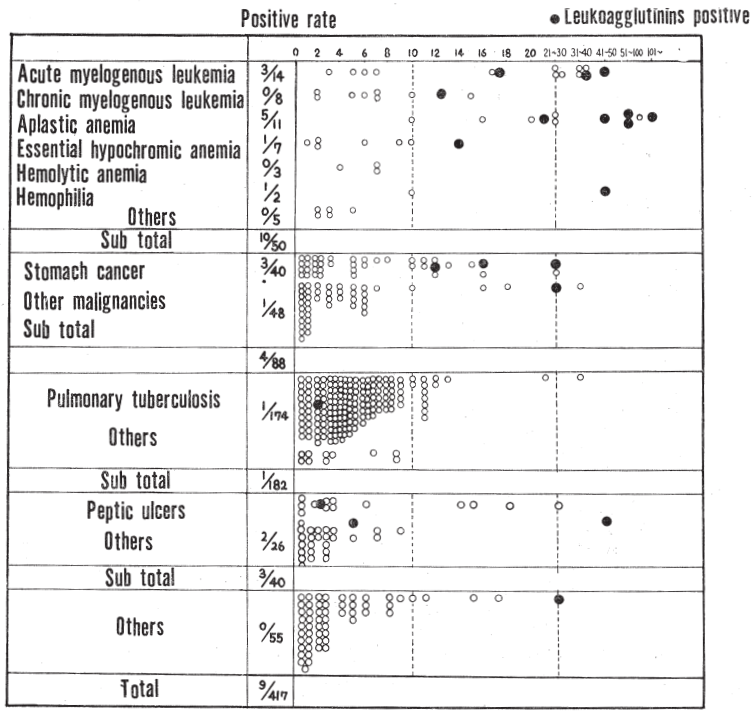

FIG. 4. Cases with positive leuko-agglutinins in various diseases and according to frequency of transfution

TABLE 3. Leukoagglutinins and Transfusion Reaction

Leuko-agglutinisn	Transfusion reaction											
	Group with febril reaction			Group wtih febril and urticaria reaction			Group with urticaria			Group with other reaction		
	Cases	Mean		Cases	Mean		Cases	Mean		Cases	Mean	
		Frequency of transfusion	Volume of transfusion		Frequency of transfusion	Volume of transfusion		Frequency of transfusion	Volume of transfusion		Frequency of transfusion	Volume of transfusion
Positive	5	29.8	5.770	4	42.3	6.050	0	—	—	0	—	—
Negative	8	5.3	2.720	10	12.4	3.020	40	6.7	2.605	14	7.1	2.630
Total	13	14.7	3.800	14	29.3	8.850	40	6.7	2.605	14	7.1	2.630
Positve rate	38.5%			28.6%			0			0		

agglutinins positive cases in each to the groups are indicated. When the type of transfusion reaction is observed in relation to presence of agglutinins, there were 5 out of 13 (38.5%) in cases with febrile reaction, and 4 out of 14 (28.6%) in those with febrile urticarial reaction, where leuko-agglutinins were demon-

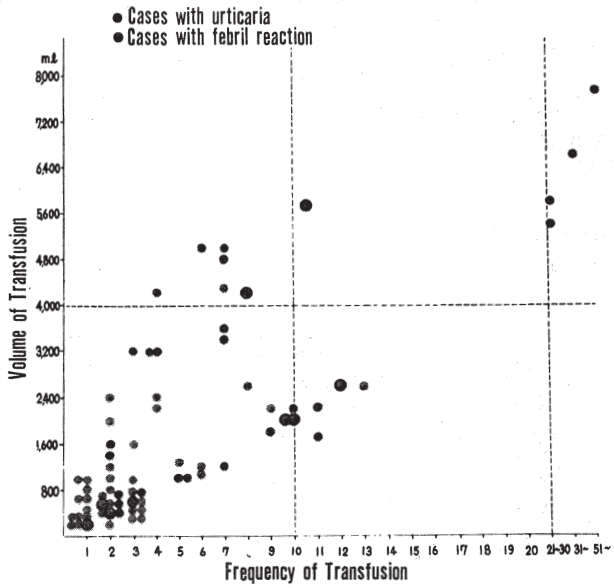

FIG. 5. Transfusion reaction in relation to frequency and volume of transfusion

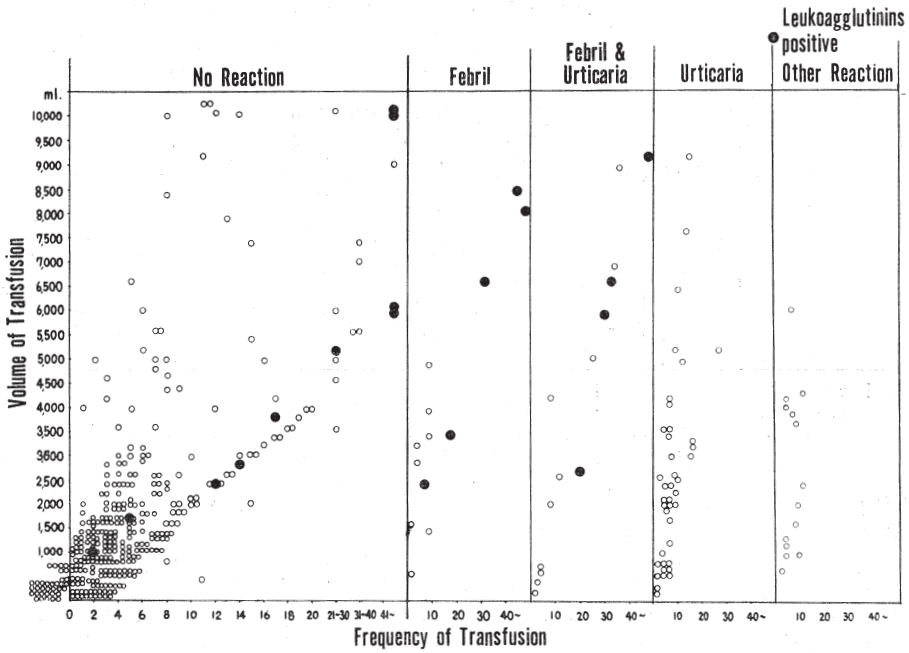

FIG. 6. Leuko-agglutinins and transfusion reaction

strated. Contrary to the above in the 40 cases of urticarial and 14 with no febrile-urticarial reaction leuko-agglutinins coule not be demonstrated.

The above results indicate that a definite relation exists between febrile reaction due to transfusion and the production of leuko-agglutinins.

B) Leuko-isoantibodies other than leuko-agglutinins

Next, studies were conducted on leukocyte antibodies related to blood transfusion by conducting the antihuman globulin consumption test, as well as investigations on the complement-fixing antileukocyte antibodies, phagocytosis inhibition factor and leuko-precipitins.

The subjects studied were cases with positive leuko-agglutinins, and various types of transusion reaction positive cases, and transfused cases other than the above two selected at random.

1) Antihuman globulin (AHG) consumption test.

The experimental technique of this test is shown in Fig. 7.

Results: Cases with positive AHG consumption test were 18 out of 66 examined (27.2%), and are shown in Fig. 8. There was found no clear relation to frequency of transfusion. Also there was seen no relation between transfusion reaction and the presence of this antibody.

2) Complement fixing antileucocyte antibodies.

The technique of the complement fixation test is described in Fig. 9.

Results: As shown in Fig. 10, positive results were obtained in 6 out of 39 cases (15.3%).

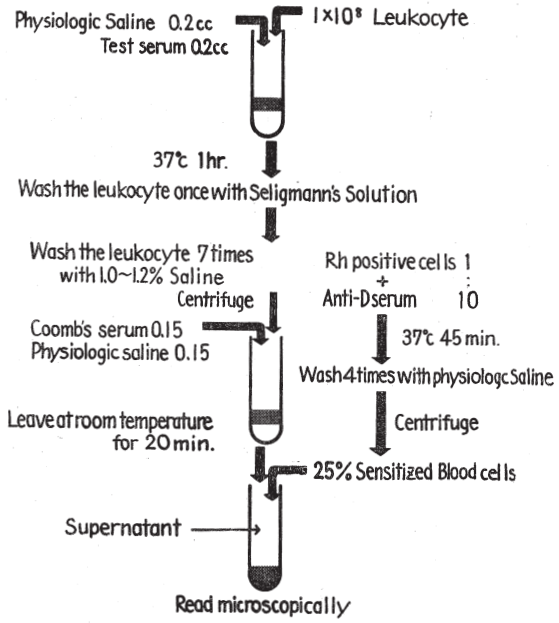

FIG. 7. Anti-humanglobulin (AHG) consumption test

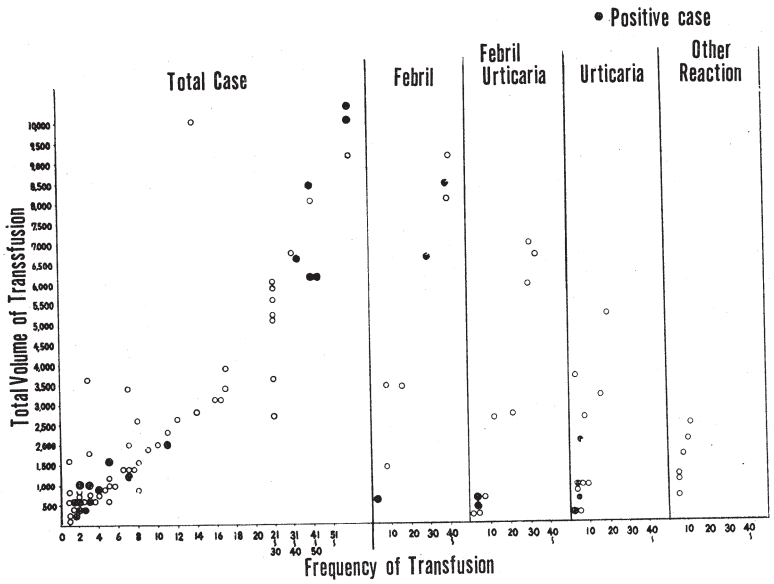

FIG. 8. Antiglobulin consumption test
In group with leuko-agglutinins, trunsfusion reaction and polytransfusion

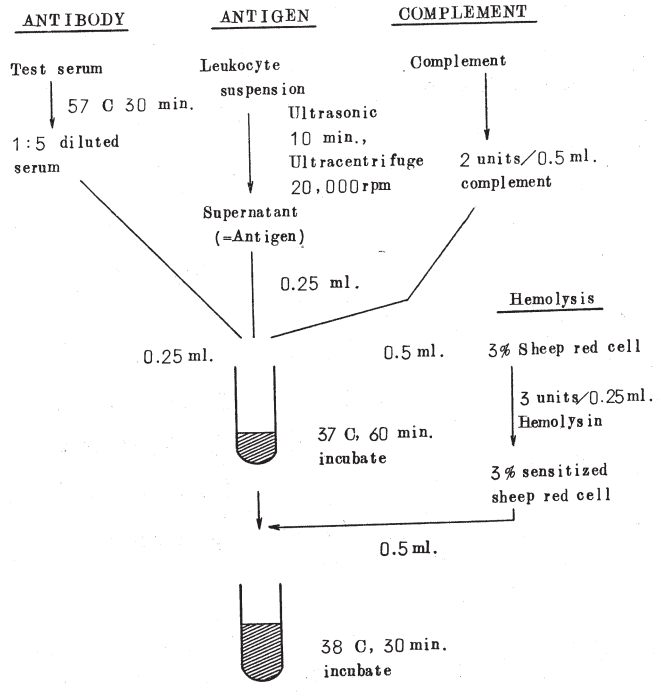

FIG. 9. Complement-fixing antileukocyte antibodies

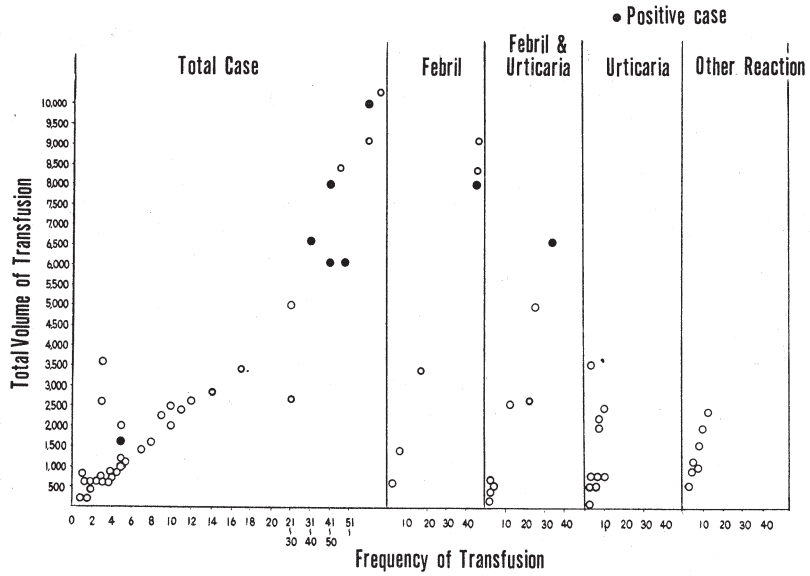

FIG. 10. Complement fixing antileukocyte antibodies
In group with leuko-agglutinins, transfusion reaction and polytransfusion

Of the 6 positive cases 5 belonged to the group that received more than 26 transfusions, a finding that deserves attention. But no relation was seen between the presenece of this antibody and transfusion reaction.

3) Phagocytosis inhibition factor.

The techique employed is shown in Fig. 11.

Results: As may be seen from Fig. 12, positive cases were 25 out of 76 (32.9%), and there was seen no relation to frequency of transfusion. Also, no definite relation was seen between the positive cases and type of transfusion reaction.

Test serum diluted 1 : 4 in donor plasma

↓

Diluted serum+citrated normal blood 0.3 ml

↓ 37°C, 60 min

A drop of blood is placed on a slide glass smeared by Indian ink and embedded with paraffin

↓ 37°C, 30 min

The percentage of neutrophils containg phagocytized Indian ink. (The value is compared with controls made by substituting normal human sera for the test serum)

↓

In a typical positive experiment the number of phagocytized neutrophils should be at least 50% less in test than in control sera.

FIG. 11. Inhibition of leukocyte phagocytic activity

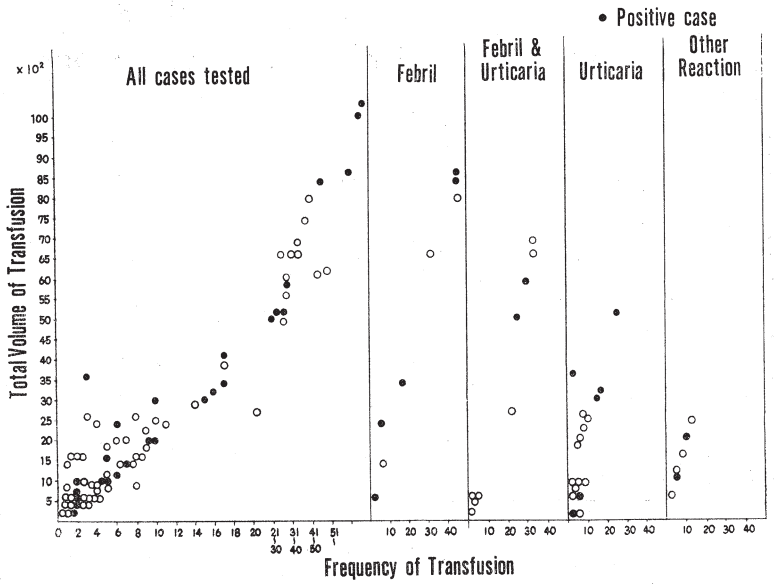

FIG. 12. Leukocyte phagocytosis inhibiting factor

4) Leucoprecipitins

The technique is shown in Fig. 13.
Results: All 202 cases were negative.

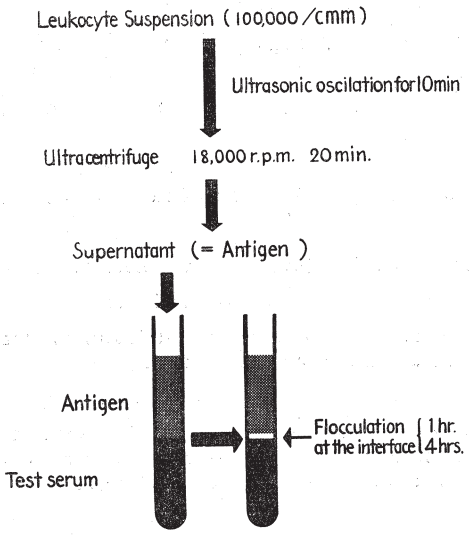

FIG. 13. Leukoprecipitins test

C) Correlation between the various types of leuko-antibodies

Table 4 shows the correlation of positive rates when two each of the leuco-antibodies were compared. There was seen a most prominent correlation between leuko-agglitinins and the complement fixation reaction, followed by

TABLE 4. Correlation between the Various Types of Leuco-Antibodies

	Cases	Correlation coefficiency	x^2 test
Agglutinins and complement fixing antibodies	44	0.755	9.67
Agglutinins and AHG consumption test	71	0.624	7.24
Complentent fixing antibodies and AHG consumption test	36	0.602	1.81
Agglutinins and phagocytosis inhibiting factor	86	0.517	4.57
AHG consumption test and phagocytosis inhibiting factor	05	0.400	1.37
Complement fixing antibodies and phagocytosis inhibiting factor	40	0.385	0.03

leuko-agglutinins and the AHG consumption test.

Correlation was lowest between phagocytosis inhibition factor and complement fixation reaction.

A case is described of severe post-transfusion febrile reaction believed to be due to the presence of leuko-antibodies (Fig. 14).

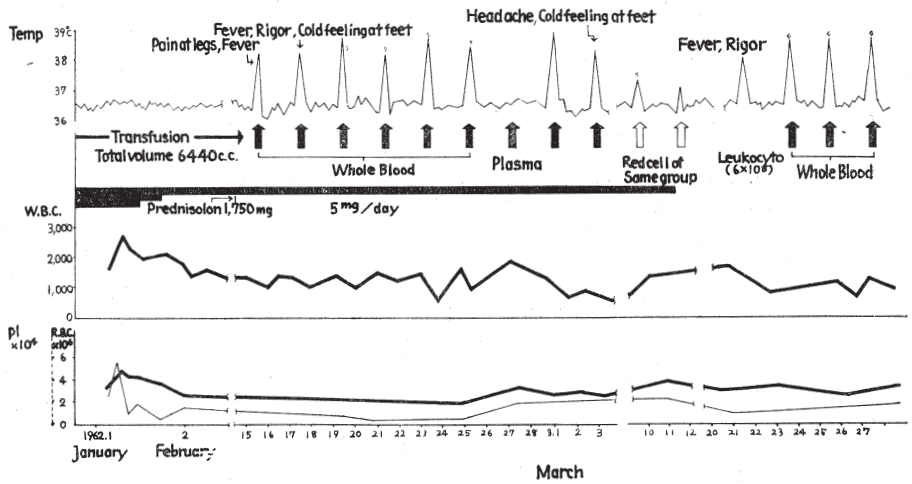

FIG. 14. A case with severe post-transfusion febrile reaction due to the occurance of leukoantibodies

Case of aplastic anemia; Age: 50 years; male.

Since November 1961, received 34 transfusions of blood of the same group prepared by siliconiged apparatus with total volume of 6,440 ml. In February 5, 1962, the 35th transfusion was made when rigor followed by fever of 38.2°C resulted after the injection of 20 ml. of blood. Since then, rigor and a fever of 38 2°～39.1°C was constantly seen following each of the 8 transfusions made. In each case, a few minutes after the commencement of the transfusion there

occurred flushing of the face, tachycardia, and 30 minutes after the end of the transfusion, rigor accompanied by high fever which remained constant for 4 to 5 hours occurred and fell gradually thereafter.

In February 27, plasma obtained from the same group of blood was injected intravenously, when fever and other side-effects failed to occur.

Again, no reaction resulted following transfusion 2 times of red cell suspension only after removing the leukocytes (on March 4 and 12).

Next, a suspension of leucocytes (cell count $6\times10^8/0$) was injected intravenously when a typical febrile reaction occurred (March 22). The immunohematological studies of the patient's serum are indicated in Table 5. It will be seen that the results were entirely negative with both red cell and platelet series, and leuco-agglutinins, AHG consumption tests and phogacytosis inhibition factor were all positive.

Based on the above findings, it was presumed that this case was one that presented violent febrile reaction due to leuco-antibodes.

TABLE 5. Immunohematological Examination of the Reported Case

I. Leukocyte Antibodies

Leukocyte antibodies	Results
Agglutinins	(+)
AHG test	(+)
Conplement fixing antibodies	±
Phagocytosis inhibiting factor	+
Precipitins	−
Antiplatelet antibodies	−

II. Blood group and Rh type B group -CcDEe

III. Direct Coom's test (−)

IV. Indirect Coom's test

Blood group for sensitization	Serum method		Coomb's method
	Prompt	Heated	
O-CeDEeMLe^a(+)K(+)	(−)	(−)	(−)
O-CCDeeMNLe^a(−)K(+)	(−)	(−)	(−)
O-ccDEENLe^a(+)K(+)	(−)	(−)	(−)
O-CcDeeNLe^a(−)K(+)	(−)	(−)	(−)
O-Ccdee	(−)	(−)	(−)
O-ccdEe	(−)	(−)	(−)
Patient own B-CcDEe	(−)	(−)	(−)
Anti D sensitized O-CcDEe	(−)	(+)	(卌)

II. EXPERIMENTAL STUDIES ON LEUCOCYTE ANTIGENS AND ANTIBODIES

1) Antigenic differences and similarities of different parts of leukocytes

a) Method: Leucocytes obtained from patient with chronic myelogenous leukemia were subjected to fractionation of leucocyte, by the technique illustrated in Fig. 15. The nucleus, mitochondria, neutrophilic granules, microsomes and supernatant protein component fractions obtained were used as antigens. The purity of each of these cellular fractions was confirmed by

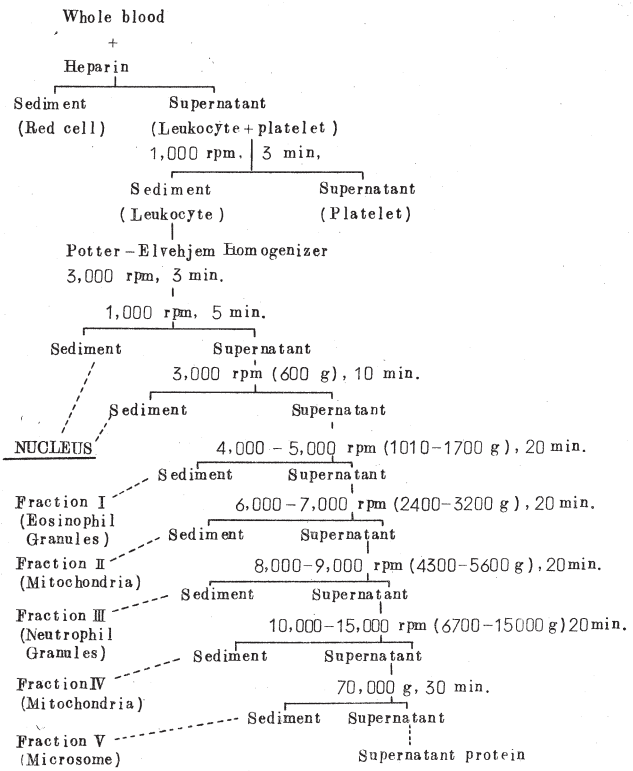

FIG. 15. Procedure for fractionation of leukocyte

electron-microscopy. Next, in order to compare accurately the grades of antigenicity of each of these fractions, estimations of the nitrogen content were made by the indophenol micromethod, and the weight ratios of nitrogen are shown in Table 6. Based on the above the amount of antigen for immunization was corrected.

TABLE 6. Ntrogen Ratio in Fractions of Leukocyte
(By indophenol method)

	γ/0.05 g	Weight ratio/N
Nucleus	110.0	1.84
Mitochondria	251.0	3.86
Neutrophilic granules	65.0	1.00
Microsome	71.7	1.10
Supernatant protein	199.9	3.06

Each of the fractions were injected intravenously into rabbits 4 times ordinarily at interbals of 5 days. On the 5th day following the last injection blood was collected and immune sera were obtained.

Results: Table 7 shows the titers of leuco-agglutinins of each of the hyper-

TABLE 7. Leuko-agglutinins of the Hyperimmune Rabbit Sera against the Fraction of Human Leukocytes

Test sera		Titers								
		1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	1 : 2048
Nucleus	#1	+	±	0	0	0	0	0	0	0
	#2	+	+	±	0	0	0	0	0	0
Mitochondria	#1	###	###	###	###	###	##	+	±	0
	#2	###	###	###	##	##	##	+	±	0
Neutrophilic granules	#1	###	###	##	##	+	±	0	0	0
	#2	###	###	###	##	##	##	+	±	0
Microsome	#1	###	###	##	##	+	±	0	0	0
	#2	###	##	##	+	±	0	0	0	0
Supernatant fraction	#1	###	##	##	+	+	+	0	0	0
	#2	###	###	##	##	+	+	±	0	0
Normal rabbit sera	#1	−	0	0	0	0	0	0	0	0
	2	−	0	0	0	0	0	0	0	0
	3	−	0	0	0	0	0	0	0	0
	4	−	0	0	0	0	0	0	0	0
	5	−	0	0	0	0	0	0	0	0
	6	−	0	0	0	0	0	0	0	0

immune rabbit sera against the various cellular fractions. It will be seen that the agglutinin titer against fraction is extremely low. Contrary to this the cytoplasmic fractions showed high agglitinintiters, the maximum of the mitochondrial fraction being 1024×, of the neutrophile granular 512×, of the microsome 256×, and of the supernatant protein component 1024×. A finding calling for attention is the presence of antigenicity in the neutrophilic granule fraction, hither-to unreported.

b) Method: Guinea pig leucocytes were collected by the method indicated in Fig. 16, and the cellular fractions were obtained by the method of fractionation described already. Rabbits were immunized with each of the fractions, and the antisera obtained were examined for the agglutinin titers. Next, 5 ml. of the antisera were injected into guinea pigs in order to induce a granulocytosis, experimentally.

Results: 1) As indicated in Table 8, agglutinins were found in cytoplasmic fractions other than of the nuclei, as in case of human leucocytes already described.

2) Next, antisera for the mitochondria, protein supernatant and neutropile granules were injected each into guinea pigs, and as may be seen from the results indicated in Fig. 17, there resulted a decrease of 70~80% in the number of leucocytes 1 to 2 hours after the injection. Recovery however, was seen thereafter, and the normal value was regained at the end of 72 to 96 hours.

Next, 30 minutes before the injection into guinea pigs of anti-sera against guinea pig leucocytes, 6 mg./kg. and 12 mg./kg., of prednisolone, 30 mg./kg. of folic acid and 5 mg./kg. of glycyllithin were injected, and as control, physiologic

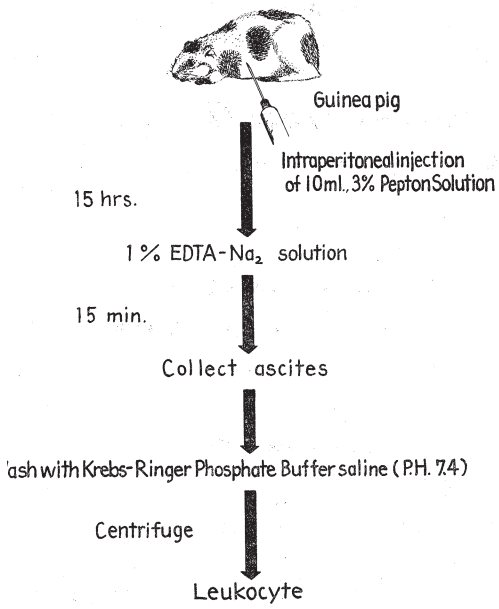

FIG. 16. Collection of guinea pig leukocytes

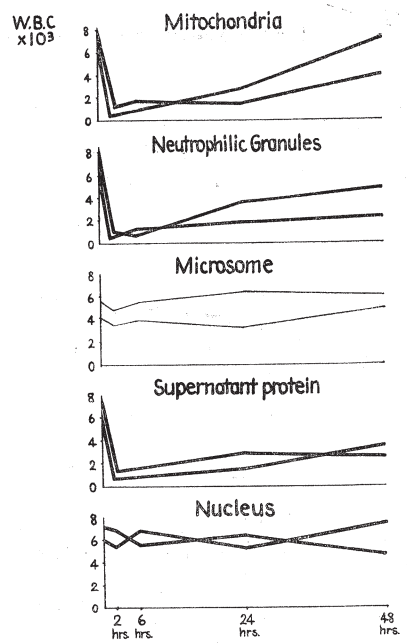

FIG. 17. Leukopenic action of hyperimmune rabbit sera against the fraction of guinea pig leukocytes

TABLE 8. Leuko-agglutinins of the Hyperimmune Rabbit Sera Against the Fractions of Guinea Pig Leukocytes

Test sera	Titers								
	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	1 : 2048
Nucleus	0 +	0 ±	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Mitochondria	### ###	### ###	### ##	### ##	## ##	## +	+ ±	± 0	— 0
Neutrophilic granules	### ###	### ###	## ##	## ##	+ ##	± +	— ±	0 0	0 0
Microsome	### ###	### ##	## ##	## +	+ +	± —	— —	0 0	0 0
Supernatant fraction	### ###	### ###	### ##	## ##	## +	## ±	+ —	± 0	— 0
Normal rabbit sera	—	0	0	0	0	0	0	0	0

saline was injected. Following the above, the hyperimmune rabbit serum was injected, when as is shown in Fig. 18, there was seen a prominent decrease of leucocytes, especially disappearance of neutrophiles in the control group, but

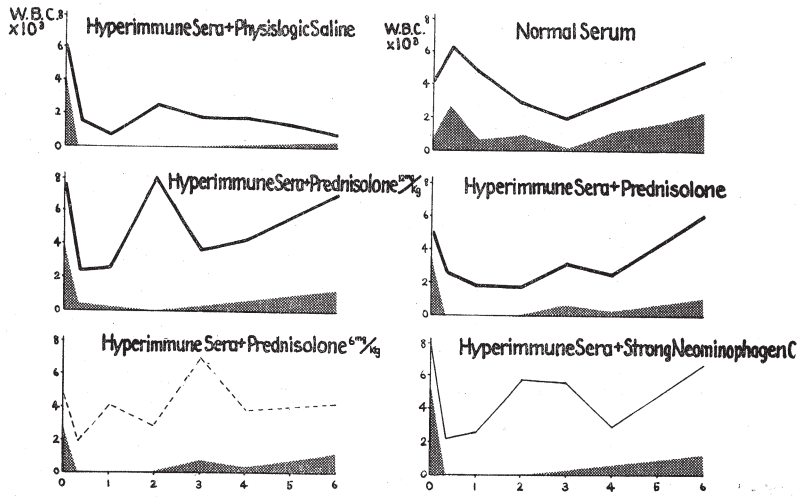

FIG. 18. Effects of various drugs on leukopenic action of hyperimmune rabbit sera against guinea pig leukocyte

in the group given the various drugs the decrease in leucocytes and loss of neutrophiles rapidly recovered.

3) *Leukocyte antibodies and homograft reaction*

Experimental method: i) Fig. 19 shows the method of transfer of lymph node cells according to the technique of Haris and Harris.

ii) A definite amount (2 mg. dry weight) of shigellaza was injected into the hind foot pad of a rabbit. On the 4th day the sensitized local lymph node was excised, and a suspension of the lymph node cells was injected intravenously into a different rabbit, and the bacterial agglutinin titers were estimated according to passage of time.

iii) Rabbits were immunized by leucocytes with Freund's adjuvant (the leucocytes were obtained by introduction of 1% NaCl solution intraperitoneally into rabbits). Next, at intervals of 5 days the suspension of lymph node cells of (ii) was injected 4 times intravenously into the rabbit and the bacterial agglutinin titers were estimated according to passage of time. By the above the effects of leucocyte antibodies on lymph node cells were examined.

Results: i) In Fig. 20, are shown the distribution of the bacterial agglutinin titers of rabbits injected with 300 million shigella sensitized lymph node cells, and the geometric mean curve.

As reported by Harris and Harris, it will be seen that agglutinin titers began to rise from the day of injection, with a relatively acute rise up to the 5th or 6th day, followed by a gradual fall thereafter and disappeared usually on about the 30th day.

In Fig. 21 are shown the results of agglutiniation tests following the injection of lymph node cells sensitized with shigella bacilli into rabbits previously sensitized by 100 million, 10 million and 1 million rabbit leucocytes.

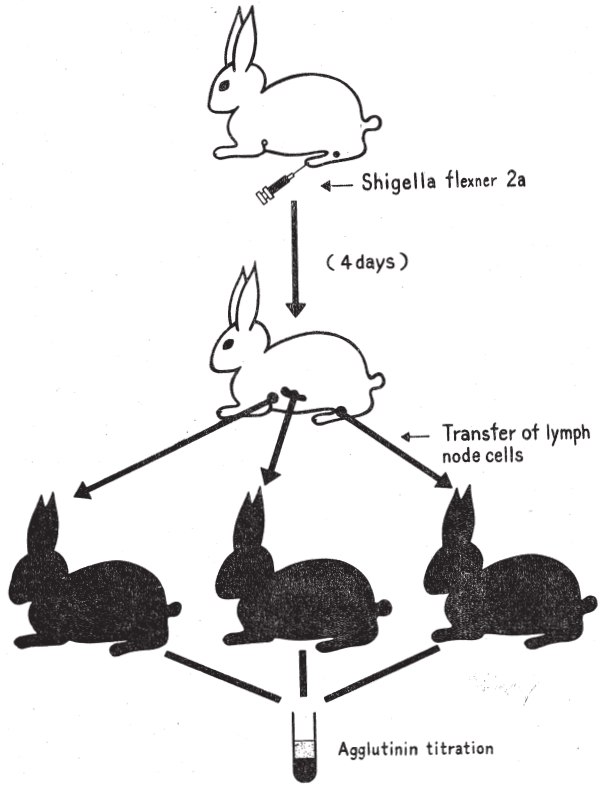
Shigella flexner 2a
(4 days)
Transfer of lymph node cells
Agglutinin titration

FIG. 19.

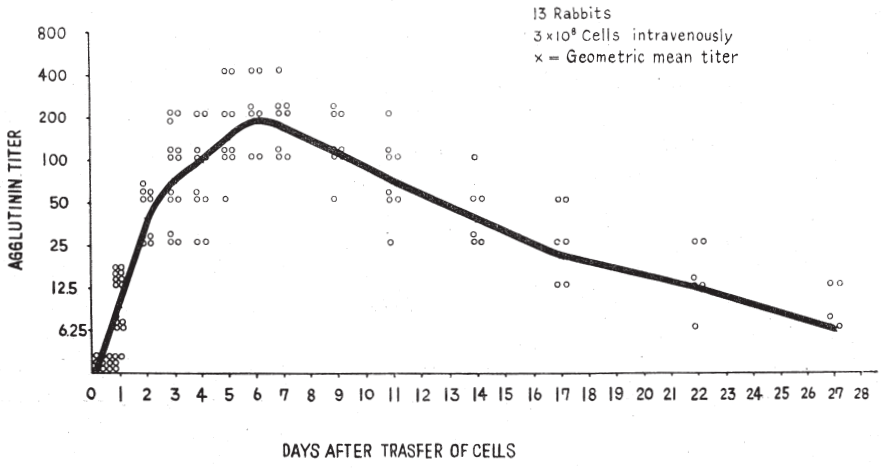
13 Rabbits
3×10⁸ Cells intravenously
× = Geometric mean titer
AGGLUTININ TITER
800
400
200
100
50
25
12.5
6.25
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
DAYS AFTER TRASFER OF CELLS

FIG. 20

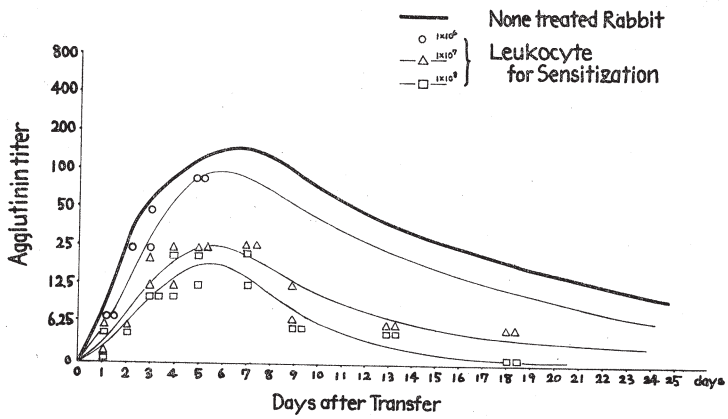

FIG. 21. Serum titers of leukocyete sensitized recipient of lymphnode cells obtained from donors 4 days after injection of shigella 2 a

In rabbits immunized with 1 million leucocytes there was seen a low grade inhibition of agglutinin titer when compared with the non-immunized control group but in the 10 million group there was seen a maximum inhibition of 25 times, and in the 100 million leucocyte sensitized group the inhibitory effect was further enhanced.

From the data obtained above, leucocyte antibodies are not necessarily specific to leucocytes, *i.e.* they are not tissue specific, but show the presence of reaction with lymph node cells, a finding that may be said to be highly interesting.

Conclusions: 1) Examinations were made on leuco-agglutinins, anti-human globulin consumption test, complement fixing antileucocyte antibodies, phagocytosis-inhibition factor and leucopreciptins in cases with histories of blood transfusion.

2) The occurence of leuco-agglutinins rises in proportion to number of transfusions. It is not influenced much by the total volume of transfusion.

3) Of the transfusion reactions, there seems to exist a relation between febrile reaction and leuco-agglutinins.

4) The frequency of appearance of other leucocyte antibodies seems to be less related to both total volume and frequency of transfusion, as well as to transfusion reaction than leuko-agglutinins.

5) Leucocyte antigens were extremely rare in the nuclear fraction. They were abundant in all fractions of the cytoplasm, while antigenicity was demonstrated in neutrophile granules.

6) Leucocyte iso-antibodies are not necessarily specific to leucocytes themselves, and evidence was obtained that reaction occurs with lymph node cells.

REFERENCES

1. BRAUNSTEINER, H.: *Wien Ztschr. f. Innere Med.* **35**: 470, 1954.
2. BUTLER, J.: *J. Clin. Invest.* **35**: 1150, 1956.
3. BUTZENGEIGER, K. H. AND GARTZ, M.: *Klin. Wchnschr.* **58**: 495, 1950.
4. BUTZENGEIGER, K. H.: *La Semoine des Hop.* **31**: 141, 1955.
5. DAUSSET, J.: *Vox Sang.* **4**: 190, 1954.
6. DAUSSET, J.: *Le Sang* **25**: 683, 1954.
7. DAUSSET, J.: *Compte. Rend. Soc. de Biol.* **148**: 41, 1954.
8. DAUSSET, J., NENNA, A. AND H. BRECY.: *Blood* **9**: 696, 1954.
9. DAUSSET, J., QUIJADA, H. AND H. BRECY. *Sixth Internat. Cong., Internat. Soc. Hem.*, Aug. 27-Sept. 1, 1956, p. 493.
10. DOAN, C. A.: *J.A.M.A.* **86**: 1593, 1926.
11. FINCH, S. C., J. F., J. F. ROSS AND F. G. EBAUGH.: *J. Lab. and Clin. Med.* **42**: 555, 1953.
12. FLECK, L.: *Acta haemat.* **8**: 282, 1952.
13. FRANCKE, E.: *Klin. Wchnschr.* **19**: 1053, 1940.
14. COUDSCHMIT, R., AND VAN J. J. LOGHEM.: *Vox Saug.* **3**: 58, 1953.
15. GOUDSCHMIT, R. AND VAN J. J. LOGHEM.: *Vox Saug.* **3**: 89, 1953.
16. JANDL, J. H. AND W. B. CASTLE.: *J. Lab. and Clin. Med.* **47**: 669, 1956.
17. LAPIN, J. AND A. HORONICK.: *Blood* **11**: 225, 1956.
18. MATOTH, Y., E. ELIAN, D. NELKEN AND A. C. NEVO.: *Blood* **11**: 735, 1956.
19. MATSUNO, M.: *Tohoku J. Exper. Med.* **19**: 168, 1932.
20. MEDAWAR, P. B.: *Brit. J. Exper. Path.* **27**: 15, 1946.
21. MIDDLEBROOK, G. AND R. J. DUBOS.: *J. Exper. Med.* 88: 521, 1948.
22. MIESCHER, P.: *Sixth Internat. Cong., Internat. Soc. Hem.*, Aug. 27-Sept. 1, 1956, p. 495.
23. MIESCHER, P.: AND M. FAUCONNET.: *Schweiz. med. Wchnschr.* **84**: 1036, 1954.
24. MINOR, A. H. AND L. BURNETT.: *Blood* **7**: 693, 1952.
25. MOESCHLIN, S.: *Le Sang* **26**: 32, 1955.
26. MULLER, W. AND J. WEINREICH.: *Klin. Wchnschr.* **34**: 505, 1956.
27. PALMER, J. W. AND T. D. GERLOUGH.: *Science* **93**: 155, 1940.
28. PIROFSKY, B.: *J. Lab. and Clin. Med.* **48**: 745, 1956.
29. RIIS, P.: *Acta Haemat.* **14**: 302, 1955.
30. SELIGMAN, M., P. GRABAR AND J. BERNARD.: *Vox Sang* **4**: 181, 1954.
31. SELIGMAN AND P. GRABAR.: *La Presse Med.* **62**: 1700, 1954.
32. SKOOG, W. A. AND W. S. BECK.: *Blood* **11**: 436, 1959.
33. STERNBERGER, L. A., S. M. FEINBERG AND M. E. CLARKE.: *J. Exper, Med.* **103**: 523, 1956.
34. TAKIKAWA, T., T. ITO, J. KATO, T. YOSHIDA, H. KONDO AND I. MIYATA: *Acta. Haemat.* **18**: 179, 1957.
35. THOMPSON, J. S. *Blood* **10**: 1228, 1955.
36. TULLIS, J. L. New York, Academic Press Inc., 1953.
37. VALLEE, B. L., W. JR. HUGHES AND J.: BIBSON.: *Blood*, SpecialIssue No. **1**: 82, 1947.
38. WALFORD, R. L.: *Clin. Res. Proc.* **5**: 55, 1957.
39. WASASTJERNA, C.: *Scand. J. Clinand Lac. Invest.* 8: 14, 1956.
40. WEINREICH, J. AND W. MULLER.: *Actahaemat.* **16**: 376, 1956.
41. WILSON, M. W. AND B. H. PRINGLE. *J.*: *Immunol.* **73**: 232, 1954.