FORMALINIZED RED CELLS IN DIAGNOSTIC VIROLOGY

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DIFFICULTY in obtaining a constant supply of fresh red blood cells for daily hemagglutinating (HA) tests has been a major inconvenience in many laboratories. Methods of preserving red cells have been devised, but none seem as effective as the formalin treatment suggested by Flick (*1*), Gold and associates (*2*), and others. Formalinized red cells have been used in both the indirect and direct hemagglutination tests. Daniel and associates (*3*) and Sell (*4*) have shown that tanned, formalinized cells sensitize with a protein antigen agglutinate in the presence of specific antiserums. The Waaler-Rose test for rheumatoid arthritis was modified by Milgrom and associates (*5*) by using formalinized sheep erythrocytes in place of fresh cells. Since this method of preserving red cells was first described, its potential for use in virus identification has been demonstrated by several investigators (*1*, *6–8*). The present study was undertaken to determine if formalinized red blood cells from four animal species and three groups of human cells were as sensitive in the viral HA test as were fresh cells from the same animal and human sources.

Materials and Methods

The type, strain designation, and source of the viruses tested in this study are summarized in table 1. Human red blood cells, groups A, B, and O, collected in acid-citrate-dextrose, were obtained from the U.S. Naval Hospital blood bank, Great Lakes, Ill. Sheep, rat, chicken, and monkey cells were collected in Alsever's solution. All cells were formalinized within 3 to 5 days after collection. Several lots of each of the different red blood cells were tested. The fresh cells were at least 3 days old when tested.

Formalinization of the cells was performed according to the method suggested by Gold and associates (*2*) with the following modifications. Equal volumes of unwashed red cells and formalin-citrate-saline solution (29 volumes of 5 percent tri-sodium citrate in 0.9 percent saline and 1 volume of 36 percent weight-per-volume formalin added before use) were mixed together and incubated overnight at 37° C. After incubation, the cells were washed five times in distilled water, five times in physiological saline (0.85 percent), and then resuspended to a 10 percent suspension in physiological saline. Each day, cells for use in the hemagglutinating test were diluted to an 0.8 percent suspension in 1 percent normal rabbit serum saline (*3*). Before use, the normal rabbit serum was absorbed for 30 minutes at 37° C. with the cells to be diluted. As suggested by Daniels and associates (*3*), one-half percent bovine serum albumin (Dade Reagents, Inc.) in saline was also used as a diluent. If the 10 percent suspension of formalinized cells was stored longer than 2 weeks before diluting, it was necessary to resuspend the cells in a blender for 3 to 5 minutes to eliminate nonspecific agglutination. Since these cells were resistant to lysis by physical methods, resuspending them in a blender causes little, if any, hemolysis of the cells.

Sample portions of each cell type were stored at 4° C. and tested periodically with aliquots of influenza A_2, B, and adenovirus type 9 to see if any loss of hemagglutinating ability did occur. Human and monkey cells were stored

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for 6 months and rat and chicken cells, for 5 months.

The HA tests were performed in plexiglas microplates (Cooke Engineering Co.) as recommended by Sever (*9*). One percent normal rabbit serum or 0.5 percent bovine serum albumin in saline was used as the diluent. All the viral HA tests were incubated at room temperature except the influenza C tests which were incubated at 4° C.

Normal tissue culture and allantoic fluids were included with each series of tests. The

Table 1. Type, strain designation, and source of viruses used in the hemagglutination tests

Type of virus	Strain and source	Preparation
Myxoviruses:		
Influenza A	Swine/1976/31—CDC	Allantoic fluid.
Influenza A1	FM1/47—CDC	Do.
Influenza A2	Jap/305/57—CDC	Do.
Influenza B	GL/1739/54—GL	Do.
Influenza C	GL/1167/54—GL	Amniotic fluid.
Parainfluenza 1	C–43—MBA	Monkey kidney tissue culture.
Parainfluenza 2	Greer (University of Chicago)	Do.
Parainfluenza 3	C–35—ATCC	Do.
Parainfluenza 1 (Sendai)	CDC	Allantoic fluid.
Newcastle disease virus	Roakin (University of Wisconsin)	Do.
Mumps	Enders—ATCC	Do.
Reoviruses:		
I	MBA	Monkey kidney tissue culture.
II	MBA	Do.
III	MBA	Do.
Herpes simplex	MK/HF–378—ATCC	H.Ep-2 tissue culture.
Measles	Edmonton, Davenport (University of Michigan).	KB tissue culture.
ECHO 28	2060—GL	Do.
Respiratory synctial virus	Long (University of Chicago)	H.EP–2 tissue culture.
Adenovirus 1–10, 14	ATCC	KB tissue culture.

CDC—Communicable Disease Center, Public Health Service, Atlanta, Ga.; GL—Naval Medical Research Unit No. 4, Great Lakes, Ill.; MBA—Microbiological Associates, Bethesda, Md.; ATCC—American Type Culture Collection, Washington, D.C.

Table 2. Results of hemagglutination tests with myxoviruses using 6 types of fresh and formalinized red blood cells

Viruses	Human cells						Rat cells		Monkey cells		Chicken cells	
	Blood group A		Blood group B		Blood group O							
	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized
Influenza A	256	256	32	16	256	256	64	128	—	—	128	128
Influenza A1	128	64	32	32	512	256	16	128	—	—	512	512
Influenza A2	64	64	64	64	64	128	32	64	64	64	128	64
Influenza B	256	128	32	64	128	64	32	64	64	64	256	256
Influenza C	256	[1]	64	—	256	—	1, 024	1, 024	64	—	512	—
Parainfluenza 1	128	128	64	16	64	64	32	16	64	64	64	—
Parainfluenza 2	16	8	16	—	16	16	8	—	32	32	16	—
Parainfluenza 3	16	16	16	16	32	16	8	8	16	8	32	—
Parainfluenza 1 (Sendai)	1, 024	512	8	16	1, 024	1, 024	64	64	512	256	32	64
Newcastle Disease	128	64	16	16	32	8	16	—	—	—	64	64
Mumps	32	32	8	8	128	64	[1]	—	64	64	64	32

[1] Less than 1:8 virus dilution.

NOTE: Results are expressed as reciprocal of virus dilutions. Minus signs indicate negative results.

Table 3. Results of hemagglutination tests with adenoviruses using 6 types of fresh and formalinized red blood cells

Adenovirus	Human cells						Rat cells		Monkey cells		Chicken cells	
	Blood group A		Blood group B		Blood group O							
	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized	Fresh	Formalinized
1	(1)	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	8	8	—	—	—	—
3	—	—	—	—	—	—	—	—	32	—	—	—
4	—	—	—	—	—	—	16	8	—	—	—	—
5	—	—	—	—	—	—	16	16	—	—	—	—
6	—	—	—	—	—	—	8	8	—	—	—	—
7	—	—	—	—	—	—	—	—	16	—	—	—
8	128	256	16	16	128	64	1, 024	1, 024	—	—	—	—
9	128	256	32	64	512	512	128	128	—	—	8	8
10	16	16	8	—	32	32	128	128	—	—	—	—
14	—	—	—	—	—	—	8	—	8	—	—	—

[1] >1: 8 virus dilution.

NOTE: Results are expressed as reciprocal of virus dilutions. Minus signs indicate negative results.

fresh and formalinized cells were tested at the same time, using the same aliquot of virus. Cell controls for each type of cell were also included.

Results

The formalinized cells generally reacted with the myxoviruses to the same titer or one dilution lower when compared to the fresh control cells (table 2). A notable exception was influenza C, which hemagglutinated formalinized rat cells and none of the other formalinized cell types. A second exception was the lack of reaction between formalinized chicken cells and the three parainfluenza viruses. The Sendai strain of parainfluenza 1 virus did agglutinate formalinized chicken cells.

Adenoviruses types 2, 4, 5, and 6 hemagglutinated only fresh and formalinized rat cells (table 3). Fresh monkey cells showed a reaction with adenovirus types 3 and 7, while type 14 hemagglutinated both fresh rat and monkey cells. Adenovirus types 8, 9, and 10 demonstrated a reaction with fresh and treated human and rat cells. Type 9 was the only adenovirus that agglutinated both treated and untreated chicken cells.

Among a miscellaneous group of viruses, only reoviruses hemagglutinated fresh human cells. Since the three reoviruses did not hemagglutinate either fresh or treated animal cells, these results are not included in table 4. The reoviruses reacted with formalinized group O cells, but not with group B cells. Reovirus I and II also agglutinated treated group A cells. Herpes simplex, measles, ECHO 28, respiratory synctial virus, normal tissue culture, and allantoic fluid controls did not hemagglutinate either human or animal cells. Neither

Table 4. Results of hemagglutination tests with reoviruses, using fresh and formalinized human erythrocytes

Erythrocytes	Reovirus I	Reovirus II	Reovirus III
Blood group A:			
Fresh	64	32	16
Formalinized	32	16	—
Blood group B:			
Fresh	32	32	32
Formalinized	—	—	—
Blood group O:			
Fresh	16	32	32
Formalinized	64	32	32

NOTE: Results are expressed as reciprocal of virus dilutions. Minus signs indicate negative results.

Table 5. Viral hemagglutination titers of stored formalinized erythrocytes

Storage time (months)	Human cells									Rat cells			Monkey cells			Chicken cells		
	Blood group A			Blood group B			Blood group O											
	A2	B	9	A2	B	9	A2	B	9	A2	B	9	A2	B	9	A2	B	9
0	64	128	256	64	64	64	128	64	512	64	64	128	64	64	—	64	128	8
1	64	64	256	64	64	64	128	64	512	64	128	128	64	64	—	64	128	8
3	64	128	128	64	128	64	64	64	256	128	64	128	64	128	—	64	256	8
5	----	----	----	----	----	----	----	----	----	64	64	128	----	----	----	128	256	8
6	64	128	256	64	64	64	128	128	256	----	----	----	64	64	—	----	----	----

NOTE: A2—influenza A2, B—influenza B, and 9—adenovirus type 9. Results are expressed as reciprocal of virus dilutions. Minus signs indicate negative results; leaders (----) indicate not tested.

fresh nor formalinized sheep cells were agglutinated by any of the viruses tested.

After 5 months' storage of formalinized rat and chicken erythrocytes and 6 months' storage of formalinized human and monkey cells, little, if any, loss of hemagglutinating activity was observed. These results are presented in table 5.

It was observed that normal rabbit serum from several lots contained small amounts of nonspecific hemagglutination inhibitors to myxoviruses, as has been previously reported (*10*). If necessary, this inhibitor could be removed by treating the serum with potassium periodate. One percent normal rabbit serum in saline did not, however, demonstrate any inhibition.

Discussion

The results indicated that use of formalinized red cells was feasible in presumptive identification of some myxoviruses, adenoviruses, and perhaps other groups of hemagglutinating viruses. Differences in agglutinating ability among the human and animal cells were constant in tests of cells from three to five donors.

The storage of formalinized cell types did not appear to be a problem. Human and monkey cells were stored up to 6 months at 4° C. and rat and chicken cells, up to 5 months with no discernible loss of reactivity.

The mechanism by which formalin treatment changes the agglutinating ability of a red cell for certain viruses is not presently known (*3*). Moskowitz and Cobb have shown, however, that formalinized blood group A and B erythrocytes will not agglutinate with homologous antibody, but will absorb anti-A or anti-B from this serum (*11*).

It has been reported that formalinized human O cells cannot be used in the hemagglutination test because of nonspecific reactions (*6*). Our work has shown that if 1 percent normal rabbit serum in saline or one-half percent bovine serum albumin in saline was used as the diluent and the cells were thoroughly resuspended in a blender, this nonspecific agglutination did not occur. It must be emphasized, however, that the cells were thoroughly suspended before they were diluted.

Summary

Hemagglutination tests with 29 viruses using seven types of fresh and formalinized red blood cells of human and animal origin showed that human and animal cells stored for 5 to 6 months could be employed provided proper precautions were observed. Influenza, parainfluenza, Newcastle disease, and mumps viruses, as well as adenoviruses, reoviruses, and a miscellaneous group, were tested on human cells of blood groups A, O, and B and on cells of rats, monkeys, chickens, and sheep.

Use of fresh and formalinized red cells in a presumptive identification of certain hemagglutinating viruses now appears to be feasible.

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Employment of Psychiatrists

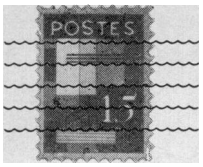
Almost half the psychiatrists in America are employed either full time or part time in Federal, State, or local government agencies and private organizations, according to a recent survey by the National Institute of Mental Health, Public Health Service, in cooperation with the American Psychiatric Association.

Eighty-eight percent of the 18,740 psychiatrists reported information about themselves. Slightly more than half of all psychiatrists are engaged in private practice, but even of those who are self-employed, only 40 percent are in full-time private practice. The others combine private practice with teaching, administration, consultation, or research.

The survey pinpointed several manpower shortages in psychiatry. Only 9 percent, or 1,346, of the respondents specialize in child psychiatry, and only 144 consider mental retardation their main field of interest.

Psychiatrists gravitate toward big cities. Nearly one-third practice in cities of 3 million or more where only 17 percent of the population is located. More than half of these specialists are found in five States: 21 percent in New York, 13 percent in California, 6 percent each in Pennsylvania and Massachusetts, and 5 percent in Illinois.

The complete report, a major activity of a 5-year manpower study on psychiatrists, will be published in mid-1966 by the National Institute of Mental Health.



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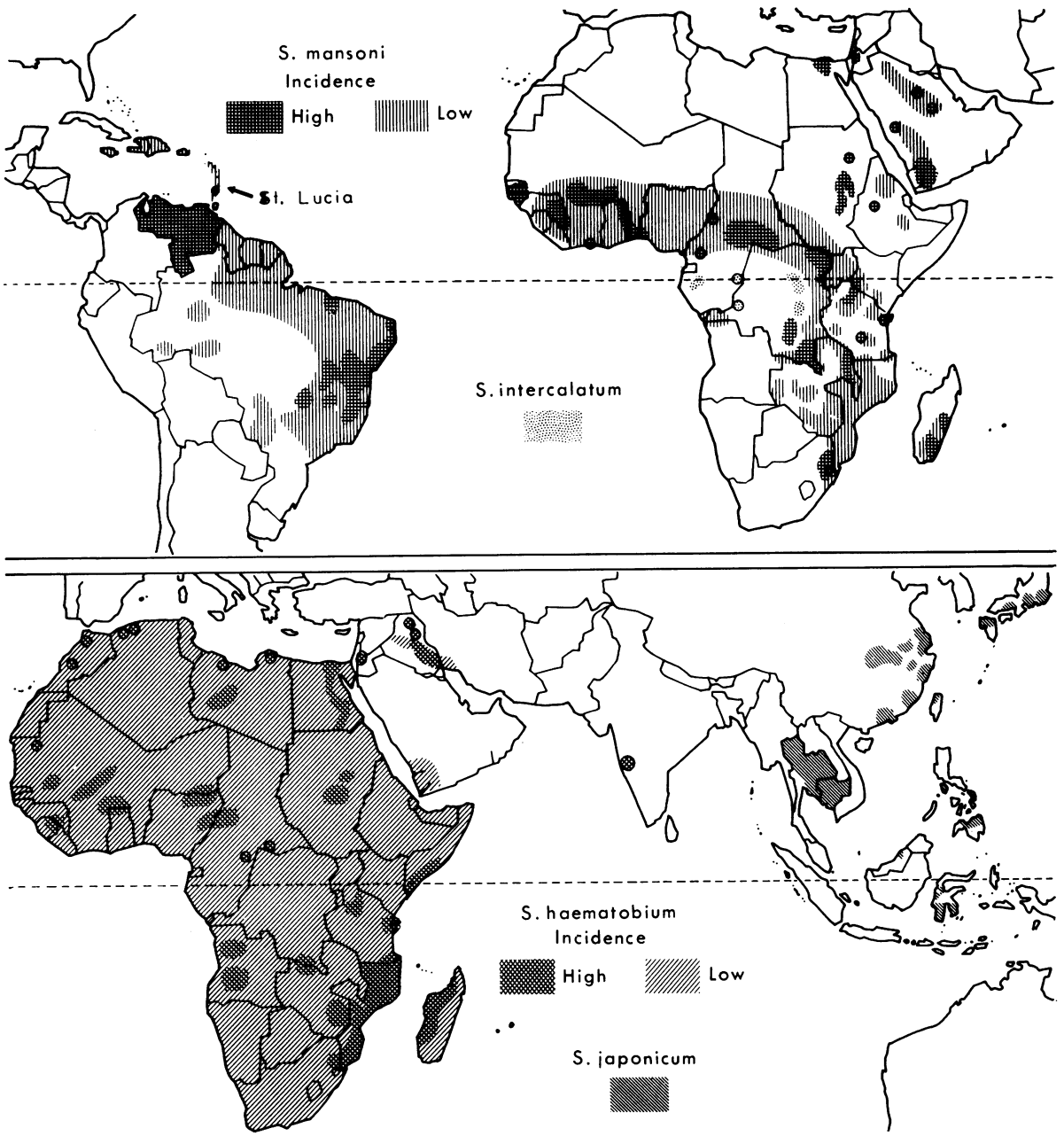

World distribution of species of schistosomes

SOURCE: This map was adapted from WHO Chronicle 13: 2, January 1959.

Control of Schistosomiasis

The Rockefeller Foundation will undertake field studies in St. Lucia, West Indies, aimed at the control of schistosomiasis. In its several forms, schistosomiasis is estimated to affect 200 million or more people in Africa, South America, the Caribbean islands, and the Near and Far East. An occupational disease of the rural poor, it constitutes a major obstacle to increased food production in many parts of the world.

St. Lucia in the Windward Islands, part of a chain that stretches from Puerto Rico (see map), has been chosen as the site for studies which may lead to measures for controlling the disease and for training personnel in research and control techniques. It is an ideal island laboratory for a substantial pilot project where current knowledge and techniques about treatment, snail eradication, and sanitation controls can be demonstrated.

St. Lucia's isolated river valleys, each forming a virtually untouched ecological entity, create sharply definable areas almost perfectly suited to experimental and operational work under controllable conditions. Almost all of the island's 100,000 people and their cattle are exposed to schistosomiasis, yet St. Lucia, with an area of 233 square miles, is sufficiently compact so that the effects of control measures can be calculated.

The Foundation and the government of St. Lucia have allocated funds and assigned trained personnel to start the cooperative project. The island government will supply land, labor, and subprofessional personnel. Professional specialists and funds for importing necessary scientific equipment and constructing a small laboratory will be furnished by the Foundation. The project will take at least 5 years to yield significant results.

The economic loss caused by schistosomiasis is immeasurable. It retards agricultural progress through irrigation projects in many developing areas. For example, in one irrigation project in Rhodesia the disease beeame so severe that the project had to be dropped only 10 years after its inauguration. Since the success of the Foundation's program to produce more food in developing countries will depend upon irrigated crops in areas where schistosomiasis is endemic, the need for control is apparent.

Schistosomes related to those which afflict humans can also parasitize horses and cattle. In Japan and Formosa such animal parasitism today is perhaps more important than human parasitism. In Africa especially, flatworms disseminated like the human schistosomes produce enormous cattle losses through death and through making the meat unfit for human consumption. One of these animal parasites, *Fasciola hepatica,* occurs throughout St. Lucia.

International Cancer Research Agency

A new International Agency for Cancer Research has been established at Lyons, France. Originally sponsored by the governments of France, West Germany, Italy, United Kingdom, and the United States, the agency now includes representatives from Australia and the U.S.S.R.

Its governing council is comprised of emissaries from the agency's five founding nations, and the director-general of the World Health Organization, Dr. M. G. Candau, who also heads the agency secretariat. Prof. Eugene Aujaleu of France is chairman, and Dr. Kenneth M. Endicott, director of the National Cancer Institute, Public Health Service, is the U.S. representative.

The agency is intended to serve as a means through which participating countries and the World Health Organization, in liaison with the International Union Against Cancer and other interested organizations, may cooperate in the stimulation and support of all phases of cancer research. In addition to educating and training cancer research personnel, its permanent program includes collecting and disseminating information about cancer epidemiology research throughout the world. Special projects may encompass pilot demonstrations of cancer prevention activities and support of research at the national level. Although the agency conducts no research, it will make research grants from the $150,000 contributed every year by each of its seven members.

Research projects will be recommended by a 12-member scientific advisory council. Advisers are Prof. G. Klein, Sweden; Dr. D. Metcalf, Australia; Prof. O. Múhlbock, Holland; and Prof. P. N. Wahi, India, who were appointed for 1 year. Designated to serve 2 years were Prof. N. N. Blohin, U.S.S.R.; Dr. W. R. S. Doll, United Kingdom; Prof. B. Kellner, Hungary; and Dr. G. Mathé, France. Prof. I. Berenblum, Israel; Prof. P. Bucalossi, Italy; Prof. H. Hamperl, West Germany; and Dr. A. M. Lilienfeld, United States, have 3-year terms.