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Use of Yeast-phase Antigens in a Complement Fixation Test for Histoplasmosis

III. Preliminary Results With Human Sera

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Recent reports from this laboratory have described a complement fixation test for the detection in immune rabbit sera of antibodies against *Histoplasma capsulatum*. In one study whole yeast-phase organisms were used as antigen (*1*); in the other, ground yeast-phase antigens were employed (*2*). Since ground antigens offered greater stability and clarity, they were employed in the studies with human sera.

In evaluating the complement fixation test for human sera, we were confronted with the following questions:

1. What is the potential value of the test as an aid to the diagnosis of histoplasmosis?
2. Is there any relationship between histoplasmin skin sensitivity and humoral antibodies against the yeast phase of *H. capsulatum*?
3. Does the skin test *per se* affect antibody levels?
4. Do the cross reactions between *H. capsulatum* and *Blastomyces dermatitidis* noted in studies with immune rabbit serum (*1*, *2*, *3*, *4*) and in guinea pigs (*5*, *6*) occur in human material, and, if so, to what extent would they constitute misleading diagnostic criteria?

Materials and Methods

Sera. Sera were classified into three groups. Group I consisted of sera selected at random from specimens submitted for routine Wassermann and Kahn tests for which no skin sensitivity data were available. Group II sera were obtained from normal volunteers immediately

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prior to and 3 weeks after skin-testing with histoplasmin. All sera in group III were from skin-test-positive persons with proved cases of histoplasmosis. Antibody titrations were made within 24 hours of receipt of the sera, which were inactivated at 56° C. for 30 minutes before use.

Skin tests. Tests and readings were made in accordance with the directive issued with lot H–40 histoplasmin.[1] Each of the volunteers in Group II received an intracutaneous injection (on the anterior surface of the forearm) of 0.1 ml. of a 1:1,000 dilution of test material. Readings were made 48 hours later and reactions showing induration or edema measuring 5 mm. or more in diameter were considered positive.

Complement fixation test. The complement fixation test was conducted as described in our original report (*2*), except for the modifications noted below.

Antigens. Optimal dilutions of ground histoplasma antigens were determined by titration in the presence of strong positive serum obtained from persons in the acute phase of histoplasmosis. This antigen was prepared from the G-2 [2] strain of *H. capsulatum*. In cross-reaction studies the A-5 [3] strain of *B. dermatitidis* was employed as antigen. The optimal dilution for this antigen was determined by titration in the presence of serum from a proved case of systemic blastomycosis.

Incubation. For comparative purposes, 4- and 18-hour periods of incubation at 3° to 6° C. were allowed for complement fixation in the first experiment. For reasons described below, all subsequent tests were incubated for the shorter period.

Results

Group I. In this experiment, 316 sera which had been submitted for routine Kahn and Wassermann tests were examined for antibody content. The sera were incubated for 18 hours at 3° to 6° C. Eighty-five (26.9 percent) of these specimens fixed complement in serum dilutions varying from 1:10 to 1:40. Thirty-eight (12 percent) of the sera fixed complement through serum dilutions of 1:10; 35 (11 percent) through 1:20; and 12 (3.8 percent) through 1:40. This number of "positives" in "normal" sera seemed rather high for a specific test.

The studies of Kent and Rein in amebiasis (*7*) demonstrated that the incidence of false positive reactions was considerably reduced when 4-hour, rather than 18-hour, incubation periods were used. On the basis of these findings, 135 additional sera were titrated simultaneously in duplicate tests in which an incubation period of 4 hours at 3° to

[1] The histoplasmin was prepared, standardized and supplied through the generosity of Dr. Arden Howell, Jr., Public Health Service, Duke University.

[2] Strain 715 (*H. capsulatum*) from the collection of Dr. N. F. Conant, Duke University.

[3] Strain 930 (*B. dermatitidis*) from the collection of Dr. Conant.

6° C. was allowed for fixation in one set, and 18 hours in the second. Optimal dilutions of antigen were previously determined with positive serum under the two different conditions of the test. As noted in table 1, after 4 hours' incubation, only 8 or 5.9 percent of the sera fixed complement, as compared to 47 or 34.7 percent after 18 hours' incubation. In the 4-hour group, 6 (4.4 percent) serum specimens reacted at 1:10 dilution; 2 (1.5 percent) at 1:20; and none at 1:40. The positive serum control in the 4-hour test fixed complement in a dilution of 1:320, while in the 18-hour test, the titer was 1:640. The results tabulated in table 1 demonstrate that the titer of the positive serum control was reduced by only one dilution in the 4-hour test, while the number of test sera reacting at any dilution was considerably lower than in the 18-hour test. These findings suggest that the "positive" reactions appearing in the lower serum dilutions during the longer period of incubation were nonspecific. Therefore, in our subsequent studies with human sera, the time allowed for fixation of complement was limited to 4 hours.

TABLE 1.—*Results of duplicate tests with 135 sera from normal volunteers, allowing 4 and 18 hours at 3°–6° C. for fixation of complement, and employing an optimal dilution of G–2 ground antigen*

Serum dilution	18 hrs. at 3°–6° C.			4 hrs. at 3°–6° C.		
	Number of sera reacting	Percentage of sera reacting	Positive control serum titer	Number of sera reacting	Percentage of sera reacting	Positive control serum titer
1:10	20	14.8	1:640	[1] 6	4.4	1:320
1:20	21	15.5		[2] 2	1.5	
1:40	6	4.4		0		
Total	47	34.7		8	5.9	

[1] After 18 hours' incubation, 4 of the 6 sera had given titers of 1:40, while 2 had reacted at 1:20.
[2] Both sera had titers of 1:40 after 18 hours' incubation.

Group II. The sera for group II were obtained from 207 normal volunteers who were skin-tested simultaneously with histoplasmin. Of this number, 118 were healthy civilian persons employed in the laboratories of the Army Medical Department Research and Graduate School and 89 were orthopedic patients at Walter Reed General Hospital. This group represented a diverse geographic population including natives of 40 States, Canada, the Philippine Islands, Turkey, and Brazil. Only 13 were natives of the District of Columbia.

As noted in table 2, 73 (35.2 percent) persons were skin-test-positive, and the sera of 16 (7.7 percent) fixed complement in low dilutions (1:10 to 1:40). Considering the group as a whole, 10 (13.7 percent) of the reacting sera were from the 73 skin-test-positive persons, and 6 (4.5 percent) were from the 134 skin-test-negative individuals. Further analysis of these data reveals that among the sera from the civilian

group of 118 those which fixed complement in low dilution were from skin-test-positive persons originally living in areas considered endemic for histoplasmosis. None of the negative reactors in this set showed complement-fixing antibodies. On the other hand, all of the six serum specimens from skin-test-negative individuals which fixed complement in low dilution were from Army patient personnel. This latter group, because of service tours in Army installations throughout many parts of the world, has undoubtedly been subject to a number of natural and protective immunizations not represented in the civilian group. However, since this latter observation is difficult to evaluate, calculations for this study (table 2) were based on the total number of 207 volunteers.

TABLE 2.—*Classification of 207 normal volunteers according to histoplasmin sensitivity and complement fixation capacity of sera*

Number of persons	Skin sensitivity to H-40	Number of sera fixing complement			Total	Percent positive
		Serum dilutions				
		1:10	1:20	1:40		
73	Positive	6	2	2	10	13.7
134	Negative	5	1	0	6	4.5
Total 207		11	3	2	16	

Twenty days after the intracutaneous administration of histoplasmin, second specimens of sera were obtained from the 118 healthy civilian volunteers in group II. These were again titrated for antibodies with ground yeast-phase antigens of *H. capsulatum* The results of the complement-fixation tests were exactly the same as those observed with the first serum specimens taken from this group. Sera which did not fix complement before skin-testing were also negative 20 days later, and the titers of the "positive" sera showed no increase. If a detectable antibody increase had occurred in the interim because of skin-testing with histoplasmin, it was of extremely short duration. Nevertheless, it can be assumed that the single skin test *per se* did not stimulate any antibodies which could be detected approximately 3 weeks later.

Group III. Sera from seven skin-test-positive, proved cases of histoplasmosis were titrated for antibodies against *H. capsulatum*. Three of the patients were in the acute phase of the disease. As shown in table 3, the serum of case 1, taken 15 days after the onset of illness, fixed complement through a dilution of 1:1280. Twenty-seven and 42 days later, second and third specimens, respectively, showed a drop in titer to 1:640. A fourth specimen obtained on the seventy-eighth

TABLE 3.—*Results of complement fixation tests employing an optimal dilution of G–2 histoplasma antigen, and related data in 7 cases of histoplasmosis with positive skin tests*

Case number	Phase of disease	Serum specimen number	Intervals between specimens (days)	Complement fixation titers, serum dilutions								
				1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
				Percent								
1	Acute	1	(1)	0	0	0	0	0	0	0	0	70
		2	12	0	0	0	0	0	0	10	85	100
		3	15	0	0	0	0	0	0	50	100	100
		4	36	0	0	0	50	100	100	100	100	100
		5	56	10	20	45	100	100	100	100	100	100
2	do	1	(2)	0	0	50	100	100	100	100	100	100
		2	27	0	0	0	0	45	100	100	100	100
3	do	1		0	0	0	0	0	60	100	100	100
4	Chronic	1		0	20	95	100	100	100	100	100	100
		2	30	0	35	100	100	100	100	100	100	100
5	do	1		0	0	40	95	100	100	100	100	100
		2	30	0	45	95	100	100	100	100	100	100
6	do	1		(3) ac								
		2	30	(3) ac								
7	do	1		100	100	100	100	100	100	100	100	100
		2	7	100	100	100	100	100	100	100	100	100
		3	7	100	100	100	100	100	100	100	100	100
		4	7	100	100	100	100	100	100	100	100	100
		5	7	100	100	100	100	100	100	100	100	100
		6	7	100	100	100	100	100	100	100	100	100

[1] 15th day of illness. [2] 13th day of illness. [3] Anticomplementary.
0=no hemolysis (complete fixation); 100=complete hemolysis (no fixation); the titer was taken as being the highest dilution of serum showing 50 percent or less of hemolysis.
Controls: Antigen—100 percent; complement—100 percent; cells—0 percent.

day of illness had a titer of only 1:80, while on the one hundred and thirty-fourth day the serum fixed complement up to a 1:40 dilution. After the initial onset of the disease, this patient had shown gradual clinical improvement.

The first serum specimen taken from case 2 on the thirteenth day of illness was positive at 1:40, 27 days later the titer of his serum had increased to 1:160. A single serum sample from case 3 taken in the acute phase of the disease fixed complement completely through a dilution of 1:160.

Two serum specimens, collected 1 month apart, were obtained from three chronically ill patients (cases 4, 5, 6) whose diagnosis of histoplasmosis was of 2 to 5 years' duration. The first serum samples from cases 4 and 5 fixed complement through 1:20 and 1:40 dilutions, respectively, while that from case 6 was anticomplementary. Second specimens from cases 4 and 5 each fixed complement through 1:20 dilutions of serum. Serum specimens obtained from case 7 at weekly intervals for 8 weeks consistently failed to fix complement in serum dilutions of 1:10.

The titers of the above sera from the three persons in the acute phase of the disease were significantly greater than any of the reactions observed either in the sera from the chronic cases of histoplasmosis or in normal sera. This observation is essentially in accord

with that made when sera were tested for antibodies with histoplasmin by means of the collodion agglutination technique (*8*).

Because of the limited amount of serum available from this group, the cross relationship between *H. capsulatum* and *B. dermatitidis* which had been noted in immune rabbit sera (*1*, *2*, *3*, *4*) could be studied only with five human sera. These were titrated for antibodies with dilutions of ground yeast-phase antigens of both *H. capsulatum* and *B. dermatitidis*. As shown in table 4, one serum specimen having a titer of 1:160 with histoplasma antigen (223) fixed complement through a dilution of 1:40 with the blastomyces antigen. Two other

TABLE 4.—*Cross reactions in the complement fixation test employing optimal dilutions of ground antigens from the G–2 strain of* H. capsulatum *and the A–5 strain of* B. dermatitidis *and sera from proved cases of histoplasmosis and blastomycosis*

Sera	G–2 antigen									Serum titer
	Serum dilutions						Controls			
	1:10	1:20	1:40	1:80	1:160	1:320	Ant.	Comp.	Cells	
	Percent									
206 [1]	0	0	75	100	100	100	100	100	0	1:20
223 [1]	0	0	0	0	40	95				1:160
225 [1]	0	0	0	60	100	100				1:40
325 [2]	70	100	100	100	100	100				0
327 [2]	95	100	100	100	100	100				0

Sera	A–5 antigen									Serum titer
	Serum dilutions						Controls			
	1:10	1:20	1:40	1:80	1:160	1:320	Ant.	Comp.	Cells	
	Percent									
206 [1]	10	90	100	100	100	100	100	100	0	1:10
223 [1]	0	10	20	95	100	100				1:40
225 [1]	0	90	100	100	100	100				1:10
325 [2]	40	100	100	100	100	100				1:10
327 [2]	50	100	100	100	100	100				1:10

[1] Serum from proved cases of histoplasmosis.
[2] Serum from proved case of blastomycosis.
0=no hemolysis (complete fixation); 100=complete hemolysis (no fixation); the titer was taken as being the highest dilution of serum showing 50 percent or less of hemolysis.

sera (206, 225) which had reacted to histoplasma antigen in 1:20 and 1:40 dilutions, respectively, fixed complement in 1:10 dilutions when blastomyces antigens were used. Two serum samples (325, 327) from different cases of systemic blastomycosis with specific titers of only 1:10 did not react in the presence of the histoplasma antigens. These results, although unavoidably very limited in scope, were analagous to those observed in rabbit sera.

Discussion

The primary purpose of this study was to develop a test for the detection of antibodies against *H. capsulatum* which would insure maximum specificity. Kent and Rein (*7*), in complement fixation studies of amebiasis, reported the value of a 4-hour incubation period in reducing the number of false positive reactions. The use of this time factor, rather than one of 18 hours, in the present work proved valuable in decreasing the reactivity of "normal" sera from 26.9 percent at the longer incubation period to 5.9 percent at 4 hours. Thus, the 4-hour incubation period was confirmed in this study as a means of improving the specificity of the complement fixation test.

Next, this study attempted to determine whether any relationship exists between skin sensitivity to histoplasmin (a filtrate of the mycelial phase of *H. capsulatum*) and humoral antibodies against the yeast phase of the organism. Results of this investigation suggest that such antibodies are more likely to occur in skin-test-positive persons. The positive serum reactions in this study, however, were far weaker than those observed in sera of persons with acute histoplasmosis.

The possible value of the complement fixation test as a diagnostic tool in histoplasmosis was suggested by the observation that the serum titers of three patients in the early acute phase of the disease was considerably higher than any reaction noted in the sera of "normal" persons. In one case, the gradual decrease of serum antibody from 1:1280 on the 15th day of illness to 1:40 on the 134th day, as well as the low titers observed in two chronic cases, and the complete absence of detectable antibody in a third, suggests that the humoral response of the host is transitory. The titers of the chronically ill persons known to have had the disease for 2 to 5 years did not differ appreciably from those observed in a very low percentage of individuals in a normal population which may or may not have been previously exposed to histoplasmosis. Thus, it is possible to assume that a high antibody titer against *H. capsulatum* may be of value to the clinician as an aid in diagnosis. In fact, culture for the organism in two cases in this series was instigated only after the marked antibody content of the serum had been discovered. The ease with which the organism was isolated by blood culture in this instance suggests that the test may also be of value in indicating the most propitious time for attempting to isolate the organism, thereby facilitating rapid and certain diagnosis. Since similar results were recorded in our studies employing the collodion agglutination technique (*8*), it is our belief that the continuation of serologic studies of an accumulated number of cases will add substantially to the understanding of the exact nature of histoplasmosis.

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There is relatively little data on complement fixation studies available for comparative purposes. Salvin (*9*), using yeast-phase antigens, reported titers of 1:32 and 1:128, respectively, in single serum specimens from two patients with histoplasmosis. Furcolow, Bunnell, and Tenenberg (*10*) have reported interesting results in complement fixation studies employing histoplasmin as antigen. An accurate comparison between their data and ours is difficult, however, because of several factors: (1) Ground yeast-phase antigens were employed in our test, while histoplasmin was used in the above study; (2) our test is quantitative in that it employs the 50 percent end-point of hemolysis and serial serum dilutions, instead of a graded intensity of reactions with undiluted serum; (3) the stages of the disease have not been specifically designated in the cited studies. Despite these differences, results indicate a similar trend. Furcolow et al. report that 242 miscellaneous "control" cases yielded 5.4 percent reactions (3+ and 2+). Eight (5.9 percent) of our 135 random serum samples, and 16 (7.7 percent) of the 207 volunteers were positive in low dilution, resulting in a total of 24 or 7 percent positive sera in a group of 342 persons. These figures of 5.4 percent and 7 percent are remarkably close for such small groups. The demonstration of antibodies in 8 of 9 proved cases and in 10 of 36 suspected cases of histoplasmosis by the Kansas group and the results obtained in this laboratory suggest the potential value of serologic tests as an aid to diagnosis. The accumulation of more data will be required before the tests can be adequately evaluated, however.

The cross reaction between *H. capsulatum* and *B. dermatitidis* noted in our earlier studies with rabbit antisera (*1*, *2*, *3*, *4*) was observed to occur as well in human sera, where the cross was again more marked between blastomyces antigen and histoplasma serum than between histoplasma antigen and blastomyces antisera. It should be noted that none of the available titers of blastomyces sera was higher than 1:10. In Martin's summary of 22 cases (*11*), including both cutaneous and generalized blastomycosis, the highest complement fixation titer observed was 1:32. Since we have found the immunogenic capacity of *B. dermatitidis* to be much less than *H. capsulatum* in rabbits (*1*), it is possible that the organism does not incite a marked antibody increase in the human host during any stage of the disease. If such a postulate is correct there is little danger that serologic data based on the use of either blastomyces or histoplasma antigen alone could be misinterpreted. However, until it is proved otherwise, it should be assumed that at some stage of blastomycosis the serum titer may be markedly increased, just as in histoplasmosis, and that in this stage of the disease the cross reaction with histoplasma antigen would also be increased. The serologic picture resulting from the use of histo-

plasma antigen alone would, in such an instance, be completely misleading.

Histoplasma sera apparently react with blastomyces antigens in a ratio proportionate to the specific titer of the serum. As the titer increases with specific antigen, it increases also with blastomyces antigen. When both antigens are employed simultaneously it is immediately obvious that the titer with specific antigen is always several dilutions higher than with blastomyces antigen, and results are easily interpreted. Conversely, it is conceivable that a histoplasma serum of high titer used only with blastomyces antigen could, as in the above, obscure exact diagnosis. For these reasons, both antigens will in the future be used in this laboratory with all sera suspected of having antibodies against either *H. capsulatum* or *B. dermatitidis*.

During the development and use of serologic methods, the question often arises as to whether skin-testing agents *per se* stimulate antibody production. Three weeks after "normal" persons were given a single intradermal injection of histoplasmin, no stimulatory effect was noted. The sera of those persons previously showing some complement-fixing antibodies evidenced no detectable increase in titer; also, sera which had been negative prior to skin testing were likewise negative 20 days later. If any antibody response had occurred, it was of such a transient nature as to be absent approximately 3 weeks after skin testing with histoplasmin.

Summary

1. A complement fixation test for histoplasmosis utilizing ground yeast-phase antigens of *H. capsulatum* has been described.

2. Four-hour incubation periods at 3° to 6° C. for fixation of complement were less likely to yield nonspecific reactions than 18-hour periods.

3. Sera from three cases of acute histoplasmosis fixed complement in high titer, while sera from three chronic cases did not vary appreciably from the titers found in a small percentage (5.9 to 7.7 percent) of a "normal" population.

4. In a group of 207 volunteers, humoral antibodies occurred in 10 of 73 skin-test-positive persons and 6 of 134 skin-test-negative individuals.

5. Skin testing with histoplasmin did not incite any antibody response that was detectable 20 days after the tests.

6. The cross reaction between *H. capsulatum* and *B. dermatitidis* was further demonstrated.

7. The potential value of complement fixation studies as an aid to the diagnosis of histoplasmosis was demonstrated.

REFERENCES

(1) Saslaw, S., and Campbell, C. C.: The use of yeast phase antigens in a complement fixation test for histoplasmosis. I. Preliminary results with rabbit sera. J. Lab. & Clin. Med. **33**: 811 (1948).
(2) Campbell, C. C., and Saslaw, S.: The use of yeast phase antigens in a complement fixation test for histoplasmosis. II. Results with ground antigens. Ibid. **33**: 1207 (1948).
(3) Saslaw, S., and Campbell, C. C.: A method for demonstrating antibodies in rabbit sera against histoplasmin by the collodion agglutination technic. Proc. Soc. Exper. Biol. & Med. **68**: 559 (1948).
(4) Saslaw, S., and Campbell, C. C.: A Comparison between histoplasmin and blastomycin by the collodion agglutination technic. Pub. Health Rep. **64**: 290 (1949).
(5) Emmons, C. W., Olson, B. J., and Eldridge, W. W.: Studies in the role of fungi in pulmonary disease. I. Cross-reactions of histoplasmin. Pub. Health Rep. **60**: 1383 (1945).
(6) Howell, A. Jr.: Studies of fungus antigens. I. Quantitative studies of cross-reactions between histoplasmin and blastomycin in guinea pigs. Pub. Health Rep. **62**: 631 (1947).
(7) Kent, J. F., and Rein, C. R.: Serodiagnosis of amebiasis. Science **103**: 598 (1946).
(8) Saslaw, S., and Campbell, C. C.: A collodion agglutination test for histoplasmosis. Pub. Health Rep. **64**: 424 (1949).
(9) Salvin, S. B.: Complement fixation studies in experimental histoplasmosis. Proc. Soc. Exper. Biol. & Med. **66**: 342 (1947).
(10) Furcolow, M. L., Bunnell, I. L. and Tenenberg, D. J.: A complement fixation test for histoplasmosis. II. Preliminary results with human sera. Pub. Health Rep. **63**: 169 (1948).
(11) Martin, D. S.: Cited from Manual of Clinical Mycology, National Research Council, Philadelphia, W. B. Saunders Co., 1945, p. 46.

Persistence of Fluoroscopic Screens

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Radiographic intensifying and fluoroscopic screens, in common with other devices using phosphors for the conversion of radiant energy into visible light, exhibit the phenomenon known as persistence or lag. This means that the phosphor continues to emit light for an appreciable time after the exciting radiation has stopped. It has long been recognized that the light emission from a phosphor consists of two distinct types: fluorescence during excitation, and phosphorescence after excitation. These two types of emission differ from each other both in the mechanism by which they are produced and in their characteristics of wave length and duration. The phosphorescent emission is of longer wave length than the corresponding fluorescent emission and lasts for some time after the termination of the exciting exposure; whereas fluorescence occurs only during, or, at the most, for a few micro-seconds after excitation. The mechanism of these phenomena

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has been discussed by Mott and Gurney (*1*), Hirschlaff (*2*), Riehl (*3*) and others.

The screens used in roentgenography may be devided conveniently into three groups; the intensifying screens, fluoroscopic screens used for visual examination, and screens used in photofluorography. There is overlapping of the last two groups, since many screens are used both for fluoroscopy and photofluorography. Persistence in screens of each of these groups presents different aspects and assumes different degrees of importance.

In the case of intensifying screens, persistence is negligible. In fact, the period of phosphorescence in those intensifying screens studied by this laboratory is so short that it cannot be measured by the methods to be described.

Fluoroscopic screens are available with almost any degree of persistence ranging from a few hundredths of a second to many minutes or even longer. Fluoroscopic screens having a long persistence are objectionable for diagnostic examinations since afterimages, due to lag, tend to obscure the outlines being observed whenever the screen is moved. Likewise the outlines of moving structures under observation are blurred. In view of the inefficient viewing conditions prevailing in fluoroscopy, therefore, it is important that screens used for this purpose have low lag.

Persistence in screens intended for photofluorographic use has been the subject of much discussion and many complaints. With the development of techniques allowing or requiring exposures in rapid succession, the importance of persistence in screens becomes of greater moment. It is the purpose of this study to present quantative data on the persistence characteristics of commonly used screens and the effects of this persistence on the film.

Two years ago the Electronic Laboratory of the Public Health Service measured the rate of decay of the persistence of a large number of screens (*4*). However, no attempt was made at that time to determine the extent of objectionable effects resulting from screen lag. What is required in order to evaluate a particular screen is not only the rate of decay of phosphorescence, but also the total quantity of light emitted during the period of persistence.

In order to measure and record the intensity and rate of decay of phosphorescence in an excited screen, the following apparatus was devised. A photoelectric cell of the multiplier type was arranged to receive the light emitted from the screen under examination. Since the light emitted during the period of excitation is several hundred times as intense as that emitted during the greater part of the period of phosphorescence, a shutter of neutral density was arranged to operate in conjunction with the exposure control switch. During the

exciting exposure this shutter is interposed between the screen and the photocell and is withdrawn immediately upon completion of the exposure. The output of the photocell is fed to the galvanometer string of an electrocardiograph which serves as a recording microammeter. The electrocardiograph was used as the recording instrument merely because it was available and served the purpose. Obviously any other device of comparable sensitivity and low inertia would serve as well. For recording persistence of duration longer than a few seconds, a Micromax recorder of 5 millivolt sensitivity was used. Schematic diagrams of the apparatus and circuit are shown in figure 1. A typical recording is shown in figure 2. The length and intensity of

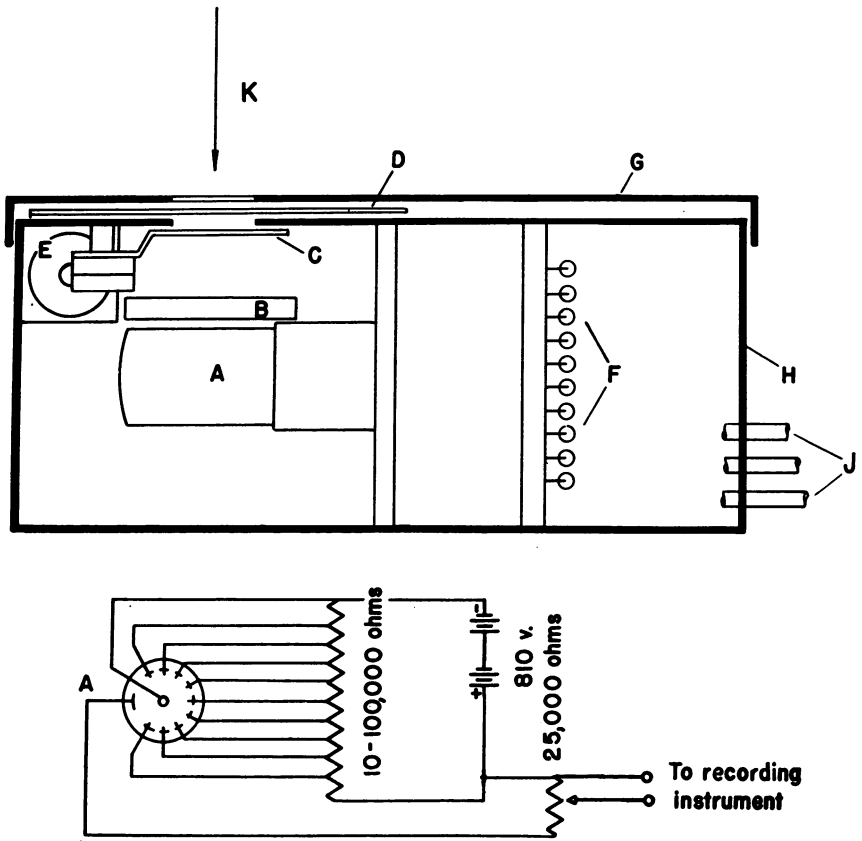

Figure 1

the exciting exposure are recorded as the broad horizontal trace, and the persistence intensity is shown by the curve. The density of the shutter used was 2.48, corresponding to a transmission of 1/300. Hence the intensity of illumination during exposure is the average

CORRECTION

insert as page 562a in

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The legends for figures 1 to 10 in the article "Persistence of Fluoroscopic Screens," by Willard W. Van Allen were omitted. The legends follow:

Figure 1. Schematic diagram of apparatus used for measuring the phosphorescence of fluoroscopic screens. A, photoelectric cell; B, lead glass filter; C, neutral density shutter; D, screen under test; E, solenoid to operate shutter; F, voltage dividing resistors for photocell; G, light-tight cover; H, light-tight case and electrostatic shield; J, cables to batteries, main contactor, and recorder; K, exciting radiation.

Figure 2. Typical record of the decay of posphorescence as recorded on an electrocardiograph.

Figure 3. Curves showing the decay of phosphorescence for four different screens. A, after 0.1-second exposure; B, after 0.3-second exposure; C, after 0.5-second exposure; D, after 1.0-second exposure. All exposures made at 85 kv., 100 ma., through a phantom of 10 cm. of Masonite presdwood and at a target-screen distance of 40 inches.

Figure 4. Schematic diagram of apparatus used to measure photographically the persistence exposure. A, unrestricted port to allow film to receive total exposure; B, shutter-controlled port to restrict film exposure to that due to persistence; C, shutter; D, solenoid to operate shutter.

Figure 5. Curves showing the relation between the conditions of excitation and the quantity of light emitted by screens during the period of phosphorescence.

Figure 6. Curves showing the average exposure due to persistence expressed as percentage of the exciting exposure. A, older D-type screen showing long persistence; B, recent D-type screen; C, 666D screen; D, E–2 type screen; E, B-type screen.

Figure 7. Characteristic Density—Log Exposure curve for a typical photofluorographic film. Solid curve shows densities for normal exposure. Dashed curves show corresponding densities for exposures of 10%, 20% and 30%, respectively, of the exposures referred to the solid line.

Figure 8. Curves showing the progress with time of exposures due to persistence, expressed as percent of the total persistence exposure. B, long-persistence screens, type D; C, medium-persistence screens, type 666D; D, short-persistence screens, type E–2 and B. (Curves labeled to correspond to figure 6.)

Figure 9. Curves showing the rate of decay of phosphorescence after a series of 3 exposures per minute for 1 hour. A, after the first exposure on a rested screen; B, after the last exposure of the series. All exposures are 0.3 second at 85 kv., 100 ma., through 10 cm. of presdwood at a target-screen distance of 40 inches.

Figure 10. Curves showing the exposure due to persistence after a series of exciting exposures. 0.1-second exposures at the rate of 12 per minute for 5 minutes. 0.3-second exposures at the rate of 3 per minute for 1 hour. All exposures at 85 kv., 100 ma., through a 10 cm. phantom of presdwood at a target-screen distance of 40 inches. A, long-persistence D-type screen; B, short persistence D-type screen; C, 666D screen; D, E–2 screen; E, B-type screen.

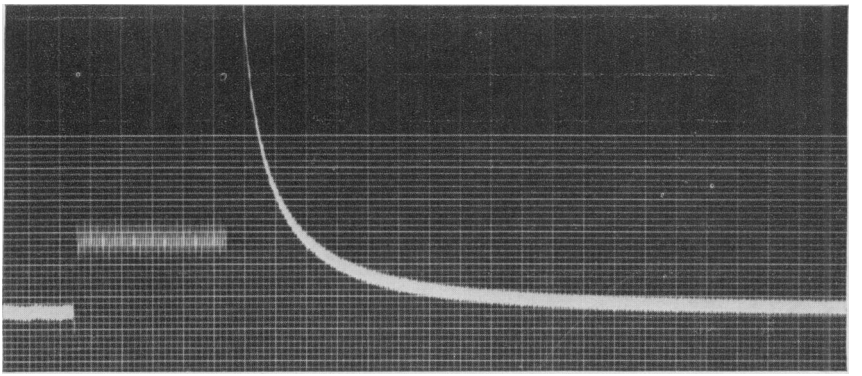

Figure 2

ordinate of the horizontal trace above the zero line multiplied by 300. The persistence intensities, expressed as percent of the exciting intensity, may then be readily determined for any time following termination of the exposure. Preliminary tests established the linearity of the system, and the inherent time delay of the apparatus and circuit as less than 0.02 second.

Using this apparatus, curves showing the decay of persistence are readily obtained for any type of screen and under any desired conditions of excitation. Figure 3 shows decay curves obtained in this manner for four typical fluoroscopic screens. For the examples in figure 3, exposures were made at 85 kilovolts peak, 100 milliamperes through a phantom of 10 centimeters of Masonite presdwood [1] and at a target-screen distance of 40 inches. Curve *A* shows the decay of phosphorescence following a 0.1-second exposure, and curves *B*, *C*, and *D* show the decay following a 0.3-, 0.5-, and 1.0-second exposure respectively. In each case the intensity of illumination is expressed as percent of the intensity during exposure. It will be noted from these curves that there is a wide variation in the persistence of phosphorescence among these screens, at least during the period of low intensity. Attention should be paid not only to the time required for the intensity to fall to a particular fraction of the original brightness, but also to the slope of the decay curve at this point. For example, the B-type screen requires about 1 second to fall to 0.01 percent of its original brightness and the phosphorescence at this level is still decreasing rapidly. On the other hand, by comparison, the other screens require a longer time to reach this level of brightness and the rate of decay at this point is much slower, indicating that phosphorescence of low intensity will continue for a longer time.

[1] A phantom of 10 cm. of Masonite presdwood has been shown by Chamberlain to be equivalent to the average chest. See Chamberlain, E. W.: Fluoroscopes and fluoroscopy. Radiology, 38: 383 (1942).

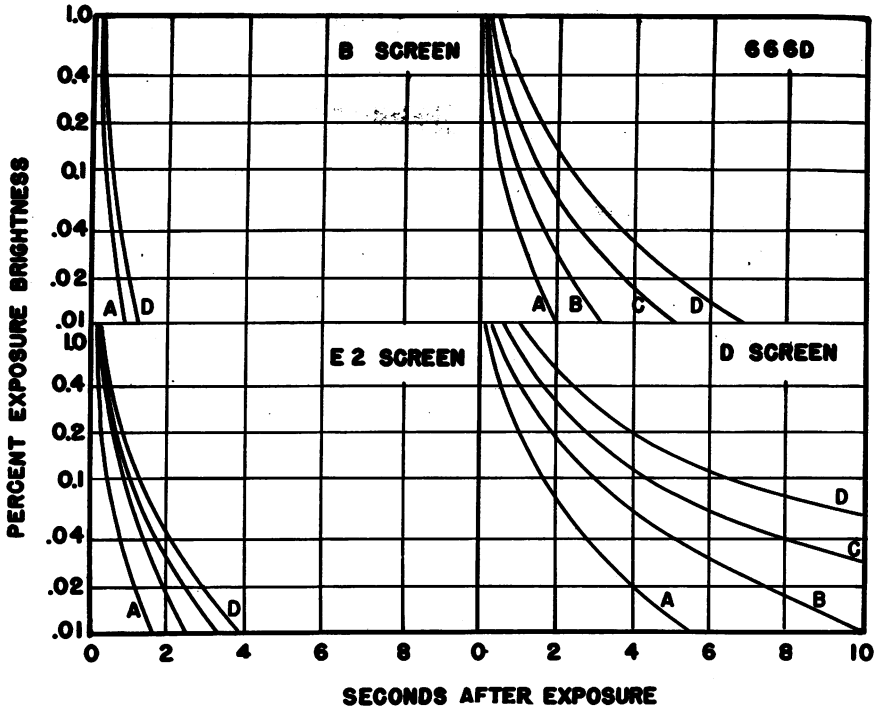

Figure 3

It is important, however, to consider the total quantity of light emitted during the period of phosphorescence, since it is this total quantity of light which determines the magnitude of adverse effects on the film exposed to it. Mathematically, this quantity of light is expressed by $\int i\,dt$, where i is the intensity of phosphorescence at any time t, and represents the area under the decay curve. This area may be measured directly by means of a planimeter, or the integral evaluated mathematically, provided the decay function can be discovered. The quantity of light may also be measured photographically. Examination of the decay curves shows that the decay during the first few hundredths of a second may be closely approximated by an exponential function of the form

$$i=i_0e^{-ct}$$

while the greater part of the period of decay follows closely the hyperbolic form

$$i=at^{-b}$$

where: i is the intensity at any time t,
i_0 is the original brightness during excitation, and
a, b, c are constants readily determined from graphs.

Using these equations, values may be obtained by integration for the quantity of light emitted during phosphorescence.

The following method was devised in order to obtain direct measurements by photographic methods of the light emitted during phosphorescence, both as a check on the mathematical approximations described above and to take into account possible variations in the effect on the film due to quality of the light emitted.

Apparatus, shown diagrammatically in figure 4, was constructed, consisting essentially of two sheets of steel pierced, as shown, by two

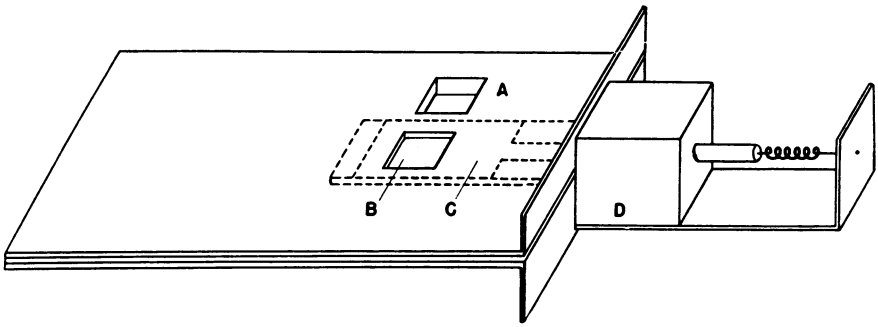

Figure 4

holes to allow transmission of light. A light-opaque shutter is arranged between the two metal sheets and operated by a solenoid in such manner that during the exciting exposure one of the two apertures in the plate is closed. The shutter opens immediately upon termination of the exciting exposure. The screen under examination is placed on one side of the holder, a film on the other side and a suitable light-tight cover fitted in place. This cover is of thin aluminum to allow passage of the exciting radiation. Thus the film receives the total exposure through the unrestricted port, but only the exposure due to persistence through the other. Since the intensity of the total exposure under these conditions with film and screen in close proximity is much greater than it would be for the same exciting energy in a photofluorograph, it was necessary to place neutral filters of known transmission over the unrestricted port in order to reduce the exposure to a value which could be recorded on the film in its useful range. Also corrections are necessary for density produced on the film by direct roentgen exposure through the ports and shutter. The speed of the shutter was determined, and it was found that the shutter required 0.02 second to open after termination of the exposure.

Using this apparatus, exposures were made under the same conditions of time, kilovoltage, current, and filtration as with the photoelectric recording system. The densities recorded by the film through

the two ports were measured and each density corrected for fog, filtration, and direct roentgen exposure. From the characteristic $D=\log E$ curve of the film the ratio of the exposure due to persistence to the exciting exposure was determined. These ratios were much lower than those predicted by the mathematical and mechanical determinations. The discrepancy was due to the time lag in the shutter and led to a greater appreciation of the rapidity with which the persistence exposure progresses, as will be shown below. When the exposure during the first 0.02 second was subtracted from the mathematically determined quantity, the results obtained photographically checked closely with the other determinations. In fact, the values obtained by mechanical integration with the planimeter, by mathematical integration of the assumed functions, and photographically, were so close that confidence could be placed in the adequacy of any one of the three methods.

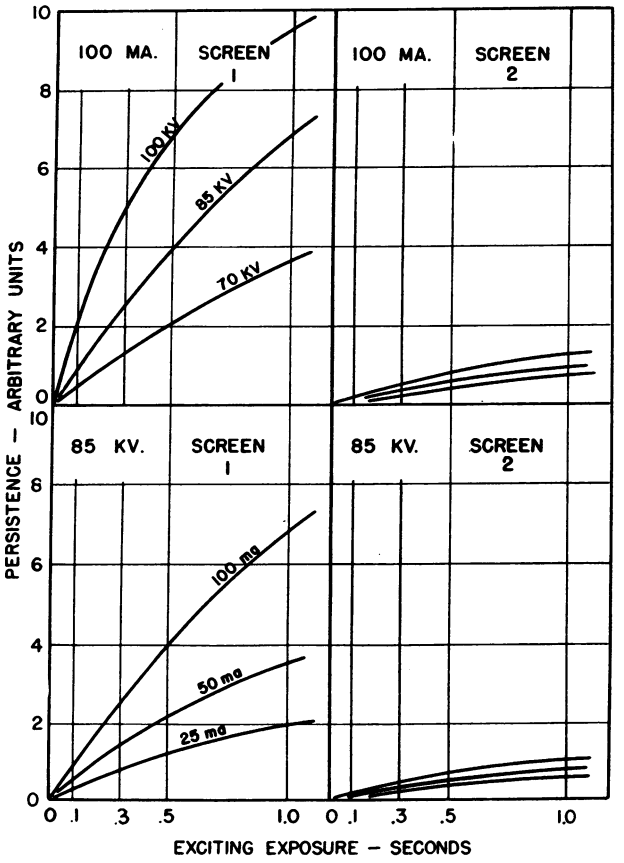

Figure 5

In order to investigate the factors determining the amount of persistence encountered under various conditions, tests were made according to the experimental techniques outlined above at 70, 85, and 100 kilovolts, 25, 50, and 100 milliamperes and with exciting exposures of 0.1-, 0.3-, 0.5-, and 1.0-second duration. In each case the exposures were made at a target-screen distance of 40 inches through a phantom of 10 centimeters of presdwood. Curves showing the relation between exposure and persistence for two types of screens are shown in figure 5. It will be noted that the persistence increases with kilovoltage, milliamperage, and time. In other words, the persistence increases as the intensity of excitation and the length of excitation increase. Furthermore, the increase in persistence with excitation intensity—that is, the brightness of the screen during exposure—is roughly proportional. These curves show the total quantity of light emitted during the period of phosphorescence.

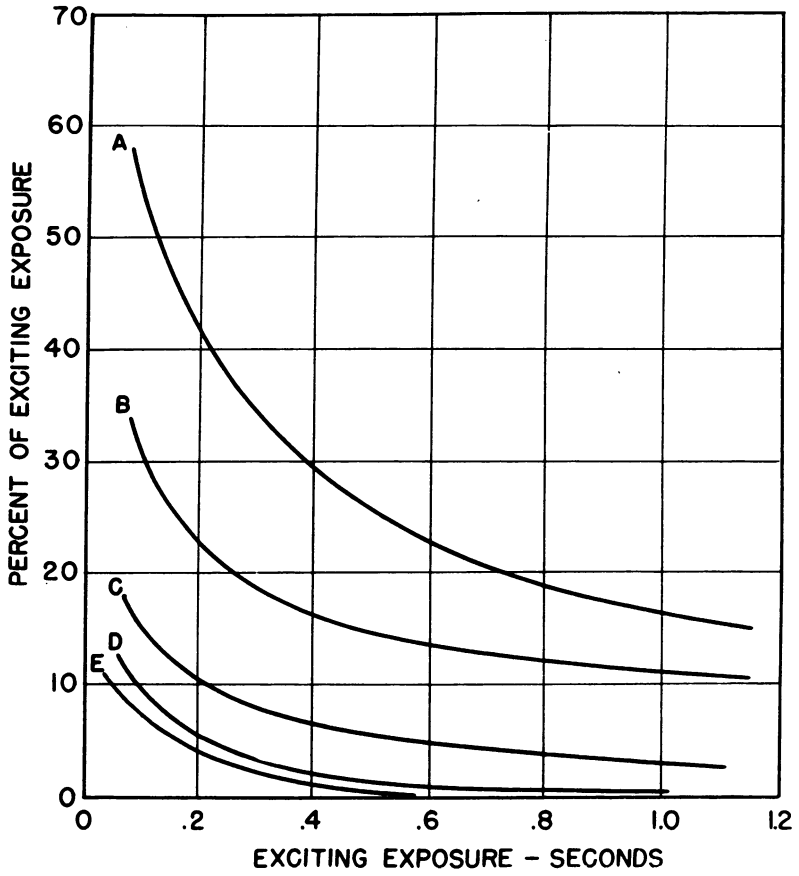

Figure 6

Of greater interest, however, is the amount of persistence expressed as percent of the exciting exposure. Since, as pointed out in figure 5, the persistence is roughly proportional to the intensity of excitation, it is not surprising to find that, for exposures of equal duration, the exposure due to persistence is very nearly the same percent of the exciting exposure regardless of the intensity of excitation, at least within the range investigated. Figure 6 shows the average exposure due to persistence following exciting exposures in the range from 0.1 to 1.0 second expressed as percent of the exciting exposure. Five types of screens are used as illustrations; curve *A*, for an older type of D screen showing considerable persistence; curve *B*, for a recent D-type screen; curve *C*, for a 666D screen; curve *D*, for an E-2 screen; and curve *E*, for a B-type screen. It will be observed that for short exposures the persistence exposure is relatively high, decreasing in importance as the length of the exciting exposure increases. This is of special interest in view of the present trend toward shorter and shorter exposures of increasing intensity. Thus exposures of 0.1 second on D-type screens may produce persistence exposures of from 30 to 50 percent of the exciting exposure, whereas the same screens at exposures of 1.0 second produce persistence exposures of less than 20 percent of the original.

By reference to the curves of figure 6 and to the characteristic $D=\log E$ curve of the film, it is possible to predict the effect on the film of this persistence exposure. The solid curve in figure 7 shows the characteristic $D=\log E$ curve of a typical photofluorographic film. The curves in dashed lines show the densities produced on a film for exposures of 10 percent, 20 percent and 30 percent, respectively of the exposures for the solid curve. That is, if a given exposure produces a density of 2, an exposure 30 percent as great would produce a density of about 1.2 and an exposure 10 percent as great would produce a density of about 0.35. Thus, if the amount of persistence is known, the density produced by it can be read approximately from the appropriate curve directly below the corresponding exposure density on the solid curve. For example, suppose an exposure of 0.2 second using a screen of persistence shown by curve *C* in figure 6 produces a density of 1.5 on the film. From figure 6 it is found that this exposure results in a persistence exposure of 10 percent. From figure 7 it is found that 10 percent of an exposure producing a density of 1.5 results in a density of about 0.2. In this case, therefore, the persistence effects are negligible.

Whether persistence exposures are negligible, as in the above example, or much higher, they will produce objectionable effects only if the film is moved from position after exposure. Obviously, if the film remains in position after exposure, the only effect of persistence

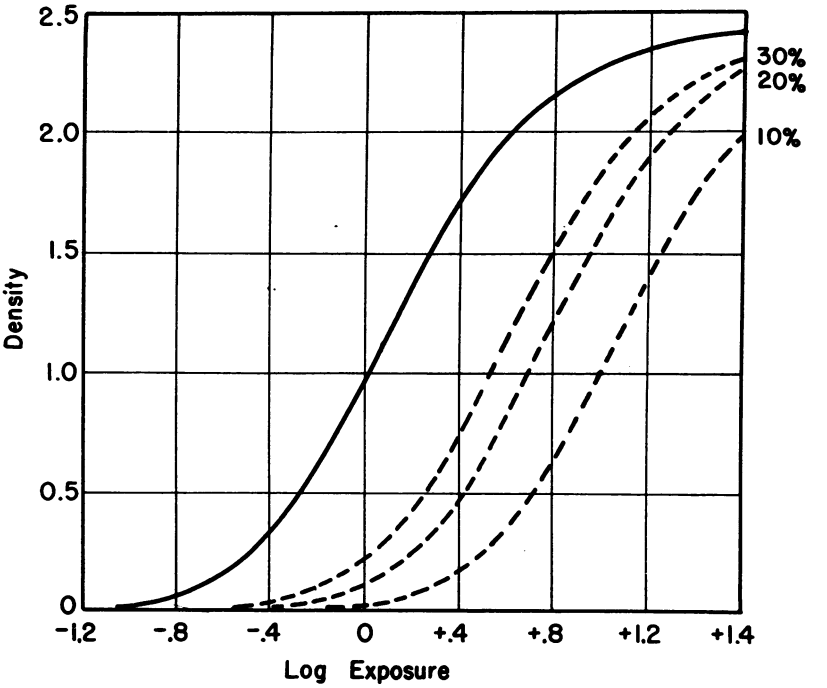

Figure 7

is to increase slightly the original exposure. It is after the film is moved or while it is in motion that fogging or afterimages can be produced. It is important, therefore, to see how the persistence exposure progresses with time.

It has already been pointed out that the decay of phosphorescence during the first few hundredths of a second is very rapid, and only persistence of low level remains for any length of time. This was clearly demonstrated by the fact that a delay of 0.02 second in opening the shutter in the apparatus used for measuring persistence exposure photographically, resulted in a loss of from 20 to 60 percent of the persistence exposure. That is, from 20 to 60 percent of the persistence exposure, depending on the intensity and rate of decay of persistence, is completed in something like 0.02 second.

Figure 8 shows the progress of the exposure due to persistence, expressed as the percent of the total persistence exposure which has occurred after a given length of time. Curve *D* is for screens of short persistence such as the B and E–2 type; curve *C* for 666D; and curve *B* for the D-type screens. Obviously, each type of persistence requires its own curve; however, the curves shown indicate the general order of magnitude of the effect.

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Since it requires a definite time to move the film from position after exposure, these curves are important in considering the effect of persistence exposure on a film. In the case of short-persistence screens, curves *C* and *D*, over 30 percent of the persistence exposure has occurred at the end of 0.01 second and over 70 percent at the end of 0.1 second. Therefore, except in cases where high film speed is deliberately planned or required, as for example, in cine-roentgenography, it is almost impossible to move the film from position until it has received at least half of the total persistence exposure. If the total persistence exposure amounts to, say, 20 percent, only 10 percent exposure is left to produce harmful effects. Reference to figure 7 shows that this exposure is practically negligible. The same argument applies to the longer persistence type of screen, curve *B*, figure 8, provided the delay in moving the film is of the order of 0.1 second.

So far only the persistence due to single exposures on a rested screen has been considered. It is characteristic of many screens, especially those of longer persistence, that, if exposures are repeated rapidly after intervals too short to allow the screen to recover completely from phosphorescence, the persistence increases with each successive exposure until it reaches a value several times greater than for the first exposure of the series. The extent of this growth of persistence depends upon the exciting exposure and the rate of repetition. It is impossible to give here more than two examples of this effect, but they will serve to indicate the general nature of the phenomenon. In one case, the screens were given a series of exposures

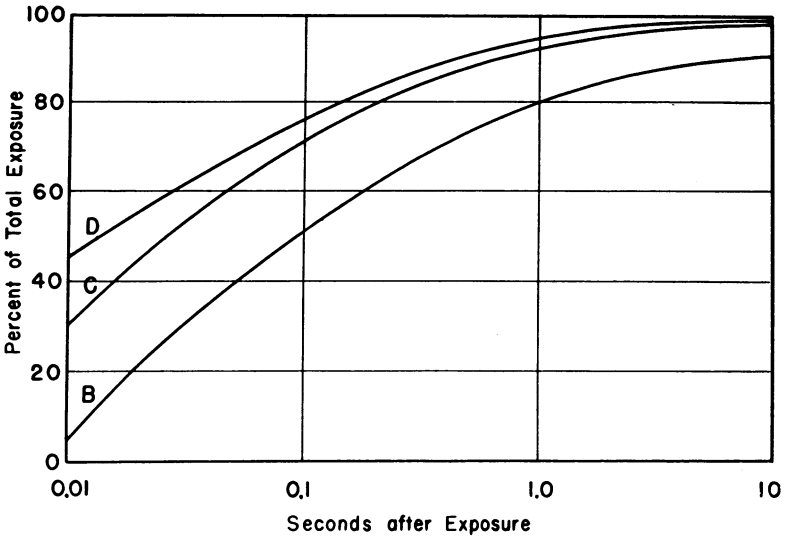

Figure 8

of 0.1 second at intervals of 5 seconds for 5 minutes. In the other case, the screens were given a series of exposures of 0.3 second at 20 second intervals for 1 hour. All exposures were made at 85 kilovolts, 100 milliamperes, at a target-screen distance of 40 inches and through a 10-centimeter presdwood phantom. The persistence after the last exposure of the series was then measured as described above. The resulting decay curves are shown in figure 9. Curve *A* shows the

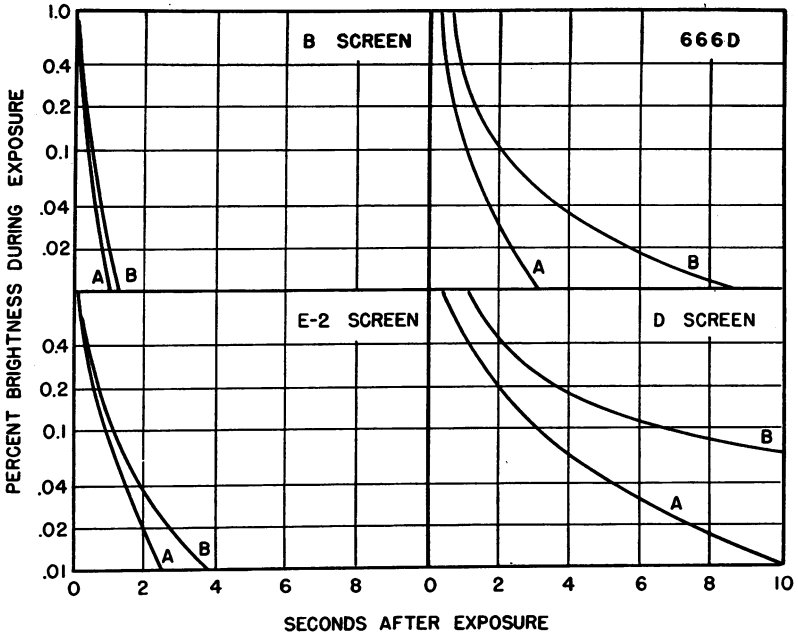

Figure 9

rate of decay after the first exposure, and curve *B* shows the rate of decay after the last exposure of the series. It will be noted that the increase in persistence is slight for the low-persistence screens B and E–2, somewhat greater for 666D and very much greater for the longer persistence D screens. Furthermore, the slope of the decay curve for the longer persistence screens indicates that low intensity phosphorescence may be expected to continue for some time; in many cases, hours. This is, of course, well known to those who have inadvertently exposed such screens to bright daylight.

Figure 10 shows the exposures resulting from this persistence plotted against the elapsed time after the exposure. It will be observed that the short-persistence screens produce exposures of the same order as when rested and which are complete in a relatively short time, as indicated by the horizontal lines, curves *D* and *E*.

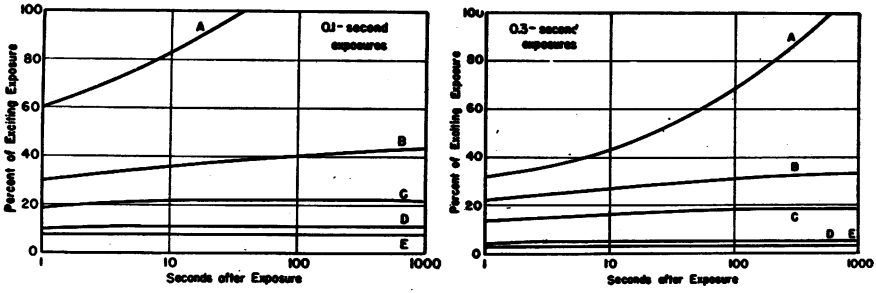

Figure 10

Screens of the 666D and short-persistence D-type, curves *C* and *B* respectively, show persistence exposures which are essentially complete in 1 second, while the persistence of the older type D-screen is so great that it continues to increase for many minutes. Thus the exposure due to persistence of such a screen may amount to 50 percent of the exciting exposure during the 20-second interval between exposures, but will produce an exposure of 100 percent or more of the original after the last exposure of the series or if the series is interrupted during its course. Reference to figure 7 again makes possible a prediction of the extent of objectionable effects to be expected from such persistence exposures.

Finally, limits of tolerance of persistence may be established from the curves of figure 7, and the choice of a screen for a particular application guided thereby. Two examples will suffice to show the reasoning. The limits chosen are for example only and may or may not represent practical cases. Suppose that the maximum density resulting from the primary exposure is 2.0 and that fogging due to persistence must be less than 0.5. Further, assume that only single isolated exposures of 0.3-second duration are required. From figure 7 it is seen that for a primary exposure density of 2.0 an exposure about 15 percent as great will result in a density of 0.5. Figure 6 shows that screens represented by curves *C*, *D*, and *E* produce persistence exposures less than 15 percent and would therefore be acceptable. However, the persistence shown by curve *B* is only slightly higher than 15 percent for this exposure and reference to figure 8 shows that 50 percent of this exposure has been completed in 0.1 second. Hence this screen also would be acceptable provided the film was not moved from position for about 0.1 second, actually less.

For the second example, suppose that a series of exposures of 0.3 second at the rate of 3 per minute is to be made with the same conditions of maximum density and fog density as above. Also assume that any exposure after the series is complete may be disregarded (by sacrificing the next frame if necessary). Reference to figure 7

again sets a limit of 15 percent on the persistence exposure. From figure 10 we see that for exposures stipulated, only screens of the type illustrated by curves *D* and *E* provide persistence below the level indicated, although the exposure resulting from the screen shown by curve *C* is only slightly above this value. If the film remains in position, say 0.1 second before being moved, this screen also should prove satisfactory.

Summary

Experimental methods for determining the rate of decay and total quantity of persistence of phosphorescent emission from fluoroscopic screens are described. Graphs showing the rate of decay of phosphorescence for several typical screens used in fluoroscopy and photofluorography and under various conditions of exposure are presented. The effects on a film of exposures resulting from persistence are discussed and the effect of repeated exposures on the persistence characteristics of screens is described. Factors influencing the limits of tolerance of persistence in screens are illustrated.

REFERENCES

(*1*) Mott, Nevill F., and Gurney, R. W.: Electronic Processes in Ionic Crystals. New York, Oxford University Press, 1940.

(*2*) Hirschlaff, Ernest: Fluorescence and Phosphorescence. Brooklyn, Chemical Publishing Company, 1938.

(*3*) Riehl, Nickolaus: Physik and technische Anwendung der Lumineszenz. Berlin, Springer, 1941 (in Technische Physik, Bd. 3) published by license of the alien property custodian by J. W. Edwards and Edwards Bros., Inc., Ann Arbor, Michigan, 1945.

(*4*) Van Allen, Willard W.: The persistence of fluoroscopic screens. Pub. Health Rep. **61:** 1583 (1946).

Demonstration of Tubercle Bacilli in Sputum Smears

Selection of choice particles compared with various sodium hydroxide concentration techniques*

By GEORGE A. SPENDLOVE, M. D., MARTIN CUMMINGS, M. D., and ROBERT A. PATNODE**

For purposes of differential diagnosis in tuberculosis, there can be no doubt that cultivation or animal inoculation of pathological materials are far more reliable than microscopic methods. There are situations, however, where the latter methods can be used profitably, for example, in following the course of a patient after original diagnosis by cultivation and/or animal inoculation; or in the mass screening of large numbers of patients where cultivation techniques are not readily available. In such situations, the question of the most efficient smear technique arises.

A few decades ago the preferred method of making sputum smears was to pour the specimen into a Petri dish on a black background and, with a platinum loop, to select bits of caseous debris or purulent material. This was done according to Koch's suggestion (*1*): "It will not do to take any chance streak of mucus from the sputum, which consists not only of secretion from the diseased parts of the lungs, but also of that from the bronchial tubes, saliva and mucus from the mouth and nose. We want to examine the secretion furnished by the diseased lung, and must therefore confine our attention to the ball-like yellowish masses, often to be found floating singly in the frothy viscous liquid, often however, forming the greater part of the sputum." Within recent years, this method has been abandoned by many laboratories in favor of one of the numerous concentration techniques.

Petroff (*2*), Hanks (*3*), Cameron (*4*), Nagy (*5*), and many others have advocated a change from the direct smear to a portion of the treated sputum in which the bacilli have been concentrated. Theoretically such concentration of the bacilli seems practical; yet, in the case of small volumes of sputum and large volumes of digestants, concentration may not actually take place. Benzancon and Philibert (*6*) estimate the specific gravity of the tubercle bacillus to be between 1.010 and 1.080, which leaves little advantage in centrifugation for a digestant such as 4 percent NaOH with a specific gravity of about 1.040, or for 23 percent (10 percent anhydrous) trisodium phosphate which has a specific gravity of more than 1.060.

*This study is based on duplicate examination of four series of sputum specimens numbering 500, 263, 244, and 716, respectively.

**S. A. Surgeons and S. A. Sanitarian (R), respectively, from the Tuberculosis Section, Communicable Disease Center, Public Health Service, Atlanta, Ga., in cooperation with the Division of Tuberculosis, Washington, D. C. Assisting in the preparation of this report were Drs. M. Frobisher, Jr., Stuart Willis and Carroll Palmer.

Centrifugation is not the only procedure of doubtful value. Steenken and Smith (*7*) state: "In preparing smears, routine decolorization with acid-alcohol containing 5 percent nitric acid may fail to detect bacilli that are only slightly acid-fast. In smears of material that has been previously digested with sodium hydroxide, the acid-fast property is prone to attenuation. This may be the explanation for the occasional case of a positive sputum or urine culture after negative smears."

Robinson and Stovall (*8*) say: "NaOH has the disadvantage of being injurious to the organisms if left too long in contact with them and may destroy their acid-fast character."

Using accepted sodium hydroxide digestion and concentration methods, Mishulow, Melman, and Romano (*9*) examined 369 specimens of sputum from 268 suspected and definite cases of tuberculosis. In each instance the result on examinations of direct smears prepared from *selected flecks* was as good as that prepared by concentration. These authors concluded: "It would therefore seem that by carefully selecting the material and thorough examination of the smears, more positive results may be obtained on routine examination without resorting to concentration of sputums."

To determine the relative efficiency of smears made by selection of choice particles and smears made by sodium hydroxide concentration, a comparison of the two methods under routine diagnostic conditions was undertaken.

Materials and Methods

Specimens were obtained from three sources, as follows:

1. *Lawson Veterans' Administration Hospital tuberculosis wards.* These specimens were usually freshly collected, and direct smears and sodium hydroxide concentrates were prepared on the same day.

2. *Georgia Department of Public Health.* These specimens were sent to the Health Department by practicing physicians throughout the State. After examination in the laboratories of the State Health Department, the remainders of the specimens were forwarded to the Tuberculosis Evaluation Laboratory. These were usually 2 to 3 days old when received.

3. *Battey Hospital, a State sanatorium in Rome, Ga.* Specimens were mailed to us from a distance of about 100 miles, and usually were 3 to 4 days old when prepared.

Two methods were employed in making the smears. In the first, the smears were prepared directly from the specimens by placing the sputum in a Petri dish on a black background, and selecting small bits of necrotic material, or cheesy, rusty, or bloody portions from various regions of the specimen. These choice particles were separated by means of sterile applicator sticks, placed on a slide, crushed and

smeared by the rotary motion of another slide. With a minimum of experience, workers were able to detect and avoid food particles and various other artifacts unlikely to contain the bacilli. The smears thus prepared were thicker than the usual bacteriological smears, but care was taken to make them thin enough for complete decolorization during the staining process.

In the second method, which employed the concentration and sodium hydroxide digestion technique, 2–5 ml. of the specimen were transferred to a centrifuge tube and about 10 ml. of 4 percent NaOH were added. The tubes were shaken vigorously and then incubated at 37° C. for 20 minutes, during which time they were shaken once every 5 minutes. After centrifugation, the sediment was neutralized with 2 drops of 2N HCl (without indicator) and used for smear. The methods of digestion and concentration were those used by the State Serum Institute at Copenhagen as reported by Holm and Lester (*10*).

The smears of concentrated sputum, as well as the direct smears of selected flecks, were fixed on a warm plate at 75° C. for 30 minutes, then flamed and placed on a staining rack. The smears were covered with filter paper, then saturated with basic carbolfuchsin, and heated until the stain steamed gently. The slides were allowed to stand for 5 minutes, after which the filter papers were removed, the smears washed in water and decolorized about 2 minutes in acid alcohol (3 ml. conc. HCl to 95 ml. 95 percent alcohol). The slides were again washed in water, counterstained 15 to 30 seconds with Loeffler's alkaline methylene blue, rinsed with water and dried.

After examining a similar number of fields (approximately 10 minutes per slide) the results were summarized as negative and positive. In all, 500 specimens of sputum were examined. In the series of comparisons between direct smears and smears of concentrates, shown below, better results were consistently obtained by examining direct smears made of selected sputum flecks.

	Number	*Percent*
Positive direct smear—positive concentrate	107	21. 4
Positive direct smear—negative concentrate	92	18. 4
Negative direct smear—positive concentrate	8	1. 6
Negative direct smear—negative concentrate	293	58. 6
Total	500	100. 0

Because of the great difference between the results obtained from examining smears made by the two methods, it was suspected that faulty technique (such as excessive dilution, prolonged exposure to NaOH, or improper neutralization), was responsible for the discrepancy. With this in view, a new series of 263 specimens was examined in which the concentration method was modified so as to achieve a more exact neutralization of the sediment.

The supernatant fluid was decanted after centrifugation, a small piece of sterile nitrazine paper was dropped in the tube and dilute hydrochloric acid added drop by drop until the paper indicated neutrality. One drop of this concentrate was placed on a slide, dried, and stained as previously described. Each specimen had one smear made of select flecks and one made of the digested sediment as described. Results of examination of 263 sputum specimens comparing direct smears with smears of the concentrates (exact neutralization) are given below.

	Number	*Percent*
Positive direct smear—positive concentrate	72	27. 4
Positive direct smear—negative concentrate	13	5. 0
Negative direct smear—positive concentrate	0	0. 0
Negative direct smear—negative concentrate	178	67. 6
Total	263	100. 0

The results of this study show a much closer correlation of positives for direct smear and positives for concentrated smears. Presumably, improved results in the case of the concentrated smears were due to the more careful neutralization, which suggests a possible relationship between exposure to sodium hydroxide and acid-fastness as mentioned by Steeken and Smith (*7*) and Robinson and Stovall (*8*) previously quoted.

In view of the better results of more exact neutralization of concentrates, other methods of improving the concentration technique were attempted. Instead of using only 2 ml. of sputum to 10 ml. of NaOH, the proportions were changed to equal parts of specimen and NaOH, and the specimens were shaken in a paint-conditioning machine (*11*) for 10 minutes at room temperature (instead of 20 minutes at 37° C). A total of 244 specimens were digested for 10 minutes by equal volumes of 4 percent sodium hydroxide, concentrated by centrifugation at 3,000 r. p. m. for 15 minutes, decanted and the sediment neutralized as previously described, using sterile nitrazine paper as an indicator.

As with the previous series, two smears were made from each specimen, one directly from selected flecks and one from the concentrated sediment. Results of examination of 244 sputum specimens comparing direct smears with smears of the concentrates (equal parts sputum and digestant) follow:

	Number	*Percent*
Positive direct smear—positive concentrate	58	23. 7
Positive direct smear—negative concentrate	19	7. 7
Negative direct smear—positive concentrate	3	1. 2
Negative direct smear—negative concentrate	164	67. 4
Total	244	100. 0

In this study, concentration yielded fewer positives than direct smears. The data par se are of doubtful significance in such a small series, but the cumulative value of the several experiments is considerable.

A test of the effectiveness of the macroscopic selection method was made after one further attempt to improve the concentration technique. To avoid the possibility of washing the concentrated smears off the slides, a fixative agent was used. Equal parts of egg albumin and glycerine were mixed, filtered through gauze, and applied by touching the surface of the slide with an applicator stick wet with the fixative. This very small drop of reagent was smeared evenly over the surface and allowed to dry. After heat fixation of the specimen, the slides were stained in the usual manner, altering the previous procedures only by allowing the slide to cool for 5 minutes in a refrigerator before decolorizing and destaining without first washing in water as suggested by Stempa (*12*).

A total of 716 sputa were so treated, making 2 smears of each as previously described. Results of examination of the 716 sputum specimens comparing direct smears with smears of the concentrates (fixative, slide cooled, no washing) follow:

	Number	*Percent*
Positive direct smear—positive concentrate	204	28. 5
Positive direct smear—negative concentrate	36	5. 0
Negative direct smear—positive concentrate	8	1. 1
Negative direct smear—negative concentrate	468	65. 4
Total	716	100. 0

Discussion

In the last series of experiments, the described changes in techniques yielded a significantly greater number of positive results from concentrates than were obtained without such changes. This improvement was attributed to several factors, including the use of relatively larger volumes of specimen, shorter exposure to digestant, more exact neutralization, the use of a smear fixative, and more attention to staining procedures (cooling the slides before decolorizing and using care to prevent dislodgement of the bacilli during washing or staining). These results indicate, too, the necessity for more reliable techniques than are usually used in the preparation of smears by sodium hydroxide concentration methods. It is clear, however, that even with the improvements in concentration technique, the direct smear method, properly used, consistently yields superior results.

It should be noted that all experiments were performed by the same bacteriologists, so that any change due to personal experience was eliminated. Often, a second team of workers reviewed these smears and similar results were obtained.

An impartial evaluation of the procedures used was made during the work of a training class (*13*) when 7 different workers, trained in as many different dependable laboratories, independently prepared direct and concentrated smears from 121 sputum specimens. Using the techniques described for the third series of comparisons described above, these bacteriologists obtained, on the average, 47.1 percent positives by the macroscopic selection of material for smears, as compared to 40.5 percent for concentrated smears.

It would appear, therefore, that under the conditions described, the simpler technique—direct smears of carefully selected material—is superior as a routine procedure to smears made from NaOH concentrates.

References have already been made to articles written by Robinson and Stovall (*8*), and Steenken and Smith (*7*), wherein the authors mention the possible disappearance of the acid-fast properties of the tubercle bacilli when subjected to sodium hydroxide digestion. It is possible that this accounts for much of the differences in results between the methods studied. This would appear to be borne out by the fact that most of the improvement came with shorter exposure to sodium hydroxide and more careful neutralization.

There is a probability that differences in specific gravity between the bacilli and sodium hydroxide mixtures are not sufficient to insure concentration on centrifugation. Hanks, Clark, and Feldman (*3*) wrote of finding tubercle bacilli in the supernatants discarded during the routine examination of specimens digested and centrifuged in the usual manner. On occasions these authors even found more bacilli in the flocculation concentrate of the supernatant than were found in the original sodium hydroxide concentrate.

Whitehead (*14*), using Hanks' method, examined 3,046 specimens and concluded that: "Concentration, centrifugalization and smears of the sediment did not seem to increase the number of organisms seen in the smears."

In our opinion, the inferiority of NaOH concentration methods could probably be attributed to: (a) the loss of bacilli during centrifugation as suggested by Hanks, and (b) loss of acid-fastness, as suggested by Steenken.

The chief use of concentration methods is to supplement the direct smear when a specimen is negative by the latter examination. A study of the last table reveals this to be true, but only in a small number of cases. It is therefore suggested that if one goes to the trouble of concentrating the specimen it should be planted on culture media where small numbers of tubercle bacilli may be more readily detected.

It is by no means suggested that direct smears made by the selec-

tion technique are as reliable as cultivation methods of isolating tubercle bacilli from pathological material. One must remember that many thousands of tubercle bacilli must be present in pathological material before the microscopist has a reasonable chance of finding them. It must also be stressed that one cannot reliably differentiate between pathogenic and nonpathogenic acid-fast bacilli microscopically.

Where the choice of method is limited, however, to microscopic examination, it is of value to make smears of choice particles of pathological material and to learn to select those particles which, from the pathologist's viewpoint, are most apt to contain the tubercle bacilli which are present in a given specimen.

Summary and Conclusions

1. Four series of sputa, consisting of 500, 263, 244, and 716 specimens, have been studied.

2. Smears made of selected particles gave more positive results in each series than smears made following concentration of the same specimens by the various methods of sodium hydroxide digestion used.

3. Sodium hydroxide digestion and concentration techniques are quite exacting in their requirements.

4. Macroscopic selection is dependable and probably superior to sodium hydroxide digestion and concentration by the methods described.

REFERENCES

(*1*) Koch, R.: Die aetiologie der tuberkulose, Mitteilungen aus dem Kaiserlichen Gesundheitsamte. **ii:** 1–88 (1884). Translated by Stanley Boyd in "Recent Essays by Various Authors on Bacteria in Relation to Disease," New Sydenham Society, London, 1886.

(*2*) Petroff, S. A.: A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J. Exper. Med. **21:** 38–42 (1915).

(*3*) Hanks, J. H., Clark, H. F., and Feldman, H.: Concentration of tubercle bacilli from sputum by chemical flocculation methods. J. Lab. and Clin. Med. **23:** 736–746 (1938).

(*4*) Cameron, G. M. and Castles, R.: Comparison of methods adaptable to production line examination of sputum for tubercle bacilli. J. Lab. and Clin. Med. **30:** 163–168 (1945).

(*5*) Nagy, A. H.: Evaluation of the tubercle bacillus concentration methods of Petroff, Pottenger, and chemical flocculation. J. Lab. and Clin. Med. **25:** 67–71 (1939).

(*6*) Ref. cited by Calmette, A.: Tubercle Bacillus Infection and Tuberculosis in Man and Animals, Williams and Wilkins Co., 1923. Translation by Soper, W. B. and Smith, G. H. p. 22–23.

(*7*) Steenken, W., Jr., and Smith, M. M.: Culture of tubercle bacilli. Am. Rev. Tuberc. **38:** 503–513 (1938).

(*8*) Robinson, L. and Stovall, W. D.: Factors influencing the demonstration of tubercle bacilli by concentration methods. J. Lab. and Clin. Med. **27:** 84–91 (1941).

(*9*) Mishulow, L., Melman, M. and Romano, M.: An improved method of direct smear examination for acid-fast bacilli in sputums. Science **80:** 143–144 (1934).

(*10*) Holm, J. and Lester, V.: Diagnostic demonstration of tubercle bacilli. Abstract from Acta Tuberculosea Scandinavica **16:** 3–4 (1941). In Pub. Health Rep. **62:** 847–854 (1947).
(*11*) Steenken, W., Jr., and Smith, M. M.: A shaking machine and containers for shaking sputums and other body fluids during the process of homogenization. J. Lab. and Clin. Med. **27:** 1582–1585 (1942).
(*12*) Stempa, H.: The variations of the tubercle bacillus in clinical specimens. Am. Rev. Tuberc. **22:** 443–448 (1930).
(*13*) Tuberculosis Evaluation Laboratory Training Course. Unpublished data, 1948.
(*14*) Whitehead, H. G.: Bacteriological diagnosis of tuberculosis. Am. Rev. Tuberc. **42:** 795–800 (1940).

Characteristics of Commercial X-ray Screens and Films—V

By Willard W. Van Allen, B. Sc.*

As reported in a previous issue [1] the Electronics Laboratory is engaged in the evaluation of characteristics of commercial X-ray films, screens, and developers in appropriate combinations. The results of this work are summarized in these pages from time to time in tables giving the speed of screen-film-developer combinations, the contrast (gamma) of the films in different developers, and the fog developed by the films in these developers. These tables are cumulative and include the results of previous determinations, corrected and revised where necessary.

Table 1.—*Speed of fluoroscopic screen-film-developer combinations* [1]

Screens	Film and developer [2]							
	Ansco Fluorapid film		DuPont Fluorofilm		Eastman Blue Photoflure		Eastman Green Photoflure	
	Eastman X-ray	Ansco Liquadol	Eastman X-ray	Ansco Liquadol	Eastman X-ray	Ansco Liquadol	Eastman X-ray	Ansco Liquadol
D sample 1	120	105	95	90	95	85		
D sample 2	150	125	115	110	115	105		
D sample 3	155	140	130	120	130	115		
666D sample 1	100	75	80	65	75	65		
666D sample 2	125	100	100	85	100	85		
B sample 1							60	55
B sample 2							70	55
B–2							95	85
E–2							140	120

[1] Speeds determined with film and screen in direct contact and therefore do not represent the speed of the same combinations when used in a photofluorograph. Subsequent reports will contain data on additional developers used in combination with the films and screens shown in this table. These will include: G. E. Supermix, Eastman Liquid X-ray, Eastman Rapid X-ray, and Buck X-ray.
[2] Development time (as recommended by manufacturer of developer): Eastman X-ray Developer (dry ingredients) 8 minutes at 68° F.; Ansco Liquadol, 4 minutes at 68° F.

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[1] Pub. Health Rep. **64:** 430 (1949)

TABLE 2.—*Speed of intensifying screen-film-developer combinations* [1]

Screens	Film and developer [2]				
	Ansco High-speed film [3]	DuPont No. 508 film		Eastman Blue Brand film	
	Ansco Liquadol	Eastman X-ray	Ansco Liquadol	Eastman X-ray	Ansco Liquadol
Buck:					
Xtra speed	70	55	50	85	90
Midspeed	60	50	45	70	75
Definition	50	40	40	60	65
Eastman:					
Ultra speed	110	90	85	140	145
Fine grain	85	70	65	110	110
Definition	60	50	45	80	75
Patterson:					
High speed	115	80	85	120	130
Parspeed	60	55	50	90	80
Detail	20	20	15	25	25

[1] Subsequent reports will contain data on additional developers used in combination with the films and screens shown in this table. These will include: G. E. Supermix, Eastman Liquid X-ray, Eastman Rapid X-ray, and Buck X-ray.
[2] Development time (as recommended by manufacturer of developer): Eastman X-ray Developer (dry ingredients) 4½ minutes at 68° F.; Ansco Liquadol, 3 minutes at 68° F.
[3] Test with Eastman X-ray Developer to be reported in a subsequent issue.

TABLE 3.—*Average values of fog and contrast (gamma)*[1]

Film	Fog densities—Developer [2]		Contrast (gamma)—Developer [2]	
	Eastman X-ray	Ansco Liquadol	Eastman X-ray	Ansco Liquadol
Photofluorographic:				
Ansco Fluorapid	0.19	0.09	2.1	1.8
DuPont Fluorofilm	.21	.15	1.9	2.0
Eastman Blue Photoflure	.07	.04	1.8	1.8
Eastman Green Photoflure	.17	.11	2.0	2.1
Roentgenographic:				
Ansco High speed		.10		2.8
DuPont No. 508	.18	.20	2.6	2.7
Eastman Blue Brand	.11	.08	2.8	3.0

[1] Values obtained with open-tank development and continuous mechanical agitation at 68° F.
[2] Development time as given in tables 1 and 2. Subsequent reports will present similar data for additional developers used in combination with the films shown above. These will include: G. E. Supermix, Eastman Liquid X-ray, Eastman Rapid X-ray, and Buck X-ray.

Correction

The article "Tuberculosis Mortality in the United States, 1947," Public Health Reports, vol. 64, No. 13, April 1, 1949, contained the following errors:

Page 413, table 5, under headings Percentage Change: Male: 1939-41 to 1947, −49.9 should be −49.4.

Page 418, table 7, under headings, Percentage change in rate, 1946 to 1947: Idaho, −5.5 should be +5.5.

Page 421, table 9, under headings, Type of service: Tuberculosis hospitals: Wisconsin, 32 should be 323.

INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED APRIL 16, 1949

Except for very slight increases in the East North Central and Mountain Areas, the reported incidence of measles declined during the week in all of the nine geographic divisions. A total of 27,750 cases was reported, as compared with 32,909 last week and a 5-year (1944–48) median of 25,616. Of the 19 States reporting more than 500 cases, only 6, with reports as follows (last week's figures in parentheses), showed increases: Massachusetts 1,023 (1,001), Ohio 976 (678), Georgia 655 (575), Oklahoma 537 (445), Texas 3,013 (2,799), Washington 565 (475). The total for the year to date is 343,715 cases, as compared with a 5-year median of 250,733, reported for the corresponding period last year.

The total of 2,606 cases of influenza reported, as compared with 2,658 last week and a 5-year median of 1,917, is the largest reported for a corresponding week of the past 5 years except that of 23,536 for the same week in 1947. Only 4 States as follows, reported currently more than 101 cases (last week's figures in parentheses): Virginia 242 (249), South Carolina 304 (471), Alabama 386 (170), Texas 1,009 (1,120). The total to date is 61,035, as compared with a 5-year median of 179,321 and 324,632 reported for the period in 1944.

Of 45 cases of poliomyelitis reported (last week 41, 5-year median 31), 12 occurred in Texas (last week 5), 4 in New Jersey, and 3 in California. No other State reported more than 2 cases. The total reported for the past 4 weeks, since the average week of seasonal low incidence, is 180, as compared with a 5-year median of 109.

During the week, 3 cases of anthrax were reported, 2 in Pennsylvania and 1 in New York; 3 cases of Rocky Mountain spotted fever, 1 each in North Carolina, South Carolina, and Montana; and 2 cases of smallpox, 1 in Kansas and 1 in Mississippi.

A total of 9,232 deaths was recorded during the week in 94 large cities in the United States, as compared with 9,495 last week, 9,009 and 9,751, respectively, for the corresponding weeks of 1948 and 1947, and a 3-year (1946–48) median of 9,127. The total for the year to date is 147,369, as compared with 151,611 for the corresponding period last year. Infant deaths during the week totaled 608, last week 611, 3-year median 659. The cumulative figure is 9,915 as compared with 10,417, for the corresponding period last year.

Telegraphic case reports from State health officers for week ended April 16, 1949

[Leaders indicate that no cases were reported]

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Poliomyelitis	Rocky Mt. spotted fever	Scarlet fever	Smallpox	Tularemia	Typhoid and paratyphoid fever [d]	Whooping cough	Rabies in animals
NEW ENGLAND														
Maine	1		101	396		29			14			1	2	
New Hampshire			4	63					1				6	
Vermont				278					4				13	
Massachusetts	8	1		1,023	1		1		190			1	58	
Rhode Island				129		10			9				3	
Connecticut	5		1	1,250	3	50			30				6	
MIDDLE ATLANTIC														
New York	5		[b] 2	2,475	11	320	1		187			2	75	7
New Jersey	6		3	1,586		58	4		120			2	40	1
Pennsylvania	15	2	([b])	2,056	3				263			1	73	
EAST NORTH CENTRAL														
Ohio	2		1	976	5	50			324			2	47	19
Indiana	4		6	203		8			61			1	10	24
Illinois			31	152	4	104	1		116		2		42	2
Michigan [c]	2		3	613	3	20	2		386			1	24	2
Wisconsin			33	1,994	2	13	1		61				35	
WEST NORTH CENTRAL														
Minnesota	5			75	2	5	1		29		1	1		
Iowa	2			74		6	2		9				1	7
Missouri	2		5	279	2	19	2		24		2		3	
North Dakota				10					3		2		5	
South Dakota				72					1	([1])				
Nebraska			9	93					4					
Kansas				999	2	20			17	1			11	
SOUTH ATLANTIC														
Delaware	1			31			1		12					
Maryland [c]	2		3	555	2	46			[c] 35				6	
District of Columbia	1			146	1	7			7				4	
Virginia	1		242	929	2	51			17			2	11	4
West Virginia	3		13	110	1	6			9				14	
North Carolina	3			851	3			1	13		3	1	26	
South Carolina	1	1	304	336		122		1	4				22	1
Georgia	2		24	655	1	40	1		6		3		2	13
Florida	6		10	105	1	18	2		3			1	1	2

EAST SOUTH CENTRAL														
Kentucky	3		4	148	2	1	1		28				7	12
Tennessee	3		89	472	3	74	1		20			1	15	
Alabama	3		386	592	1	66			7			1	3	11
Mississippi [a]	4		15	166	1	35	1		9	1	2	2	5	
WEST SOUTH CENTRAL														
Arkansas	3		87	615		65			4		6	2	12	3
Louisiana	1		27	140	6	64	1		3		3	5	1	
Oklahoma		3	34	537	1	33			5				5	3
Texas	20		1,009	3,013	11	306	12		22			9	105	24
MOUNTAIN														
Montana				49	1			1	9				1	
Idaho	1		5	163		3	1		[c] 18				1	
Wyoming				27		7	1		3					
Colorado	1		26	396		18			13			1	2	
New Mexico				138		11			6			1	4	
Arizona			101	206		8			4				3	
Utah [a]				73		6	1		9				10	
Nevada				22			1							
PACIFIC														
Washington	1			565	4		1		21				12	
Oregon			1	266	1	26	2		17			2	12	
California	10		27	1,598	9	36	3		[c] 101			2	37	2
Total	127	7	2,606	27,750	89	1,761	45	3	2,263	2	24	42	775	
Median, 1944–48	230	7	1,917	25,616	131		31	2	3,971	10	12	53	2,149	
Year to date 15 weeks	2,495	116	61,035	343,715	1,286	35,635	1,102	18	42,015	[1] 27	398	630	14,971	
Median, 1944–48	4,231	119	179,321	250,733	2,837		521	10	52,463	149	267	689	32,906	
	(27th)		(30th)	(35th)	(37th)		(11th)		(32d)	(35th)		(11th)	(39th)	
Seasonal low week ends	July 10		July 31	Sept. 4	Sept. 18		Mar. 19		Aug. 14	Sept. 4		Mar. 19	Oct. 2	
Since seasonal low week	7,609		97,305	396,108	2,130		180		64,713	[1] 42		170	25,004	
Median, 1944–48	11,797		299,112	285,679	4,341		109		91,034	225		210	58,674	

[a] Period ended earlier than Saturday.
[b] New York City and Philadelphia only, respectively.
[c] Including cases reported as streptococcal infection and septic sore threat.
[d] Including paratyphoid fever, reported separately, as follows: New York 1; New Jersey 2; Virginia 2; Louisiana 1; Texas 1; Colorado 1; Oregon 1; California 2; salmonella infections, not included, were reported as follows: New York 1.
[1] Deduction: Smallpox, South Dakota, week ended Mar. 26, 1 case.
Anthrax: New York 1; Pennsylvania 2.
Alaska: Influenza 1; measles 2; streptococcal sore throat 2.
Territory of Hawaii: Diphtheria 1; influenza 6; measles 152; poliomyelitis 1.

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended March 26, 1949.—During the week ended March 26, 1949, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		4	5	296	525	22	17	42	257	1,168
Diphtheria				16	2			3		21
Dysentery:										
Amebic								3		3
Bacillary					1				1	2
German measles		1		305	48		170	13	7	544
Influenza		115			131	3	20		1	270
Measles		131	42	320	237	222	126	262	158	1,498
Meningitis, meningococcal				2	1					3
Mumps		9	5	241	250	51	39	19	65	679
Poliomyelitis				1						1
Scarlet fever		5	3	133	110	3	1	21	20	296
Tuberculosis (all forms)		4	2	100	36	18	11		29	200
Typhoid and paratyphoid fever				11					2	13
Undulant fever					1	1		2		4
Venereal diseases:										
Gonorrhea	1	4	9	92	52	31	15	29	57	290
Syphilis		7	5	106	33	11	2	8	16	188
Other forms									1	1
Whooping cough				126	22	8	3			159

JAMAICA

Notifiable diseases—4 weeks ended March 26, 1949.—For the 4 weeks ended March 26, 1949, cases of certain notifiable diseases were reported in Kingston, Jamaica, and in the island outside of Kingston, as follows:

Disease	Kingston	Other localities	Disease	Kingston	Other localities
Chickenpox	17	47	Scarlet fever	1	
Diphtheria		1	Tuberculosis (pulmonary)	43	47
Dysentery, unspecified	1	3	Typhoid fever	4	47
Erysipelas	1		Typhus fever (murine)	2	1
Leprosy		4			

NEW ZEALAND

Gilbert Islands—Butaritari (Makin)—Influenza.—An epidemic of influenza was reported on the atoll of Butaritari (Makin) in the Gilbert Islands, New Zealand, in the month of January 1949. During the month 784 cases are stated to have occurred there.

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

Note.—The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

India—Calcutta.—During the week ended April 2, 1949, 215 cases of cholera with 61 deaths were reported in Calcutta, India.

Plague

India.—During the week ended April 2, 1949, plague was reported in cities in India as follows: Cawnpore 36 cases, 8 deaths; Calcutta 12 cases, 1 death.

Smallpox

Great Britain—England—London.—Information dated April 7, 1949, state that 1 fatal case of smallpox occured in an isolation hospital in London during the first 10 days of April 1949. This fatality was the wife of a man who died on the steamship "Mooltan" before its arrival at the Port of London on April 4, apparently of smallpox. It was assumed that the disease was contracted in Bombay. According to information dated April 13, 1949, 5 confirmed cases of smallpox, 4 of them fatal, have been reported in England since the death of the above patient. Two suspected cases which have been under observation in isolation are considered to be almost certainly smallpox, while 3 other suspected cases, also under observation, are believed to be only chickenpox. These are the first cases of smallpox reported in England since the outbreak in the first half of the year 1947.

India.—During the week ended April 9, 1949, smallpox was reported in cities in India as follows: Bombay 158 cases, 43 deaths, Madras 36 cases, 5 deaths, New Delhi 21 cases, 4 deaths.

Java—Batavia.—In the current outbreak of smallpox in Batavia, Java, 1,425 cases with 108 deaths had been reported in the city up to March 16, 1949, 153 cases with 16 deaths within a 30-mile radius, and 1,022 cases with 100 deaths to the west of Batavia in Bantam District. During the period March 6–April 9, 1,260 cases were reported in the city.

Typhus Fever

Chile—Santiago.—During the period March 20–April 2, 1949, 21 cases of typhus fever were reported in Santiago, Chile.

Ethiopia.—Delayed report: During the period October 19–November 1, 1948, 27 cases of typhus fever were reported in Ethiopia.

Transjordan.—During the week ended April 2, 1949, 17 cases of typhus fever were reported in Transjordan.

Yellow Fever

No reports of yellow fever were received during the current week.

DEATHS DURING WEEK ENDED APR. 9, 1949

[From the Weekly Mortality Index, issued by the National Office of Vital Statistics]

	Week ended Apr. 9, 1949	Corresponding week, 1948
Data for 94 large cities of the United States:		
Total deaths	9,496	9,749
Median for 3 prior years	9,749	
Total deaths, first 14 weeks of year	138,137	142,602
Deaths under 1 year of age	611	719
Median for 3 prior years	714	
Deaths under 1 year of age, first 14 weeks of year	9,307	9,758
Data from industrial insurance companies:		
Policies in force	70,476,744	71,084,296
Number of death claims	13,506	15,613
Death claims per 1,000 policies in force, annual rate	10.0	11.5
Death claims per 1,000 policies, first 14 weeks of year, annual rate	9.8	10.6