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IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY. VI.
TECHNICAL PROCEDURES

Warren R. Sanborn

Fort Detrick
Frederick, Maryland

March 1968

AD

AD 579032

MISCELLANEOUS PUBLICATION 20

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MARCH 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

MISCELLANEOUS PUBLICATION 20

IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY

VI. TECHNICAL PROCEDURES

Warren R. Sanborn

March 1968

Technical Information Division
AEROBIOLOGY AND EVALUATION LABORATORY

FOREWORD

The use of immunofluorescence, or fluorescent antibody, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The volume of literature related to immunofluorescence or fluorescent antibody and covering use of this technique has expanded explosively over the relatively few years since its inception. This expanding literature volume bears witness to the basic value of the technique. Through 1954, only about 40 articles had been published. In the next two years, 58 were added. During 1957 and 1958 there were 83 and 96, respectively. By 1961 the annual figure had reached more than 260 articles. For this supplementary second edition, the figures for 1963, 1964, and 1965 are 551, 764, and 678, respectively. These totals are testimony to Dr. Coons' genius.

Although it would be virtually impossible to cite every article that refers to the use of immunofluorescence, an attempt has been made to approach that limit. To that end, more than 445 journals were searched. In addition, six abstracting journals and the computer system of the National Library of Medicine, MEDLARS, were employed. Fifteen languages are represented. Translations were provided by colleagues of the compiler, government translating services, abstractors, and the compiler. The earliest entry in the original edition was 1905. In the present edition, entries covering the years 1963, 1964, and 1965 are the primary ones included, but there are also a few earlier entries not listed in the first edition. Further entries for 1966 and 1967 are now being compiled; these will be incorporated into further revisions of this bibliography.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. This entire second edition, Miscellaneous Publication 20, has the same overall title, "Immunofluorescence, an Annotated Bibliography," as the first edition (Miscellaneous Publication 3). The present edition also has the same six-volume structure: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending

undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author. A seventh volume, "Author and Subject Indexes," has been added to further aid the investigator in his search for articles relevant to his interest area.

Abstracts for citations in this edition have been prepared or modified in keeping with the central theme, the application of immunofluorescence to various problems. If the primary emphasis in the original article was immunofluorescence and the author's summary reflected this, the summary was generally left unchanged, except for minor changes and abbreviations simply to save space. In other instances, it was necessary to write a new abstract in order to indicate the proper place of immunofluorescent technique in the study. At the same time, the main point of such articles was maintained in abbreviated form in the abstract. Hopefully, this approach will be successful in bringing the application of immunofluorescence to the attention of the reader, while preserving each author's ideas at the same time. It is further hoped that this bibliography will aid investigators in avoiding duplication of effort and thus contribute to even greater and more imaginative applications of immunofluorescence.

Accession numbers have been assigned consecutively to citations throughout all six volumes of this edition. The plan for further future volumes allows this simple system. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in each volume.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

To avoid excess duplication and unwieldy size, the second parts of Volumes V and VI contain only basic citations for articles printed in the other four volumes. However, titles of articles are included to assist the reader in selection of those citations of possible interest. As in the other volumes, the references are placed in subject categories and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous forms. For unmistakable identification, they are listed below.

| | |
|------|---|
| BSA | bovine serum albumin |
| DANS | a. 1-dimethylaminonaphthalene-5-sulfonic acid |
| | b. 5-dimethylamino-1-naphthalene sulfonic acid or chloride form. |

| | |
|---------|--|
| FA | fluorescent antibody |
| FIC | fluorescein isocyanate |
| FITC | fluorescein isothiocyanate |
| FTA | fluorescent treponemal antibody |
| FTA abs | fluorescent treponemal antibody absorbed |
| FTA-200 | a modification of the above based on serum dilution |
| PAP | primary atypical pneumonia |
| PAS | para-aminosalicylic acid |
| PBS | phosphate-buffered saline |
| RB 200 | a. lissamine rhodamine RB 200 b. lissamine rhodamine B 200 c. lissamine rhodamine B d. sulphorhodamine B e. acid rhodamine B |
| TPFA | <u>Treponema pallidum</u> fluorescent antibody |
| TPI | <u>Treponema pallidum</u> immobilization |

Generally, the citations follow the format prescribed by the second edition of Style Manual for Biological Journals, American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations," Z39.5-1963, American Standards Association Incorporated, New York.

The compiler began to collect this immunofluorescence literature in 1957 while he was stationed at U.S. Navy Preventive Medicine Unit No. 2, Norfolk, Virginia. The literature collection became more intense and organized after 1959 when he was transferred to Fort Detrick, Frederick, Maryland. Following his further transfer to the Microbiology Department of the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he continued this work with the encouragement and support of both of these latter installations. Work on the second edition began in 1964, and it has continued through support from both the U.S. Army and the Bureau of Medicine and Surgery of the U.S. Navy. This volume was completed while the compiler was assigned to U.S. Navy Medical Research Unit No. 3, FPO, New York, 09527, where he is currently serving as head of the Bacteriology Department.

The information in these volumes was originally recorded on coded marginal punch cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701, for improvement of the subsequent editions. Reader assistance in this area will be deeply appreciated.

ACKNOWLEDGMENTS

The essential team effort required for development of this immunofluorescence bibliography cannot be overstressed. As with many projects of this nature, the talents, advice, guidance, and assistance of many people led to the completion of this second edition. The compiler is deeply grateful to the many people who have contributed.

Financial support for this project at first was absorbed by the Pathology Division and the Walter Reed Army Medical Unit, Fort Detrick. However, completion of the first edition (through 1962) was made possible by special financial assistance from Physical Defense Division, Fort Detrick, under Dr. Charles R. Phillips. I am extremely grateful to him for his aid. Expenses for this second edition were primarily met through a generous grant from U.S. Navy Bureau of Medicine and Surgery, Preventive Medicine Division, under CAPT J. Millar, MC, USN. Many administration expenses also were borne by the Naval Medical Research Institute and by Fort Detrick.

A number of libraries kindly donated their services. In spite of the unusual requests required by this project, these libraries were very helpful and willingly assisted, often providing valuable suggestions. Libraries primarily involved were the Technical Library, Fort Detrick, under Mr. Charles N. Bebee and later Miss Joyce A. Wolfe, and the Technical Reference Library, Naval Medical Research Institute, Mrs. T.P. Robinson, librarian. Much valuable assistance was also rendered by the National Institutes of Health Library, Miss R. Connelly, reference librarian, the National Library of Medicine, and the library of the Walter Reed Army Medical Unit, Fort Detrick. The staff members of these libraries were both helpful and patient. Without such fine assistance, the work could not have been completed.

It is a pleasure to acknowledge the highly competent secretarial help. Secretaries providing their capable and untiring talents were: Miss Sandra Rosenblatt, Miss Linda L. Zimmerman, Mrs. Marguerite M. Matovich, Mrs. Gene Heaven, Mrs. Linda Franklin, Mrs. Alberta Brown, Mrs. Margaret Raheb, and a number of others. Valuable assistance in double-checking problem references was provided by Mrs. Catherine F. Eaves and Mrs. Mary J. Gretzinger. Dr. George H. Nelson was a willing consultant for classification problems. Dr. Harold W. Batchelor provided an essential key to the development of this work by introducing the compiler to marginal punch card systems and guiding him in their application.

The Technical Information Division, under Mr. Gerald W. Beveridge, continually provided all types of assistance in addition to a home base from which to work. My gratitude for this cannot be fully expressed.

Last, but by no means least, the essential editorial work receives my highest praise. The tireless efforts, patience, and driving force supplied by these people were the prime factors in bringing this edition to completion. Mrs. Madeline Warnock Harp, in charge, Mrs. Mary D. Nelson, and Mrs. Ruth P. Zrudzinski all spent many hard weeks of work on this project. I shall always be indebted to them.

ABSTRACT

This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume VI is divided into two major sections. The first section contains 292 annotated citations to general procedures in the use of immunofluorescence, arranged by subject areas. The second section contains 360 cross-reference to citations in the other volumes that describe specific applications of this technique; these are arranged to correspond with the subject areas of the other volumes. A complete author index for these 652 citations is included.

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I. GENERAL PROCEDURES

A. ANTISERUM PRODUCTION

7436

Andersen, F.L.; Lowder, L.J.; Hammond, D.M.; Carter, P.B. 1965. Antibody production in experimental Eimeria bovis infections in calves. Exp. Parasitol. 16:23-35.

Two experiments were conducted to investigate the possible presence of humoral antibodies in calves experimentally infected with Eimeria bovis. Ten calves received 25,000 oocysts and 1 million oocysts 26 or 27 days later; eight calves were inoculated once with 1 million oocysts. Blood samples were taken periodically and the sera collected were tested against first-generation merozoites by indirect FA and the slide agglutination test, and against an oocyst extract by the precipitin ring test. In vitro observations were also made on the effect of immune serum upon the first-generation merozoites. Fluorescence of the merozoites was first observed in serum collected 10 to 22 days after inoculation. The calves that received only one large inoculum attained, on the average, higher titers in less time than did the calves inoculated twice. Agglutinating antibody was first detected 7 to 17 days after inoculation; however, in this case calves that received a second inoculum developed higher titers than did those inoculated once. The results of the precipitin ring tests paralleled closely those of the slide agglutination tests.

7437

Cebra, J.J.; Goldstein, G. 1965. Chromatographic purification of tetramethylrhodamine-immune globulin conjugates and their use in the cellular localization of rabbit gamma globulin polypeptide chains. J. Immunol. 95:230-245.

Rabbit spleen imprints were double-stained with pairs of fluorescein and tetramethylrhodamine conjugates derived from the following goat reagent globulins: anti-Fragment III, anti-gamma-globulin heavy chain, anti-light chain, and anti-Fragment I. Bright specific staining could be obtained with the subfractionated conjugates at concentrations as low as 32 to 43 ug of antibody per ml of conjugate. A series of simple Kodak Wratten filters, K2, 23A, and 45A, were used to differentiate between singly and doubly stained fluorescent cells. Staining with anti-light chain and anti-III, conjugated with contrasting fluorochromes, resulted in double labeling of 63 to 78 per cent of all fluorescent cells. A distinct minority of the fluorescent cells, 21 to 34 per cent of the population, stained only with the anti-light chain reagent.

A similar class of cells, comprising 14 to 26 per cent of all fluorescent cells, stained only with the anti-I reagent when it was used as a counter-stain with anti-III reagent. Anti-light chain reagents detected more immunoglobulin-containing cells than did anti-I reagents. Double staining with anti-I and anti-light chain yielded 63 to 85 per cent doubly stained cells, 15 to 32 per cent of cells stained with the anti-light chain alone, and 0 to 10 per cent of cells stained with anti-I alone.

7438

Georgala, D.L.; Boothroyd, M. 1965. Preparation of fluorescent polyvalent Salmonella antisera. *Nature* 205:521-522.

The conjugated polyvalent antisera described here are being assessed in routine immunofluorescence Salmonella tests on raw meat. Experience so far confirms that the direct technique may have worthwhile advantages over the indirect technique used in our earlier investigations. If necessary, a series of such conjugated polyvalent antisera could be prepared, using Group O antigens from all existing Salmonella groups.

7439

Goldin, R.B.; Krasnik, F.I. 1963. Specific staining of ornithosis virus by fluorescein-labeled incomplete antibodies. *Acta Virol.* 7:561.

Sera from pigeons recovered from ornithosis served as source of antibodies. The presence in these sera of complete and incomplete antibodies was estimated by the direct and indirect modifications of the complement fixation reaction. The conjugates were all equally sufficiently specific, clearly stained homologous viral bodies, and induced no fluorescence of antigenically distinct microorganisms. Conjugates prepared from incomplete antibodies stained homologous antigens more rapidly, in higher dilution, and stained more particles than conjugates prepared from complete antibodies.

7440

Kaplan, W.; Clifford, M.K. 1964. Production of fluorescent antibody reagents specific for the tissue form of Coccidioides immitis. *Amer. Rev. Resp. Dis.* 89:651-658.

Five different lots of rabbit Coccidioides immitis antiglobulins were tagged with fluorescein isothiocyanate. These reagents brightly stained endospores and contents of spherules formed in vivo. The labeled antibodies also cross-reacted with Histoplasma capsulatum, Blastomyces dermatitidis, and other heterologous fungi. Absorption of these conjugates with yeast cells of H. capsulatum eliminated all nonspecific activity. Two of the absorbed reagents reacted strongly with the tissue forms of C. immitis.

These conjugates had been prepared from globulins produced by rabbits infected with the fungus and by rabbits immunized with killed arthrospores. By dilution, the former conjugate was also rendered specific for tissue forms of C. immitis. The specific conjugates were successfully used to detect C. immitis in clinical materials from confirmed cases of coccidioidomycosis.

7441

Karakawa, W.W.; Sedgwick, A.K.; Borman, E.K. 1964. Typing of Haemophilus influenzae with fluorescent antibody reagent. Health Lab. Sci. 1:114-118.

Immunization of rabbits over a 4-week period with vaccine harvested from 6-hour cultures on Levinthal agar of Haemophilus influenzae Type B yields antiserum of high type specificity. Antibodies to somatic antigens common to all types are present in minimal concentration as compared with homologous-type, anticapsular antibodies. This antiserum yields fluorescein-labeled conjugates that stain smooth Type B strains in high titer and are rendered completely type-specific by minimal adsorption with heterologous-type cells from smooth cultures or by use of a blocking anti-rough serum in a two-step inhibition technique. Rapid identification of H. influenzae in clinical material, particularly the demonstration of Type B in spinal fluid, is readily and surely achieved by use of carefully prepared and standardized FA reagents. Other available serological techniques are more susceptible to uncontrollable errors arising from less than optimal antigen-antibody ratios, a factor not operative in the direct FA method.

7442

Kishko, Ya.H. 1965. Use of isothiocyanate-labelled fluorescein anti-complementary serum for the Goldwasser-Shepard test. Mikrobiol. Zh. Akad. Nauk Ukr. RSR 27:5:67-70. In Russian.

An anticomplementary conjugate, labeled by FITC, was obtained by immunizing rabbits with guinea pig serum. Following globulin extraction, it was labeled in a proportion of 25 mg of preparation per 100 mg of 2.5 to 3.0 per cent protein. BA-47-108567.

7443

Lee, C.L.; Davidsohn, I.; Takahashi, T. 1965. Prolonged immunization; II. Effect on complement-fixing, incomplete, and combining antibodies in rabbit anti-mouse erythrocyte serum. *J. Immunol.* 94:423-429.

Rabbit anti-mouse erythrocyte sera from each of seven courses of immunization were studied with nine immunologic techniques, namely, agglutinin titrations with antiglobulin serum, in serum-albumin medium, with papain-treated cells, and in saline medium; hemolysin and complement-fixation titrations; determination of combining ability with fluorescein and iodine-125-labeled sera; and production of anemia in mice. Formalinized erythrocytes can be used successfully to study certain immunologic reactions. In vitro reactions, except for complement fixation and coating with fluorescent antibodies, did come near their peak levels in two-course sera; after four or more courses, titers of all in vitro reactions were essentially parallel to the base line. Whether these tests are demonstrating different reactions of the same antibody or reactions of different antibodies requires further investigation. On the other hand, the anemia-producing ability showed marked decrease in sera collected after more than three courses of immunization. No in vitro reaction has yet been found to serve as a reliable indicator of the in vivo potency of a rabbit anti-mouse erythrocyte serum.

7444

Lennette, E.H.; Woodie, J.D.; Nakamura, K.; Magoffin, R.L. 1965. The diagnosis of rabies by fluorescent antibody method employing immune hamster serum. *Health Lab. Sci.* 2:24-34.

Antirabies immune sera were prepared by the inoculation of hamsters with three or four serial doses of beta-propiolactone - inactivated suspension of CVS strain of fixed rabies virus in Arlacel-mineral oil adjuvant followed by two doses of live virus suspension. Fluorescein-conjugated immune globulins prepared from these hamster sera were consistently superior in staining quality to the immune horse serum conjugates previously employed in fluorescent antibody tests for rabies. An evaluation of the FA test, employing hamster immune serum conjugates, included examination of approximately 4,200 specimens. About three-fourths of the specimens were shipped in glycerine-saline preservative; the remainder were fresh or frozen. An array of species was represented, with dogs, cats, skunks, and bats being the most frequent. From the combined results of FA tests, examinations for Negri bodies, and the inoculation of mice, 363 of the approximately 4,200 specimens were positive for rabies; 99.4 per cent were detected by the FA test, 98.3 per cent by inoculation of mice, and 65.8 per cent by the presence of Negri bodies. When fluorescein-globulin conjugates of good staining quality are employed, FA is as sensitive as mouse inoculation.

7445

Marshall, J.D., Jr. 1964. The use of immunofluorescence for the identification of members of the genus Pasteurella in chemically fixed tissues. Diss. Abstr. 24:4921.

FA, cultural, and histologic methods were used to determine the effectiveness of FA for diagnosis of pasteurellosis. Specimens were obtained from animals infected with Pasteurella gallinarum, P. haemolytica, P. multocida, P. novicida, P. pestis, P. pseudotuberculosis, and P. tularensis. FA provided rapid, reliable identification of all species in pure or mixed cultures or from untreated clinical material. P. pestis and P. pseudotuberculosis cross-reacted serologically. Fraction I antiserum from P. pestis was specific. Ethanol (95 or 70 per cent), methanol, chloroform, or 10 per cent formalin were satisfactory fixatives for all species except P. pestis and P. pseudotuberculosis. Methanol was the only satisfactory fixative for these. Histopathologic studies using FA are described.

7446

Matuhasi, T. 1962. Fluorescent blood cells and anti-fluorescein antibodies. Proc. Congr. Int. Soc. Blood Transfus. 9:578-581.

Blood cells can be directly conjugated with FITC. Anti-fluorescein antibodies are produced in rabbits by injection of fluorescent blood cells or serum globulin conjugated with FITC. The fluorescent blood cells can be so clearly distinguished from normal blood cells that they are detected in normal blood cells for a long time even after they are reinjected into the blood stream and that the life span of the fluorescent blood cells can be easily measured. The life span of the fluorescent red blood cells was about 35 days in normal rabbits, but it was much reduced in rabbits having anti-fluorescein antibodies.

7447

Moody, M.D.; Jones, W.L. 1963. Identification of Corynebacterium diphtheriae with fluorescent antibacterial reagents. J. Bacteriol. 86:285-293.

Conditions are described in which fluorescent antibody reagents can be prepared and used to identify Corynebacterium diphtheriae in pure and mixed cultures and in clinical materials. The use of O and OK antigens for immunization of rabbits to prepare the antibody was compared. The most satisfactory reagents were those made from serum of rabbits injected with live OK suspensions of C. diphtheriae. Such fluorescent reagents were used successfully in direct and indirect fluorescent

antibody tests to identify both toxinogenic and atoxinogenic C. diphtheriae but not to differentiate the two kinds of organisms.

7448

O'Berry, P.A. 1964. A comparison of three methods of serum fractionation in the preparation of Vibrio fetus fluorescent antibody conjugates. Amer. J. Vet. Res. 25:1669-1672.

Lapine and bovine hyperimmune sera were prepared by parenteral injection of two venereal strains of Vibrio fetus. These sera and those from rabbits and cows negative for V. fetus agglutinins were fractionated by procedures utilizing ethanol, ammonium sulfate, and ethodin. Globulin fractions were conjugated to fluorescein isothiocyanate, studied electrophoretically, and found to be composed of gamma globulins with a trace of beta globulins. Homologous and heterologous V. fetus cell suspensions and smears were stained with each fluorescent antibody conjugate. Conjugates prepared from serum fractionated with ammonium sulfate were superior. Bright staining was observed more frequently with bovine serum conjugates than with rabbit serum conjugates. Although the staining of cell suspensions was rapid and simple, better results were obtained by staining smears.

7449

Strauss, A.J.L.; Lichter, E.A.; Vannier, W.E.; Goodman, H.C. 1963. Purification of human gamma globulin; production of specific anti-gamma globulin sera; gamma globulin fragmentation on long dialysis. Federation Proc. 22:991:326.

Human plasma, sodium sulfate-precipitated globulin, and Rivanol-prepared globulin were compared by column chromatography. The DEAE-cellulose columns were eluted with Tris buffer, pH 8.6. The final products were compared for integrity, purity, and yield. Protein fractions, collected up to the maximum optical density at 280 m μ were pure gamma globulin according to analytical ultracentrifugation, immunodiffusion, and immunoelectrophoresis. Rabbits immunized with these products in Freund adjuvant produced antihuman gamma globulin antisera, which also reacted with beta-2A and beta-2M globulin determinants shared with gamma globulin. Dialysis of sodium sulfate-precipitated globulin for 6 to 30 days against Tris buffer caused fragmentation of the gamma globulin. Sedimentation rates and both fast and slow electrophoretic components were noted.

7450

Sulzer, A.J.; Kagan, I.G. 1964. Fluorescent antibody (FA) test for schistosomiasis and trichinosis. *J. Parasitol.* 50(Suppl.):23-24.

The indirect FA test for schistosomiasis has been under investigation in this laboratory for several years and more recently the test for trichinosis. Techniques for rapid efficient methods of conjugating antiserum with FITC and the preparation of an antigen for trichinosis, stable under storage at 4 C, have been developed. Certain aspects basic to the FA reaction have been elucidated. One of the variables investigated was the effect of multiple washes of antigen after addition of positive serum and before addition of labeled antiglobulin conjugate. With some positive sera the FA test increased from a negative reaction with one wash to a brilliant 4 plus reaction with four washes. These results indicate that under certain conditions schistosome or trichina antigens that have been sensitized with gamma globulin from a positive serum will react weakly or negatively in the test. This effect may be overcome by use of antigen washed several times or by conjugate at relatively high concentration. This may clarify why a prozone effect has been reported in the FA test for trichinosis. Dependable methods were developed for production of labeled antiserum in less than 2 weeks from initial inoculation of the animal. Complete article.

7451

Thiede, H.A.; Choate, J.W.; Bindschadler, D.D. 1963. Chorionic gonadotropin localization in the human placenta by immunofluorescent staining: I. Production and characterization of antihuman chorionic gonadotropin. *Obstet. Gynecol.* 22:310-315.

The production of a potent and specific antiserum to HCG in rabbits is described. The in vitro activity of the antibody was characterized by ring precipitin titers, agar-gel diffusion, and immunoelectrophoresis. These studies established the presence of a potent precipitating antibody that reacted specifically with HCG. The antigen was concentrated in the Beta-1 fraction and formed a single precipitation line with the rabbit antibody in agar gel diffusion. The antihormonal activity of the antiserum was demonstrated by quantitative bioassay. The problems associated with the application of such an antiserum for detection of HCG, including its use in immunofluorescence studies, are discussed.

7452

Yudate, H. 1963. Application of the fluorescent antibody technique in obstetrics and gynecology with emphasis on fundamental and clinical studies in immunological and mycological diagnosis. J. Jap. Obstet. Gynecol. Soc. 15:131-140. In Japanese.

Application of FA for rapid diagnosis of infectious diseases in the fields of gynecology and obstetrics, was made to improve differential diagnosis of fungi on smears from clinical and experimental sources. With four antisera produced against Candida albicans, C. stellatoidea, C. guilliermondii, and Torulopsis glabrata, specific identification of these four fungi was possible. No cross-reaction was observed with absorbed antiserum. With absorbed antisera, an attempt was made to identify these four fungi in vaginal smears and smears obtained from oral swabs of newborn. C. albicans and T. glabrata were detected but not C. stellatoidea and C. guilliermondii. The rate of detection by direct swab of the lesion, cultivation in Bouillon medium for a short period, and by centrifugation was improved by these methods in this order. Among FA, biological, and serological methods, biological and FA technique gave equally good results. The identification of these four fungi was possible even when mixed with other fungi. Rabbit antiserum could be produced easily using heated antigens. The method could also be applied to study of the spread of C. albicans in experimentally induced infections and to the detection of antigen in impression smears of organs and in frozen tissue sections.

B. CONJUGATE PREPARATION

7453

Axt, J.; Jentzsch, K.D. 1962. Study of brucellae with fluorescent antibodies: 1. Conjugation of immune globulin with DANS. Arch. Exper. Veterinaarmed. 16:1309-1316. In German.

This is a description of Brucella antiserum with DANS. This process decreased the agglutinin titer considerably. Preservation of antisera with 0.5 per cent phenol did not affect the conjugation.

7454

Bates, H.A.; Pomeroy, B.S.; Seal, U.S.; Jay, A.R. 1965. Ornithosis: Experimental immunofluorescent studies. Avian Dis. 9:24-30.

Ion exchange chromatography and gel filtration yielded a turkey serum fraction that had apparently lost little of its DCF antibody activity and, after conjugation to fluorescein isothiocyanate, stained with specificity tissues obtained from infected mice and turkeys. This positive fluorescent staining could be correlated with gross lesions and isolation of an ornithosis agent from these tissues.

7455

Baumstark, J.S. 1964. Separation of gamma globulin from human serum: A preparative method. Federation Proc. 23:2675:547.

It is well known that when human serum is subjected to chromatography on DEAE-cellulose at low ionic strength and a pH of 7.5 to 8.5, the gamma globulin fraction will be found in the breakthrough volume. This was true also for a 2.5 x 36 cm column of DEAE Sephadex equilibrated with 0.01 M phosphate buffer, pH 6.5. Also, the continued collection of effluent past the gamma globulin peak failed to elute any additional protein. This finding led to the batch method described below. Ten grams of DEAE Sephadex previously equilibrated with 0.01 M phosphate buffer to pH 6.5 is mixed with 50 ml of undialyzed serum and allowed to stand at 4 C for 1 hour. The mixture is then washed on a Buchner funnel with four 25-ml samples of 0.01 buffer and taken to dryness after the last 25-ml rinse. The filtrate is then mixed with a second 10 grams of DEAE Sephadex and stirred at 4 C for 1 hour. The preparation is filtered as described above. Almost quantitative yields of gamma globulin have been obtained by this method. The product thus obtained produced a single peak in the analytical ultracentrifuge with a sedimentation coefficient of 6.5. The preparation produced a single arc of precipitate when subjected to immunoelectrophoresis at a level of 2 to 5 mg per ml. Complete article.

7456

Braune, M.O.; Gentry, R.F. 1965. Standardization of the fluorescent antibody technique for the detection of avian respiratory viruses. Avian Dis. 9:535-545.

Each phase of the FA technique was investigated. Gamma globulin solutions prepared from specific antisera against Newcastle disease virus, infectious bronchitis virus, and infectious laryngotracheitis virus having neutralization indices of 3.5 or greater were satisfactory for conjugation. The optimum conjugation ratio of FITC to protein was 0.03 mg dye per mg protein at a conjugation time of 12 hours. Reactions of a high fluorescent intensity were observed in infected tracheal smear preparations that were fixed in acetone at -20 C for 10 minutes and reacted for 30 minutes with conjugates adjusted to contain twice the protein concentration of the FA titration endpoint. A washing time of 20 minutes or more in phosphate-buffered saline did not affect the intensity of specific reactions. Infected tracheas could be stored at 4 C under moist conditions for 72 hours and retain their reactivity.

7457

Brooks, J.B.; Lewis, V.J.; Pittman, B. 1965. Separation of fluorescent antibody conjugates into 7S and 19S globulin components by gel filtration. Proc. Soc. Exp. Biol. Med. 119:748-751.

Gel filtration proved to be a simple, effective method for separation of fluorescein-labeled antiglobulin for E. coli into 7S and 19S fractions. Both fractions were found to contribute considerable nonspecific staining, but specific staining was associated predominantly with the 7S globulin. The ratio of specific staining titer to nonspecific staining activity was threefold greater with a pool of fractions in which only 7S globulin was detected than with the unfractionated conjugate when the two preparations were tested at the same protein concentration.

7458

Cebra, J.J.; Goldstein, G. 1965. Chromatographic purification of tetramethylrhodamine-immune globulin conjugates and their use in the cellular localization of rabbit gamma globulin polypeptide chains. J. Immunol. 95:230-245.

Rabbit spleen imprints were double-stained with pairs of fluorescein and tetramethylrhodamine conjugates derived from the following goat reagent globulins: anti-Fragment III, anti-gamma-globulin heavy chain, anti-light chain, and anti-Fragment I. Bright specific staining could be obtained with the subfractionated conjugates at concentrations as low as 32 to 43 ug of antibody per ml of conjugate. A series of simple Kodak Wratten

filters, K2, 23A, and 45A, were used to differentiate between singly and doubly stained fluorescent cells. Staining with anti-light chain and anti-III, conjugated with contrasting fluorochromes, resulted in double labeling of 63 to 78 per cent of all fluorescent cells. A distinct minority of the fluorescent cells, 21 to 34 per cent of the population, stained only with the anti-light chain reagent. A similar class of cells, comprising 14 to 26 per cent of all fluorescent cells, stained only with the anti-I reagent when it was used as a counterstain with anti-III reagent. Anti-light chain reagents detected more immunoglobulin-containing cells than did anti-I reagents. Double staining with anti-I and anti-light chain yielded 63 to 85 per cent doubly stained cells, 15 to 32 per cent of cells stained with the anti-light chain alone, and 0 to 10 per cent of cells stained with anti-I alone.

7459

Cerottini, J.C.; Jaton, J.; Froidevaux, T.; Isliker, H. 1964. Immunofluorescence by means of fragments of antibodies. *Helv. Physiol. Pharmacol. Acta* 22:C9-C12. In French.

The gamma globulin fraction of rabbit serum immunized against Histoplasma capsulatum was separated into three parts by means of papain digestion and chromatography on a carboxymethyl-cellulose column. FITC was added to the fractions, and the fractions added to the antigen as outlined. Fraction I and II always are fixed on the antigen giving a positive fluorescence. Fraction I from a non-immune animal does not show fluorescence. Fraction III does not react whether the animal is immune or not. It might be better to use Fractions I or II instead of the total gamma globulin to avoid unspecific fluorescence. BA-46-12110.

7460

Clark, H.F.; Shepard, C.C. 1963. A dialysis technique for preparing fluorescent antibody. *Virology* 20:642-644.

A dialysis method is described for conjugating serum proteins with FITC. Antisera against rabies, polio, rickettsiae, and mycobacteria were used. Human, monkey, guinea pig, sheep, and rabbit globulins were conjugated by this method. Good specific FA staining with minimal nonspecific staining was obtained.

7461

Corstvet, R.E.; Sadler, W.W. 1964. The diagnosis of certain avian diseases with the fluorescent antibody technique. *Poultry Sci.* 43:1280-1288.

A modified method of conjugate preparation produced conjugates that were satisfactory for differentiating various avian pathogens in artificial media and tissues. This simplified method saved considerable time over previously published methods by eliminating fractionation of the unconjugated serum, dialysis, and concentration. This was desirable since indirect FA did not function with colony imprints. FA can be very useful in evaluating mycoplasma cultures as to purity and greatly simplifies the identification of Mycoplasma spp. or serological types as well as other avian pathogens. The results also showed that tissue imprints of trachea, air sac, and proventriculus could be used in identifying NDV by FA.

7462

Dedmon, R.E.; Holmes, A.W.; Deinhardt, F. 1965. Preparation of fluorescein isothiocyanate - labeled gamma globulin by dialysis, gel filtration, and ion-exchange chromatography in combination. *J. Bacteriol.* 89:734-739.

Antiviral immune gamma globulin isolated from rabbit and guinea pig sera were labeled through dialysis membranes with fluorescein isothiocyanate and purified in several ways to eliminate nonspecific staining. Gel filtration of the conjugate with Sephadex G-25 coarse beads followed by column fractionation with diethylaminoethyl-Sephadex yielded consistently highly specific staining materials. Fluorescein-protein ratios varied between 1.0 and 4.0. This technique has proved to be simple and reliable, and is less time-consuming than previous techniques.

7463

Fletcher, S. 1964. Serology of toxoplasmosis. *Brit. Med. J.* 2:1660.

This is a report of the routine use of indirect FA for the serologic diagnosis of toxoplasmosis. The methods are briefly described.

7464

Frommshagen, L.H. 1965. The solubility and other physicochemical properties of human gamma globulin labeled with fluorescein isothiocyanate. *J. Immunol.* 95:442-445.

The solubility of human gamma globulin, unlike that of albumin and alpha-beta globulins, is modified by the addition of fluorescein isothiocyanate (FITC). The solubility, as well as electrophoretic behavior, of fluorescein-labeled gamma globulin demonstrates a considerable heterogeneity of labeling at the higher conjugation ratios and a more homogeneous labeling at low conjugation ratios. The value of optimum labeling of immune globulin preparations in order to assure specific staining was again underscored. Nonspecific staining was assayed on HeLa cells.

7465

Frommshagen, L.H.; Martins, M.J. 1963. A comparison of fluorescein-labeled gamma globulins purified by Rivanol and DEAE chromatography. *J. Immunol.* 90:116-120.

Preparations of fluorescein-labeled gamma globulins obtained by the Rivanol procedure were found to be comparable to DEAE gamma globulins in recovery of antibody and specificity of fluorescent staining. These preparations were applied to the fluorescent antibody analysis of the V antigens of influenza virus, where it was necessary to use purified, labeled gamma globulins to achieve full specificity. However, in other virus-cell systems immune globulins prepared by ammonium sulfate precipitation, conjugated at low ratios of fluorescein to protein, and passed through a Sephadex column were found to be comparable to the Rivanol and DEAE globulins in specificity and, at times, superior in stainability. In the reverse situation, where essentially no contaminating host materials are present, as with preparations of bacteria, the use of ammonium sulfate globulins conjugated at high F:P ratios seems to afford the greatest intensity of staining.

7466

Georgala, D L.; Boothroyd, M. 1965. Preparation of fluorescent polyvalent Salmonella antisera. *Nature* 205:521-522

The conjugated polyvalent antisera described here are being assessed in routine immunofluorescence Salmonella tests on raw meat. Experience so far confirms that the direct technique may have worthwhile advantages over the indirect technique used in our earlier investigations. If necessary, a series of such conjugated polyvalent antisera could be prepared, using Group O antigens from all existing Salmonella groups.

7467

Hebert, G.A.; Pittman, B. 1965. Factors affecting removal of ammonium sulfate from salt-fractionated serum globulins employing a spectrophotometric procedure for determination of sulfate. *Health Lab. Sci.* 2:48-53.

The barium chloranilate spectrophotometric procedure for determining sulfate in globulins subjected to ammonium sulfate fractionation has proved precise and sensitive. The method was employed to study factors affecting the rate of removal of ammonium sulfate from globulins in dialysis sacs. It was found that the rate of dialysis was dependent upon the amount of sulfate present, duration of dialysis period, frequency of change of dialysate, and protein content, inasmuch as high sulfate content was associated with high protein values. Factors that had little or no effect on the rate of dialysis were agitation, size of dialysis sac in comparison with the volume dialyzed, and the immune state of the animals from which the globulins were obtained.

7468

Hsu, K.C.; Rifkind, R.A. 1963. Fluorescent, electron microscopic, and immunoelectrophoretic studies of labeled antibodies. *Scienza* 142:1471-1473.

Antibodies, produced in rabbits, to each of three bacterial species have been doubly labeled with fluorescein and ferritin. Irrespective of which label was conjugated to the antibody first, immunologic activity was maintained. Moreover, these preparations gave as high a degree of specificity in fluorescent and electron microscopic studies as did singly labeled antibodies. Immunoelectrophoretic analyses and other immunologic tests further confirmed that the antibodies were conjugated to both labels without loss of specific activity. The technique thus permits the relatively simple method of immunofluorescence to be used as an aid in selecting optimum ferritin antibody conjugates for localizing antigen at the molecular level by electron microscopy.

7469

Karakawa, W.W.; Borman, E.K.; McFarland, C.R. 1964. Typing of Group A streptococci by immunofluorescence: I. Preparation and properties of Type I fluorescein-labeled antibody. *J. Bacteriol.* 87:1377-1382.

Unabsorbed, fluorescein-labeled globulins derived from rabbits immunized with acid-extracted M protein of Type I streptococci, plus adjuvant, were found to have high fluorescent antibody staining titers and to be considerably more type-specific than were similar preparations derived from whole-cell immunization. Appropriate absorption rendered the anti-M reagent entirely type-specific without appreciable loss of titer; whole-cell reagent was appreciably weakened in FA titer by comparable absorption. Type specificity was confirmed by parallel bactericidal, long-chain, and precipitin studies.

Removal of reactivity by absorption with homologous M protein was complete, confirming that the FA reaction was truly a manifestation of an M anti-M protein system. The data indicate that the development of FA reagents specific for the streptococcal types is feasible.

7470

Karakawa, W.W.; Sedgwick, A.K.; Borman, E.K. 1964. Typing of Haemophilus influenzae with fluorescent antibody reagent. Health Lab. Sci. 1:114-118.

Immunization of rabbits over a 4-week period with vaccine harvested from 6-hour cultures on Levinthal agar of Haemophilus influenzae Type B yields antiserum of high type specificity. Antibodies to somatic antigens common to all types are present in minimal concentration as compared with homologous-type, anticapsular antibodies. This antiserum yields fluorescein-labeled conjugates that stain smooth Type B strains in high titer and are rendered completely type-specific by minimal adsorption with heterologous-type cells from smooth cultures or by use of a blocking anti-rough serum in a two-step inhibition technique. Rapid identification of H. influenzae in clinical material, particularly the demonstration of Type B in spinal fluid, is readily and surely achieved by use of carefully prepared and standardized FA reagents. Other available serological techniques are more susceptible to uncontrollable errors arising from less than optimal antigen-antibody ratios, a factor not operative in the direct FA method.

7471

Khomenko, N.A.; Olshevskaya, T.R. 1965. Increase of fluorescent globulin specificity for the direct method of Shigella staining. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:7:33-36. In Russian.

The specificity of fluorescent antibodies for the detection of Shigella dysenteriae could be increased by their preparation from specific sera after meticulous sorption of heterologous agglutinins. For the same purpose fluorescent antibodies should be used in high dilutions, 1:32 to 1:64. To produce a more intensive fluorescence of bacteria, stain the smears for a more prolonged period of time, 1.5 to 2 hours at 37 C in a humid chamber.

7472

Kishko, Ya.H. 1965. Use of isothiocyanate-labelled fluorescein anti-complementary serum for the Goldwasser-Shepard test. Mikrobiol Zh. Akad Nauk Ukr. RSR 27:5:67-70. In Russian.

An anticomplementary conjugate, labeled by FITC, was obtained by immunizing rabbits with guinea pig serum. Following globulin extraction, it was labeled in a proportion of 25 mg of preparation per 100 mg of 2.5 to 3.0 per cent protein. BA-47-108567.

7473

Klugerman, M.R. 1965. Chemical and physical variables affecting the properties of fluorescein isothiocyanate and its protein conjugates. *J. Immunol.* 95:1165-1173.

The effect of pH, time, temperature, and buffer systems on solutions of fluorescein isothiocyanate and its protein conjugates was examined fluorometrically. The fluorescence of the isothiocyanate and of the protein conjugate was maximal at about pH 8.7 and pH 10.7, respectively. The stability of their fluorescence above pH 7 was affected adversely by increases in pH or temperature. The protein conjugate, however, showed maximum stability at pH 10.5 and above. The type of buffer - carbonate, phosphate, borate, Tris or barbiturate - did not affect the fluorescence of the free dye significantly. On the other hand, increasing the molarity of the buffer caused a decrease in stability of fluorescence of the free dye but did not seriously affect the fluorescence of the conjugate. The absorbance of fluorescein isothiocyanate and its conjugates increased with increasing pH. With increased pH or temperature of the reaction mixture during conjugation, fluorescein isothiocyanate reacted more readily with the protein. Conditions may be selected to obtain the desired degree of label with short conjugation periods. Conjugation of a bovine anti-Brucella abortus globulin sample for 30 minutes at pH 9.45 and room temperature was as effective as conjugation at pH 8.75 for 18 hours at 5 C. No apparent loss of biologic activity was observed as the result of conjugation.

7474

Lewis, V.J.; Jones, W.L.; Brooks, J.B.; Cherry, W.B. 1964. Technical considerations in the preparation of fluorescent antibody conjugates. *Appl. Microbiol.* 12:343-348.

Ammonium sulfate, hydrochloric acid, Ethodin, and ethanol were compared for fractionation of rabbit antiserum prior to conjugation with fluorescein isothiocyanate. Fractionation with the salt was the method of choice from the standpoints of simplicity and recovery of antibody effective in conjugates prepared from the fractions. Effects of pH, temperature, dye-protein ratio, and molarity and type of buffer upon conjugation were studied. These technical factors were adjusted to produce conjugates for Corynebacterium diphtheriae that possessed higher specific titers than did reagents obtained by previously employed techniques.

7475

McDevitt, H.O.; Coons, A.H. 1964. Methods for preparation of fluorescent proteins, p. 142-148. In H.N. Eisen (ed.) *Methods in Medical Research*, Vol. 10, Section II. Year Book Medical Publishers, Inc., Chicago, Ill.

With the exception of DEAE cellulose chromatography, the methods described here have been in use for several years, and they have been found to be effective when gauged by the ultimate but rough test of staining. However, very few quantitative data are available correlating fluorescein-to-protein ratio (or antibody titer) with staining intensity. The necessity for evaluating DEAE column fractions in comparison with tissue powder - absorbed conjugates points up this lack of precise knowledge. Fluorescent protein conjugates should be routinely evaluated after purification and removal of nonspecific staining with regard to F/P ratio, protein concentration, and antibody concentration. Accumulation of information of this kind will permit selection of labeling and purification procedures on a rational basis and will greatly increase the reliability of preparation of fluorescent proteins.

7476

McKinney, R.M.; Spillane, J.T.; Pearce, G.W. 1964. Determination of purity of fluorescein isothiocyanates. *Anal. Biochem.* 7:74-86.

Quantitative infrared measurements were applied for a simple and accurate means of determining the FITC content of FITC products. Paper chromatography affords a method of identification and semiquantitative determination of the isomer or isomers present. Chloride analysis is helpful in determining whether the product is present in part or wholly as the hydrochloride. A combination of these methods provides a means of thoroughly evaluating FITC products for use in immunofluorescent staining.

7477

McKinney, R.M.; Spillane, J.T.; Pearce, G.W. 1964. Fluorescein diacetate as a reference color standard in fluorescent antibody studies. *Anal. Biochem.* 9:474-476.

Procedures are given for setting up a reference standard to measure the amount of fluorescein bound to protein in a conjugate. They are simple and easy to apply.

7478

McKinney, R.M.; Spillane, J.T.; Pearce, G.W. 1964. Factors affecting the rate of reaction of fluorescein isothiocyanate with serum proteins. *J. Immunol.* 93:232-242.

Only FITC of the highest purity should be used for spectrophotometric reference standards. The extent of reaction of FITC products with rabbit gamma globulin under standardized conditions showed very close correlation with the purity of the isothiocyanate products as determined by infrared measurements. Rabbit and bovine alpha globulins were found to react considerably more rapidly than horse alpha globulin with FITC. In all species studied, the albumin fraction reacted much more rapidly than the gamma globulin fraction. A systematic study of the effects of temperature, concentration, and pH on the rate and extent of reaction of FITC with normal rabbit gamma globulin indicated that the rate can be greatly accelerated by modifying the reaction conditions. At the reaction temperature of 25 C, pH 9.5, protein concentration 2.5 per cent, and buffer salt concentration of 0.05 M, conjugation of FITC with rabbit gamma globulin was essentially completed in 30 minutes. With these reaction conditions, the ratio of fluorescein to protein of the conjugate was very reproducible and could be controlled by the initial amount of dye added. Conjugation of FITC with gamma globulin at 25 C, pH 9.5, yielded an excellent product.

7479

Mikhailov, I.F.; Pers, I.F. 1964. Isolation of antibodies from antigen-antibody complex by ultrasound. *Zh. Mikrobiol. Epidemiol. i Immunobiol.* 41:112-119. In Russian.

Antibodies were separated from their specific complex with somatic antigens by ultrasound and then eluted in saline. Antibody preparations were free of nonspecific protein, heterologous antibody, and the antigen. Antibodies retained their immunologic specificity. An antiflexneri conjugate labeled with fluorescein isocyanate was among the sera used.

7480

Myers, J.; Sargent, A.U.; Cohen, J.J. 1965. Whole antiserum versus the gamma globulin fraction of antiserum in the indirect fluorescent antibody technique. *Immunology* 9:101-105.

Two antisera were compared with their gamma globulin fractions for effectiveness as the middle or unlabeled layer in the indirect fluorescent antibody technique. The gamma globulin fraction did not produce better results in either case than the equivalent concentration of whole serum. Control sections were prepared with normal rabbit serum substituted for the whole

antiserum and with normal rabbit gamma globulin substituted for the gamma globulin fraction of the antiserum. The control sections with gamma globulin showed considerably more nonspecific fluorescence than those with whole serum. In one case the nonspecific fluorescence in the gamma globulin control interfered with interpretation of the experimental section.

7481

O'Berry, P.A. 1964. A comparison of three methods of serum fractionation in the preparation of Vibrio fetus fluorescent antibody conjugates. Amer. J. Vet. Res. 25:1669-1672.

Lapine and bovine hyperimmune sera were prepared by parenteral injection of two venereal strains of Vibrio fetus. These sera and those from rabbits and cows negative for V. fetus agglutinins were fractionated by procedures utilizing ethanol, ammonium sulfate, and ethodin. Globulin fractions were conjugated to fluorescein isothiocyanate, studied electrophoretically, and found to be composed of gamma globulins with a trace of beta globulins. Homologous and heterologous V. fetus cell suspensions and smears were stained with each fluorescent antibody conjugate. Conjugates prepared from serum fractionated with ammonium sulfate were superior. Bright staining was observed more frequently with bovine serum conjugates than with rabbit serum conjugates. Although the staining of cell suspensions was rapid and simple, better results were obtained by staining smears.

7482

Ocklitz, H.W.; Weppe, C.-M. 1964. The bacteriological diagnosis of pertussis; fluorescence serology and culturing compared: II. The fluorochromatization of antibodies of Bordetella pertussis. Zentralbl. Bakteriol. 104 103-114. In German.

A report is made on personal experiences with the preparation of anti-pertussis serum suitable for fluorochromatization of antibodies, and also on the concentration, coupling, purification, and testing of this serum. Finally, the preparation and critical examination of the microscopical preparations are described.

7483

Olenchnowitz, A.-F.; Kokles, R.; Urbaneck, D. 1965. Experiences in the preparation of purified fluorescent antibodies. Arch. Exp. Veterinaarmed. 19:139-148. In German.

Globulins from bovine foot-and-mouth disease hyperimmune serum are precipitated with ammonium sulfate and coupled with FITC. The conjugates are purified by gel filtration and chromatography. The dye-protein ratio largely determines the staining properties of the conjugates. The importance of their extent of coupling and antibody content for the specificity of antibody determination is discussed and optimal conditions for preparing conjugates without unspecific fluorescence are postulated. BA-46-80842.

7484

Peters, H. 1963. Construction and use of a small Sephadex column for the separation of fluorescent antibodies. Stain Tech. 38:260-262.

Column construction and use is fully described.

7485

Rivera de Sala, A.; Cancio, M.; Rodriguez-Molina, R. 1965. Ion exchange chromatography in the separation of circumoval precipitins. Bol. Asoc. Med. Puerto Rico 57:210-212.

A simplified procedure has been developed for the isolation of the protein fraction containing the circumoval precipitin antibodies from the serum of patients infected with Schistosoma mansoni. The procedure is eight to ten times faster than other procedures previously used and makes unnecessary the use of special laboratory facilities. The globulins obtained have been utilized for the performance of the fluorescent circumoval precipitin technique.

7486

Schell, H.D.; Ghetie, V. 1964. Labelling of serum proteins with 1-dimethylaminonaphthalene-5-sulphonyl chloride and the purification of the fluorescent conjugates. Rev. Roumaine Biochim. 1:2:203-208. In German.

The labeling of serum proteins with DANS has revealed a competition between the different fractions for fluorochrome. Albumin always collects the largest quantity of fluorochrome. For labeling antibodies the use of purified gamma globulin preparations is preferable. The chromatographic technique on DEAE-cellulose is the most efficient method for removing the by-products from fluorescent conjugates.

7487

Sulzer, A.J.; Kagan, I.G. 1964. Fluorescent antibody (FA) test for schistosomiasis and trichinosis. J. Parasitol. 50(Suppl.):23-24.

The indirect FA test for schistosomiasis has been under investigation in this laboratory for several years and more recently the test for trichinosis. Techniques for rapid efficient methods of conjugating antiserum with FITC and the preparation of an antigen for trichinosis, stable under storage at 4 C, have been developed. Certain aspects basic to the FA reaction have been elucidated. One of the variables investigated was the effect of multiple washes of antigen after addition of positive serum and before addition of labeled antiglobulin conjugate. With some positive sera the FA test increased from a negative reaction with one wash to a brilliant 4 plus reaction with four washes. These results indicate that under certain conditions schistosome or trichina antigens that have been sensitized with gamma globulin from a positive serum will react weakly or negatively in the test. This effect may be overcome by use of antigen washed several times or by conjugate at relatively high concentration. This may clarify why a prozone effect has been reported in the FA test for trichinosis. Dependable methods were developed for production of labeled antiserum in less than 2 weeks from initial inoculation of the animal. Complete article.

7488

Summers, W.A. 1965. A rapid procedure for preparing fluorescein-isothiocyanate-labeled anti-anaplasma gamma globulin. Amer. J. Vet. Res. 26:1459-1462

A method for the conjugation of anti-anaplasma bovine gamma globulin with FITC was described. This procedure reduced the chance of protein denaturation, decreased the time required for preparation, and resulted in a product that imparted brilliant fluorescence when applied to Anaplasma marginale in infected erythrocytes.

7489

Wolf, P.L.; Pearson, B.; Rosenblatt, M.; Jacobsitz, E. 1963. A new highly specific method for immunofluorescence. Amer. J. Clin. Pathol. 40:430.

Striking specificity was noted in immunofluorescent reactions, in which only the 2S gamma globulin fraction of immune serum was conjugated with fluorescein isothiocyanate and then used as the outer layer in indirect immunofluorescence. Total gamma globulin was first separated from sheep antirabbit serum with ammonium sulfate. Dialyzed gamma

globulin was then placed in an A-50 diethylaminoethanol-Sephadex column and eluted. The first 54 per cent of the total eluate contained only the 7S fraction of the gamma globulin. This fraction was then conjugated with fluorescein isothiocyanate by conventional methods. A group of 7-week-old mice were injected intravenously with bovine serum albumin and were killed 2.5 hours after the injections. Their spleens were removed, frozen, and sectioned in a cryostat. The sections were overlaid with rabbit anti-BSA serum and, subsequently, with the fluorescein-conjugated 7S fraction of the antirabbit serum. Fluorescence was specifically confined to discrete groups of splenic cells. Controls manifested no fluorescence. It is postulated that the specificity of fluorescent antibody reactions is based on removing 19S antibodies that are likely to be nonspecific in their predilection and using the highly specific antibody of 7S variety.

7490

Wood, B.T.; Thompson, S.H.; Goldstein, G. 1965. Fluorescent antibody staining: III. Preparation of fluorescein isothiocyanate - labeled antibodies. *J. Immunol.* 95:225-229.

A rapid method of preparing bright and specific staining fluorescent antibodies is described. The method is discussed, emphasizing the need for chromatographically homogeneous antibody globulin, the importance of pH in the coupling reaction, and the advantages of fractionating the fluorescent antibody reaction mixture in DEAE-cellulose.

7491

Zaccheo, D.; Grossi, C.E. 1963. Analysis of the characteristics of immune sera in relation to their labeling with fluorochromes. *Boll. Soc. Ital. Biol. Sperim.* 39:1562-1584. In Italian.

The modification of physical-chemical properties of the protein molecules of a serum due to introduction of fluorescent radicals was studied during different phases of preparation of the conjugates. The alpha-precipitation test, immunodiffusion (Ouchterlony) test, and immunoelectrophoretic analysis were employed. A lowering of the isoelectric point of the protein with a resulting relative acidification of the serum takes place. The total protein content of the sera decreased, as a result of labeling, by 20 to 35 per cent of the initial value. This decrease sometimes corresponded to a loss of antibodies.

C. SPECIMEN PREPARATION

7492

Allen, J.C. 1963. Immunofluorescence applied to protein solutions. *J. Lab. Clin. Med.* 62:517-524.

Protein antigen in aqueous solution may be gelled in agar, frozen, sectioned, and stained with fluorescein-labeled antibody. By this means identification and quantitation of protein antigen present in solution may be accomplished, and the applicability of immunofluorescence may be extended from its classical limits of identification of antigen held to or within tissues or microorganisms.

7493

Aoyama, Y. 1965. The pretreatment of the frozen section for the fluorescent antibody technique. *Jap. J. Exp. Med.* 35:50P.

One of the reasons for the poor staining of brain sections with FAT might be the presence of a large quantity of lipids in the materials. The pretreatment of section with acetone for 10 minutes at 5 C or 20 C eliminated cholesterol only, while use of ether, carbon fluoride, and carbon tetrachloride showed massive removal of cholesterol, cephalin, lecithin, and small amount of sphingomyelin. Chloroform-methanol dissolved more lipids than the solvents above mentioned. For preservation of antigen activity, pretreatment of section with carbon tetrachloride for 30 to 60 minutes at 5 C was preferable. Chloroform-methanol destroyed viral antigenicity within less than 5 minutes. The improvement of staining results with the suitable pretreatment of sections was due to the removal of lipids bound to the antigens in cytoplasm of nerve cell and of lipids in myelin sheath interfering with the contact with the labeled antibody.

7494

Balaeva, N.M.; Korn, M.Ya.; Kulberg, A.Ya. 1963. Detection of antibodies to Rickettsia prowazeki by the fluorescent serological method. *Zh. Mikrobiol. Epidemiol. i Immunobiol.* 40:1:52-57. In Russian.

This work was undertaken to elaborate optimal conditions for the detection of antibodies to Rickettsia prowazeki in typhus patients with the aid of the fluorescent serological method. The specificity of this reaction was studied by comparing its results with those of the complement fixation test and neutralization reaction of the toxic substance of rickettsiae. The significance of the pH of physiological saline used for sera dilution, as well as the effect of serum inactivation and of the methods of rickettsia antigen fixation on a slide, was ascertained.

7495

Ball, J.; Bahgat, N.E.D.; Taylor, G. 1964. Effect of aldehyde fixation on cellular rheumatoid factor and certain tissue antigens. *J. Histochem. Cytochem.* 12:737-739.

Rheumatoid factor, nuclei, and gamma-2 globulins can be identified by immunofluorescence titers on alcohol-fixed paraffin-embedded sections, although wax of low melting point is required for preservation of nuclear antigens. Rheumatoid factor also resisted aldehyde fixatives; hence, it should be possible to investigate the ultrastructural characteristics of cells containing it.

7496

Berenznitskaya, E.R. 1964. A method for selective extinction of the parafluorescence of cocci in preparations stained with pertussis and parapertussis fluorescent sera. *Zh. Mikrobiol. Epidemiol. i Immunobiol.* 41:71-74.

A method has been developed for selective extinction of the parafluorescence of cocci in preparations first stained with fluorescent pertussis and parapertussis sera, and then Gram-stained. Treatment of the preparations with gentian violet and iodine, serving as fluorescence extinction agents, has made it possible to eliminate the fluorescence of the Gram-positive parafluorescent cocci, and to retain at the same time the fluorescence of the Gram-negative pertussis and parapertussis bacteria.

7497

Braune, M.O.; Gentry, R.F. 1965. Standardization of the fluorescent antibody technique for the detection of avian respiratory viruses. *Avian Dis.* 9:535-545.

Each phase of the FA technique was investigated. Gamma globulin solutions prepared from specific antisera against Newcastle disease virus, infectious bronchitis virus, and infectious laryngotracheitis virus having neutralization indices of 3.5 or greater were satisfactory for conjugation. The optimum conjugation ratio of FITC to protein was 0.03 mg dye per mg protein at a conjugation time of 12 hours. Reactions of a high fluorescent intensity were observed in infected tracheal smear preparations that were fixed in acetone at -20 C for 10 minutes and reacted for 30 minutes with conjugates adjusted to contain twice the protein concentration of the FA titration endpoint. A washing time of 20 minutes or more in phosphate-buffered saline did not affect the intensity of specific reactions. Infected tracheas could be stored at 4 C under moist conditions for 72 hours and retain their reactivity.

7498

Burnham, T.K.; Fine, G.; Neblett, T.R. 1964. Tumor imprints as a source of nuclear substrate for the detection of antinuclear factors. *J. Invest. Dermatol.* 42:7-9.

Sera of patients with positive lupus erythematosus cell tests, scleroderma, and various dermatoses were investigated for antibodies to skin components and antinuclear factors by the indirect fluorescent antibody technique. Speckled nuclear fluorescence in the epidermis was seen with several of the scleroderma sera. Epidermal nucleolar fluorescence occurred with one scleroderma serum; homogeneous epidermal nuclear fluorescence was seen with several of the lupus erythematosus cell positive sera. Tumor imprints were far superior for the detection of antinuclear factors to the conventional blood smears and tissue sections. Nuclear fluorescence was much more conspicuous because of greater number, size, and delineation of nuclei and the greater quantity of some of the nuclear antigens.

7499

Chadwick, P.; Abbott, L. 1964. Specificity and sensitivity of a microcolony technique for fluorescent antibody identification of pathogenic Escherichia coli serotypes. *Can. J. Microbiol.* 10:853-859.

The specificity and sensitivity of a fluorescent antibody technique applied to growing microcolonies has been investigated, using serotypes of Escherichia coli responsible for infective enteritis as a model. Microcolonies of ten E. coli serotypes showed bright fluorescence when treated with homologous conjugated antiserum but no fluorescence when treated with heterologous conjugated antisera. Microcolonies of Enterobacteriaceae strains of other genera or of E. coli strains not associated with infective enteritis showed no fluorescence when treated with conjugated antisera prepared against the enteritis serotypes. Experiments with artificially infected fecal suspensions showed that the sensitivity of the microcolony technique was approximately 100 times greater than that of the direct smear method. A number of other advantages and possible disadvantages of the microcolony technique are discussed and its usefulness in epidemiological work is suggested.

7500

Cookson, L.O.C. 1964. Some modifications of the fluorescent antibody test in human bilharziasis. Bull. WHO 31:799-813.

Although the FA test for human bilharziasis has proved of great value, its use involves certain difficulties that the author has attempted to obviate. The paper describes a cheap and reproducible method for producing a cercarial antigen conjugated with rhodamine B 200 for use in the indirect FA test. The second part deals with a new modification in which the conjugated cercarial antigen is employed with a bentonite-absorbed FITC antihuman globulin serum and discusses the advantages of this test over the normal FA test. Experience has shown that the use of rhodamine-albumin-coated cercariae, conjugated cercariae, or normal fixed cercariae as antigens does not always give valid results when compared with those obtained with the FA test or the ordinary complement fixation test in bilharziasis. The author describes a modification of the complement fixation test involving the use of a bentonite-absorbed fluorescent anti-guinea-pig serum and the RB 200 - conjugated cercariae described earlier. This test has given reproducible results in known positive control human sera that have been valid when compared with the Sadun FA test, the conjugated cercarial FA test, and the bentonite fluorescent antibody test described in the second part of this paper.

7501

Dacres, W.G. 1963. Fluorescein-labeled antibody technique for identification of leptospiral serotypes: Refixation of formalin-fixed organisms with osmic acid vapor. Amer. J. Vet. Res. 24:1321-1323.

Leptospire fixed with osmic acid vapor were stained specifically with fluorescein-labeled antibody conjugates. Leptospire fixed with formalin underwent a great degree of cross-staining. Those fixed with formalin and subsequently treated with osmic acid vapor stained specifically.

7502

Danielsson, D. 1965. A membrane filter method for the demonstration of bacteria by the fluorescent antibody technique: 1. A methodological study. Acta Pathol. Microbiol. Scand. 63:597-603.

Bacteria suspended in tap water or cultured in broth, and then trapped on non-fluorescent membrane filters, could be identified within one hour by means of the fluorescent antibody method. For this purpose the fluorescence microscope was equipped for incident illumination. The technique described allowed a quantitative determination of the bacteria identified serologically.

7503

Danielsson, D.; Laurell, G. 1963. Rapid detection of small numbers of bacteria in water by means of fluorescent antibodies. *Acta Pathol. Microbiol. Scand.* 58:159.

FA technique combined with membrane filter technique has been used to study bacterial contamination of water. Known concentrations of the test bacteria, enteropathogenic E. coli, were added to fixed volumes of water. As a rule 1 liter was filtered through a membrane filter (Millipore HAWG, 47 mu). At bacterial densities of 500 to 1,000 bacteria per liter identification was accomplished in 2 hours. At lower concentrations the membrane filters were incubated for various periods in broth and subsequently centrifuged to concentrate the bacteria. A concentration of 50 bacteria per liter could be detected in 5 to 6 hours, 15 to 20 in 8 to 10 hours, and 2 to 5 in 12 to 16 hours, respectively. The technique was also quantitative at different ratios (0.2 to 100) between concentration of contaminating bacteria and test bacteria. By conventional methods, diagnosis could be made after 48 hours at the earliest. At a high ratio of contaminating bacteria to test bacteria, the latter could often not be isolated. Promising attempts have been made to detect bacteria directly on nonfluorescent membrane filters (Millipore HABG). Samples of the water of a river in central Sweden were examined for enteropathogenic E. coli. By the use of FA ten different strains of these bacteria were detected. Six of these were also isolated by conventional tests. Complete article.

7504

Danilova, T.A.; Korn, T.Ya. 1964. The possibility of elimination of cross-reactions between streptococci of various groups and staphylococci in applying the fluorescent antibody method. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 41:13-16.

In the direct method of fluorescent antibodies the fluorescent serum to Group A Streptococcus stained not only strains of the homologous group, but also cultures of C and G groups and some Staphylococcus strains. The sorption of a labeled serum with the live Streptococcus Group C culture eliminated the specific staining of strains of the Groups C and G; however, the sorbed serum retained its capacity to stain Staphylococcus. Treatment of fixed smears in a trypsin solution has made it possible to eliminate the Staphylococcus staining without disturbing the specific fluorescence of the Group A Streptococcus.

7505

Demissie, A. 1964. The isolation of enteropathogenic salmonellae from the River Fyris and their detection by the fluorescent antibody method. Acta Pathol. Microbiol. Scand. 60:299-300.

In an investigation aimed at isolating enteropathogenic salmonellae from the River Fyris, a modified swab technique and filtration through membrane filters, supercel, and Seitz filters were compared. Negative results were obtained when impure river water was filtered through membrane and Seitz filters. Nine salmonellae strains were isolated. One, S. typhimurium, NS phage type, was isolated by the supercel method. Swabs were made from strips of gauze compactly rolled around rectangular wire frames, 15 by 20 cm and about 5 to 7 inches thick. These swabs immersed in the river for 48 to 96 hours were effective traps for salmonellae. One strain of S. paratyphi B, 3AI var. 2, one of S. blockley, and one of S. enteritidis were isolated by this method.

7506

Diggs, C.L.; Sadun, E.H. 1965. Serological cross-reactivity between Plasmodium vivax and Plasmodium falciparum as determined by a modified fluorescent antibody test. Exp. Parasitol. 16:217-223.

The cross-reactivity between P. vivax and P. falciparum was studied by indirect fluorescent antibody. Two modifications were employed: use of liquid nitrogen for preservation of parasitized whole blood for the source of antigen, and use of Evans blue as a counterstain. Six of 29 sera from natural P. vivax infections reactive with P. vivax antigen were also reactive with P. falciparum antigen. Eleven of 21 serologically positive sera from natural P. falciparum infections were reactive with P. vivax antigen, two of these with the P. vivax antigen only. Sera from human volunteers with either P. vivax or P. falciparum infections were titrated in parallel tests with homologous and heterologous antigen. Geometrical mean reciprocal titers with P. falciparum sera were 28.3 for the homologous antigen and 6.3 for the heterologous antigen. For P. vivax sera the values were 17.2 and 9.3 for the homologous and heterologous antigens. Values for the P. falciparum sera were 132 with the homologous antigen and 20.0 with the heterologous antigen; the P. vivax sera gave values of 30.0 and 11.9 with the homologous and heterologous antigens. Two hundred and forty-six sera from subjects presumably free of malaria revealed 28 positives. There are both a species-specific and a group-specific component in these two organisms. The usefulness of the filter paper method of blood collection was confirmed.

7507

Eveland, W.C. 1964 Use of a fluorescein-labeled sonically disrupted bacterial antigen to demonstrate antibody-producing cells J Bacteriol 88.1476-1481.

Cells obtained by primary tissue culture of the spleens of chickens immunized with sonically disrupted Escherichia coli 0111 organisms were stained with a fluorescein-labeled homologous antigen by use of direct immunofluorescent methods. Brilliant staining of the cytoplasm in cells from immunized birds appeared to be diffuse in certain cells and rather globular in others. In contrast, cells from nonimmunized birds showed no staining at all. The cells involved in the specific reaction appeared to be those of the lymphocyte-monocyte-plasma cell types, as shown when stained by the May-Grunwald-Giemsa method. Preparations stained by the methyl green-pyronin technique revealed an increase in the pyroninophilic cells in the preparations from the immunized birds, thus demonstrating increased amounts of ribonucleic acid in these cells, which in turn is consistent with the presence of antibody globulin. Specificity of the reaction was confirmed also by staining antibody-coated E. coli 0111 organisms with the conjugate, precipitin reaction with specific antibody, and specific agglutination with circulating antibody from the immunized birds.

7508

Guthe, T.; Vaisman, A.; Paris-Hamelin, A. 1964 Fluorescent antibody technique using dried and eluted blood. Study of the preservation and shipment of samples at high temperatures Bull WHO 31 87-94
In French

The possible effect of shipment by airmail to tropical countries and of storage of the blotting-paper discs at temperatures ranging from 21 to 43 C for 32 to 80 days was investigated. The results show that such transport and storage do not, in the conditions described, significantly affect the results of FTA-100 testing of disc eluents. The test results with disc eluents after actual air shipment and storage in tropical conditions also showed good agreement with the results of FTA testing of sera examined immediately after collection. A slight degradation of reactive venipuncture sera appeared when the sera were stored in the laboratory at 18 to 20 C and then retested in comparison with air-shipped discs or discs stored during the same period in the laboratory.

7509

Johnson, G.D.; Holborow, E.J. 1963. Immunofluorescent test for rheumatoid factor in serum. *Lancet* 2:1142-1143.

A method for demonstrating rheumatoid factor by immunofluorescence, using sheep blood smears sensitized with hemolysin, is described. Correlation was found between positive staining and differential agglutination titer.

7510

Joncas, J. 1964. The direct fluorescent antibody technique studied with reovirus Type I. *Rev. Can. Biol.* 23:333-338.

A fluorescent antibody technique is described that consists of a few technical modifications of the standard method. Reovirus Type I was the antigen used. Tissue culture medium devoid of antibiotics and of phenol red has been found to decrease the intensity of the autofluorescence of the tissue culture. The length of the staining period has been shortened to 10 minutes at room temperature with improved results. A homemade tray is mentioned that greatly facilitates the handling of the small cover slips, and the Columbia staining dishes have been found most helpful. Finally, photographs were taken without any change in filters, using an Ansochrome 200 daylight color film on a Leitz microscope.

7511

Kaplan, W.; Kaufman, L. 1963. Specific fluorescent antiglobulins for the detection and identification of Blastomyces dermatitidis yeast-phase cells. *Mycopathol. Mycol. Appl.* 19:173-180.

Two lots of rabbit anti-Blastomyces dermatitidis globulins were conjugated with fluorescein isothiocyanate. These reagents brightly stained elements of the yeast and mycelial phases of ten strains of B. dermatitidis. In addition, the labeled antibodies cross-reacted with elements of the yeast and mycelial phases of seven strains of Histoplasma capsulatum and cells of numerous other heterologous fungi. Adsorption of one lot of labeled antibodies twice with yeast cells of H. capsulatum and once with elements of Coccidioides immitis rendered the conjugate specific for the yeast phase of B. dermatitidis. Three adsorptions with yeast cells of H. capsulatum followed by a single adsorption with elements of C. immitis rendered the second conjugate specific for yeast-phase cells of B. dermatitidis. The specific reagents did not react with the mycelial phase of this fungus.

7512

Kartman, L. 1960. The role of rabbits in sylvatic plague epidemiology, with special attention to human cases in New Mexico and use of fluorescent antibody technique for detection of Pasteurella pestis in field specimens. Zoonoses Res. 1:1-27.

The epidemiological role of rabbits in sylvatic plague was evaluated to define their position in the natural plague cycle and in public health. A review of the literature indicated that little is known, but that numerous observations, especially in South America, suggest that rabbits become infected during epizootics and may convey the infection to man. The fluorescent antibody staining technique was used to identify plague organisms in animals found dead in the field. Bone marrow provided the most useful tissue for application of this method to material from cadavers of varying degrees of decomposition.

7513

Komminos, G.N.; Tompkins, V.N. 1963. A simple method of eliminating the cross-reaction of Staphylococcus in the fluorescent antibody technic. Amer. J. Clin. Pathol. 40:319-324.

Rabbit immune sera against various species of bacteria, when tested by the direct or indirect fluorescent antibody method, cross-react with certain staphylococci. When staphylococci on a slide were pretreated with papain, then anti-meningococcal, anti-streptococcal A, or anti-Escherichia coli immune serum failed to cross-react. When similarly treated slides were stained with anti-Haemophilus influenzae, anti-pneumococcal, or anti-Listeria monocytogenes immune serum, papain eliminated cross-reaction only after these sera were absorbed with a staphylococcal strain. The difference in the effectiveness of papain suggests that in the case of the first three immune sera the cross-reaction factor was solely a papain-sensitive substance and that in the latter three immune sera two factors were involved--a heterogenetic antigen requiring the corresponding absorption and the papain-sensitive substance. A practical application of these findings is discussed.

7514

Kundin, W.D.; Liu, C. 1963. Effects of acetone, ultraviolet irradiation, and formalin on West Nile virus infectivity and immunofluorescent antigenicity. Proc. Soc. Exp. Biol. Med. 114:359-360.

Infectivity of West Nile virus in frozen sections was inactivated by UV light in a matter of minutes, but antigenicity persisted for several hours. Immersion of infected frozen sections in acetone inactivated 99 per cent of virus infectivity in minutes, yet antigenicity was not impaired for as long as 2 weeks at room temperature. In our hands fixation by formalin was unsatisfactory.

7515

LaBrec, E.H. 1965. The effects of fixation and postfixation treatment of nonspecific fluorescence in tissues. Bacteriol. Proc. M130:61.

The protein granules of polymorphonuclear eosinophil and neutrophil (PMN) leukocytes exhibit a striking affinity for fluorescein-labeled antibody. Despite improvements in reagents and techniques, nonspecific fluorescent (NSF) staining of these granules remains a problem in using FA methods. The effects of fixation and postfixation methods on NSF staining and a method for eliminating NSF staining of PMN granules are reported. Frozen sections of tissues or bone marrow smears were fixed in various nonpolar solvents, dried, immersed in 0.05 M HCl, washed in buffered saline, and stained with FA. Under these conditions, PMN granules were not stained by FA; they were stained by FA if the sections or smears were fixed in 10 per cent formalin prior to HCl treatment. Postfixation treatment with 0.05 M HCl did not affect specific antigen-FA reactions in various systems examined. The effects of fixation and postfixation on staining of PMN granules by eosin YS (tetrabromofluorescein) and related dyes were the same as those seen with FA. These staining reactions and others to be reported suggest that the fluorescein molecule conjugated to serum protein retains its property to react with tissue like a free dye molecule.

7516

Leibovitz, A.; Oberhofer, T.R.; Meacham, J.T.; Diestelhorst, T.N. 1963. Enhancement of specificity of the fluorescent treponemal antibody test as compared with the TPI test. Amer. J. Clin. Pathol. 40:480-486.

The production of Treponema pallidum antigen that readily adheres to glass slides is further resolved by suspension of organisms treated with sodium hypochlorite in normal saline solution that contains 5 per cent inactivated normal rabbit serum. This solution also permits the storage of treponemes in the deep freeze without diminution of fluorescence. The fluorescent treponemal antibody (FTA) 100 test was as specific and as sensitive as the Treponema pallidum immobilization test to rule out biologic false positive reactors. Paired serum specimens enhanced the reliability of both tests in rendering a definite diagnosis.

7517

Maestrone, G. 1963. Demonstration of leptospiral and viral antigens in formalin-fixed tissues. *Nature* 197 409-410

Modifications of tissue preparation procedures for FA staining are described. These modifications warrant further investigation because of the following practical implications: Elimination of the hazard of handling infectious material; simplification of collection, shipment, and storage of suspected specimens; investigation by FA of specimens submitted for routine histopathological examination; and possibility of a more extensive use of this relatively new tool

7518

Marshall, J.D., Jr. 1964. The use of immunofluorescence for the identification of members of the genus Pasteurella in chemically fixed tissues. *Diss. Abstr.* 24 4921.

FA, cultural, and histologic methods were used to determine the effectiveness of FA for diagnosis of pasteurellosis. Specimens were obtained from animals infected with Pasteurella gallinarum, P. haemolytica, P. multocida, P. novicida, P. pestis, P. pseudotuberculosis, and P. tularensis. FA provided rapid, reliable identification of all species in pure or mixed cultures or from untreated clinical material. P. pestis and P. pseudotuberculosis cross-reacted serologically. Fraction I antiserum from P. pestis was specific. Ethanol (95 or 70 per cent), methanol, chloroform, or 10 per cent formalin were satisfactory fixatives for all species except P. pestis and P. pseudotuberculosis. Methanol was the only satisfactory fixative for these. Histopathologic studies using FA are described.

7519

Miller, J.N.; Whang, S.J.; Boak, R.A.; Carpenter, C.M. 1964. Complexities of the fluorescent treponemal antibody test, and its preliminary evaluation in the serologic diagnosis of syphilis. *Tech. Bull. Regist. Med. Technol.* 34 37-39. Also, *Amer. J. Clin. Pathol.* 41 337-339.

The use of Treponema pallidum antigen suspended in Nelson's medium in the fluorescent treponemal antibody (FTA) test resulted in a reduction of 90 to 95 per cent in the numbers of organisms present after the test was performed, as well as a fragmentation of many organisms. When the treponemes were suspended in sterile 0.85 per cent saline solution and preserved with 1:10,000 final dilution of Merthiolate, neither loss of antigen nor fragmentation was observed. The contents of each individual vial of labeled antihuman globulin should be titered.

and stored at -20 C. Subjectiveness involved in determining the intensity of the fluorescent reaction necessitated close comparison of the results of the test with positive control sera. Of 35 diagnostic problem sera that were reactive in the TPI test, 29 (82.9 per cent) were FTA-reactive. In the same category 94.2 per cent were FTA-nonreactive. Thus, the FTA test failed as a means of detecting 17.1 per cent of the patients with latent syphilis. Greater experience and extensive research are essential before the FTA test can be used as a dependable procedure for the diagnosis of syphilis.

7520

Miura, T.; Kashi, T. 1964. Autofluorescence of pathogenic fungi. *Tohoku J. Exp. Med.* 82:158-163.

Autofluorescence of pathogenic fungi was investigated. Smears were made from the surface of the growth on 4 per cent glucose agar and were fixed by heating, absolute alcohol, or formalin and were placed under the fluorescence microscope. The fungi tested showed various kinds of autofluorescence. There were considerable differences in brightness or in color tone of autofluorescence due to the difference of species, but none due to difference of strains. Of all fungal species tested, Microsporum japonicum and Epidermophyton floccosum gave the most brilliant autofluorescence; its color tone was pale blue. Fixation methods affected the brightness of autofluorescence, and heating was most effective in allowing the most brilliant autofluorescence.

7521

Miura, T.; Kashi, T. 1964. Difference in result of the fluorescent antibody staining of dermatophytes due to the difference of fixing procedure. *Tohoku J. Exp. Med.* 84:72-80.

The probable relationship between the intensity of the fluorescent antibody staining reaction of dermatophytes and fixing procedure of the smear was investigated. Smears of dermatophytes, including Trichophyton rubrum, T. interdigitale, T. asteroides, Epidermophyton floccosum, and Microsporum japonicum, were fixed by heating, by ethanol, or by formalin, or were left to be air-dried without fixation. They were stained with either anti - T. rubrum or anti - T. interdigitale fluorescent antibody. The experiments have led to the conclusion that, of the fixing techniques employed, heating was the most desirable. Smears fixed by heating could hardly be stained with any of the conjugates, and they gave the most brilliant autofluorescence.

7522

Nairn, R.C.; Ghose, I.; Porteous, I.B.; Urquhart, J.A. 1963. Detection of autoantibodies to cytoplasmic and nuclear antigens in freeze-dried thyroid. *J. Clin. Pathol.* 16:281-282.

The most important single factor for success with the present freeze-drying method is the suitability of the original thyroid tissue to provide antigenically active microscopical preparations. The thyroid should be hyperplastic to ensure an abundance of cytoplasmic microsomal antigen and a predominance of small acini to retain colloid. If satisfactory embedded tissue blocks were available commercially, the technique for cellular and colloid antigens described in this and the previous publication could be used for most routine laboratory investigations of clinically significant autoantibodies.

7523

Ocklitz, H.W.; Weppe, C.-M. 1964. The bacteriological diagnosis of pertussis; fluorescence serology and culturing compared: II. The fluorochromatization of antibodies of *Bordetella pertussis*. *Zentralbl. Bakteriol.* 194:103-114. (German).

A report is made on personal experiences with the preparation of anti-pertussis serum suitable for fluorochromatization of antibodies, and also on the concentration, coupling, purification, and testing of this serum. Finally, the preparation and critical examination of the microscopical preparations are described.

7524

Paronetto, F. 1963. The fluorescent antibody technique applied to titration and identification of antigens in solutions or antisera. *Proc. Soc. Exp. Biol. Med.* 113:394-397.

Cellulose acetate discs or agar are useful supporting media for titration or identification of antigen or antibody. The cellulose acetate fluorescent spot method is simple to perform and read and requires only a few lambda of antiserum or antigen; the fluorescein-impregnated agar method is essentially the same as the fluorescent antibody technique applied to tissue. In the titration of antiovine serum albumin, antihuman gamma globulin, and antihorse serum, both fluorescent methods were more sensitive than the ring precipitin test, when the indirect staining was used. In addition, the methods permitted selection of proper fixatives for a given antigen and determination of the proper concentration of labeled antisera for demonstration of tissue antigen.

7525

Paton, A.M. 1964. The adaptation of the immunofluorescence technique for use in bacteriological investigations of plant tissue. *J. Appl. Bacteriol.* 27:237-243.

The preparation of plant tissue for examination by FA is described. The procedures suggested are intended to overcome some of the major obstacles encountered and the efficacy of the method for plant investigations is upheld by its successful application in a simple test system.

7526

Post, R.S. 1963. Formation of lipid-protein complexes in renal epithelial cells in the course of protein reabsorption. *J. Lab. Clin. Med.* 62:1001-1002.

Serum proteins penetrate normal glomerular structures separating capillary lumen and Bowman's space at a variable rate. Protein loading in the animal and human results in an increased loss of protein from the glomerulus. Morphologic changes occur that suggest intracellular localization of protein in a system of ducts and vacuolar structures that, as determined by electron microscopy, are membrane-lined. The author has been able to demonstrate, with FA, bovine serum albumin in discrete structures within epithelial cells of the proximal tubules and glomeruli of rats within 24 hours of injection of the protein. In addition, histochemical studies and observed variation in fluorescence intensity within the glomerular cytoplasmic structures correlate to suggest biochemical alteration of the contained protein with formation of lipid-protein complexes. With the same technique, rat globulin was demonstrated after bovine albumin loading within both types of epithelial cells in amounts greater than normal. These latter observations cast some doubt on the specificity of globulin localization in the glomerulus. A special tissue preparation technique is described.

7527

Ramirez-De Pita, V.; Velez-Pratt, G.; Biagi, F. 1965. Immunofluorescence detectable antigens in calcareous corpuscles of Cysticercus cellulosae. *Rev. Fac. Med. Univ. Nac. Autonoma. Mex.* 7:379-383. In Spanish.

A procedure for obtaining tissue-free calcareous corpuscles of Cysticercus cellulosae is described. The antigenic protein material appears in the homogenized fluid. It is absent from the NaOH and HCl treated corpuscles. The homogenized fluid gives gel precipitation bands with serum from rabbits inoculated with Cysticercus cellulosae antigen. BA-47-38435.

7528

Ryschenkow, E.; Wertlake, P.T. 1965. Visualization of fungi and other microorganisms on Millipore filter by fluorescent antibody technique. *Bacteriol. Proc.* M23:43.

A technique has been developed applicable to fungi and other microorganisms in fluid that yields microorganisms with specific fluorescence that contrasts crisply with a dark Millipore filter background. Both direct and indirect fluorescent methods were successful. For the direct method, three volumes of homologous fluorescent antisera were added to one volume of washed saline suspension of organisms, mixed, and allowed to stand for 30 minutes at 24 C. The mixture was then washed two times with buffered saline, resuspended in saline, and passed through a 0.45-micron Millipore filter with a Swinny hypodermic adapter. The filter was dried at 37 C for 30 minutes and placed on a slide with immersion oil and cover slip for examination. The indirect method was similar except that the suspension was originally exposed to untagged homologous antisera, and fluorescent antiglobulin was added to the washed, sedimented cells. These procedures are especially recommended for rapid identification of small numbers of microorganisms in body fluids that are normally sterile, or in early or scant broth cultures when it is important to prevent loss of cells.

7529

Sinha, R.C.; Black, L.M. 1963. Wound-tumor virus antigens in the internal organs of an insect vector. *Virology* 21:183-187.

A simple method of dissecting out the various internal organs of Agallia constricta Van Duzee is described. Wound-tumor virus antigens were detected by FA in hemolymph smears of viruliferous leafhoppers of both sexes and in the smears of the fat body from female transmitters. The fat body of only one male transmitter of ten tested was positive. When organs of the same kind from 11 to 20 exposed or viruliferous leafhoppers were pooled and extracts tested for wound-tumor soluble antigens by the precipitin ring test, a positive reaction was given by the fat body, intestine, and salivary glands of both sexes and by the ovaries. Malpighian tubules and the testes failed to show a positive reaction. Although the tests suggested that the highest virus antigen concentration is in the fat body, it seems probable that the initial site of infection is the intestinal tract. By squeezing a small drop of hemolymph onto a slide and applying the fluorescent smear test it is possible to determine, without damaging any of the organs, whether an insect is viruliferous.

7530

Sinha, R.C.; Reddy, D.V.R.; Black, L.M. 1964. Survival of insect vectors after examination of hemolymph to detect virus antigens with fluorescent antibody. *Virology* 24:666-667.

Hemolymph examination facilitates certain types of experiments in which the identity of young individual viruliferous and nonviruliferous exposed vectors is desirable. FA was used to detect viral antigen in the hemolymph.

7531

Stout, G.W.; Falcone, V.H.; Moore, M.B., Jr. 1964. Protocols for testing reagents employed in serologic tests for syphilis. *Health Lab. Sci.* 1:119-128.

As a portion of this, procedures for FA reagents are given.

7532

Sulzer, A.J. 1965. Indirect fluorescent antibody tests for parasitic diseases: I. Preparation of a stable antigen from larvae of Trichinella spiralis. *J. Parasitol.* 51:717-721.

A stable antigen was developed for the indirect fluorescence test for Trichinella spiralis antibodies. The antigen was prepared by digesting fresh larvae of T. spiralis with pepsin for 36 hours and fixing the cuticle in 10 per cent formalin - 0.5 per cent bovine serum albumin. The antigen can be stored for several months without loss of activity or specificity. Evidence is advanced to support the hypothesis that specific antigenic sites are a constituent part of the larval cuticle.

7533

Sulzer, A.J.; Kagan, I.G. 1964. Fluorescent antibody (FA) test for schistosomiasis and trichinosis. *J. Parasitol.* 50(Suppl.):23-24.

The indirect FA test for schistosomiasis has been under investigation in this laboratory for several years and more recently the test for trichinosis. Techniques for rapid efficient methods of conjugating antiserum with FITC and the preparation of an antigen for trichinosis, stable under storage at 4 C, have been developed. Certain aspects basic to the FA reaction have been elucidated. One of the variables investigated was the effect of multiple washes of antigen after addition of positive serum and before addition of labeled antiglobulin conjugate. With some positive sera the FA test increased from a negative reaction with one wash to a brilliant 4 plus reaction with four washes. These results indicate that under certain conditions schistosome or trichina antigens that have been sensitized with

gamma globulin from a positive serum will react weakly or negatively in the test. This effect may be overcome by use of antigen washed several times or by conjugate at relatively high concentration. This may clarify why a prozone effect has been reported in the FA test for trichinosis. Dependable methods were developed for production of labeled antiserum in less than 2 weeks from initial inoculation of the animal. Complete article.

7534

Toussaint, A.J.; Anderson, R.I. 1965. Soluble antigen fluorescent antibody technique. *Appl. Microbiol.* 13:552-558.

An indirect fluorescent antibody procedure employing soluble antigen fixed onto an artificial matrix was developed, and a mechanical means for reading test results was devised. The method employs two small cellulose acetate paper discs for each test. One disc contains soluble antigen diluted in 1 per cent bovine serum albumin (BSA); the other contains 1 per cent BSA only and serves as a control. After testing by the indirect FA procedure, the results of the tests are read on a fluorometer fitted with a paper chromatogram door. The instrument is set at zero with the control disc as a blank, and the specific fluorescence of the antigen disc is determined. Findings obtained with homologous and heterologous antisera indicated that the method yields excellent results. The soluble antigen fluorescent antibody technique has definite advantages over the conventional indirect FA procedures. The investigator may objectively select the antigen to be employed. It is possible to obtain objective mechanical reading of test results rather than the highly subjective readings required by conventional methods. The system compensates for any nonspecific fluorescence contributed either by the serum or by free fluorescein in the conjugated antiserum.

7535

Vaisman, A.; Hamelin, A.; Guthe, T. 1963. Use of the fluorescent antibody technique for dried blood eluents: Comparison of the FTA, TPI, and the improved lipid antigen application to serum. *Bull. WHO* 29.1-6.

Past attempts to use dried finger-puncture blood for lipoidal antigen testing of the eluent were successful when blotting paper was used as the adsorption medium. With the introduction of the immunofluorescent technique in syphilis and other treponematoses, it was decided to undertake a fluorescent treponemal antibody (FTA) testing study of dried blood eluents using blotting paper as the absorption medium, since there is need for a simple procedure in areas where information on specific serotesting for treponematoses is required and where

venipuncture is impracticable. The authors describe the preliminary results of their FTA tests. The serological reactivity to FTA, TPI, and lipoidal antigen was also examined in venipuncture sera from the same individuals. The variations found in sensitivity, specificity, and reproducibility of the blotting paper disc FTA-100 procedure were not significant, and the results were practically the same as those obtained independently by FTA-100 examination of venipuncture sera from the same individuals. The advantages of finger-puncture blood sampling are outlined.

7536

Zaman, V. 1965. The application of fluorescent antibody test to Balantidium coli. Trans. Roy. Soc. Trop. Med. Hyg. 59:80-82.

The fluorescent antibody test on Balantidium coli is described. Experience with unfixed and fixed specimens has shown that there is no nonspecific staining with the unfixed specimens; the controls remain completely negative. With the fixed specimens the controls show a certain amount of nonspecific fluorescence. It is, however, possible to differentiate the test slides from the controls by the difference in the intensity of the fluorescence. In terms of brightness, acetone gave the best results among the five different fixatives tried.

D. NONSPECIFIC FLUORESCENCE

7537

Alexander, W.R.M.; Potter, J.L. 1963. Rhodamine-conjugated papain as a counterstain in fluorescence microscopy. *Immunology* 6:450-452.

A new method of counterstaining unfixed tissue for fluorescence microscopy is described. The method depends upon the reaction of crude papain, labeled with a fluorescent dye, with nuclear and cytoplasmic constituents of tissue cells.

7538

Berenznitskaya, E.R. 1964. A method for selective extinction of the parafluorescence of cocci in preparations stained with pertussis and parapertussis fluorescent sera. *Zh. Mikrobiol. Epidemiol. i Immunobiol.* 41:71-74.

A method has been developed for selective extinction of the parafluorescence of cocci in preparations first stained with fluorescent pertussis and parapertussis sera, and then Gram-stained. Treatment of the preparations with gentian violet and iodine, serving as fluorescence extinction agents, has made it possible to eliminate the fluorescence of the Gram-positive parafluorescent cocci, and to retain at the same time the fluorescence of the Gram-negative pertussis and parapertussis bacteria.

7539

Bradstreet, P. 1965. Bacterial agglutinating antisera and immunofluorescence. *Brit. Med. J.* 1:440.

A warning is given against indiscriminate use of diagnostic agglutinating antisera for FA studies. FA detection of antigens may easily be too sensitive to use sera intended for other purposes.

7540

Brooks, J.B.; Lewis, V.J.; Pittman, B. 1965. Separation of fluorescent antibody conjugates into 7S and 19S globulin components by gel filtration. *Proc. Soc. Exp. Biol. Med.* 119:748-751.

Gel filtration proved to be a simple, effective method for separation of fluorescein-labeled antiglobulin for *E. coli* into 7S and 19S fractions. Both fractions were found to contribute considerable nonspecific staining, but specific staining was associated predominantly with the 7S globulin.

The ratio of specific staining titer to nonspecific staining activity was threefold greater with a pool of fractions in which only 7S globulin was detected than with the unfractionated conjugate when the two preparations were tested at the same protein concentration.

7541

Cerottini, J.C.; Jaton, J.; Froidevaux, T.; Isliker, H. 1964. Immunofluorescence by means of fragments of antibodies. *Helv. Physiol. Pharmacol. Acta* 22:C9-C12. In French.

The gamma globulin fraction of rabbit serum immunized against Histoplasma capsulatum was separated into three parts by means of papain digestion and chromatography on a carboxymethyl-cellulose column. FITC was added to the fractions, and the fractions added to the antigen as outlined. Fraction I and II always are fixed on the antigen giving a positive fluorescence. Fraction I from a non-immune animal does not show fluorescence. Fraction III does not react whether the animal is immune or not. It might be better to use Fractions I or II instead of the total gamma globulin to avoid unspecific fluorescence. BA-46-12110.

7542

Clark, H.F.; Shepard, C.C. 1963. A dialysis technique for preparing fluorescent antibody. *Virology* 20:642-644.

A dialysis method is described for conjugating serum proteins with FITC. Antisera against rabies, polio, rickettsiae, and mycobacteria were used. Human, monkey, guinea pig, sheep, and rabbit globulins were conjugated by this method. Good specific FA staining with minimal nonspecific staining was obtained.

7543

Crandall, C.A.; Echevarria, R.; Arean, V.M. 1963. Localization of antibody-binding sites in Ascaris suum by means of fluorescent techniques. *J. Parasitol.* 49(Suppl):33.

Embryonated eggs, second-stage larvae, and larvae isolated from the livers and lungs of experimentally infected animals were incubated in fluorescein-labeled antiglobulin. Controls consisted of incubation and staining with fluorescein-labeled normal globulin. Rhodamine bovine albumin was added to the fluorescent globulins in some cases to decrease nonspecific staining. Specific staining was observed in oral, anal, excretory pore, and cuticular precipitates of larvae isolated from tissues. No precipitates were formed on second-stage larvae incubated in immune globulins. The cuticle of whole and sectioned second- and third-stage larvae stained specifically. Specific fluorescent staining of the larval cuticle was observed in

sectioned embryonated eggs whereas the 'fertilization' membrane stained nonspecifically. No staining of the cuticle of sectioned adult worms resulted with either immune or normal fluorescent globulin. Complete article.

7544

Danielsson, D. 1963. The demonstration of N. gonorrhoea with the aid of fluorescent antibodies: I. Immunological studies of antigenococcal sera and their fluorescein-labeled globulins, with particular regard to specificity. *Acta Dermatovenerol.* 43:451-464.

Antigenococcal sera were prepared in rabbits and their immunological activity was measured by agglutination, complement fixation, and fluorescent antibody techniques. The fluorescein isothiocyanate - conjugated globulins gave strong reactions with meningococci, with certain strains of S. aureus, and with Group G streptococci. Weak reactions were noted with certain Neisseria (flava, flavescens, sicca, and catarrhalis), certain Group A and Group C streptococci, S. aureus, S. albus, and a few types of pneumococci. Cross-reactions with S. aureus were eliminated by absorbing the conjugate with such S. aureus as gave strong reactions. The cross-reactions noted with other cocci, exempting meningococci, were at the same time reduced to a minimum. The immunological significance and the practical consequence of these observations are discussed.

7545

Danielsson, D. 1965. The demonstration of Neisseria gonorrhoeae with the aid of fluorescent antibodies: 5. A comparison of different techniques--absorption, one-step inhibition, and counterstaining--for elimination of cross reactions. *Acta Dermato-Venerol.* 45:61-73.

FITC conjugated rabbit antigenococcus globulins and rabbit normal globulin were tested for reactions with Neisseria strains, staphylococci, and streptococci. Strong reactions were observed between antigenococcus conjugates and meningococci. Both the antigenococcus and normal globulin conjugates gave strong reactions with some of the staphylococcus and Group G streptococcus strains. These could be eliminated by absorbing with strongly reacting strains of Staphylococcus aureus. At the same time FA titers of the antigenococcus conjugates with gonococci and meningococci were lowered. The staining of staphylococci was somewhat inhibited by adding human serum or normal rabbit serum to antigenococcus conjugates. Adding antistaphylococcal rabbit serum to FITC-labeled antigenococcus globulins had a more pronounced inhibiting effect. RB 200-labeled globulin of the antistaphylococcal rabbit serum had the same effect. The application of delayed FA tests on clinical specimens showed that it was necessary to take precautions against nonspecific reactions. In males, delayed FA tests and culture were of equal value for diagnosis of gonococci. In females, delayed FA tests gave a higher yield than culture.

7546

Danilova, T.A.; Korn, T.Ya. 1964. The possibility of elimination of cross-reactions between streptococci of various groups and staphylococci in applying the fluorescent antibody method. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:13-16.

In the direct method of fluorescent antibodies the fluorescent serum to Group A Streptococcus stained not only strains of the homologous group, but also cultures of C and G groups and some Staphylococcus strains. The sorption of a labeled serum with the live Streptococcus Group C culture eliminated the specific staining of strains of the Groups C and G; however, the sorbed serum retained its capacity to stain Staphylococcus. Treatment of fixed smears in a trypsin solution has made it possible to eliminate the Staphylococcus staining without disturbing the specific fluorescence of the Group A Streptococcus.

7547

Demissie, A. 1965. Immunofluorescence identification of Salmonella in fecal specimens. Acta Pathol. Microbiol. Scand. 65:271-286.

An investigation into the usefulness of the FA method in detecting salmonellae in feces and river water was undertaken. Cross serological reactivity was a serious handicap. All the isolated strains belonged to the family Enterobacteriaceae. Absorption techniques were employed to minimize cross-reactivity, and the results indicate that this may be the solution of the problem. Sera of satisfactory specificity were produced for the Salmonella Groups B, C, and D. Absorbed sera for Group E still gave some cross-reaction.

7548

Engelhardt, N.V. 1964. The use of antibodies against gamma globulin in the indirect fluorescent antibody test. Biul. Eksp. Biol. Med. 57:67-70. In Russian.

The antibodies against rabbit gamma globulin were obtained from both native and labeled antirabbit serum with the aid of polystyrene immunosorbent. These antibodies when used in the indirect fluorescent antibody method gave the opportunity to exclude all the cross-reactions of the labeled serum with the sections; nonspecific staining of the sections also essentially decreased.

7549

Fletcher, S. 1964. Serology of toxoplasmosis. *Brit. Med. J.* 2:1660.

This is a report of the routine use of indirect FA for the serologic diagnosis of toxoplasmosis. The methods are briefly described.

7550

Frommagen, L.H. 1965. The solubility and other physicochemical properties of human gamma globulin labeled with fluorescein isothiocyanate. *J. Immunol.* 95:442-445.

The solubility of human gamma globulin, unlike that of albumin and alpha-beta globulins, is modified by the addition of fluorescein isothiocyanate (FITC). The solubility, as well as electrophoretic behavior, of fluorescein-labeled gamma globulin demonstrates a considerable heterogeneity of labeling at the higher conjugation ratios and a more homogeneous labeling at low conjugation ratios. The value of optimum labeling of immune globulin preparations in order to assure specific staining was again underscored. Nonspecific staining was assayed on HeLa cells.

7551

Fry, C.S.; Wilkinson, A.E. 1963. A note on the use of Evans blue as a background stain in the fluorescent treponemal antibody test. *Brit. J. Vener. Dis.* 39:190-191.

The FTA test was improved by using Evans blue as a counterstain that quenched background fluorescence.

7552

Fuerst, D.E.; Jannach, J.R. 1965. Autofluorescence of eosinophils: A bone marrow study. *Nature* 205:1333-1334.

Autofluorescence of eosinophils obtained from patients with various diseases was studied. Degrees of fluorescence varying with disease were obtained. No correlation with drug therapy was found. The results of this study may clarify some problems of autofluorescence obscuring specific FA reactions.

7553

Funckes, A.J.; Ly, H.; Gates, E.H.; Imagawa, D.T. 1963, Procedures to decrease nonspecific fluorescent staining of measles-infected tissue. *Federation Proc.* 22:217:197.

Because of problems associated with nonspecific fluorescence in immunofluorescent techniques, a study was made to determine the significance of some physical properties of media. Whole sera were labeled with fluorescein isothiocyanate, which was fractionated by low-temperature ethanol procedures. Fluorescein-labeled antibody fractionated with glycine-ethanol mixtures contained only traces of free fluorescein. These labeled antibodies are absorbed by normal tissue homogenate to reduce nonspecificity. The effect of pH, ionic strength, and dielectric constant of media during these absorptive procedures was found to be important. During the staining procedure, alteration of pH, ionic strength, and dielectric constant from the procedures used during absorption can significantly affect nonspecific staining. Nonspecific fluorescence was significantly reduced as shown by comparative photomicrography in measles-infected mouse brain smears and in measles-infected HeLa cell cultures. Complete article.

7554

Haglund, J.R.; Ayers, J.C.; Paton, A.M.; Kraft, A.A.; Quinn, L.Y. 1964. Detection of Salmonella in eggs and egg products with fluorescent antibody. *Appl. Microbiol.* 12:447-450.

Organisms of the genus Salmonella are detected in eggs and egg products within 24 hours in the presence of Pseudomonadaceae and other Enterobacteriaceae by combining selective cultural methods with fluorescent antibody techniques. These techniques are specific for Salmonella when H antibodies are used. Absorption techniques are necessary before the O antibodies give specific reactions for Salmonella. No cross-reactions appear when H antiserum is used. Absorption and interference techniques indicate the test is specific for Salmonella.

7555

Henle, G.; Henle, W. 1965. Cross-reactions among gamma globulins of various species in indirect immunofluorescence. *J. Immunol.* 95:118-124.

Several preparations of fluorescein isothiocyanate - conjugated rabbit antibodies to guinea pig or human gamma globulin fractions were found to be capable of staining the characteristic mumps virus inclusions in infected HeLa cells previously sensitized with virus-specific antibodies derived from heterologous animal species. Brilliant immunofluorescence was elicited by labeled antibodies to heterologous gamma globulins only when the antiviral sera used for the primary stage of the reaction were

used undiluted, or at the most in 10-fold dilution. The rabbit anti-guinea pig gamma globulin sera from which effective fluorescein conjugates were prepared yielded from one to three lines of precipitation in Ouchterlony tests with sera from several heterologous species with the exception of rabbits, whereas those sera from which strictly species-specific conjugates were obtained produced no precipitates with heterologous sera. Cross-reacting antisera to guinea pig gamma globulin also reduced the viral neutralizing capacity of human poliomyelitis convalescent serum. No attempts were made to identify the cross-reacting serum components. Such broadly reactive anti-gamma-globulins might be useful for the indirect technique of immunofluorescence in that they overcome the usually observed species limitation of this method.

7556

Hulten, J.; Ponten, J. 1964. Staining of normal, atypical, and cancerous colon epithelium by ovalbumin - fluorescein isothiocyanate at different pH levels. *Acta Pathol. Microbiol. Scand.* 60:112.

Sections from normal colon, colon polyps, and colon carcinoma have been stained with egg albumin - fluorescein isothiocyanate conjugates at pH 3 to 11. At pH 7 to 8 the cytoplasm of normal epithelium stained strongly, the polyp epithelium stained very weakly, and cancerous epithelium not at all. At pH 3 to 5 the egg albumin - fluorescein isothiocyanate complex stained epithelial mucins very intensively, paralleling the intensity of a PAS-stain. At the same pH, mast cells were left unstained, although they were strongly PAS-positive. Neutrophilic and eosinophilic leukocytes stained strongly at pH 7 to 8. No structures were stained at pH 10 to 11. At an acid pH the egg albumin - fluorescein isothiocyanate method provided a valuable stain for epithelial mucin. At neutral or slightly alkaline pH the stain clearly differentiated between neoplastic and normal epithelial cells in the colon. While not FA, the findings reported have great value in demonstrating some causes and controls for nonspecific fluorescence.

7557

Joncas, J. 1964. The direct fluorescent antibody technique studied with reovirus Type I. *Rev. Can. Biol.* 23:333-338.

A fluorescent antibody technique is described that consists of a few technical modifications of the standard method. Reovirus Type I was the antigen used. Tissue culture medium devoid of antibiotics and of phenol red has been found to decrease the intensity of the autofluorescence of the tissue culture. The length of the staining period has been shortened to 10 minutes at room temperature with improved results. A homemade tray is mentioned that greatly facilitates the handling of the small cover slips, and the Columbia staining dishes have been found most helpful. Finally, photographs were taken without any change in filters, using an Ansochrome 200 daylight color film on a Leitz microscope.

7558

Kaplan, W.; Clifford, M.K. 1964. Production of fluorescent antibody reagents specific for the tissue form of Coccidioides immitis. Amer. Rev. Resp. Dis. 89:651-658.

Five different lots of rabbit Coccidioides immitis antiglobulins were tagged with fluorescein isothiocyanate. These reagents brightly stained endospores and contents of spherules formed in vivo. The labeled antibodies also cross-reacted with Histoplasma capsulatum, Blastomyces dermatitidis, and other heterologous fungi. Absorption of these conjugates with yeast cells of H. capsulatum eliminated all nonspecific activity. Two of the absorbed reagents reacted strongly with the tissue forms of C. immitis. These conjugates had been prepared from globulins produced by rabbits infected with the fungus and by rabbits immunized with killed arthrospores. By dilution, the former conjugate was also rendered specific for tissue forms of C. immitis. The specific conjugates were successfully used to detect C. immitis in clinical materials from confirmed cases of coccidioidomycosis.

7559

Karakawa, W.W.; Krause, R.M.; Borman, E.K. 1965. Immunochemical aspects of the cross-reactivity between Groups A and C streptococci as detected by the fluorescent antibody technique. J. Immunol. 94:282-288.

The cross-reactivity as detected by the fluorescent antibody method between Group A sera and Group C streptococci is dependent upon a rhamnose moiety that is shared by both Groups A and C carbohydrates and that, under certain circumstances may be serologically reactive. The cross-reactivity is inhibited by A-variant carbohydrate, and by a rhamnose disaccharide isolated from A-variant carbohydrate by the rhamnosidase of McCarty. Cross-reactivity between Groups A and C carbohydrates is dependent, in part, upon the fact that this rhamnose disaccharide is a structural feature common to both antigens.

7560

Khomenko, N.A.; Olshevskaya, T.R. 1965. Increase of fluorescent globulin specificity for the direct method of Shigella staining. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:7:33-36. In Russian.

The specificity of fluorescent antibodies for the detection of Shigella dysenteriae could be increased by their preparation from specific sera after meticulous sorption of heterologous agglutinins. For the same purpose fluorescent antibodies should be used in high dilutions, 1:32 to 1:64. To produce a more intensive fluorescence of bacteria, stain the smears for a more prolonged period of time, 1.5 to 2 hours at 37 C in a humid chamber.

7561

Komninos, G.N.; Tompkins, V.W. 1963. A simple method of eliminating the cross-reaction of Staphylococcus in the fluorescent antibody technic. *Amer. J. Clin. Pathol.* 40:319-324.

Rabbit immune sera against various species of bacteria, when tested by the direct or indirect fluorescent antibody method, cross-react with certain staphylococci. When staphylococci on a slide were pretreated with papain, then anti-meningococcal, anti-streptococcal A, or anti-Escherichia coli immune serum failed to cross-react. When similarly treated slides were stained with anti-Haemophilus influenzae, anti-pneumococcal, or anti-Listeria monocytogenes immune serum, papain eliminated cross-reaction only after these sera were absorbed with a staphylococcal strain. The difference in the effectiveness of papain suggests that in the case of the first three immune sera the cross-reaction factor was solely a papain-sensitive substance and that in the latter three immune sera two factors were involved--a heterogenetic antigen requiring the corresponding absorption and the papain-sensitive substance. A practical application of these findings is discussed.

7562

Korn, M.Ya.; Mayorova, G.F. 1963. On some causes of Staphylococcus staining with heterologous fluorescent sera. *Zh. Mikrobiol. Epidemiol. i Immunobiol.* 40:11:51-56. In Russian.

Staphylococcus staining with heterologous fluorescent sera was studied. As demonstrated, the presence in the sera of the normal antibiotics to Staphylococcus served as one of the causes of this phenomenon.

7563

LaBrec, E.H. 1965. The effects of fixation and postfixation treatment of nonspecific fluorescence in tissues. *Bacteriol. Proc.* M130:61.

The protein granules of polymorphonuclear eosinophil and neutrophil (PMN) leukocytes exhibit a striking affinity for fluorescein-labeled antibody. Despite improvements in reagents and techniques, nonspecific fluorescent (NSF) staining of these granules remains a problem in using FA methods. The effects of fixation and postfixation methods on NSF staining and a method for eliminating NSF staining of PMN granules are reported. Frozen sections of tissues or bone marrow smears were fixed in various nonpolar solvents, dried, immersed in 0.05 M HCl, washed in buffered saline, and stained with FA. Under these conditions, PMN granules were not stained by FA; they were stained by FA if the sections or smears were fixed in 10 per cent formalin prior to HCl treatment. Postfixation treatment with 0.05 M HCl did not affect

specific antigen-FA reactions in various systems examined. The effects of fixation and postfixation on staining of PMN granules by eosin YS (tetrabromofluorescein) and related dyes were the same as those seen with FA. These staining reactions and others to be reported suggest that the fluorescein molecule conjugated to serum protein retains its property to react with tissue like a free dye molecule.

7564

Mayorova, G.F.; Korn, M.Ya. 1963. A study of the antipertussis fluorescent serum specificity. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:9:42-48. In Russian.

In studies on the specificity of fluorescent serological examination of H. pertussis with the aid of antipertussis fluorescent serum, it was impossible to detect H. pertussis with the aid of heterologous sera. Antipertussis fluorescent serum stained some species of microorganisms nonspecifically: P. pestis, E. coli, Bacillus brucellosis, P. tularensis, and others. Staphylococci and streptococci were stained specifically. The method of fixation also influenced the results of the investigation. Rough fixation by flame provoked microbial staining of H. pertussis with heterologous fluorescent sera. Identification of H. pertussis in practical conditions by FA at present is fraught with some difficulties, since the Staphylococcus and Streptococcus may be stained simultaneously. It was impossible to distinguish H. parapertussis from H. pertussis by the indirect method.

7565

Mikhailov, I.F. 1963. Criteria of specific fluorescence of bacteria stained with fluorescent antibodies. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:7:94-97. In Russian.

As established, the only authentic criterion of specific fluorescent sera interaction with homologous bacteria possessing a membrane was a characteristic marginal fluorescence of a microbial cell. The intensity of this fluorescence is relative. Uniform fluorescence of bacteria possessing cellular membrane may be caused by nonspecific factors such as autofluorescence or secondary fluorescence. Patchy fluorescence of bacteria is connected with specific staining of localized antigens and points to the antigenic commonness with bacteria against which the fluorescent serum was prepared.

7566

Mikhailov, I.F.; Pers, I.F. 1964. Isolation of antibodies from antigen-antibody complex by ultrasound. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:112-119. In Russian.

Antibodies were separated from their specific complex with somatic antigens by ultrasound and then eluted in saline. Antibody preparations were free of nonspecific protein, heterologous antibody, and the antigen. Antibodies retained their immunologic specificity. An anti-flexneri conjugate labeled with fluorescein isocyanate was among the sera used.

7567

Miura, T.; Kasai, T. 1964. Autofluorescence of pathogenic fungi. Tohoku J. Exp. Med. 82:158-163.

Autofluorescence of pathogenic fungi was investigated. Smears were made from the surface of the growth on 4 per cent glucose agar and were fixed by heating, absolute alcohol, or formalin and were placed under the fluorescence microscope. The fungi tested showed various kinds of autofluorescence. There were considerable differences in brightness or in color tone of autofluorescence due to the difference of species, but none due to difference of strains. Of all fungal species tested, Microsporium japonicum and Epidermophyton floccosum gave the most brilliant autofluorescence; its color tone was pale blue. Fixation methods affected the brightness of autofluorescence, and heating was most effective in allowing the most brilliant autofluorescence.

7568

Mizuno, S.; Yodate, H. 1963. Rapid diagnostic method for fungi by means of fluorescent antibody technique. Rinsho Byori 11:391-399. In Japanese.

For the FA technique used as a serological procedure in diagnosis of fungi, preparation of antigens from Candida, Saccharomyces, and Torulopsis varieties, using rabbits, preparation of specific immune sera, and preparation of fluorescent-labeled antibodies are described. C. albicans, C. stellatoidea, C. guilliermondii, and T. glabata were diagnosed specifically by the FA method. From vaginal smear samples, C. albicans was detected in 65 of 96 cases; T. glabata in 21 of 96, C. stellatoidea and C. guilliermondii in none. Ten of 96 were undetectable. These results compared favorably with those obtained by other methods. Good results in identification of C. albicans were also obtained from smear samples from newborn infants. Methods of eliminating nonspecific fluorescence are suggested.

7569

Myers, J.; Sargent, A.U.; Cohen, J.J. 1965. Whole antiserum versus the gamma globulin fraction of antiserum in the indirect fluorescent antibody technique. *Immunology* 9:101-105.

Two antisera were compared with their gamma globulin fractions for effectiveness as the middle or unlabeled layer in the indirect fluorescent antibody technique. The gamma globulin fraction did not produce better results in either case than the equivalent concentration of whole serum. Control sections were prepared with normal rabbit serum substituted for the whole antiserum and with normal rabbit gamma globulin substituted for the gamma globulin fraction of the antiserum. The control sections with gamma globulin showed considerably more nonspecific fluorescence than those with whole serum. In one case the nonspecific fluorescence in the gamma globulin control interfered with interpretation of the experimental section.

7570

Neblett, T.R.; Merriam, L.R.; Burnham, T.K.; Fine, G. 1964. A source of false-positive fluorescent treponemal antibody reactions. *J. Invest. Dermatol.* 43:439-440.

Non-syphilitic sera that demonstrated antinuclear factor with tumor imprints also produced a reactive fluorescent treponemal antibody (FTA) test whose intensity seemed to parallel that of nuclear immunofluorescence. Such reactive FTA results were rendered negative by absorption with human tumor homogenates; they were diminished partially by normal tissue homogenate absorption, but remained unaffected by animal tissue powder absorptions. Patients furnishing such sera were considered non-syphilitic. Known syphilitic sera absorbed with tumor or normal tissue homogenates could not be rendered negative to the FTA, and these did not produce nuclear immunofluorescence. False-positive FTA test results may indicate the presence of an autoimmune disorder.

7571

Okada, T.S. 1964. Cellular distribution of some microsomal antigens in embryonic chicken kidneys. *Exp. Cell Res.* 33:584-587.

Distribution of constituents of deoxycholate extracts of chicken kidney microsomes were studied by FA. Fraction K45 proteins were precipitated from the extracts by 25 and 45 per cent saturation ammonium sulfate. Fraction E refers to those from 60 and 80 per cent saturation ammonium sulfate precipitation. Antisera to K45 and E were conjugated and used.

Methods for fixation of specimens and elimination of nonspecific fluorescence are detailed. Staining patterns using one or both conjugates on tissue sections are described. FA demonstrated differing cellular distribution of K45 and E antigens.

7572

Olenchnowitz, A.-F.; Kokles, R.; Urbaneck, D. 1965. Experiences in the preparation of purified fluorescent antibodies. Arch. Exp. Veterinaarmed. 19:139-148. In German.

Globulins from bovine foot-and-mouth disease hyperimmune serum are precipitated with ammonium sulfate and coupled with FITC. The conjugates are purified by gel filtration and chromatography. The dye-protein ratio largely determines the staining properties of the conjugates. The importance of their extent of coupling and antibody content for the specificity of antibody determination is discussed and optimal conditions for preparing conjugates without unspecific fluorescence are postulated. BA-46-80842.

7573

Pagnes, P. 1963. The influence of complement in the FTA. Minerva Dermatol. 38:337-339. In Italian.

Alexin is not involved in this reaction, and it probably does not contribute to false-positive results.

7574

Peacock, W.L.; Thayer, J.D. 1964. Direct FA technique using Flazo orange counterstain in identification of Neisseria gonorrhoeae. Public Health Rep. 79:1119-1122.

Laboratory diagnosis of gram-stained cervical smears from women suspected of having gonorrhoea was so unproductive in the past that when the culture method was used the stained smear procedure was not. Even after immunofluorescent methods had greatly improved diagnosis of direct smears, results were still about 50 per cent inferior to those obtained with the delayed fluorescent antibody procedure or with the culture method. By the use of Flazo orange as a counterstain, nonspecific background fluorescence was quenched without obscuring points of reference, such as pus cells. Eye strain was notably reduced. One hundred fifty-six female contacts of male gonorrhoeal patients were examined. In the more rapid FA procedure the method of smear preparation and counterstain resulted in specific diagnoses of gonorrhoea to within 2 per cent of results by the culture method.

7575

Porter, B.M.; Thomas, B.K.; Furcclow, M.L.; Varga, D.T. 1963. Comparison of two Histoplasma capsulatum fluorescent antibody conjugates based on 800 clinical specimens. *Bacteriol. Proc.* M141:90.

On the basis of 800 clinical specimens, two differently prepared conjugates were compared. In both cases, antiserum prepared against the yeast phase of Histoplasma capsulatum was tagged with fluorescein isothiocyanate. In one case, the conjugate was absorbed three times with tissue powders, once with monkey liver powder and Candida sp. yeast powder, and twice with mouse liver powder. In the second case, the conjugate was absorbed with packed, formalin-killed yeast cells of Blastomyces dermatitidis according to procedures adapted from those outlined by Kaplan and Kaufman in 1961. The conjugate absorbed with packed B. dermatitidis is more specific, requires fewer reagents, and can be diluted for use with clinical specimens.

7576

Quan, S.F.; Knapp, W.; Goldenberg, M.I.; Hudson, B.W.; Lawton, W.D.; Cnen, T.H.; Kartman, L. 1965. Isolation of a strain of Pasteurella pseudotuberculosis from Alaska identified as Pasteurella pestis: An immunofluorescent false positive. *Amer. J. Trop. Med. Hyg.* 14:424-432.

A gram-negative, bipolar-staining rod, isolated from a snowshoe hare in Alaska, was identified as Pasteurella pestis. This identification was of particular importance because plague is under international quarantine and has never been reported from Alaska. Subsequent work has established that the organism is a strain of P. pseudotuberculosis Type I B that possesses an antigenic substance very closely related to the Fraction I antigen of the plague bacterium. The presence of this antigen resulted in the fluorescent antibody test yielding a false-positive finding, and has raised the question as to the current emphasis in differentiating between these two bacterial species on the basis of the presence or absence of Fraction I. The determination of P. pestis must include other testing procedures, such as the isolation of the organism or the demonstration of its presence in tissues of test animals.

7577

Rappaport, B.Z.; Walker, J.M.; Booker, B.F. 1964. Purification of highly conjugated precipitins and globulins containing reagins with silk hydrolysate. *J. Immunol.* 93:782-791.

Highly conjugated human globulins containing reaginic antibodies against egg albumin, ragweed pollen, and cottonseed and conjugated rabbit precipitins against egg albumin and ragweed pollen were purified by adsorption with silk hydrolysate. The purified antibodies were still highly conjugated and gave satisfactory specific staining of antigen in allergic human skin. The reduction in nonspecific staining was probably due to adsorption of noncovalently bound dye by the silk hydrolysate. The presence of antibody-bound antigen in epithelial cells of challenged, allergic skin was confirmed. The epithelial cells of histamine-challenged, allergic skin were capable of binding specific antigen when this was supplied in vitro. The human reaginic globulins and the rabbit precipitins gave similarly effective staining and inhibition results, and the localization of antigen demonstrable with either antibody was the same.

7578

Rivenson, S.; Planes de Banchero, E.; Zakin, M.M. 1962. Identification of the aphthous virus using the fluorescent antibody technique. *Rev. Invest. Ganaderas* 15:275-284.

Using FA, it was possible to see the antigen-antibody reaction between the three types of foot-and-mouth disease virus (O, A, and C) and their homologous sera. Smears of bovine tongue epithelium and monolayers of pig kidney cells were used. FA was not a satisfactory method for the detection of specific antibody in the sera of cattle convalescent from the disease. FITC was more satisfactory than RB 200 as a labeling agent. The superiority of the gamma globulins of the rabbit sera to total serum and complete globulins was demonstrated. The gamma globulins were fractionated by precipitation with ammonium sulfate 50 per cent saturation and by passage on DEAE cellulose, without notable differences. The labeled gamma globulins were purified by passage on DEAE cellulose and by adsorption on tissue powder. With the first method nonspecific fluorescence was eliminated.

7579

Sodeman, W.A., Jr.; Jeffery, G.M. 1964. Immunofluorescent staining of sporozoites of Plasmodium gallinaceum. J. Parasitol. 50:477-478.

Sporozoites of P. gallinaceum were stained by direct FA. Immune serum was obtained from an infected chicken, and its preparation is described. Nonspecific background fluorescence of exoskeleton fragments was quenched by counterstaining with either Evans blue, RB 200-BSA, or Flazo orange. No sporozoite staining was seen using conjugated human antiserum against P. vivax, P. falciparum, or P. cynomolgi. Brightly fluorescing masses were seen on the tip end and lateral wall of some sporozoites. These areas appeared to correspond with the apical cup and the micropyle.

7580

Sommerville, R.G.; MacFarlane, P. 1964. The rapid diagnosis of virus infections by immunofluorescent techniques applied to blood leukocytes. Lancet 1:911-912.

The application of the fluorescent antibody technique to peripheral blood leukocytes offers a new method for the rapid diagnosis of virus infections.

7581

Staak, C. 1961. Studies on staining brucellae with the aid of fluorescent antibodies. Inaugural Dissertation. Institut für Veterinar-Hygiene, Freien Univ. Berlin. Journal No. 357. 59p. In German.

Anti-Brucella antisera were used in a study of conjugation procedures. Both RB 200 and FITC were used. When amounts of dye in reaction mixtures increased, protein conjugation was increased. Excess dye conjugation did not result in better staining. Non-specific fluorescence made it impossible to distinguish brucellae in mixtures of brucellae and guinea pig blood. Culture of Brucella on agar containing fluorochrome revealed that acridine orange, 1:10,000, was the best combination. The bacteria took up the dye. Mixture of the stained brucellae with guinea pig blood resulted in increased fluorescence of lymphocytes, granulocytes, and other blood components.

7582

Trouet, A.; Stadtsbaeder, S. 1964. Demonstration of group antigens by immunofluorescence technique. Rev. Belge Pathol. Med. Exp. 30:53-64. In French.

Group antigens, particularly D antigens, can be identified by indirect technique FA. The reaction is specific for the majority of red cells, but a variable percentage (1/2,000 to 1/20,000) fluoresce aspecifically. The aspecific reactions were eliminated by use of a fluorescent anti-globulin fraction obtained by chromatography on DEAE-cellulose and adsorbed with normal human red cells and with the supernatant fraction of human serum precipitated with 50 per cent saturated ammonium sulfate. The causes of aspecific reactions are discussed.

7583

White, L.A.; Kellogg, D.S., Jr. 1965. Neisseria gonorrhoeae identification in direct smears by a fluorescent antibody counterstain method. Appl. Microbiol. 13:171-174.

Direct smears from female patients have been considered unreliable for the detection of Neisseria gonorrhoeae by FA methods because of the inadequate background contrast of the fluorescein-stained smears and a scarcity of organisms on the smear. Evans blue dye employed as a counterstain eliminated the nonspecific background staining and increased the reliability of the direct FA procedure. Direct smears demonstrating positive fluorescence were obtained from 86 per cent of a group of culturally positive females. The FA-counterstain technique is as sensitive as the presently recommended cultural procedures.

7584

White, R.G. 1963. The applications of fluorescent antibody techniques in bacteriology and virology; the fluorescent antibody technique in the detection of localization of bacterial antigens: Application to mycobacteria, Nocardia, and corynebacteria. Proc. Roy. Soc. Med. 56:474-478.

The use of a double-layer fluorescent antibody technique with rabbit antiserum to Mycobacterium smegmatis results in a pattern of staining of corynebacteria, nocardias, and some mycobacteria species that conforms with previously reported results of cell-wall analyses for sugars, amino sugars, and amino acids and of cell wall agglutination tests. It presumably depends upon the existence of a common antigenic determinant identical with that present in the glycopeptide moiety of wax D of human strains of M. tuberculosis. The failure of the fluorescent antibody method to stain M. smegmatis, M. avium, M. lepraemurium,

and a strain of *M. phlei* is attributed to the inaccessibility of this antigen to antibody at the surface of these organisms. Antigen is accessible in cell-wall preparations. The widespread occurrence of this antigen throughout mycobacteria, corynebacteria, and nocardia species is of importance in the specific diagnosis of antigens or bacteria in these genera by the fluorescent antibody method.

7585

Wolf, P.L.; Pearson, B.; Rosenblatt, M.; Jacobschitz, E. 1963. A new highly specific method for immunofluorescence. *Amer. J. Clin. Pathol.* 40:430.

Striking specificity was noted in immunofluorescent reactions, in which only the 7S gamma globulin fraction of immune serum was conjugated with fluorescein isothiocyanate and then used as the outer layer in indirect immunofluorescence. Total gamma globulin was first separated from sheep antirabbit serum with ammonium sulfate. Dialyzed gamma globulin was then placed in an A-50 diethylaminoethanol-Sephadex column and eluted. The first 54 per cent of the total eluate contained only the 7S fraction of the gamma globulin. This fraction was then conjugated with fluorescein isothiocyanate by conventional methods. A group of 7-week-old mice were injected intravenously with bovine serum albumin and were killed 2.5 hours after the injections. Their spleens were removed, frozen, and sectioned in a cryostat. The sections were overlaid with rabbit anti-BSA serum and, subsequently, with the fluorescein-conjugated 7S fraction of the antirabbit serum. Fluorescence was specifically confined to discrete groups of splenic cells. Controls manifested no fluorescence. It is postulated that the specificity of fluorescent antibody reactions is based on removing 19S antibodies that are likely to be nonspecific in their predilection and using the highly specific antibody of 7S variety.

7586

Wolf, P.L.; Pearson, B.; Rosenblatt, M.; Vazquez, J.; Jarkowski, T. 1965. A rapid method to diminish nonspecific reactions in immunofluorescence with the use of tagged 7S gamma globulin immune sera. *Amer. J. Clin. Pathol.* 43:47-52.

A method has been perfected to obviate nonspecific staining in indirect immunofluorescence. The method involves utilizing fluorescein-tagged 7S gamma globulin immune sera as the outer layer in indirect immunofluorescence. The 7S gamma globulins are obtained by column chromatography, utilizing A-50-DEAE Sephadex. Free fluorescein is readily removed by a G-25 Sephadex column. The four test systems used demonstrate that the preparation of our sera reveals no or very little nonspecific fluorescent material.

7587

Young, S.; Cowan, D.M. 1965. The apparent immunofluorescence of tissue mast cells. *Brit. J. Exp. Pathol.* 46:649-652.

Both rat mast cells and rat eosinophils exhibited fluorescence in blue violet light after treatment with FITC-conjugated rabbit anti-rat gamma-G globulin and in each cell this fluorescence was immunologically nonspecific. Whereas eosinophils fluoresced after treatment with fluorescein alone, mast cells fluoresced only after treatment with a protein conjugate containing gamma-G globulin. The phenomenon was observed in cells from different organs and the fluorescence was seen to be associated with the cytoplasmic granules normally present in these cells.

E. LABELING COMPOUNDS

7588

Grossi, C.E.; Mayersback, H. von. 1964. Technical factors affecting immunohistological methods: I. The influence of different pH and buffer salts for unspecific reactions. *Acta Histochem.* 19:382-398.

For unspecific reactions the type of buffer in labeled antibody solution plays an important role. Two reasons are electrical charge of tissue proteins, and extraction of cell components. In immunohistology the generally used phosphate buffer is not very suitable and causes quite a loss of cell components. Cells can only be colored with difficulty after incubation with buffered sera.

7589

Hsu, K.C.; Rifkind, R.A.; Zabriskie, J.B. 1964. Immunochemical studies with fluorescein and ferritin doubly labeled antibodies. *J. Histochem. Cytochem.* 12:7.

A technique is described for the double labeling of antibody with fluorescein and ferritin that makes it possible to employ the same conjugate for both fluorescence and electron microscopy. Studies with microorganisms and tissues demonstrate that the specificity of the antibody is not affected by the double labeling. Pneumococci, Types 2 and 18, were treated with homologous ferritin-conjugated antibody or fluorescein and ferritin-conjugated antibody and examined under the electron microscope; capsular swelling with penetration of ferritin granules through the capsular material up to the cell wall was seen. The same doubly labeled antisera used with fluorescence microscopy demonstrated specific 'staining' of the same organisms. Control experiments included blocking of the reaction with unlabeled antibody. Both the ferritin conjugates and the doubly labeled conjugates presented the same immunologic pattern of activities by Ouchterlony agar-gel diffusion and immunoelectrophoretic analysis. Complete article.

7590

Klugerman, M.R. 1965. Chemical and physical variables affecting the properties of fluorescein isothiocyanate and its protein conjugates. *J. Immunol.* 95:1165-1173.

The effect of pH, time, temperature, and buffer systems on solutions of fluorescein isothiocyanate and its protein conjugates was examined fluorometrically. The fluorescence of the isothiocyanate and of the protein conjugate was maximal at about pH 8.7 and pH 10.7, respectively.

The stability of their fluorescence above pH 7 was affected adversely by increases in pH or temperature. The protein conjugate, however, showed maximum stability at pH 10.5 and above. The type of buffer - carbonate, phosphate, borate, Tris or barbituate - did not affect the fluorescence of the free dye significantly. On the other hand, increasing the molarity of the buffer caused a decrease in stability of fluorescence of the free dye but did not seriously affect the fluorescence of the conjugate. The absorbance of fluorescein isothiocyanate and its conjugates increased with increasing pH. With increased pH or temperature of the reaction mixture during conjugation, fluorescein isothiocyanate reacted more readily with the protein. Conditions may be selected to obtain the desired degree of label with short conjugation periods. Conjugation of a bovine anti-Brucella abortus globulin samp. for 30 minutes at pH 9.45 and room temperature was as effective as conjugation at pH 8.75 for 18 hours at 5 C. No apparent loss of biologic activity was observed as the result of conjugation.

7591

Lewis, V.J.; Brooks, J.B. 1964. Comparison of fluorochromes for the preparation of fluorescent antibody reagents. *J. Bacteriol.* 88:1520-1521.

Conjugates of anti-Corynebacterium diphtheriae and anti-Escherichia coli antisera were prepared using four dyes, FITC, RB 200, DANS, and tetramethylrhodamine isothiocyanate. When titers were compared, FITC was superior.

7592

McKinney, R.M.; Spillane, J.T.; Pearce, G.W. 1964. Factors affecting the rate of reaction of fluorescein isothiocyanate with serum proteins. *J. Immunol.* 93:232-242.

Only FITC of the highest purity should be used for spectrophotometric reference standards. The extent of reaction of FITC products with rabbit gamma globulin under standardized conditions showed very close correlation with the purity of the isothiocyanate products as determined by infrared measurements. Rabbit and bovine alpha globulins were found to react considerably more rapidly than horse alpha globulin with FITC. In all species studied, the albumin fraction reacted much more rapidly than the gamma globulin fraction. A systematic study of the effects of temperature, concentration, and pH on the rate and extent of reaction of FITC with normal rabbit gamma globulin indicated that the rate can be greatly accelerated by modifying the reaction conditions. At the reaction temperature of 25 C, pH 9.5, protein concentration 2.5 per cent, and buffer salt concentration of 0.05 M, conjugation of FITC with rabbit gamma globulin was essentially completed

in 30 minutes. With these reaction conditions, the ratio of fluorescein to protein of the conjugate was very reproducible and could be controlled by the initial amount of dye added. Conjugation of FITC with gamma globulin at 25 C, pH 9.5, yielded an excellent product.

7593

Moritz, K.U.; Ambrosius, H. 1964. Sulforhodamine B, a good labeling stain suitable for immunohistology. Acta Histochem. 19:191-194. In German.

Sulforhodamine B chloride proved to be a good staining material in immunohistology, as compared with fluorescein isothiocyanate. The advantages of sulforhodamine B are easy availability, good durability, and simple coupling.

F. STAINING PROCEDURES

7594

Alexander, W.R.M.; Potter, J.L. 1963. Rhodamine-conjugated papain as a counterstain in fluorescence microscopy. *Immunology* 6:450-452.

A new method of counterstaining unfixed tissue for fluorescence microscopy is described. The method depends upon the reaction of crude papain, labeled with a fluorescent dye, with nuclear and cytoplasmic constituents of tissue cells.

7595

Beutner, E.H.; Holborow, E.J.; Johnson, G.D. 1965. A new fluorescent antibody method: Mixed antiglobulin immunofluorescence or labelled antigen indirect immunofluorescence staining. *Nature* 208:353-355.

This method is a multiple layer technique. Fluorescently labeled antigens are bound to antigens in tissue by antibodies. The technique is described and illustrated. Controls and parameters are discussed for quantifying the method.

7596

Buffe, D.; Burtin, P.; Grabar, P. 1964. Intracellular localization of the low molecular weight chains and high molecular weight chains of gamma globulins. *Compt. Rend.* 258:4629-4631. In French.

With the aid of specific antisera labeled with FITC and sulforhodamine dyes, new evidence is shown that long- and short-chain gamma globulins are synthesized within the same cell, but in different parts.

7597

Camargo, M.E. 1965. Preparation of microscopical slides to simplify immunofluorescence serological titrations. *Rev. Inst. Med. Trop. Sao Paulo* 7:39-40.

A simple technique makes possible running as many as ten immunofluorescence reactions per microscopic slide so as to facilitate routine work.

7598

Danielsson, D. 1965. The demonstration of Neisseria gonorrhoeae with the aid of fluorescent antibodies: 5. A comparison of different techniques--absorption, one-step inhibition, and counterstaining--for elimination of cross reactions. *Acta Dermato-Venerol.* 45:61-73.

FITC conjugated rabbit antigonococcus globulins and rabbit normal globulin were tested for reactions with Neisseria strains, staphylococci, and streptococci. Strong reactions were observed between antigonococcus conjugates and meningococci. Both the antigonococcus and normal globulin conjugates gave strong reactions with some of the staphylococcus and Group G streptococcus strains. These could be eliminated by absorbing with strongly reacting strains of Staphylococcus aureus. At the same time FA titers of the antigonococcus conjugates with gonococci and meningococci were lowered. The staining of staphylococci was somewhat inhibited by adding human serum or normal rabbit serum to antigonococcus conjugates. Adding antistaphylococcal rabbit serum to FITC-labeled antigonococcus globulins had a more pronounced inhibiting effect. RB 200-labeled globulin of the antistaphylococcal rabbit serum had the same effect. The application of delayed FA tests on clinical specimens showed that it was necessary to take precautions against nonspecific reactions. In males, delayed FA tests and culture were of equal value for diagnosis of gonococci. In females, delayed FA tests gave a higher yield than culture.

7599

Deinhardt, F.; Dedmon, R.E. 1965. Simplified method for fluorescent antibody staining of tissue cultures. *Nature* 205:1122-1123.

A simple staining chamber and support rack for cover glasses are described.

7600

Grossi, C.E.; Mayersback, H. von. 1964. Technical factors affecting immunohistological methods: I. The influence of different pH and buffer salts for unspecific reactions. *Acta Histochem.* 19:382-398.

For unspecific reactions the type of buffer in labeled antibody solution plays an important role. Two reasons are electrical charge of tissue proteins, and extraction of cell components. In immunohistology the generally used phosphate buffer is not very suitable and causes quite a loss of cell components. Cells can only be colored with difficulty after incubation with buffered sera.

7601

Henle, G.; Henle, W. 1965. Cross-reactions among gamma globulins of various species in indirect immunofluorescence. *J. Immunol.* 95:118-124.

Several preparations of fluorescein isothiocyanate - conjugated rabbit antibodies to guinea pig or human gamma globulin fractions were found to be capable of staining the characteristic mumps virus inclusions in infected HeLa cells previously sensitized with virus-specific antibodies derived from heterologous animal species. Brilliant immunofluorescence was elicited by labeled antibodies to heterologous gamma globulins only when the antiviral sera used for the primary stage of the reaction were used undiluted, or at the most in 10-fold dilution. The rabbit anti-guinea pig gamma globulin sera from which effective fluorescein conjugates were prepared yielded from one to three lines of precipitation in Ouchterlony tests with sera from several heterologous species with the exception of rabbits, whereas those sera from which strictly species-specific conjugates were obtained produced no precipitates with heterologous sera. Cross-reacting antisera to guinea pig gamma globulin also reduced the viral neutralizing capacity of human poliomyelitis convalescent serum. No attempts were made to identify the cross-reacting serum components. Such broadly reactive anti-gamma-globulins might be useful for the indirect technique of immunofluorescence in that they overcome the usually observed species limitation of this method.

7602

Hiramoto, R.; Bernecky, J.; Jurand, J.; Hamlin, M. 1964. The use of paired fluorescent labeled antibodies. *J. Histochem. Cytochem.* 12:14-15.

Tetramethylrhodamine, lissamine rhodamine, 1-dimethylaminonaphthalene-5-sulfonyl chloride (DSC), and fluorescein were used to determine their suitability for paired labeled fluorescent antibody studies. The possibilities of quenching fluorescent labels with change of pH was also investigated. Horse anti-rabbit globulin was labeled with the various dyes and served as the reagent to detect rabbit antibodies. The fluorescent reagents contained 5.6, 7.9, and 7.0 residue per mole protein respectively. Sensitized rat kidney served as the tissue for study. This was prepared by injecting rats intravenously with rabbit anti-rat kidney globulin. Two labels used simultaneously on a single kidney section showed yellow fluorescence when both the red (rhodamine) and the green (fluorescein) reacted at the same antigenic sites. Quenching the fluorescence for fluorescein could be accomplished by dropping the pH of the medium to 3. Partial reactivation of fluorescence occurred when the medium was made alkaline. Fluorescein fluoresced well from pH 11 to 7. Appreciable quenching occurred at pH 5 and 4. From pH 4 complete reactivation occurred when the medium was

made alkaline. Tetramethylrhodamine fluoresced well from pH 11 to 4; some loss of fluorescence was seen at pH 3. Lissamine rhodamine fluoresced best at pH 7 and 6. DSC was a poor label. At pH 2 all showed irreversible quenching. Complete article.

7603

Hiramoto, R.N.; Hamlin, M. 1964. Detection of two antibodies in single plasma cells by the paired fluorescence techniques. *Federation Proc.* 23:1456:343.

Cohn Fraction II human gamma globulin was column-purified and employed to immunize guinea pigs. The globulin fraction as well as papain digests were assayed against individual guinea pig hyperimmune serum by immunoelectrophoresis and Ouchterlony plates. Animals that showed one precipitin band against the whole gamma globulin fraction but two antigenically distinct bands against the digests were selected for study to determine whether these two distinct antibodies to the gamma globulin Fragments, I and II, were being synthesized within single cells. Rabbit antihuman gamma globulin sera were labeled with either aminotetramethylrhodamine or fluorescein isothiocyanate. Plasma cells containing two antibodies were detected by the indirect staining method. Frozen sections of spleens were treated with isolated Fragment I, then stained with aminotetramethylrhodamine conjugate capable of reacting with this fragment. The same section was treated with Fragment II and stained with fluorescein conjugate specific for this fragment. Observations showed orange, green, and yellow fluorescence, indicating that some plasma cells can synthesize one and others may synthesize two distinct types of antibodies. Gradation of staining indicated greater content of one type of antibody over the other. Complete article

7604

Hiramoto, R.N.; Hamlin, M. 1965. Detection of two antibodies in single plasma cells by the paired fluorescence technique. *J. Immunol.* 95:214-224.

The concept that a single antibody-forming cell may have the capacity to synthesize more than one antibody simultaneously was tested. This problem was approached by using a single antigen on which two determinant groups could be detected. Guinea pigs were hyperimmunized with a column-purified human gamma globulin preparation. Animals showing antibodies that reacted with a single band to the whole antigen but with two bands to the papain-digested antigen by immunoelectrophoresis were sacrificed and the spleen sections studied to see if any single cell was responding with two antibodies to both fragments of the whole antigen. A multiple antibody response in individual cells was detected by the sensitive indirect paired fluorescence technique. From a total of 482 antibody-containing

cells counted, 30 per cent had antibodies directed toward the CP-I fragment and 24 per cent to the CP-II fragment. It appeared that about 45 per cent of the cells contained antibodies directed to both the CP-I and CP-II fragments. The gradation of colors caused by the combination of tetramethylrhodamine and fluorescein conjugates in the multiple antibody-producing cells and the selective quenching studies of fluorescence at pH 4.0 indicate that different cells contained different amounts of one type of antibody over the other.

7605

Hiramoto, R.; Hamlin, M. 1965. Multiple antibodies in single cells. *Federation Proc.* 24:1399:381.

The capacity of a single immunocyte to synthesize more than one antibody simultaneously has been a subject of prime interest. An approach to this problem was made by using a single antigen, DEAE-purified human gamma globulin. Sera from guinea pigs made hyperimmune with this antigen were assayed immunoelectrophoretically. Animals showing antibodies that react with production of a single band to the whole antigen but with two bands to the digested antigen were tested at the cellular level by the indirect paired fluorescence method to see if any single cell is responding with double antibodies to both fragments of the globulin antigen. Indeed, a large proportion of cells in response to the stimulus of a single antigen could produce two types of antibodies. From a total of 482 antibody-containing cells counted, 30 per cent had antibodies directed toward the F fragment and 24 per cent to the S fragment. It appeared that about 45 per cent of the cells contained antibodies directed to both the F and S fragments. The gradation of colors caused by the combination of tetramethylrhodamine and fluorescein conjugates in the multiple-antibody-producing cells and the selective quenching studies of fluorescence at pH 4.0 indicate that different cells contained different amounts of one type of antibody over the other. Complete article.

7606

Hsu, K.C.; Rifkind, R.A.; Zabriskie, J.B. 1964. Immunochemical studies with fluorescein and ferritin doubly labeled antibodies. *J. Histochem. Cytochem.* 12:7.

A technique is described for the double labeling of antibody with fluorescein and ferritin that makes it possible to employ the same conjugate for both fluorescence and electron microscopy. Studies with microorganisms and tissues demonstrate that the specificity of the antibody is not affected by the double labeling. Pneumococci, Types 2 and 18, were treated with homologous ferritin-conjugated antibody or fluorescein and ferritin-conjugated antibody and examined under the electron microscope; capsular swelling with penetration of ferritin granules through the capsular

material up to the cell wall was seen. The same doubly labeled antisera used with fluorescence microscopy demonstrated specific 'staining' of the same organisms. Control experiments included blocking of the reaction with unlabeled antibody. Both the ferritin conjugates and the doubly labeled conjugates presented the same immunologic pattern of activities by Ouchterlony agar-gel diffusion and immunoelectrophoretic analysis. Complete article.

7607

Lind, K. 1965. Role of fresh human or guinea pig serum in the indirect fluorescent antibody test for Mycoplasma pneumoniae antibodies in human sera. Acta Pathol. Microbiol. Scand. 63:639-640.

Evidence is provided suggesting that the observed inhibition by fresh guinea pig serum as a diluent for human sera tested with conjugated horse antihuman globulin is due to a partial blocking of the M. pneumoniae antibodies by guinea pig complement. The inhibition by fresh guinea pig serum was a constant observation, although varying in degree with the different conjugates employed. Fresh human serum, however, either enhanced or inhibited the reaction, depending on the conjugate used. These results call for more comprehensive and detailed studies concerning the role of the conjugate in the indirect fluorescent antibody test, especially with respect to the interaction of complement.

7608

Llanes-Rodas, R.; Liu, C. 1965. A study of measles virus infection in tissue culture cells with particular reference to the development of intranuclear inclusion bodies. J. Immunol. 95:840-845.

The sequential morphologic changes of tissue culture cells infected with Edmonston measles were followed by combining infectivity titration, direct immunofluorescent, acridine orange and hematoxylin-eosin staining methods. The initial site of measles virus multiplication appeared to be in the perinuclear region of the cell cytoplasm. As the infection progressed, viral antigen spread into the nucleus, first as small granules, and later coalesced into large masses. During the end stage of infection, the content of measles antigen and nucleic acid decreased markedly, leaving a morphologic residue represented by intranuclear eosinophilic inclusion bodies. In this study the direct immunofluorescent staining method is found to be superior to the indirect technique in demonstrating the antigenic development within the measles-infected cells.

7609

Mikhailov, I.F. 1963. The first phase of serological reactions studied by the method of fluorescent antibodies. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:3:14-17. In Russian.

Fluorescent antibodies were used in the study of the first phase of serological reactions. The existing views on the effect of various conditions on the latter were clarified. A combination of fluorescent antibodies with antigens and the formation of stable specific complexes occurred at wide temperature and pH ranges (4.0 to 10.0). The specific complex dissociated at pH 3.5 to 3.4. Electrolyte concentration had no effect on the process of antibody-antigen combination.

7610

Mohanty, S.B.; DeVolt, H.M.; Faber, J.E. 1964. A fluorescent antibody study of infectious bronchitis virus. Poultry Sci. 43:179-182.

Growth of IBV in chicken embryo kidney cell cultures was studied by FA. Viral antigen appeared first in the nucleus at 7 hours and later in the cytoplasm. The entire nucleus contained a granular fluorescence at 24 hours. Some antigen was released at that time. By 36 hours antigen was detected in the cytoplasm. A second virus multiplication began at that time. Fiazoo orange was a successful counterstain.

7611

Myers, J.; Sargent, A.U.; Cohen, J.J. 1965. Whole antiserum versus the gamma globulin fraction of antiserum in the indirect fluorescent antibody technique. Immunology 9:101-105.

Two antisera were compared with their gamma globulin fractions for effectiveness as the middle or unlabeled layer in the indirect fluorescent antibody technique. The gamma globulin fraction did not produce better results in either case than the equivalent concentration of whole serum. Control sections were prepared with normal rabbit serum substituted for the whole antiserum and with normal rabbit gamma globulin substituted for the gamma globulin fraction of the antiserum. The control sections with gamma globulin showed considerably more nonspecific fluorescence than those with whole serum. In one case the nonspecific fluorescence in the gamma globulin control interfered with interpretation of the experimental section.

7612

Paronetto, F. 1963. The fluorescent antibody technique applied to titration and identification of antigens in solutions or antisera. Proc. Soc. Exp. Biol. Med. 113:394-397.

Cellulose acetate discs or agar are useful supporting media for titration or identification of antigen or antibody. The cellulose acetate fluorescent spot method is simple to perform and read and requires only a few lambda of antiserum or antigen; the fluorescein-impregnated agar method is essentially the same as the fluorescent antibody technique applied to tissue. In the titration of antiovine serum albumin, antihuman gamma globulin, and antihorse serum, both fluorescent methods were more sensitive than the ring precipitin test, when the indirect staining was used. In addition, the methods permitted selection of proper fixatives for a given antigen and determination of the proper concentration of labeled antisera for demonstration of tissue antigen.

7613

Peacock, W.L.; Thayer, J.D. 1964. Direct FA technique using Flazo orange counterstain in identification of Neisseria gonorrhoeae. Public Health Rep. 79:1119-1122.

Laboratory diagnosis of gram-stained cervical smears from women suspected of having gonorrhea was so unproductive in the past that when the culture method was used the stained smear procedure was not. Even after immunofluorescent methods had greatly improved diagnosis of direct smears, results were still about 50 per cent inferior to those obtained with the delayed fluorescent antibody procedure or with the culture method. By the use of Flazo orange as a counterstain, nonspecific background fluorescence was quenched without obscuring points of reference, such as pus cells. Eye strain was notably reduced. One hundred fifty-six female contacts of male gonorrheal patients were examined. In the more rapid FA procedure the method of smear preparation and counterstain resulted in specific diagnoses of gonorrhea to within 2 per cent of results by the culture method.

7614

Pital, A.; Janowitz, S.L. 1963. Enhancement of staining intensity in the fluorescent antibody reaction. J. Bacteriol. 86:888-889.

Carbonate buffer, pH 9.0, produced superior FA staining results when used for washes and to buffer glycerol in place of PBS, pH 7.2. Conjugates used were those against Bacillus anthracis, Brucella abortus, and Serratia marcescens.

7615

Sordat, B. 1965. Use of nuclear fluorochromes in the determination of cells labeled with fluorescent antibodies. *Exp. Cell Res.* 40:193-195. In French.

The procedure for nuclear staining with acridine orange or berberine sulfate, contrasting with cytoplasmic labeling produced by FITC or RB 200 conjugates, is described.

7616

Stout, G.W.; Falcone, V.H.; Moore, M.B., Jr. 1964. Protocols for testing reagents employed in serologic tests for syphilis. *Health Lab. Sci.* 1:119-128.

As a portion of this, procedures for FA reagents are given.

7617

Streiff, F.; Peters, A.; Genetet, B.; Vincent, D. 1964. Technique of marking red blood corpuscles by fluorescent antibody: Application to fetal-maternal transfusions. *Transfusion* 7:383-387. In French.

Anti-A, anti-B, and anti-D sera were conjugated with FITC. Excess color was removed by passage through Sephadex and amberlite columns. A 2 per cent suspension of red blood cells 'rbc' of a Type O mother with an A or B child was incubated with an equal volume of labeled A, B, or D antisera. A highly fluorescent aggregate of labeled rbc occurred. Fluorescence did not occur when the corresponding antigen was absent. Fluorescent cells were visible in experimental mixtures of 1/1000 of O, D, AB, and D that correspond to the transplacental passage of 0.5 ml of fetal blood. Eighteen cases of Type O women at the time of delivery were negative by this technique, but the children were O. Maternal-fetal incompatibility occurred in ten additional cases. Of the total 28 cases, fluorescent cells occurred five times, including a Group B child. BA-46-99405.

7618

Voller, A. 1963. Immunofluorescent observations on Trypanosoma cruzi.
Trans. Roy. Soc. Trop. Med. Hyg. 57:232.

T. cruzi was FA stained. The slide antigen was acetone-fixed smears. Both blood and culture forms fluoresced especially strongly in the region of the kinetoplast and around the nucleus, the central part of which did not stain. Heterologous conjugates from antisera to plasmodia or helminths did not react with T. cruzi. When excess unlabeled serum was mixed with the homologous conjugate, the fluorescent staining of T. cruzi was abolished, owing to competition for antigen sites. When normal non-immune serum was mixed with the conjugate, the reaction was not markedly diminished. This one step inhibition procedure may possibly be of use as a screening method to detect the presence of antibodies, to T. cruzi, in human serum. Complete article.

7619

Wagner, M.; Unger, H. 1963. Staining of bacterial colonies with fluorescent antibodies: A method for macroscopic observation and quantitative evaluation of immunofluorescence. Zentralbl. Bakteriol. 189:482-487. In German.

A method of staining bacterial colonies with fluorescent antibodies is described that allows their macroscopic and quantitative evaluation. The colonies grown on agar plates are stamped on a glass slide, fixed, and incubated with fluorescein-marked sera. Under oblique UV light the colonies that react with the antiserum appear green and can be counted. In this way the quantitative determination of separate types of microorganisms in a mixed colony is made possible.

G. TISSUE CULTURE (NOT VIRUS)

1. General Tissue Culture Studies

a. Antibody Production in vitro

7620

Bach, F.; Hirschhorn, K. 1963. Gamma globulin production by human lymphocytes in vitro. *Exp. Cell Res.* 32:592-595.

Studies of cultured lymphocytes demonstrated that virtually all the cells would fluoresce following direct FA staining with anti-human 7S gamma globulin. Fluorescence was mainly ring-like, but some cells showed cytoplasmic or nuclear fluorescence. Various control combinations yielded negative stain results. Five to 35 per cent of cells from a tuberculin positive patient fluoresced under the above conditions. Cells from a tuberculin negative patient did not fluoresce. Mixtures of cells from various disease conditions could be distinguished by FA. Cells that fluoresced were generally those that became enlarged under the experimental conditions. Results indicated that the small lymphocyte is an immunologically competent cell.

7621

Cremer, N.E. 1963. Competition of soluble antigens by tissue culture assay. *J. Immunol.* 90:685-696.

Antibody formation by spleen cells in vitro was studied. Hemocyanin and bovine serum albumin were the antigens employed. The time sequence of challenges and resultant antibody formation were the principal points studied. As a portion of this work, antibody in cells was detected by an FA layering procedure. Radioactively labeled bovine serum albumin was also employed.

7622

Elves, M.W.; Roath, S.; Taylor, G.; Israels, M.C.G. 1963. The in vitro production of antibody lymphocytes. *Lancet* 1:1292-1293.

Lymphocytes from a previously immunized donor have been shown to transform and simultaneously produce antibody in vitro under antigenic stimulus. These processes are believed to be related. It is suggested that the small lymphocyte is a potential producer of antibody under appropriate conditions, which shows itself in vivo in the form of the secondary response.

7623

O'Brien, T.F.; Michaelides, M.C.; Coons, A.H. 1963. Studies on antibody production: VI. The course, sensitivity, and histology of the secondary response in vitro. *J. Exp. Med.* 117:1053-1062.

The in vitro anamnestic antibody response of popliteal lymph node fragments to additions of antigen closely resembles the in vivo anamnestic antibody response in its sensitivity to antigen, in the time course of antibody production, and in the sequence of appearance and the morphology of the antibody-containing cells. Most of the cells responsible for antibody synthesis remain in the explant and do not migrate, although a few can be found in the outgrowing sheet of cells. The smallest concentration of bovine serum albumin that stimulates an anamnestic response in vitro is about 1 billionth gram per milliliter.

7624

Patterson, R.; Suszko, I.M. 1963. In vitro production of antibody by chicken spleen cells: II. Immunofluorescent studies of tissue culture preparations. *J. Immunol.* 90:836-842.

Immunofluorescent studies of chicken cells producing antibody provides a useful system for microscopic observation of the in vitro production of antibody, particularly when carried out with determinations of antibody in tissue culture media.

7625

Patterson, R.; Suszko, I.; Pruzansky, J.J. 1964. Immunofluorescent studies of the in vitro production of antibody by chicken spleen cells. *J. Histochem. Cytochem.* 12:18.

Chickens were immunized with bovine serum albumin (BSA). The spleens were removed and spleen cells grown in tissue culture. Cells were removed serially and stained for the presence of chicken antibody against BSA using rabbit anti-BSA labeled with FIC. Tissue culture media from the same preparations were studied for the presence of chicken anti-BSA using a sensitive radioisotope technique. The chicken antibody produced in vitro was detected and measured by the coprecipitation by 50 per cent saturated ammonium sulfate of BSA trace labeled with I-131. Antibody production in vitro by chicken spleen cells was followed by these parallel FA and immunoisotopic techniques. Correlations between the staining of cells for antibody in tissue culture preparations and antibody content of tissue culture medium could be made. Electrophoretic patterns indicating increased gamma globulins in tissue culture medium could also be correlated

with the demonstration of antibody in cells and antibody in tissue culture medium. Sufficient precipitating antibody was produced in some culture preparations to be demonstrable by direct precipitin techniques. Complete article.

7626

Patterson, R.; Suszko, I.M.; Pruzansky, J.J. 1964. In vitro production of chicken globulins and precipitating antibody. *Immunology* 7:440-448.

Immunofluorescent and isotope coprecipitation techniques provided methods of studying in vitro production of antibody by chicken spleen cells. In some culture preparations, precipitating antibody was produced in sufficient quantity to be measured by quantitative techniques. The in vitro production of globulins by chicken spleen cells has been demonstrated by paper and immunoelectrophoresis of tissue culture medium. The antibody in the globulin produced in the in vitro tissue culture preparations may be shown by an autoradiograph of the immunoelectrophoresis after the addition of antigen trace-labeled with radioactive iodine.

b. Identification of Cell Source

7627

Larkin, E.P. 1965. A simple method for species differentiation of cell cultures by immunofluorescence. *Growth* 29:201-206.

FA has become an important method for the determination of species of origin of cell cultures. A simple, rapid technique is reported where cells are grown on cover glasses. Live cells were examined, and only surface fluorescence was used to determine species specificity. Fifteen cell lines were studied using ten FITC conjugated globulins. Twelve of the cell lines were confirmed as to species of origin as indicated by the original investigator. Three were shown to be of human origin rather than the species indicated. This method requires a minimum of cells, eliminates trypsinization and multiple centrifugations, and maintains the viability and integrity of the test cells. No difference in specificity was noted when compared with the suspended cell method.

7628

Okada, T.S. 1965. Immunohistological studies on the reconstitution of nephric tubules from dissociated cells. *J. Embryol. Exp. Morphol.* 13:289-307.

Metanephri of 16-day chick embryos were dissociated and the process of reconstitution of the tubular structures was studied by means

of FA. Antiserum was prepared against a particular fraction isolated from microsomes of the adult kidneys and was conjugated with FITC. The labeled antibody stained only the cells derived from the secretory tubules. Sorting out of the fluorescein positive and negative cells began after one day in organ culture of the reaggregates, and selective localization was completed after 4 days. In the reaggregates of 30-hour monolayer culture, sorting out of the positive and negative cells was somewhat incomplete. In the reaggregates after 96 hours in monolayer, tubular structures were hardly reconstituted and almost all of the cells were negative.

7629

Simpson, W.F.; Stulberg, C.S. 1963. Species identification of animal strains by immunofluorescence. *Nature* 199:616-617.

The fluorescent antibody test has been used to identify a variety of established cell lines reported in the literature and used in many laboratories for experimental purposes. Certain of these lines have been selected by the Cell Culture Collection Committee as prototypes for characterization, preservation, and deposition in a national repository. Of the 46 lines, sub-lines, and variants listed, two cultures were found to contain cells of species other than originally designated. The finding that the MS line, originally alleged to be of monkey origin but that reacted only with labeled antibody specific for human cells, is in agreement with results obtained by others using different procedures. A number of unpublished cell lines of other laboratories were sent to us. Of these, five lines derived from human tissues were found to be mouse cells or mixtures of mouse and human cells. Of three designated as monkey, two were human, and one was a mixture of mouse and human. One designated as dog was found to be human.

7630

Southam, C.M.; Babcock, V.I.; De Masi, M. 1964. Growth of several human cell lines in newborn rats. *Cancer Res.* 24:345-355.

When injected intravenously into newborn rats, eight human tissue-cultured cancer cell lines--Hep-2, Detroit 6, J-111, RP 41, RP 212, Adeno Cx 1, Ovary 2, and MAC 21--grew progressively in lung and other organs of more than 50 per cent of the animals and commonly caused death after 5 to 8 weeks. Two other human cell lines, the carcinoma Hep-1 and the presumably normal Amnion B, grew less frequently and rarely caused death or illness. FA was employed to speciate cells.

7631

Stadtsbaeder, S.; Trcuat, A. 1964. Cellular and species specificity of HeLa antigens. Rev. Belge Pathol. Med. Exp. 30:236-248. In French

Fixation of anti-cellular antibodies on the walls of HeLa cells was demonstrated by FA in tissue cultures, cell suspension, and air-fixed cell preparations. Results were identical by the three methods. The possibility of intracellular penetration of cytotoxic antibodies is discussed. Among the cellular antigens demonstrated, specific antigen, more directly linked to cell wall antigens and to reactions of immunologic lysis, can be distinguished from crossing antigens, common to HeLa cell, red cells, and human serum. Among the crossing antigens, passive hemagglutination and immunodiffusion tests established an identity between a soluble antigen of HeLa cells and a fraction of human serum

c. Tissue Culture Studies Related to Autoimmune Factors

7632

Appel, S.H.; Bornstein, M.B. 1964. The application of tissue culture to the study of experimental allergic encephalomyelitis: II. Serum factors responsible for demyelination. J. Exp. Med. 119:303-312.

In the presence of a source of complement, the gamma-2 globulin fraction of rabbit experimental allergic encephalitis (EAE) serum results in complete demyelination of myelinated cultures of rat cerebellum. Exposure of the serum to homologous or heterologous brain specifically removes the myelinotoxic activity, but exposure to non-nerve tissue does not. Polylysine has no effect upon the cultures or upon the demyelination potency of EAE; heparin inhibits activity, presumably through an effect on complement. By FA the EAE globulins are specifically localized to the myelin sheaths and glial cell membranes during demyelination. As demyelination proceeds, the globulins become localized within the neuroglia in a homogeneous manner that contrasts sharply with the punctate pattern observed in control experiments. Several factors may be responsible for the pathogenesis of EAE, one of which may well be a myelinotoxic antibody.

7633

Bartfeld, H. 1965. Rheumatoid arthritic and non-rheumatoid synovia in cell culture: Morphological observations, acridine orange, and fluorescent Fraction II studies. *Ann. Rheum. Dis.* 24:31-39.

Cultures in vitro of rheumatoid synovia proliferated earlier but with few exceptions could not be subcultured as long as non-rheumatoid synovia. Multinucleated giant cells and bizarre nuclear phenomena were also more frequent in early and late rheumatoid synovium cultures. The only exceptions in the non-rheumatoid came from two patients with chronic bursitis, in which the growth pattern resembled that of rheumatoid synovia to a limited extent. Early spontaneous degeneration of the cultures that might imply that they harbored a latent virus did not occur and acridine orange preparations did not show ribonucleic acid or deoxyribonucleic acid inclusion bodies. Primary cultures of some rheumatoid synovia showed the presence of rheumatoid factor in plasma and round cells. The variations in growth pattern observed in these studies could be due to specific differences or the requirements of rheumatoid synovial cells in tissue culture.

7634

Bartfeld, H.; Juliar, J.F. 1964. 'Immunological organization' and activity of human peripheral white-blood-cell cultures. *Lancet* 2:767-769.

The small lymphocytes of human peripheral blood in cultures become transitional and lymphoplasmacytoid cells. Demonstration of rheumatoid factor in these cells by the 2nd to 4th day of culture can be interpreted as signifying that the fluorescing cells were immunologically 'committed' to produce rheumatoid factor. Conditions in the culture triggered the reaction. The specific nature of the production of rheumatoid factor was suggested by the fact that the reaction with fluorescent Fraction II was not seen in the cultures from normal controls. Some cultures, which had originally consisted of randomly dispersed peripheral white blood cells, after 2 to 4 days exhibited structural organization into lymphoplasmacytoid islets.

7635

Bornstein, M.S. 1963. A tissue-culture approach to demyelinating disorders, p. 197-211. In *Symposium on Organ Culture: Studies of Development, Function, and Disease*. National Cancer Institute Monograph No. 11.

Myelinated cultures of rat and mouse cerebellum respond with a characteristic and specific demyelinating pattern when exposed to sera withdrawn from rabbits, guinea pigs, mice, and rats subjected to experimental 'allergic' encephalomyelitis (EAE). The active factor(s) in EAE serum is an antibody(s).

The reaction is also demonstrably complement-dependent. Fluorescence studies reveal EAE globulins associated with neuroglial cell membranes and cytoplasm and the myelin sheath. Identical patterns of in vitro demyelination follow the application of some sera from patients with demyelinating disease. Rarely, sera from normal or patient controls possess similar activity. The mechanism of the human factors includes a complement dependence.

7636

Halberg, P. 1965. Thyro-cytotoxic factor: Preliminary report. Acta Med. Scand. 177:127-128.

The possible autoimmune nature of Hashimoto's disease is discussed. Hashimoto serum on all cultures demonstrated FA staining of cytoplasm that faded with aging of the cells. Antigenic deletion may take place within the cells.

7637

Ward, J.R.; Cloud, R.S.; Turner, L.M., Jr. 1964. Non-cytotoxicity of nuclear antibodies from lupus erythematosus sera in tissue culture. Ann. Rheum. Dis. 23:381-388.

The localization of binding sites of antibodies in sera from SLE patients was determined by indirect immunofluorescent technique and compared with the locus of binding of specially prepared FL cell antisera on both living and dead tissue culture cells. Anti-FL cell antibodies uniformly localized peripherally, involving only the cell membrane and cytoplasm of both viable and nonviable cells. SLE sera were found to bind with the nucleus of nonviable cells only. The failure of the SLE serum to react with viable cells was further studied by morphological examination of cells stained with hematoxylin and eosin that had been previously exposed to SLE and FL cell rabbit antisera. Viable tissue culture cells exposed to SLE antibodies were identical with normal controls. SLE serum cytotoxicity was evaluated by incubating freshly trypsinized FL cells in tubes with a series of different sera, with and without complement. SLE antibodies were nontoxic. Anti-FL cell antibodies displayed marked cytotoxicity. Antinuclear factors in SLE serum do not damage human amnion cells in tissue cultures.

d. Other Tissue Culture Studies

7638

Bjorklund, B.; Paulsson, J.-E.; Noren, B. 1965. Anti-HeLa globulin reactions with human cell antigens. *Federation Proc.* 24:279:177.

A horse anti-HeLa serum was used to study the antigenicity of pooled human normal and carcinoma tissues. Immure macroglobulin and immune gamma globulin were obtained by gel filtration with Sephadex G-200. Activity was assayed by HeLa cytotoxicity, immunodiffusion in agar, hemagglutination, and quantitative precipitation of fluorescent antibody. Cytotoxicity was caused by an immune gamma globulin, 8 micrograms of nitrogen per ml, devoid of hemagglutinating activity. Another distinct immune gamma globulin agglutinated red cells labeled with an antigen that gave gel precipitates. This antigen dominated the cancer extract and was also present in normal extract in small amounts. Fluorescence technique revealed other antigens in cytosomes from a variety of normal and malignant tissues. It is concluded that human carcinoma cells contain a pattern of antigens distinct from that of normal cells. Depending upon the nature of these antigens, their reactions with antibody will lead to various results such as precipitation, agglutination, or cytotoxic events. Complete article.

7639

Cornack, D.H.; Ambrose, E.J. 1963. Cytological studies of the entry of enzymes into cells in tissue culture. *Exp. Cell Res.* 31:566-575.

The uptake of enzymes under in vitro conditions in a selection of normal and malignant cell types has been investigated using chymotrypsin, a wheat-germ lipase preparation, and cytotoxic aliesterases isolated by column chromatography from the latter preparation. Whereas chymotrypsin was not taken up by pinocytosis, uptake of the wheat-germ enzymes was very active in all the cell types studied, suggesting that pinocytosis was stimulated by a specific interaction of the wheat-germ enzymes with the cell.

7640

Emmert, E.W.; Komins, D.R.; Miquel, J. 1963. The localization and distribution of glyceraldehyde-3-phosphate dehydrogenase in myoblasts and developing muscle fibers growing in culture. *J. Histochem. Cytochem.* 11:207-217.

The localization and increase of glyceraldehyde-3-phosphate dehydrogenase (GAPD) in the mitochondria of the developing skeletal muscle cells of chick, mouse, and human embryonic myoblasts grown in culture has been demonstrated by means of fluorescent anti-GAPD globulin. The presence of myosin in granules in the myoblast and the relationship of these to the development of the myofibril has been shown by treatment of cultures of myoblasts with fluorescent antimyosin globulin. Fluorescent antimyosin serum serves as a means of distinguishing myoblasts from fibroblasts in the same culture. Various cultures of myoblasts of increasing age stained with fluorescent anti-GAPD sera as well as cultures stained with fluorescent antimyosin have shown the progressive increase in mitochondria containing GAPD that accompanies myofibrillation. Cultures of embryonic chick heart tissue stained with fluorescent anti-GAPD globulin have shown that both muscle and the accompanying fibroblasts possess GAPD. The progressive increase in GAPD during the differentiation of the skeletal muscle fiber suggests an interrelationship of growth with glycolytic activity of the muscle cell.

7641

Fogel, M. 1965. Synthesis of an antigenic material (RAM) in rat cells cultured in vitro and in rat organs in vivo. *Exp. Cell Res.* 40:365-382.

The induction of the synthesis of an antigenic material (RAM) in in vitro cultivated rat cells and the appearance of such material in intact organs in vivo has been studied by means of the fluorescent antibody technique. Cells from organized structures that did not contain detectable RAM in vivo could be induced to synthesize this antigen when cultured in vitro. The conditions and localization are described.

7642

Fogel, M.; Sachs, L. 1964. Induction and repression of antigenic material (Forsman type) in relation to cell organization. *Develop. Biol.* 10:411-432.

The induction and repression of antigenic material in relation to cell organization has been studied with hamster cells and the fluorescent antibody technique. Cells from organized structures that did not contain the F-type antigen in vivo could be induced to synthesize this antigen by dispersing the cells and plating in vitro. The antigen was induced in cells grown as monolayers and in cells cultured as aggregated by

an organ culture technique. Dissociated embryonic kidney cells, after reaggregation and growth as an organ culture, produced the antigen while randomly distributed in the aggregate. The antigen tended to disappear in places where histological structures were formed. Embryonic liver cells were induced to synthesize F-type antigen. Histological structures were formed after reaggregation of such cells and growth as an organ culture and these structures were either free of antigen or the antigen was located as thin strips in the intercellular spaces. Synthesis of F-type antigen can be induced or repressed depending on cell organization. The antigen was induced in vitro when normal cell contacts were disturbed, and repressed when these contacts were restored.

7643

Fogel, M.; Sachs, L. 1964. The induction of Forssman-antigen synthesis in hamster and mouse cells in tissue culture, as detected by the fluorescent antibody technique. *Exp. Cell Res.* 34:448-462.

The fluorescent antibody technique has been used to determine whether hamster and mouse cells that are not synthesizing Forssman (F) antigen in vivo can be induced to synthesize this antigen in vitro and whether different normal and neoplastic cell types can be induced under these conditions. In primary cultures of normal hamster embryos, after 2 days the antigen appeared as scattered points in the cytoplasm of some cells, after 3 days in nearly all cells. In secondary hamster embryo cultures, and in cultures made from F-positive tumors, the antigen was detectable at 1 day. F-antigen synthesis after plating primary cultures in vitro was due to antigen induction in cells with originally no detectable antigen, and not due to the defective growth of F-positive cells. Induction of F antigen was also observed both in the epithelial- and fibroblast-like cells of hamster kidneys and in mouse embryo cells. A proportion of F-negative cells were found in the mouse cultures even after 12 days in vitro. In the tumor cells, the F antigen was present in seven hamster embryo clones transformed by polyoma virus (PV) and grown for 3 to 31 weeks in vitro, in a hamster embryo clone transformed by PV in vitro and grown as a tumor in adult hamsters for 4 months, and in a hamster tumor induced by PV inoculation in vivo and transplanted and grown in adult hamsters for about 4 years.

7644

Harris, C. 1965. Studies of the mechanism of antigen stimulation of DNA synthesis in rabbit spleen cultures. *Immunology* 9:529-541.

The uptake of tritiated thymidine by spleen cell suspensions from rabbits previously immunized to heterologous proteins is stimulated by spleen and peritoneal exudate cells containing the antigen. The stimulation is specific and dependent on cell contact mediated by surface antigen. It represents an increased rate of DNA synthesis and cell division by cells with the characteristics of undifferentiated cells. Macrophages become surrounded by non-dividing lymphocytes during the early period of culture and it is postulated that this reaction results in enhancement of DNA synthesis by responding cells, with the small lymphocytes acting as messengers between cells containing antigen and those dividing in response to its presence. FA is used to assist in localization of cells.

7645

Loewi, G. 1963. Effect of antibody on heterologous and homologous cells in tissue culture. *Immunology* 6:569-580.

In order to compare antibody against heterologous and homologous tissue in the same serum, rabbits were immunized with rat kidney and complete adjuvant. The resulting sera showed antibody against both rat and rabbit kidney. Cultures of rat kidney cells were killed by exposure to these sera. A concentration of 0.3 per cent gamma globulin was adequate to kill cultures in the absence of complement, but smaller concentrations were effective when guinea pig complement was added. The cell surfaces were shown by the fluorescent antibody technique to have taken up antibody. Cytoplasmic staining could be shown only in cells that had previously been injured by freezing and thawing or by fixation. Rabbit kidney cells in culture were unaffected when exposed to whole rabbit antirat serum, but were killed and their cell membranes stained on exposure to gamma globulin from such serum with the same complement fixation titer as the parent serum. Normal rabbit serum had a partial protective effect against this antibody. Antibody against heterologous tissue was found both in the ^{75}S gamma globulin and in macroglobulin fractions, but antibody against homologous tissue was confined to the latter. It is considered that the findings do not support a concept of an in vivo pathogenic role for circulating antibody.

7646

Okada, T.S. 1965. Changes in antigenic constitutions of embryonic chicken kidney cells during in vitro spreading culture. *Exp. Cell Res.* 39:591-603.

Changes in the antigenic constitutions of chicken embryo kidney cells during primary culture in monolayer were studied by means of the in vitro agar diffusion test, quantitative precipitin test, and FA. The antigenic components in the post-microsomal supernatant are maintained at least up to the final stage of primary culture, but those contained in the supernatant tend to decrease during this period. The changes occurring in the microsomal 'kidney-specific' antigens (K-antigens) were studied in more detail. The average content per individual 'K-antigen-containing' cell was decreased by about half between 0 and 7 days of primary culture. K-antigens that were localized only in the apical cytoplasm in the precultured tissue cells were found to be diffusely distributed throughout the cytoplasm in older cultures. Culture antigens were detectable exclusively in older cultures. Such changes occurring in primary culture are considered not to be due only to a selection of particular type of cells but mainly due to phenotypic changes of the cellular immunological characteristics.

7647

Weiss, L.; Lachmann, P.J. 1964. The origin of an antigenic zone surrounding HeLa cells cultured on glass. *Exp. Cell Res.* 36:86-91.

By means of FA it has been demonstrated that HeLa cells cultured on glass surfaces become surrounded by a zone containing discrete patches of species specific antigenic material. This material appears to arise by means of microruptures that occur within the cell periphery when cytoplasmic processes previously extended out to adhere to the glass are withdrawn from it.

2. Organisms in Tissue Culture (Not Virus)

7648

Clyde, W.A., Jr. 1963. Studies on Eaton agent in tissue culture. *Amer. Rev. Resp. Dis.* 88:Suppl. 212-217.

Experimental systems for working with Eaton agent have been expanded by the finding that the agent could be demonstrated directly in infected tissue cultures by means of Giemsa and fluorescent antibody techniques. The morphologic properties were those of the pleuropneumonia-like organisms. A method is described for quantitating the agent in tissue culture, permitting study of growth and inhibition of growth produced by antimicrobials and antisera. The agent was found to be susceptible in vitro

to tetracycline and its derivatives, but was resistant to penicillin. Antisera produced reduction of growth in correlation with fluorescent-stainable antibody titers of the sera, confirming the work of Eaton

7649

Clyde, W.A., Jr. 1963. Studies on growth of Eaton agent in tissue cultures. Proc. Soc. Exp. Biol. Med. 112:905-909.

A method for quantitative studies of Eaton agent with a tissue culture system is described. The agent grew slowly in monkey kidney cell cultures, unlike many PPLO, but resembled other members of the genus Mycoplasma in being resistant to penicillin and sensitive to the tetracyclines. Demonstration of growth-inhibiting effects of both rabbit antiserum and sera of patients convalescent from atypical pneumonia, in confirmation of the work of Eaton, provides another parameter for studying immune response and relating Eaton agent to human disease.

7650

Hahn, N.; Cooke, K.O. 1965. Fluorescent cell-counting neutralization test for psittacosis. J. Bacteriol. 89:1465-1471.

A sensitive, precise, and specific serological procedure, the fluorescent cell-counting neutralization test, was developed to detect and to measure quantitatively psittacosis serum-neutralizing antibodies within 24 hours. The test is based on the reduction of fluorescent cells in McCoy cell monolayers resulting from the neutralization of infective agent particles by specific antiserum. Small but significant rises in neutralizing titers were measured in serum specimens from monkeys previously exposed to the psittacosis agent and from humans with diagnoses of subclinical or established psittacosis infections.

7651

Hahn, N.; Nakamura, R.M. 1964. Quantitative assay of psittacosis virus by the fluorescent cell-counting technique. Virology 23:203-208.

An assay technique for psittacosis virus is described that is based on the enumeration of fluorescent cells within 24 hours after infection of McCoy cell monolayers. The distribution of infected cells is random; the relationship of cell counts to virus concentration is linear. The quantity of psittacosis virus neutralized by dilutions of antiserum is shown by this assay technique to be a linear function.

7652

Herman, R. 1965. Fluorescent antibody studies on the intracellular form of Leishmania donovani grown in cell culture. *Exp. Parasitol.* 17:218-228.

Leishman-Donovan bodies of Leishmania donovani from an infected hamster's spleen were injected intraperitoneally into the previously saline-stimulated abdominal cavities of hamsters. Macrophages containing intracellular parasites were harvested from these hamsters and maintained in vitro on cover slips in Leighton tubes in a balanced salt solution - serum medium. These parasitized cells on cover slips were used as the antigen in FA studies. The parasitized cells were used to detect and titrate the anti-leishmanial antibody from sera of infected mice by both the direct and indirect FA techniques. These infected macrophages were also used to detect and titrate the anti-leishmanial antibody in sera from proven cases of kala-azar.

7653

Herman, R. 1964. Fluorescent antibody studies with the intracellular form of Leishmania donovani in cell culture. *J. Parasitol.* 50(Suppl.):18.

In vitro immunological study of Leishmania donovani poses certain problems connected with the parasite's intracellular position in the mammalian host. As a possible solution to these problems, Leishman-Donovan bodies (LDs) were grown in hamster macrophages in cell culture. To obtain infected macrophages, saline-stimulated hamsters were reinoculated intraperitoneally with a partially purified suspension of LDs from an infected hamster's spleen. After 30 hours, infected macrophages were harvested with a Hanks BSS 60 per cent inactivated serum 40 per cent medium and explanted into Leighton tubes, each containing a cover slip. After 1 or 2 days at 37 C, the cover slips were removed from the tubes and air dried. Both fixed (cold acetone) and nonfixed cover slips from such cultures were stored at -20 C. Cover slips with infected cells were well suited as antigens for the demonstration of antibody in immune globulin by FA. Infected mice provided the immune globulin. Normal mouse globulin served for the controls. Using both direct and indirect FA, antibodies were successfully demonstrated, but not until after the 16th day. The cell culture - FA combination may help solve some diagnostic and other problems in leishmaniasis. Complete article.

7654

Krasnik, F.I. 1963. Demonstration of Rickettsia prowazekii in cell cultures by the fluorescent antibody method. Acta Virol. 7:190.

A combination of tissue culture and fluorescent antibody methods could become a valuable tool in early and rapid diagnosis of rickettsial infections.

7655

Krasnik, F.I. 1963. Use of the fluorescent antibody method for the study of Rickettsia prowazekii in tissue culture. Tr. Inst. Epidemiol. Mikrobiol. Gig. Leningrad 25:26-31. In Russian.

Application of the fluorescent antibody method to study of Rickettsia prowazekii in tissue cultures is very promising. By this method it was possible to observe the nature and intensity of multiplication of the causative agent dynamically, its localization, structure, and interrelationships with cells of various tissues. These preliminary data indicate that during the cultivation of rickettsiae in tissues the use of FA allows a more detailed study of the interrelationship between the causative agent and the cell. Along with this, further investigations in this direction may be promising for early and rapid detection of the causative agent during rickettsial infections.

7656

Malizia, W.F., Barile, M.F.; Riggs, D.B. 1961. Immunofluorescence of pleuropneumonia-like organisms isolated from tissue cell cultures. Nature 191:190-191.

Fluorescent antibody was used to detect PPL0 contamination of continuous tissue cell lines. Similar antigenicity of PPL0 strains obtained from various cell lines was demonstrated. The staining pattern morphologically resembled the intracytoplasmic granulation of contaminated tissue culture cells. Fluorescence was never observed in the nucleus.

7657

Attman, B., Hebert, G.A.; Cherry, W.B.; Taylor, G.C. 1964. Quantitative measurements of nonspecific staining by fluorescein isothiocyanate-labeled globulin employing mammalian cell cultures. Bacteriol. Proc. M110.64.

This paper describes a quantitative procedure for measuring fluorescence emission from cultured mammalian cells stained with FITC-labeled globulins. Cells in a monolayer of 60 to 120 per high dry microscopic field were

stained with high-titered rabbit antiglobulins for Escherichia coli labeled with FITC. Labeling conditions were varied to give conjugates having ratios of fluorescein to protein ranging from approximately 1 to 20. Cell preparations were stained with the conjugates; photometric measurements of fluorescence emission and cell count were made on each field. Results were expressed as photometric units per tissue cell and served as a measure of nonspecific staining. Conjugates of E. coli applied to noninfected mammalian culture cells provided a completely heterologous antigen-antibody system; any fluorescence beyond that given by the unstained cells was considered nonspecific. When the fluorescein-to-protein ratios of conjugates were plotted against photometric units per cell, a linear relationship was demonstrated. Conjugates within a certain range of ratios gave maximal specific staining titers for E. coli but nonspecific staining of intermediate values as measured on tissue cells. It is not desirable to label globulins under conditions resulting in high ratios of fluorescein to protein because nonspecific staining is increased without increase in the specific titer. Precise quantitative measurement of nonspecific staining furnishes a sound basis for the evaluation of the quality of conjugates.

7658

Richardson, M.; Holt, J.N. 1964. Multiplication of Brucella in cultured lymphoid and nonlymphoid cells. *J. Bacteriol.* 88:1163-1168.

Growth curves were established for the multiplication of Brucella abortus in cultured bovine cells. The number of viable brucellae was determined by colony count after lysis of the parasitized tissue cells. The number of brucellae dropped during the first 3 to 6 hours. This was followed by intracellular growth. Brucellae multiplied in uterine mucosal and fetal skin cells at an exponential rate with a 4-hour generation time. In contrast, only limited multiplication occurred in spleen cell cultures, usually approaching the stationary phase by 20 to 30 hours. Preliminary results indicated an average generation time of 8 hours in calf spleen cells. Differences were apparent in the ability of spleen cells from individual calves to support intracellular growth. This suggests that a relationship may exist between the establishment of intracellular pathogens in vitro and the natural resistance of the animal. By the use of fluorescein-labeled antisera, some insight was gained into the fate of brucellae in lymphoid cells. Fluorescent antisera stained intact brucellae and also revealed soluble antigen in the cytoplasm of reticular-like cells.

7659

Somov, G.P.; Shapiro, M.I.; Legkodimova, K.V. 1965. A study of the D. sibiricus reproduction in the human embryo tissue culture with the aid of the fluorescent antibody method. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:8:39-43. In Russian.

The immunofluorescent method was applied to the study of D. sibiricus rickettsia reproduction in a monolayer of trypsinized cells of human embryo kidneys. On the basis of the data obtained, combined use of this method is considered to have future prospects for investigating the interaction of rickettsiae with the cells and rapid determination of the species of rickettsiae isolated directly on the tissue culture from various objects.

7660

Terskikh, I.I.; Zairov, G.K. 1964. A comparative study of trachoma and ornithosis viruses. Vop. Virusol. 9:674-677. In Russian.

The development of some strains of ornithosis and trachoma viruses, chlamydozoa group, was studied by fluorescent microscopy in primary and continuously cultivated tissue cultures. According to histochemical and morphological findings, cytoplasmic inclusions of ornithosis and trachoma have three stages depending on the amount of RNA and DNA therein. Clear differences are observed between trachoma and ornithosis viruses in tissue culture, which can be used for comparative studies of newly isolated agents of this group and as diagnostic and laboratory tests to determine a stage of virus development in the cell.

H. CONJUGATE PURIFICATION

7661

Al-Doory, Y.; Gordon, M.A. 1963. Application of fluorescent antibody procedures to the study of pathogenic dematiaceous fungi: I. Differentiation of Cladosporium carrionii and Cladosporium bantianum. J. Bacteriol. 86:332-338.

The well-known polymorphism and confusing similarity of cultures of pathogenic dematiaceous molds and other so-called black yeasts make individual species difficult to identify and even more perplexing to classify. Serological investigations of antigen relationships have proved of value in previous attempts to resolve these taxonomic problems, but thus far no fluorescent antibody studies in this field have been reported. In the present investigations, fluorescein-labeled antisera to both Cladosporium carrionii and C. bantianum reacted with a number of dematiaceous species in addition to the homologous organisms, but selective dilution and absorption resulted in conjugates specific for each of these two species. Staining was confined to the periphery of both hyphae and spores, and age was a factor in the affinity of these structures for fluorescent antibody. There is evidence that antigen diffuses centrifugally from the hyphae during growth on agar media.

7662

Cebra, J.J.; Goldstein, G. 1965. Chromatographic purification of tetramethylrhodamine-immune globulin conjugates and their use in the cellular localization of rabbit gamma globulin polypeptide chains. J. Immunol. 95:230-245.

Rabbit spleen imprints were double-stained with pairs of fluorescein and tetramethylrhodamine conjugates derived from the following goat reagent globulins: anti-Fragment III, anti-gamma-globulin heavy chain, anti-light chain, and anti-Fragment I. Bright specific staining could be obtained with the subfractionated conjugates at concentrations as low as 32 to 43 μ g of antibody per ml of conjugate. A series of simple Kodak Wratten filters, K2, 23A, and 45A, were used to differentiate between singly and doubly stained fluorescent cells. Staining with anti-light chain and anti-III, conjugated with contrasting fluorochromes, resulted in double labeling of 63 to 78 per cent of all fluorescent cells. A distinct minority of the fluorescent cells, 21 to 34 per cent of the population, stained only with the anti-light chain reagent. A similar class of cells, comprising 14 to 26 per cent of all fluorescent cells, stained only with the anti-I reagent when it was used as a counterstain with anti-III reagent. Anti-light chain reagents detected more immunoglobulin-containing cells than did anti-I reagents. Double staining with anti-I and anti-light chain yielded 63 to 85 per cent doubly stained cells, 15 to 32 per cent of cells stained with the anti-light chain alone, and 0 to 10 per cent of cells stained with anti-I alone.

7663

Dashkevich, I.O.; Maiboroda, G.M.; Goldin, R.B. 1965. Purification of fluorescent conjugates from free fluorochrome with the aid of ionites. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:2:116-120. In Russian.

Two methods were compared for purification of fluorescent antibodies from an excess of fluorochrome: an author-developed ion exchange resin method and filtration through Sephadex. The results obtained demonstrated advantages of the first method.

7664

Dedmon, R.E.; Holmes, A.W.; Deinhardt, F. 1965. Preparation of fluorescein isothiocyanate - labeled gamma globulin by dialysis, gel filtration, and ion-exchange chromatography in combination. J. Bacteriol. 89:734-739.

Antiviral immune gamma globulin isolated from rabbit and guinea pig sera were labeled through dialysis membranes with fluorescein isothiocyanate and purified in several ways to eliminate nonspecific staining. Gel filtration of the conjugate with Sephadex G-25 coarse beads followed by column fractionation with diethylaminoethyl-Sephadex yielded consistently highly specific staining materials. Fluorescein-protein ratios varied between 1.0 and 4.0. This technique has proved to be simple and reliable, and is less time-consuming than previous techniques.

7665

Funkes, A.J.; Ly, H.; Gates, E.H.; Imagawa, D.T. 1963. Procedures to decrease nonspecific fluorescent staining of measles-infected tissue. Federation Proc. 22:217:197.

Because of problems associated with nonspecific fluorescence in immunofluorescent techniques, a study was made to determine the significance of some physical properties of media. Whole sera were labeled with fluorescein isothiocyanate, which was fractionated by low-temperature ethanol procedures. Fluorescein-labeled antibody fractionated with glycine-ethanol mixtures contained only traces of free fluorescein. These labeled antibodies are absorbed by normal tissue homogenate to reduce nonspecificity. The effect of pH, ionic strength, and dielectric constant of media during these absorptive procedures was found to be important. During the staining procedure, alteration of pH, ionic strength, and dielectric constant from the procedures used during absorption can significantly affect nonspecific staining. Nonspecific fluorescence was significantly reduced as shown by comparative photomicrography in measles-infected mouse brain smears and in measles-infected HeLa cell cultures. Complete article.

7666

Maiboroda, G.M.; Dashkevich, I.O. 1963. Purification of fluorescent conjugates from free fluorochrome with the aid of ionites: I. Purification of antimicrobial fluorescent antibodies with the aid of AB-17 anionite. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:3:55-59. In Russian.

Present methods of purifying fluorescent antibodies from excess fluorochrome do not provide complete removal of the free stain and are connected with the danger of partial denaturing of immune proteins. The authors suggest an advanced method for purification of antimicrobial fluorescent globulins with AB-17 anionite. In experiments with conjugates, labeled with 1-dimethylaminonaphthalene-5-sulfochloride or fluorescein isothiocyanate, there was complete removal of free fluorochrome without any harm to serological properties in the labeled proteins.

7667

McDevitt, H.O.; Coons, A.H. 1964. Methods for preparation of fluorescent proteins, p. 142-148. In H.N. Eisen (ed.) Methods in Medical Research, Vol. 10, Section II. Year Book Medical Publishers, Inc., Chicago, Ill.

With the exception of DEAE cellulose chromatography, the methods described here have been in use for several years, and they have been found to be effective when gauged by the ultimate but rough test of staining. However, very few quantitative data are available correlating fluorescein-to-protein ratio (or antibody titer) with staining intensity. The necessity for evaluating DEAE column fractions in comparison with tissue powder - absorbed conjugates points up this lack of precise knowledge. Fluorescent protein conjugates should be routinely evaluated after purification and removal of nonspecific staining with regard to F/P ratio, protein concentration, and antibody concentration. Accumulation of information of this kind will permit selection of labeling and purification procedures on a rational basis and will greatly increase the reliability of preparation of fluorescent proteins.

7668

McDevitt, H.O.; Peters, J.H.; Pollard, L.W.; Harter, J.G.; Coons, A.H. 1963. Purification and analysis of fluorescein-labeled antisera by column chromatography. J. Immunol. 90:634-642.

Fluorescent rabbit antiovine serum albumin antibody with bright specific and negligible nonspecific staining was prepared from the conjugated antiserum by a single elution from a diethylaminoethyl cellulose column. The yield was 40 to 60 per cent of the labeled precipitating antibody when conjugates with a molar fluorescein-to-protein ratio of 4 or less

were used. The antibody eluted under the conditions employed had a molar fluorescein-to-protein ratio of approximately 1. Absorption and emission studies of a variety of conjugates show that, after conjugation to protein, amorphous and crystalline fluorescein isothiocyanate preparations have the same emission intensity, and also that fluorescein isocyanate has approximately twice the emission intensity of the isothiocyanate.

7669

Porter, B.M.; Thomas, B.K.; Furcolow, M.L.; Varga, D.T. 1963. Comparison of two Histoplasma capsulatum fluorescent antibody conjugates based on 800 clinical specimens. *Bacteriol. Proc.* M141:90.

On the basis of 800 clinical specimens, two differently prepared conjugates were compared. In both cases, antiserum prepared against the yeast phase of Histoplasma capsulatum was tagged with fluorescein isothiocyanate. In one case, the conjugate was absorbed three times with tissue powders, once with monkey liver powder and Candida sp. yeast powder, and twice with mouse liver powder. In the second case, the conjugate was absorbed with packed, formalin-killed yeast cells of Blastomyces dermatitidis according to procedures adapted from those outlined by Kaplan and Kaufman in 1961. The conjugate absorbed with packed B. dermatitidis is more specific, requires fewer reagents, and can be diluted for use with clinical specimens.

7670

Rudolph, W. 1964. The rapid purification of fluorescence-labeled antibodies with Wofatit EZ. *Acta Histochem.* 17:186-192. In German.

The absorbent resin, Wofatit EZ, was used to successfully remove unbound dye from conjugation mixtures.

7671

Silva, M.E.; Kaplan, W. 1965. Specific fluorescein-labeled antiglobulins for the yeast form of Paracoccidioides brasiliensis. *Amer. J. Trop. Med. Hyg.* 14:290-294.

Two lots of rabbit Paracoccidioides brasiliensis antiglobulins, produced against the yeast form of this fungus, were conjugated with fluorescein isothiocyanate. Both reagents brightly stained elements of the yeast and mycelial forms of 15 isolates of P. brasiliensis. In addition, they cross-reacted with the tissue form of Histoplasma capsulatum, Blastomyces dermatitidis, Sporotrichum schenckii; once, with cells of the mycelial form of Coccidioides immitis, they rendered this conjugate specific for the yeast form of P. brasiliensis. Adsorption of the

second conjugate once with mycelial growth of C. immitis followed by a single adsorption with yeast cells of H. capsulatum and one adsorption with cells of Rhodotorula sp. rendered it specific for the yeast form of P. brasiliensis. The specific reagents stained P. brasiliensis cells in smears of clinical materials from four spontaneous human cases of South American blastomycosis. The specific reagents did not react with elements of the mycelial form of P. brasiliensis.

7672

Wood, B.T.; Thompson, S.H.; Goldstein, G. 1965. Fluorescent antibody staining: III. Preparation of fluorescein isothiocyanate - labeled antibodies. *J. Immunol.* 95:225-229.

A rapid method of preparing bright and specific staining fluorescent antibodies is described. The method is discussed, emphasizing the need for chromatographically homogeneous antibody globulin, the importance of pH in the coupling reaction, and the advantages of fractionating the fluorescent antibody reaction mixture in DEAE-cellulose.

I. HISTOLOGIC TECHNIQUES

7673

Bonomo, L.; Tursi, A.; Del Zotti, G. 1964. An embedding technique for immunofluorescent studies of minute specimens of tissue collected with a needle. Tech. Bull. Regist. Med. Technol. 34:173-174.

This paper deals with the description of an embedding technique for minute specimens of tissue collected with a needle. The method is based on the use of Sephadex-gelatin blocks.

7674

Dayan, A.D.; Morgan, H.G.; Hope-Stone, H.F.; Boucher, B.J. 1964. Disseminated herpes zoster in the reticuloses. Amer. J. Roentgenol. 92:116-123.

Four cases of generalized herpes zoster in patients suffering from a reticulosis are described. In two cases the herpes zoster was fatal and in one of them a full postmortem examination was performed. There was unusually extensive involvement of the posterior root ganglia by herpes zoster virus. The virus was identified by immunologic tests performed on tissue extracts and by a new fluorescent antibody technique applicable to tissue sections.

7675

Karasek, E. 1965. Studies on the preparation of tissue sections for immuno-histology. Arch. Exp. Veterinaermed. 19:113-121. In German.

The relative advantages of several methods of preparing tissue sections for FA demonstration of bacterial antigens were compared on organ material from experimentally infected mice. Paraffin sections were well suited for anthrax bacilli and erysipelas bacteria. Usable photographs of salmonellae could be obtained only from freeze-dried material. Fresh frozen sections were less suitable. Negative staining with rhodanil albumin reduced the nonspecific fluorescence. BA-46-108471.

7676

Post, R.S. 1963. Formation of lipid-protein complexes in renal epithelial cells in the course of protein reabsorption. J. Lab. Clin. Med. 62:1001-1002.

Serum proteins penetrate normal glomerular structures separating capillary lumen and Bowman's space at a variable rate. Protein loading in the animal and human results in an increased loss of protein from the glomerulus.

Morphologic changes occur that suggest intracellular localization of protein in a system of ducts and vacuolar structures that, as determined by electron microscopy, are membrane-lined. The author has been able to demonstrate, with FA, bovine serum albumin in discrete structures within epithelial cells of the proximal tubules and glomeruli of rats within 24 hours of injection of the protein. In addition, histochemical studies and observed variation in fluorescence intensity within the glomerular cytoplasmic structures correlate to suggest biochemical alteration of the contained protein with formation of lipid-protein complexes. With the same technique, rat globulin was demonstrated after bovine albumin loading within both types of epithelial cells in amounts greater than normal. These latter observations cast some doubt on the specificity of globulin localization in the glomerulus. A special tissue preparation technique is described.

7677

Sutherland, L.E.; Hartroft, P.M. 1963. Further studies of juxtaglomerular cells with the fluorescent antibody technique. *Federation Proc.* 22:2317:548.

Immunological studies of juxtaglomerular, JG, cells, begun by Edelman and Hartroft, were extended. A new and easier method of tissue preparation based on freeze substitution was adopted. This technique gave the advantage of thin sections of 1 micron or less, an impossibility with the cryostat, and more consistent histologic results than the freeze-dry method formerly used in this work. High-titered anti-hog renin made in dogs, conjugated with fluorescein isothiocyanate, selectively stained granules in JG cells of dogs and rabbits. In hog kidney, however, this antirenin, which had been absorbed with liver and bone marrow powders, stained tubular droplets as well. By absorbing with low-renin hog kidney powder instead, only specific staining of JG granules remained. In a search for other antigens, a substance reacting with anti-canine globulin was found in cytoplasm of canine JG cells. This staining was diffuse in contrast to discrete granule staining with antirenin. Complete article.

7678

Taffs, L.F.; Vollier, A. 1964. Fluorescent antibody staining of Ascaris suum larvae in the liver and lung of an infected guinea pig by means of the freeze-substitution technique. *Ann. Trop. Med. Parasitol.* 58:414-419.

By means of a freeze-substitution wax-embedding method, sectioned third-stage larvae of Ascaris suum, in the liver and lung of a guinea pig killed 7 days after experimental infection, were specifically stained with fluorescent antiserum by the direct and indirect fluorescent antibody techniques. Specific green fluorescence of the cuticle, digestive tract, and other internal tissues was observed. Common antigens between the third-stage larvae of both the hominid (A. lumbricoides) and the pig (A. suum) forms of Ascaris were demonstrated by the fluorescent staining of sectioned A. suum larvae after exposure to monkey anti-hominid Ascaris immune serum and to labeled goat antihuman globulin.

7679

Duplication, withdrawn.

7680

Williamson, A.P.; Blattner, R.J. 1965. Immunofluorescence of Newcastle disease virus in paraffin-embedded tissues of early chick embryos. Proc. Soc. Exp. Biol. Med. 118:576-580.

Paraffin-embedded tissues have been found to be suitable for immunofluorescent studies of Newcastle disease virus - induced defects in chick embryos inoculated at early stages of incubation. In embryos inoculated at 48 hours of incubation and harvested 16 to 24 hours later, viral antigen was observed in many tissues of the embryo proper and in cells of the extraembryonic membranes. The viral antigen was most concentrated in the organs subject to the virus-induced developmental defects, i.e., in tissues differentiating from the body ectoderm, including lens primordia, auditory vesicles, visceral arch ectoderm, and olfactory epithelium. It was also found in the visceral arch mesoderm and in the epimyocardium of the heart. However, virus was not restricted to these tissues, but was also observed in some cells of the general body ectoderm and was widely distributed in cells of the chorion and amnion and in the yolk sac endoderm. In late infections the specific viral fluorescence was also seen in mesodermal tissues directly underlying the affected ectoderm. Thus viral antigen was distributed in cells derived from all three of the primary germ layers, ectoderm, endoderm, and mesoderm.

J. QUANTIFICATION

7681

Allen, J.C. 1963. Immunofluorescence applied to protein solutions. J. Lab. Clin. Med. 62:517-524.

Protein antigen in aqueous solution may be gelled in agar, frozen, sectioned, and stained with fluorescein-labeled antibody. By this means identification and quantitation of protein antigen present in solution may be accomplished, and the applicability of immunofluorescence may be extended from its classical limits of identification of antigen held to or within tissues or microorganisms.

7682

Caron, G.; Martineau, B.; deRepentigny, J.; Sonea, S. 1964. Quantitative differentiation using fluorescent antibody against Staphylococcus isolates from serious clinical cases. Rev. Can. Biol. 23:455-459.

Pathogenic and non-pathogenic S. aureus strains recently isolated from patients could be differentiated with a quantitative FA method. There is a correlation between the severity of clinical staphylococcal infections and the intensity of immunofluorescence on infective strains with conjugated Staphylococcus antitoxin or normal human gamma globulin. The difference between the individual strains as shown by FA intensities was more pronounced than those shown by immunodiffusion, but, as a whole, the results obtained with both methods agree. Fluorescent human 'normal' gamma globulin gives sometimes brighter fluorescence intensities than fluorescent Staphylococcus antitoxin.

7683

Danielsson, D. 1965. A membrane filter method for the demonstration of bacteria by the fluorescent antibody technique: I. A methodological study. Acta Pathol. Microbiol. Scand. 63:597-603.

Bacteria suspended in tap water or cultured in broth, and then trapped on non-fluorescent membrane filters, could be identified within one hour by means of the fluorescent antibody method. For this purpose the fluorescence microscope was equipped for incident illumination. The technique described allowed a quantitative determination of the bacteria identified serologically.

7684

Danielsson, D.; Laurell, G. 1963. Rapid detection of small numbers of bacteria in water by means of fluorescent antibodies. *Acta Pathol. Microbiol. Scand.* 58:159.

FA technique combined with membrane filter technique has been used to study bacterial contamination of water. Known concentrations of the test bacteria, enteropathogenic E. coli, were added to fixed volumes of water. As a rule 1 liter was filtered through a membrane filter (Millipore HAWG, 47 mu). At bacterial densities of 500 to 1,000 bacteria per liter identification was accomplished in 2 hours. At lower concentrations the membrane filters were incubated for various periods in broth and subsequently centrifuged to concentrate the bacteria. A concentration of 50 bacteria per liter could be detected in 5 to 6 hours, 15 to 20 in 8 to 10 hours, and 2 to 5 in 12 to 16 hours, respectively. The technique was also quantitative at different ratios (0.2 to 100) between concentration of contaminating bacteria and test bacteria. By conventional methods, diagnosis could be made after 48 hours at the earliest. At a high ratio of contaminating bacteria to test bacteria, the latter could often not be isolated. Promising attempts have been made to detect bacteria directly on nonfluorescent membrane filters (Millipore HABG). Samples of the water of a river in central Sweden were examined for enteropathogenic E. coli. By the use of FA ten different strains of these bacteria were detected. Six of these were also isolated by conventional tests. Complete article.

7685

deRepentigny, J.; Sonea, S.; Frappier, A. 1963. Comparison of quantitative immunofluorescence and immunodiffusion for the evaluation of antigenic materials from Staphylococcus aureus. *J. Bacteriol.* 86:1348-1349.

Microphotometric measurements of FA-stained bacterial smears were made and compared with unstained smears. These results were compared with precipitin line results. Pathogenic and nonpathogenic S. aureus strains were differentiated. Normal human gamma globulin was better for immunofluorescent differentiation than was rabbit antitoxin serum. The reverse was true for immunodiffusion.

7686

Eisen, H.N. 1964. Determination of antibody affinity for haptens and antigens by means of fluorescence quenching. *Methods Med. Res.* 10:115-121.

This is a theoretical discussion of FA used in the study of hapten reactions. First additions of hapten were found to cause an excessive amount of quenching. The reasons for this are not fully understood.

7687

Hahn, N. 1965. Assay of variola virus by the fluorescent cell-counting technique. *Appl. Microbiol.* 13:865-871.

A quantitative assay for infective variola virus particles was developed that is based on the enumeration of cells containing fluorescent viral antigen after infection of McCoy cell monolayers. Direct FA was employed to stain cells. The efficiency of virus adsorption was markedly enhanced by centrifugation of virus inoculum onto McCoy cell monolayers at 500 x g for 15 minutes. By this procedure, a proportionality was obtained between the number of fluorescent cells and volume of inoculum. Observations on the sequential development of viral antigen within cells and counts of fluorescent cells showed that the optimal time for enumerating fluorescent cells was after an incubation period of 16 to 20 hours. A linear function existed between virus concentration and cell-infecting units. Fluorescent cells were distributed randomly in infected cover slip cell monolayers. The assay was demonstrated to be highly sensitive, precise, and reproducible.

7688

Hendrix, C.E.; Tew, R.W. 1965. A photographic method for determination of fluorescence intensity. *Bacteriol. Proc.* M129:61.

This describes a photographic method for quantitative measurement of the brightness of preparations stained with fluorescent antibody. The theory of the procedure is based on determinations of the exposure time (minimal exposure time) necessary to reach a reference point on the Hurter-Driffield curve of the photographic emulsion. The point chosen is at the toe of the curve, which for practical purposes represents the exposure necessary to give a barely discernible image. To insure comparability of results, illumination should be measured prior to each determination of fluorescence brightness. We employed the method in a study of staining time and titer. Polaroid 3000 speed film was used for photography with UV dark-field illumination and 1,200X magnification. Results of staining Bacillus globigii spores with two dilutions of a fluorescent conjugate are given in terms of staining times in minutes, visual estimates of brightness, and minimal exposure times in seconds. The data show that visual observations correlated rather well with photographic measurements.

7689

Hinuma, Y.; Miyamoto, T.; Ohta, R.; Ishida, N. 1963. Counting of Sendai virus particles by an immunofluorescent technique. *Virology* 20:405-412.

Sendai virus from L cell harvests was adsorbed to chicken erythrocytes or L cells and fixed smears were stained by the complement immunofluorescent technique. The numbers of the fluorescent particles corresponded linearly to the virus hemagglutinin units adsorbed from suspensions. Excellent correlation was found between the numbers of fluorescent units and the counts of particles adsorbed to chicken red cells as examined by electron microscopy.

7690

Hiramoto, R.N.; Hamlin, M. 1965. Detection of two antibodies in single plasma cells by the paired fluorescence technique. *J. Immunol.* 95:214-224.

The concept that a single antibody-forming cell may have the capacity to synthesize more than one antibody simultaneously was tested. This problem was approached by using a single antigen on which two determinant groups could be detected. Guinea pigs were hyperimmunized with a column-purified human gamma globulin preparation. Animals showing antibodies that reacted with a single band to the whole antigen but with two bands to the papain-digested antigen by immunoelectrophoresis were sacrificed and the spleen sections studied to see if any single cell was responding with two antibodies to both fragments of the whole antigen. A multiple antibody response in individual cells was detected by the sensitive indirect paired fluorescence technique. From a total of 482 antibody-containing cells counted, 30 per cent had antibodies directed toward the CP-I fragment and 24 per cent to the CP-II fragment. It appeared that about 45 per cent of the cells contained antibodies directed to both the CP-I and CP-II fragments. The gradation of colors caused by the combination of tetramethylrhodamine and fluorescein conjugates in the multiple antibody-producing cells and the selective quenching studies of fluorescence at pH 4.0 indicate that different cells contained different amounts of one type of antibody over the other.

7691

Hovnanian, H.P.; Botan, E.A.; Brennan, T.A.; Graff, W.P. 1964. Quantitative rapid immunofluorescence microscopy: II. Studies on streptococci of Group A. *Health Lab. Sci.* 1:170-178.

Fine distinctions between degrees of immunofluorescence are not reliable when based upon visual impressions alone. This is an attempt to define a quantitative basis for brightness in FA preparations.

7692

Hovnanian, H.P.; Brennan, T.A.; Botan, E.A. 1964. Quantitative rapid immunofluorescence microscopy. *J. Bacteriol.* 87:473-476.

An elaborate electronic-optical instrument for detection and measurement of FA stain reactions is described. It consists of a UV source, UV monochromator, UV filter system, bright-field fluorescence microscope, secondary filter system, a UV television camera, a microspot scanner, a quantitative light-reading device, television monitor, and an oscilloscope. Satisfactory tests were made with FA systems for E. coli, S. lutea, and B. globigii.

7693

Kunz, C. 1964. The use of the immunofluorescent method and microphotometry for the differentiation of arboviruses. *Virology* 24:672-674.

The combined application of the immunofluorescent method and microphotometry will serve as a sensitive and valuable tool for the differentiation of closely related viruses. A Reichert microphotometer was used. Fluorescence in cells was measured. In most instances the brightest fluorescence was obtained in homologous systems.

7694

Kunz, C. 1965. On the usability of fluorescence serology and microphotometry for the differentiation of viruses of the tick-borne encephalitis (TBE) group. *Zentralbl. Bakteriol. Parasitenk. Infektionskrankh. Hyg. Abt. I* 196:4:416-437. In German.

An attempt was made to differentiate the following viruses of the tick-borne encephalitis (TBE) group in HeLa cells by FA: Central European encephalitis (early summer meningo-encephalitis) virus, Far Eastern Russian encephalitis virus, louping ill virus, Omsk hemorrhagic fever virus, Kyasanur Forest diseases virus, and Langat virus. The direct and the indirect FA was used. By the direct method virus-specific antigen in

the course of the infection could only be demonstrated with the homologous conjugates in the cytoplasm. Distribution of antigen and the extent of the antigen aggregates showed characteristics that were not sufficiently marked for a clear differentiation of the viruses. Heterologous conjugates gave no or weaker fluorescence than homologous conjugate. Human convalescent sera proved more specific than hyperimmune sera of mice. By means of a microphotometer it was possible to measure the intensity of the specific fluorescence and thereby obtain an objective measure for the degree of the serological cross-reaction. This permitted differentiation of the six virus types. The combined application of the direct immunofluorescent method and microphotometry represents a promising new way of differentiation of the antigenic structure of closely related viruses. Indirect FA did not prove suitable for determination of differences in antigenic structure of viruses of the TBE group.

7695

McKinney, R.M.; Spillane, J.T.; Pearce, G.W. 1964. Fluorescein diacetate as a reference color standard in fluorescent antibody studies. *Anal. Biochem.* 9:474-476.

Procedures are given for setting up a reference standard to measure the amount of fluorescein bound to protein in a conjugate. They are simple and easy to apply.

7696

Niel, G.; Fribourg-Blanc, A. 1965. Quantitative immunofluorescence and syphilis serology: A recent statistical study. *Bull. WHO* 33:89-105. In French.

This deals with studies on 5,169 sera and describes modifications in the authors' technique for the performance of the FTA test. The authors have perfected optical instrumentation and developed strictly standardized reagents that permit excellent reproducibility for quantitative testing. Stress is laid on the importance of the quantitative expression of results. For this purpose it is essential that the technique be followed to the letter in every instance. In confirmation of earlier studies, the results detailed in this paper show the FTA test to be more sensitive at all stages of syphilis than the VDRL, Kline, or Kolmer cardiolipin tests or the TPI test with lysozyme. In primary syphilis, the FTA test is usually positive earlier than the cardiolipin tests, and in long-standing treated syphilis it is exceptional for sera to be both FTA-negative and TPI-positive. Although some sera do give nonspecific FTA reactions, these are always at low titer. More than 1,080 normal sera were subjected to parallel VDRL and FTA testing, and those reacting positively were further checked with the Kolmer and TPI tests. The FTA test detected twice as many syphilitic sera (confirmed by the TPI test) as did the cardiolipin reactions. The efficiency of the FTA test as a case-finding technique has been amply demonstrated.

7697

Nuster, R.L.; Woodruff, P. 1965. Quantitative specific antigen determination using fluorescent antibody. *J. Clin. Pathol.* 18:817-820.

A technique is described for the quantitation of a specific protein in the presence of other proteins. The application of this method to the assay of bacteriophage T-2 coat protein is presented and discussed.

7698

Paronetto, F. 1963. The fluorescent antibody technique applied to titration and identification of antigens in solutions or antisera. *Proc. Soc. Exp. Biol. Med.* 113:394-397.

Cellulose acetate discs or agar are useful supporting media for titration or identification of antigen or antibody. The cellulose acetate fluorescent spot method is simple to perform and read and requires only a few lambda of antiserum or antigen; the fluorescein-impregnated agar method is essentially the same as the fluorescent antibody technique applied to tissue. In the titration of antiovine serum albumin, antihuman gamma globulin, and antihorse serum, both fluorescent methods were more sensitive than the tung precipitin test, when the indirect staining was used. In addition, the methods permitted selection of proper fixatives for a given antigen and determination of the proper concentration of labeled antisera for demonstration of tissue antigen.

7699

Salenstedt, C.R. 1963. Studies on the virus of hepatitis contagiosa canis (HCC): V. A study with the aid of fluorescent antibodies on some features of HCC virus infection in the dog and guinea pig. *Acta Vet. Scand.* 4:384-394.

A statistical analysis of the difference in the number of fluorescent cells between sections from different parts of the tonsil was made. There was no significant difference between sections from the same small piece, but when the sections originated from different parts of the tonsil significant differences were obtained in some cases. A constant ratio between 'fluorescence titer' and virus titer could not be established. The role of the tonsils and small intestinal wall as portal of entry of infection in dogs was studied. Virus multiplied in epithelial cells of these loci. Viral antigen was also present in cells of phagocytic nature, in endothelial and in lymphoid cells. HCC antigen appeared in cells on the surface of the liver and spleen as well as in the peritoneal exudate in guinea pigs. A rapid method of diagnosing HCC is described. In spontaneously or experimentally infected dogs cell material was scraped from the tonsillar surface. After staining with a fluorescent anti-HCC serum the occurrence of viral antigen could be shown in cells in the collected specimens.

7700

Schapiro, H.C.; Alonso, R.; Dandiker, W.B. 1964. Thermodynamic parameters for the antigen-antibody reaction determined by fluorescence polarization. *Federation Proc.* 23:247:142.

Fluorescence polarization data for the antigen-antibody reaction have been interpreted by an extension of the classical mass law employing the Sips Equation. The results can be expressed in terms of the molar concentration of antibody-binding sites measured by the maximum amount of antigen bound. To facilitate the handling of fluorescence polarization data, a program for the IBM 7090 computer has been used to determine the best values of the parameters as well as of accessory optical constants.

7701

Schapiro, H.C. 1962. The application of fluorescence polarization to the antigen-antibody reaction. *Diss. Abstr.* 23:1507-1508.

Polarization of fluorescence has been used as a measure of molecular interactions. This method for the detection and measurement of the antigen-antibody reaction may be of great use. The nature of fluorescence and of the polarization of fluorescence is discussed. The mathematical treatment of the data yields directly the equilibrium constant for the initial reaction and the total number of binding sites. The primary experiments were done with univalent antibody fractions. Titration of these fractions with fluorescein labeled ovalbumin, the antigen, indicates a value of about 2×10^8 for the equilibrium constant. The reaction of the univalent fraction and the hapten, fluorescein, yields a constant of the same order of magnitude. The method gives an indication of the extent of the diversity of the equilibrium constants, and the possible nature of this diversity is discussed. The general application of the technique of fluorescence polarization to the study of molecular interactions is discussed.

7702

Tengerdy, R.P. 1963. Quantitative immunofluorescein titration of human and bovine gamma globulins. *Anal. Chem.* 35:1084-1086.

The method is based on the measurement of the excess fluorescent antibody in the supernatant of the precipitin reaction mixture. The results were compared with those obtained by the classical quantitative precipitin titration, using the Folin-Ciocalteu method for protein determination. BA-45-6696.

7703

Tengerdy, R.P. 1963. Quantitative aspects of the immunofluorescence technique. *Bacteriol. Proc.* M125:88.

Quantitative behavior in systems of gamma globulin and fluorescein-labeled anti-gamma globulin was studied as a basis for a general application of the indirect or sandwich labeling technique for quantitative studies. Quantitative precipitin titration curves were obtained in human gamma globulin with fluorescent rabbit antihuman gamma globulin (FRAHGG) and bovine gamma globulin with fluorescent rabbit antibovine gamma globulin (FRABGG) systems by plotting the fluorescence of the supernatant of the precipitin reaction mixture against the antigen content. The fluorescence of the unreacted FRAHGG or FRABGG, which were purified by a gel-filtration process and did not contain unconjugated dye, was measured spectrophotometrically in the centrifuged supernatant and corrected for the fluorescence of non-antibody globulins in the reagents. A linear relationship was obtained between fluorescence and antigen content in the antibody excess zone of 30 to 80 ug of antibody per 0.2 ml reaction mixture for both systems. The ratios of antibody to antigen, calculated from fluorescence measurements, agreed well with those obtained from the classical precipitin titration, performed simultaneously with the fluorescence measurements by measuring the protein content of the centrifuged specific precipitates with the Folin Ciocalteu method.

7704

Tengerdy, R.P. 1964. Quantitative aspects of the immunofluorescence technique. *Bacteriol. Proc.* M111:64.

This report deals with a technique refinement, using the labeled antigen uptake inhibition test of Weiler et al. to measure specific antigenic components in human sera. Fluorescein-labeled human gamma globulin (FHGG) and 7S human gamma globulin (HGG-7S) were reacted with rabbit antihuman gamma globulin serum (RAHS) in the presence of normal HGG. As the amount of normal HGG increased in the reaction mixture, the fluorescence of the supernatant increased. A standard reference point was selected on the fluorescence-HGG added curve where the fluorescence was 50 per cent that of the added FHGG; when an unknown serum gave the same fluorescence in the test as the standard, the HGG concentration in the unknown was equal to that of the standard. The relative error of the test was 5.1 per cent. The results were confirmed by classical quantitative precipitin titration, using the Folin Ciocalteu test for protein determination. Similar results were obtained in FHGG-7S, HGG-7S, and RAHS systems; the HGG-7S content of normal human sera was measured with practically the same precision as in paper electrophoretic separation and analysis. The relative error was 5.5 per cent for the immunofluorescence test and 5.2 per cent for the electrophoretic test.

7705

Tengerdy, R.P. 1965. Gamma globulin determination in human sera by the inhibition of the precipitation of fluorescein-labeled gamma globulin. *J. Lab. Clin. Med.* 65:859-868.

Gamma globulin was determined in human sera by a fluorescein-labeled antigen precipitation inhibition test. Human gamma globulin II and chromatographically pure human 7S gamma globulin were labeled with fluorescein isothiocyanate and used as test antigens. The gamma globulin concentration of an unknown serum was measured by the degree of inhibition the serum gamma globulin caused in the precipitation of fluorescein-labeled human gamma globulin with antihuman gamma globulin serum. The measure of the inhibition was the fluorescence of the unreacted inhibited fluorescent antigen in the supernatant of the precipitin reaction mixture. Under the conditions of the test, 0.01 mg of gamma globulin was detectable with a relative error of 5.5 per cent. The results obtained by this technique were comparable to those of other immunochemical tests, but were lower than those obtained by cellulose acetate electrophoresis. The specificity of the test is determined by the antigen used for labeling and for standard; therefore, this technique is potentially useful for the quantitative determination of various antigenic materials that can be prepared in pure form.

7706

Toussaint, A.J.; Anderson, R.J. 1965. Soluble antigen fluorescent antibody technique. *Appl. Microbiol.* 13:552-558.

An indirect fluorescent antibody procedure employing soluble antigen fixed onto an artificial matrix was developed, and a mechanical means for reading test results was devised. The method employs two small cellulose acetate paper discs for each test. One disc contains soluble antigen diluted in 1 per cent bovine serum albumin (BSA); the other contains 1 per cent BSA only and serves as a control. After testing by the indirect FA procedure, the results of the tests are read on a fluorometer fitted with a paper chromatogram door. The instrument is set at zero with the control disc as a blank, and the specific fluorescence of the antigen disc is determined. Findings obtained with homologous and heterologous antisera indicated that the method yields excellent results. The soluble antigen fluorescent antibody technique has definite advantages over the conventional indirect FA procedures. The investigator may objectively select the antigen to be employed. It is possible to obtain objective mechanical reading of test results rather than the highly subjective readings required by conventional methods. The system compensates for any nonspecific fluorescence contributed either by the serum or by free fluorescein in the conjugated antiserum.

7707

Toussaint, A.J.; Tarrant, C.J.; Anderson, R.J. 1965. Soluble antigen fluorescent antibody (SAFA) test for the serorecognition of infection with Trypanosoma cruzi. J. Parasitol. 51(Suppl.):29.

An SAFA technique was developed and evaluated with appropriate antisera and a soluble somatic protein antigen obtained from Schistosoma mansoni cercariae. This procedure permits objective selection of antigen and allows results to be read by precise fluorometric methods. The current investigations were initiated to determine the potential of the SAFA technique in Chagas' disease and to ascertain whether antigens in addition to somatic proteins could be employed. Exoantigen (glycoprotein), carbohydrate, and protein somatic antigens from T. cruzi were studied. These antigens could be employed without modifying the SAFA technique. Only the somatic protein and exoantigens were evaluated since the carbohydrate antigen had shown the least satisfactory results in CF tests. The SAFA technique readily differentiated between individuals with Chagas' disease and uninfected persons. Occasional borderline reactions were encountered. The SAFA test is somewhat more sensitive and at least as specific as CF procedures for American trypanosomiasis. Ability to employ various antigens in the technique suggests possible application of the method for serodiagnosis of many infectious diseases. Complete article.

7708

Wagner, M.; Unger, H. 1963. Staining of bacterial colonies with fluorescent antibodies: A method for macroscopic observation and quantitative evaluation of immunofluorescence. Zentralbl. Bakteriol. 189:482-487.
In German.

A method of staining bacterial colonies with fluorescent antibodies is described that allows their macroscopic and quantitative evaluation. The colonies grown on agar plates are stamped on a glass slide, fixed, and incubated with fluorescein-marked sera. Under oblique UV light the colonies that react with the antiserum appear green and can be counted. In this way the quantitative determination of separate types of microorganisms in a mixed colony is made possible.

K. PROPERTIES: FLUOROCHROMES, CONJUGATES

7709

Hiramoto, R.; Bernecky, J.; Jurand, J.; Hamlin, M. 1964. The use of paired fluorescent labeled antibodies. *J. Histochem. Cytochem.* 12:14-15.

Tetramethylrhodamine, lissamine rhodamine, 1-dimethylaminonaphthalene-5-sulfonyl chloride (DSC), and fluorescein were used to determine their suitability for paired labeled fluorescent antibody studies. The possibilities of quenching fluorescent labels with change of pH was also investigated. Horse anti-rabbit globulin was labeled with the various dyes and served as the reagent to detect rabbit antibodies. The fluorescent reagents contained 5.6, 7.9, and 7.0 residue per mole protein respectively. Sensitized rat kidney served as the tissue for study. This was prepared by injecting rats intravenously with rabbit anti-rat kidney globulin. Two labels used simultaneously on a single kidney section showed yellow fluorescence when both the red (rhodamine) and the green (fluorescein) reacted at the same antigenic sites. Quenching the fluorescence for fluorescein could be accomplished by dropping the pH of the medium to 3. Partial reactivation of fluorescence occurred when the medium was made alkaline. Fluorescein fluoresced well from pH 11 to 7. Appreciable quenching occurred at pH 5 and 4. From pH 4 complete reactivation occurred when the medium was made alkaline. Tetramethylrhodamine fluoresced well from pH 11 to 4; some loss of fluorescence was seen at pH 3. Lissamine rhodamine fluoresced best at pH 7 and 6. DSC was a poor label. At pH 2 all showed irreversible quenching. Complete article.

7710

Klugerman, M.R. 1965. Chemical and physical variables affecting the properties of fluorescein isothiocyanate and its protein conjugates. *J. Immunol.* 95:1165-1173.

The effect of pH, time, temperature, and buffer systems on solutions of fluorescein isothiocyanate and its protein conjugates was examined fluorometrically. The fluorescence of the isothiocyanate and of the protein conjugate was maximal at about pH 8.7 and pH 10.7, respectively. The stability of their fluorescence above pH 7 was affected adversely by increases in pH or temperature. The protein conjugate, however, showed maximum stability at pH 10.5 and above. The type of buffer - carbonate, phosphate, borate, Tris or barbiturate - did not affect the fluorescence of the free dye significantly. On the other hand, increasing the molarity of the buffer caused a decrease in stability of fluorescence of the free dye but did not seriously affect the fluorescence of the conjugate. The absorbance of fluorescein isothiocyanate and its conjugates increased with increasing pH. With increased pH or temperature of

the reaction mixture during conjugation, fluorescein isothiocyanate reacted more readily with the protein. Conditions may be selected to obtain the desired degree of label with short conjugation periods. Conjugation of a bovine anti-Brucella abortus globulin sample for 30 minutes at pH 9.45 and room temperature was as effective as conjugation at pH 8.75 for 18 hours at 5 C. No apparent loss of biologic activity was observed as the result of conjugation.

7711

Lind, K. 1965. Role of fresh human or guinea pig serum in the indirect fluorescent antibody test for Mycoplasma pneumoniae antibodies in human sera. *Acta Pathol. Microbiol. Scand.* 63:639-640.

Evidence is provided suggesting that the observed inhibition by fresh guinea pig serum as a diluent for human sera tested with conjugated horse antihuman globulin is due to a partial blocking of the M. pneumoniae antibodies by guinea pig complement. The inhibition by fresh guinea pig serum was a constant observation, although varying in degree with the different conjugates employed. Fresh human serum, however, either enhanced or inhibited the reaction, depending on the conjugate used. These results call for more comprehensive and detailed studies concerning the role of the conjugate in the indirect fluorescent antibody test, especially with respect to the interaction of complement.

7712

Lunzenauer, K.; Henze, K.; Seidler, E. 1965. The problem of type specificity and duration of usability of fluorescein-labeled antibodies of Shigella flexneri Type 4A. *Deut. Gesundh.* 20:277-280. In German.

The authors carried through immunological experiments with FITC-labeled antibodies. It was proved that when examining the pre-immunizing sera of the animals an almost complete specificity of the reactions can be achieved if the serum is saturated and if only optimally coupled antibodies are applied. If carefully stored without any admixtures, the coupled antibodies do not show any significant drop in intensity of fluorescence within a period of more than 6 months.

7713

McKinney, R.M.; Spillane, J.T.; Pearce, G.W. 1964. Storage stability of fluorescein isothiocyanate. *Anal. Biochem.* 8:525-526.

No significant deterioration occurred in any samples that were stored over Drierite over a 1-year period. Of the samples that were stored in screw-cap bottles on the laboratory shelf, the loss of FITC varied between 35 and 74 per cent of the original FITC content.

7714

Moritz, K.-U.; Ambrosius, H. 1964. Immunohistological detection of thyroglobin in the thyroid gland. *Biol. Zentralbl.* 83:3:337-347. In German.

Thyroglobin behaves electrophoretically like alpha globulin. Antiswine thyroglobin serum gives a slight reaction with the antigens of other mammals. The form and size of the microscopic precipitate of localization studies differ in swine and rats. Thyroxin does not inactivate the antiserum. Cytochemically, RB 200 is as satisfactory as FITC and is superior to DANS. BA-46-71874.

7715

Porro, T.J.; Dadik, S.P.; Green, M.; Morse, H.T. 1963. Fluorescence and absorption spectra of biological dyes. *Stain Technol.* 38:37-48.

Data include charts and tables of absorbance and fluorescence of four coupling agents for immunity reactions, 34 typical fluorescent dyes and reagents, and 12 dyes or reagents having no fluorescence in solution but which may be fluorescent in the dry state. Since this report consists largely of reference data, the original must be consulted for details. Spectra are given for the common FA dyes.

7716

Schell, H.D.; Ghetie, V. 1964. Labelling of serum proteins with 1-dimethylaminonaphthalene-5-sulphonyl chloride and the purification of the fluorescent conjugates. *Rev. Roumaine Biochim.* 1:2:203-208. In German.

The labeling of serum proteins with DANS has revealed a competition between the different fractions for fluorochrome. Albumin always collects the largest quantity of fluorochrome. For labeling antibodies the use of purified gamma globulin preparations is preferable. The chromatographic technique on DEAE-cellulose is the most efficient method for removing the by-products from fluorescent conjugates.

7717

Tengerdy, R.P. 1965. Properties of fluorescein-labeled human gamma globulin used in quantitative immunofluorescence tests. *Anal. Biochem.* 11:272-280.

The relative quenching of the fluorescence and the apparent dissociation constant of fluorescein-labeled human gamma globulin (FHGG) were used as criteria for the purity and stability of the conjugate. A conjugation

process with Celite-adsorbed FITC gave FHGG conjugates that, after Sephadex G-25 gel filtration, satisfied the purity and stability requirements. The relative degree of conjugation of the FHGG conjugates obtained by this method varied between 2.8 and 3.6, and their fluorescence was quenched by 80 per cent compared with an equivalent amount of FITC. Normal rabbit serum increased the fluorescence of FHGG by a maximum of 20 per cent, but reduced the fluorescence of FITC by a maximum of 19 per cent. The fluorescence of FHGG in soluble antigen-antibody complexes was reduced by 8 per cent. FHGG was similar to HGG in its immunological behavior.

7718

Zaccheo, D.; Grossi, C.E. 1963. Analysis of the characteristics of immune sera in relation to their labeling with fluorochromes. *Boll. Soc. Ital. Biol. Sperim.* 39:1562-1584. In Italian.

The modification of physical-chemical properties of the protein molecules of a serum due to introduction of fluorescent radicals was studied during different phases of preparation of the conjugates. The alpha-precipitation test, immunodiffusion (Ouchterlony) test, and immunoelectrophoretic analysis were employed. A lowering of the isoelectric point of the protein with a resulting relative acidification of the serum takes place. The total protein content of the sera decreased, as a result of labeling, by 20 to 35 per cent of the initial value. This decrease sometimes corresponded to a loss of antibodies.

7719

Zubzhitskii, Yu.N. 1964. Origin of the 'shining halo' phenomenon when fluorescent sera are used. *Dokl. Akad. Nauk SSSR* 155:4:927-929. In Russian

To stain microbial cultures, homologous O, OB, and ON fluorescent sera were used, obtained by tagging immune gamma globulins with FITC. A drop of microbial suspension was placed on a slide and mixed with a drop of homologous conjugate. In every position of the freely moving cell (rotation around the long and short axes, turning one of the poles toward the observer) the shining zone around the cell in the form of a halo was retained. The middle part of the cell appeared darker. Only when one and the same layer of FA is observed in various positions, is alternation of dark and shining zones possible. The eye will perceive the light of about 54 molecules of protein. BA-46-39566.

L. SEROLOGICAL TECHNIQUES

7720

Bennett, J.C.; Haber, E. 1963. Studies on antigen conformation during antibody purification. *J. Biol. Chem.* 238:1362-1366.

Fluorescent tagging of antigens is a useful method for detection of trace amounts of antigen remaining in combination with supposedly purified antibodies. Use of this technique allows for quantitative determinations of the degree of dissociation of ribonuclease from its antibody as helical structure is altered over a specific temperature range. It appears that, upon full unfolding, complete separation of antigen from antibody is possible. Employment of a temperature gradient under appropriate conditions should allow fractionation of antibodies of various binding capacities. These experiments emphasize the great difficulty encountered in preparation of antigen-free antibody when dealing with protein antigens or protein carriers of haptens. The importance of testing preparative procedures with a sensitive method of antigen detection is apparent.

7721

Bombara, G.; Morabito, D. 1961. Demonstration of erythrocytes modified by lipopolysaccharides using the fluorescent antibody technique. *Boll. Soc. Ital. Biol. Sper.* 37:217-220. In Italian.

Indirect FA was used to demonstrate endotoxin adsorbed onto human and sheep erythrocytes. Receptor sites were apparently homogeneously distributed over the cell surface. Possible uses in auto-antibody disease study were suggested.

7722

Bulatova, T.I. 1964. The nonspecificity of the indirect hemagglutination reaction in detection of botulinus toxin in food. *Zh. Mikrobiol. Epidemiol. i Immunobiol.* 41:4:96-101. In Russian.

To detect botulinus toxin in foodstuffs the author used indirect hemagglutination reaction. This reaction proved to be a rather rapid and sensitive method of laboratory diagnosis. It cannot, however, replace completely the biological test. In experiments with weak toxins this method appeared much more sensitive, as compared with the biological test, but in experiments with strong toxins it was inferior to the latter. With the aid of the indirect hemagglutination reaction, it was impossible to differentiate botulinus toxins of the A and B types. Besides, the same positive response was obtained in experiments both with botulinus toxin and with toxoids of the A and B types, and with nontoxic filtrates of C. sporogenes because of the presence in them of common somatic antigens. Thus, the indirect hemagglutination reaction cannot be used for detecting botulinus toxin in foodstuffs.

7723

Cookson, L.O.C. 1964. Some modifications of the fluorescent antibody test in human bilharziasis. Bull. WHO 31:799-813.

Although the FA test for human bilharziasis has proved of great value, its use involves certain difficulties that the author has attempted to obviate. The paper describes a cheap and reproducible method for producing a cercarial antigen conjugated with rhodamine B 200 for use in the indirect FA test. The second part deals with a new modification in which the conjugated cercarial antigen is employed with a bentonite-absorbed FITC antihuman globulin serum and discusses the advantages of this test over the normal FA test. Experience has shown that the use of rhodamine-albumin-coated cercariae, conjugated cercariae, or normal fixed cercariae as antigens does not always give valid results when compared with those obtained with the FA test or the ordinary complement fixation test in bilharziasis. The author describes a modification of the complement fixation test involving the use of a bentonite-absorbed fluorescent anti-guinea-pig serum and the RB 200 - conjugated cercariae described earlier. This test has given reproducible results in known positive control human sera that have been valid when compared with the Sadun FA test, the conjugated cercarial FA test, and the bentonite fluorescent antibody test described in the second part of this paper.

7724

Longhi, A.; Caleffi, M.L.; Toniutti, M. 1964. Observations on the value and significance of the FA test in the serological study of syphilis. Arch. Ital. Dermatol. Venereal. 32:256-300. In Italian.

A serologic study was made on sera obtained from syphilitics in all stages of syphilis and also normals. The antibody response was studied by the FA test, which was compared with the classical reactions (CF and flocculation test) and the TPI test. FA is a sensitive and highly specific test. It reacts very early in infection, when all of the other reactions are negative. It behaves in a manner analogous to the TPI test in secondary, tertiary, old, latent, and congenital syphilis. In recent, cured syphilis it becomes negative more slowly than in the Nelson test during the first 3 years, and becomes completely negative during the 4th year.

7725

Pagnes, P. 1963. The influence of complement in the FTA. Minerva Dermatol. 38:337-339. In Italian.

Alexin is not involved in this reaction, and it probably does not contribute to false-positive results.

7726

Tengerdy, K.P. 1965. Gamma globulin determination in human sera by the inhibition of the precipitation of fluorescein-labeled gamma globulin. *J. Lab. Clin. Med.* 65:859-868.

Gamma globulin was determined in human sera by a fluorescein-labeled antigen precipitation inhibition test. Human gamma globulin II and chromatographically pure human 7S gamma globulin were labeled with fluorescein isothiocyanate and used as test antigens. The gamma globulin concentration of an unknown serum was measured by the degree of inhibition the serum gamma globulin caused in the precipitation of fluorescein-labeled human gamma globulin with antihuman gamma globulin serum. The measure of the inhibition was the fluorescence of the unreacted inhibited fluorescent antigen in the supernatant of the precipitation reaction mixture. Under the conditions of the test, 0.01 mg of gamma globulin was detectable with a relative error of 5.5 per cent. The results obtained by this technique were comparable to those of other immunochemical tests, but were lower than those obtained by cellulose acetate electrophoresis. The specificity of the test is determined by the antigen used for labeling and for standard; therefore, this technique is potentially useful for the quantitative determination of various antigenic materials that can be prepared in pure form.

7727

Welter, C.J. 1964. Serologic stability and specificity of agglutinating Anaplasma marginale antigen. *Amer. J. Vet. Res.* 25:1058-1061.

The antigen used in the capillar tube-agglutination (CA) test for anaplasmosis was stable after prolonged exposure to pH 4.8 to 8.0, 0.2 to 0.5 per cent formalin, 0.2 to 1.0 per cent phenol, and 16.0 per cent NaCl. Thermostability of CA antigen was demonstrated at 45 C for 3 months, 37 C for 6 months, 4 C for 20 to 25 months, and 25 C for 24 months. No cross-reactions between Anaplasma CA antigen and antibodies specific for 21 other pathogens were observed. Cross-reactions were observed between CA antigen and antibodies to Anaplasma ovis in ten experimentally infected sheep. A globulin fraction was separated from sera of cattle naturally infected with Anaplasma and conjugated to fluorescein. The Anaplasma bodies of the CA antigen became fluorescent when exposed to conjugated immune globulin. The ability of unlabeled CA-positive sera and the inability of the CA-negative serum to block the fluorescent antibody reaction confirmed specificity and accuracy of the CA test.

II. REFERENCES TO PROCEDURES WITH SPECIFIC APPLICATIONS

A. BACTERIA

1. Actinomycetales

7728

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2. Bacillaceae

7730

Hendrix, C.E.; Tew, R.W. 1965. A photographic method for determination of fluorescence intensity. See MP 20, Vol. I, No. 5031.

7731

Hovnanian, H.P.; Brennan, T.A.; Botan, E.A. 1964. Quantitative rapid immunofluorescence microscopy. See MP 20, Vol. I, No. 5033.

3. Bedsonia (Miyagawanella)

7732

Bates, H.A.; Pomeroy, B.S.; Seal, U.S.; Jay, A.R. 1965. Ornithosis: Experimental immunofluorescence studies. See MP 20, Vol. I, No. 5045.

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Goldin, R.B.; Krasnik, F.I. 1963. Specific staining of ornithosis virus by fluorescein-labeled incomplete antibodies. See MP 20, Vol. I, No. 5048.

7734

Hahon, N.; Nakamura, R.M. 1964. Quantitative assay of psittacosis virus by the fluorescent cell-counting technique. See MP 20, Vol. I, No. 5050.

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Hahon, N.; Cooke, K.O. 1965. Fluorescent cell-counting neutralization test for psittacosis. See MP 20, Vol. I, No. 5051.

7736

Terskikh, I.I.; Zairov, G.K. 1964. A comparative study of trachoma and ornithosis viruses. See MP 20, Vol. I, No. 5063.

4. Brucellaceae

7737

Axt, J.; Jentzsch, K.D. 1962. Study of brucellae with fluorescent antibodies: 1. Conjugation of immune globulin with DANS. See MP 20, Vol. I, No. 5069.

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Karakawa, W.W.; Sedgwick, A.K.; Borman, E.K. 1964. Typing of Haemophilus influenzae with fluorescent antibody reagent. See MP 20, Vol. I, No. 5082.

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Kartman, L. 1960. The role of rabbits in sylvatic plague epidemiology, with special attention to human cases in New Mexico and use of fluorescent antibody technique for detection of Pasteurella pestis in field specimens. See MP 20, Vol. I, No. 5083.

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Lewis, V.J.; Brooks, J.B. 1964. Comparison of fluorochromes for the preparation of fluorescent antibody reagents. See MP 20, Vol. I, No. 5125.

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Smith, C.W.; Metzger, J.F. 1963. Identification of Listeria monocytogenes in experimentally infected animal tissue by immunofluorescence. See MP 20, Vol. I, No. 5135.

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7. Lactobacillaceae

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Mayorova, G.F.; Korn, M.Ya. 1963. A study of the antipertussis fluorescent serum specificity. See MP 20, Vol. I, No. 5275.

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9. Mycoplasma (PPL0)

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Unclassified
Security Classification

| DOCUMENT CONTROL DATA - R & D | | |
|--|------------------------|---|
| <i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i> | | |
| 1. ORIGINATING ACTIVITY (Corporate author) | | 2a. REPORT SECURITY CLASSIFICATION |
| Department of the Army Fort Detrick Frederick, Maryland 21701 | | Unclassified |
| 2. REPORT TITLE | | 2b. GROUP |
| IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY: VI. TECHNICAL PROCEDURES | | |
| 3. DESCRIPTIVE NOTES (Type of report and inclusive dates) | | |
| 4. AUTHOR(S) (First name, middle initial, last name) | | |
| Warren R. Sanborn | | |
| 5. REPORT DATE | 7a. TOTAL NO. OF PAGES | 7b. NO. OF REFS |
| March 1968 | 181 | 652 |
| 6a. CONTRACT OR GRANT NO. | | 8a. ORIGINATOR'S REPORT NUMBER(S) |
| A. PROJECT NO. None | | Miscellaneous Publication 20 |
| 6b. | | 8b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) |
| 6c. | | |
| 6d. | | |
| 10. DISTRIBUTION STATEMENT | | |
| Distribution of this publication is unlimited; it has been cleared for release to the general public. Non-DOD agencies may purchase this publication from Clearinghouse, ATTN: Storage and Dissemination Section, Springfield, Virginia, 22151. | | |
| 11. SUPPLEMENTARY NOTES | | 12. SPONSORING MILITARY ACTIVITY |
| | | Department of the Army Fort Detrick Frederick, Maryland 21701 |
| 13. ABSTRACT | | |
| <p>> This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume VI is divided into two major sections. The first section contains 292 annotated citations to general procedures in the use of immunofluorescence, arranged by subject areas. The second section contains 360 cross-references to citations in the other volumes that describe specific applications of this technique; these are arranged to correspond with the subject areas of the other volumes. A complete author index for these 652 citations is included.</p> | | |

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1 NOV 65

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Security Classification