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Principal Investigator:
William P. Murphy, Jr., M.D.

Institution:
Cordis Corporation

Detection and Measurement of Antigen-Antibody Reactions

Contract No. DA-49-193-MD-2203

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Cordis Corporation
241 N.E. 36th Street
Miami 37, Florida

30 September 1963

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ABSTRACT

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7. Descriptive abstract:

Considerable progress has been made in the development of the polarometer. Some improvement in sensitivity has been achieved by changes in optical design and incorporation of a more efficient light source. Keywords: U/A Reports, Immunology, Antigen-antibody reactions, Chemical [illegible], Medical [illegible] reactions, A special camera has been constructed to give photographic records of antigen-antibody reactions in an agar medium for subsequent Computer Techniques, Fluorescence Polarization, IBM 7090, Data Processing, Diphtheria Toxoid Antitoxin System

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quantitative assay. This is proving to be a useful tool for more efficient prosecution of the current program.

Computer techniques have been successfully applied to the analysis of fluorescence polarization information. The IBM 7090 program which has been established will facilitate processing and interpretation of the data which will be obtained in the coming months from various antigen-antibody systems.

Study of the diphtheria toxoid antitoxin system has yielded preliminary data of interest. Cross reactions with M. lysodeikticus have indicated no appreciable interference.

With the availability of improved instrumentation it has been found possible to substitute fluorescein labeling for the rhodamine previously used.

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I. INTRODUCTION

The current investigation involves applications of a fluorescence polarization technique to solutions which contain fluorescent-labeled antigens or antibodies.

Prior to initiation of the present program this technique had been developed and refined by Dr. Walter B. Dandliker, using fluorescein-labeled ovalbumin and rabbit anti-fluorescein ovalbumin. Fluorescence polarization measurements were made with a modified light-scattering apparatus. The need for more sophisticated and accurate optical equipment soon became obvious and an improved polarometer* was built by Cordis Corporation.

The success of Doctor Dandliker's results led to speculation about a wide variety of applications of the basic technique. These varied from specification of allergenic reactions to detection and identification of microorganisms. The possibilities aroused the interest of the Office of the Surgeon General and resulted in the current contractual effort with Doctor Dandliker as consultant and Cordis

*Funded under NIH
Grant RG-8970

Corporation as contractor because of the strong emphasis to be placed necessarily on instrumentation and application engineering.

Initiation of work under the contract was authorized October 1, 1961, and the first year's effort has been described in a previous report (1). In the pages which follow is an outline of work performed from October 1, 1962, to September 30, 1963.

II. APPLICATION PROGRESS

A. Diphtheria Toxoid Antitoxin System

1. Sensitivity of the fluorescence polarization Method

The ultimate sensitivity attainable by the fluorescence polarization method is one of the crucial questions in comparing its usefulness with that of other, available, classic, immunological techniques. The factors that influence the ultimate sensitivity are:

a. Intensity of the background fluorescence. This consists of the sum of the fluorescent light contribution from within the cell and instrument assembly itself with that from the serum or other fluid being tested for antigen or antibody content. Such background can be lowered by: incorporating proper design features into the instrument; and/or by purification of the material to be tested so as to remove adventitious fluorescence which would tend to raise the background. An additional gain is realized by use of fluorescent labels which emit in a spectral region where the background is inherently low. At present the instrument background is

roughly equivalent to 10^{-11} molar fluorescein solution. In other words, if the precision of an intensity measurement is $\pm$ 1%, then the fluorescence intensity (above that of background) for 10^{-11} molar fluorescein can be measured with a precision of $\pm$ 2%.

b. Precision of the intensity measurement.

This factor is largely a function of instrument design and, at present, the best precision that can be realized seems to be about $\pm$ 2%.

c. Strength or avidity of the antigen-antibody association.

The avidity or the association constant varies inversely with the concentration range of antigen and antibody which can be investigated. This limitation or restriction, however, is common to any method of studying the antigen-antibody combination, high sensitivities being associated with very tight combination and low sensitivities with relatively loose ones.

In order to investigate the sensitivity currently available with the present technique, the diphtheria system has been investigated further using rabbit antibody to diphtheria toxoid.

In the experiment outlined below, to constant amounts of fluorescein-labeled diphtheria toxoid, there were added varying amounts of diluted antisera, and the fluorescence polarization measured after mixing. The antiserum was from rabbits which had been injected with unlabeled diphtheria toxoid. The reaction mixture in the cuvette contained a final concentration of 0.1 flocculation units (Lf) per ml of fluorescein-labeled toxoid and the amount of flocculating antibody per ml of final solution ranged up to 0.8.

Results of the experiment are shown in Table 1 and Fig. 1. It may be seen that, within experimental error, the fluorescent polarization is constant when normal serum is used and that a linear relationship results between the polarization and antibody concentration when immune serum is used. The data of Fig. 1 would indicate that the lower

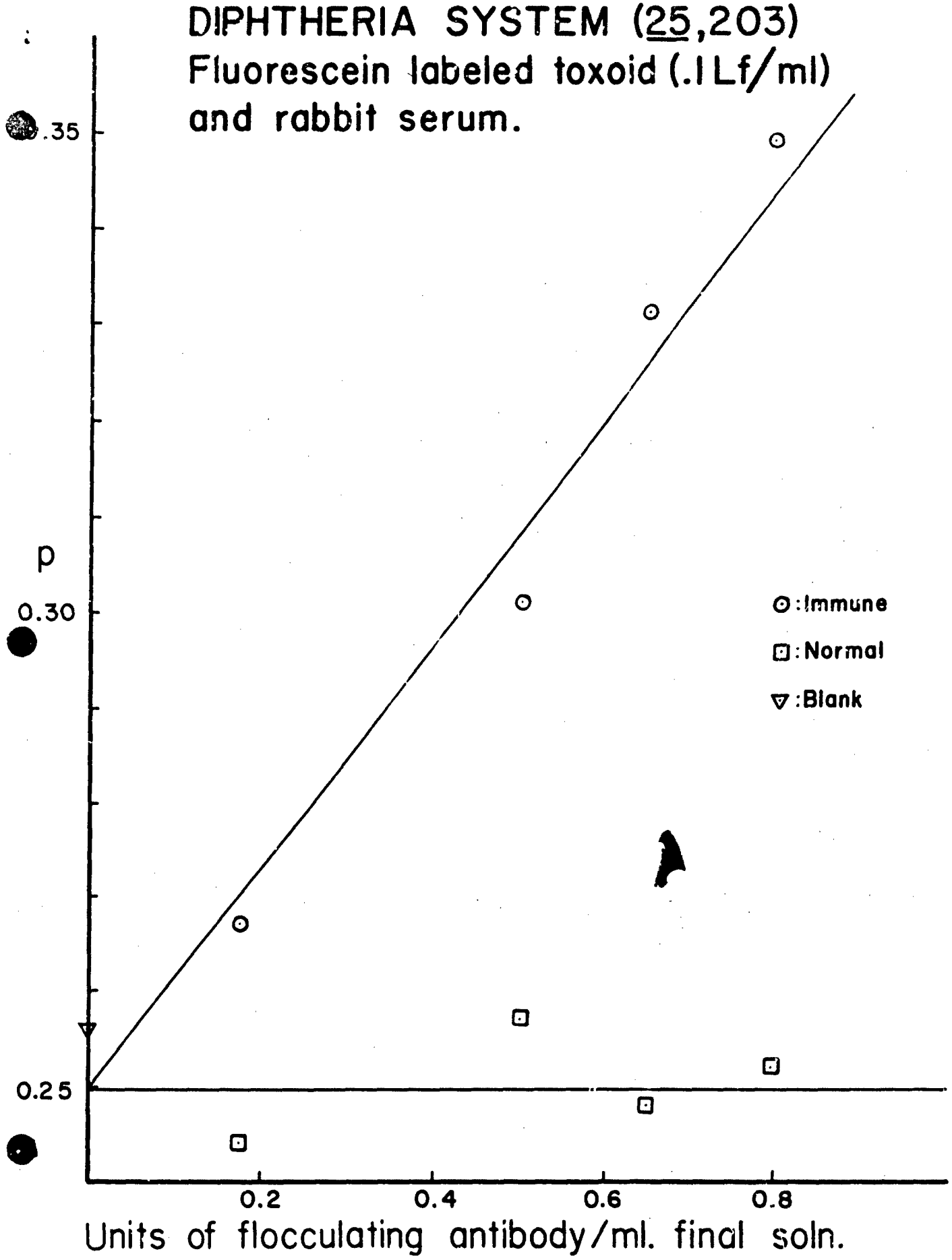
DIPHTHERIA SYSTEM (25,203)
Fluorescein labeled toxoid (.1 Lf/ml)
and rabbit serum.
.35
p
0.30
0.25
⊙ : Immune
⊡ : Normal
▽ : Blank
0.2
0.4
0.6
0.8
Units of flocculating antibody/ml. final soln.

Fig. 1

TABLE I

ml Serum per ml Final Solution (applicable to both "normal" and "immune")	Flocculation Units of Antibody/ml Final Solution (applicable to "immune" only)	Fluorescence Polarization (p)	
		Normal	Immune
0.0000	-----	0.256	0.256
0.0032	0.176	0.244	0.267
0.0091	0.500	0.257	0.301
0.0118	0.649	0.248	0.331
0.0143	0.796	0.252	0.349

These data, plotted in the accompanying figure, show the means by which fluorescence polarization is used to measure diphtheria antibody.

limit of detectability for diphtheria antibody is now at the level of 0.1 or 0.2 units/ml.

The shape of the curve attained suggests that the instrumentation limits the sensitivity of the method, at the present stage of development.

2. Effect of labeling upon reactivity

In order to explore further the possible effect of labeling upon immunological activity, three preparations of fluorescein-labeled diphtheria toxoid were prepared. These materials differed only in intensity of fluorescent label, the values, on an arbitrary scale, being in the ratio 17:12:3.6. It is to be noted, in considering a specific application, that an increase in label intensity makes the inherent sensitivity greater but, at the same time, tends to disturb the native activity of the antigen or antibody.

The three lots of labeled toxoid have been investigated, using immunodiffusion. Under the conditions so far studied, results indicate that intensity of label exerts no appreciable influence upon the reaction with

antibody. In all of the toxoid preparations investigated, including an unlabeled preparation as control, three distinct antigens were found to be present. One of these, greatly predominant, was presumed to be due to diphtheria toxoid. The proportion of the three components did not seem to change after labeling with fluorescein.

Quantitative immunodiffusion comparison of the potency of commercial horse antitoxin and rabbit antibody made to diphtheria toxoid indicated that the former was approximately four to five times as potent. In connection with the Micrococcus lysodeikticus system discussed below, cross reactions were looked for between the micrococcus and diphtheria systems. Only in one reaction was any evidence encountered to indicate cross-reaction and, in this case, only a very weak one was found.

As an additional method useful in studying the effect of varying intensity of fluorescein label, study of passive hemagglutination was initiated. While immunodiffusion showed no significant differences due to intensity of

labeling the diphtheria toxoid, it was considered important to check this finding by several different immunochemical methods. Because of the inherently high sensitivity of passive hemagglutination, it was believed possible that differences not detectable by other techniques might be uncovered.

B. The Micrococcus Lysodeikticus System

In previous progress reports, it was stated that bacterial cell walls have been successfully ruptured in the Mickle disintegrator in the presence of ballotini beads. Recently, an even better and more rapid technique has been devised, involving applications of a 20 kc Raytheon ultrasonic oscillator. This disintegrator, used alone, produced rather negligible rupture, even after several hours; however, when ballotini beads were introduced, into the sample holder, practically complete rupture of M. lysodeikticus was achieved in 90 minutes. This finding is expected to expedite production of cell-wall material needed in immunization and testing.

Further work has been carried out using M. lysodeikticus on quantitative measurement of the extent of agglutination by light transmission. The perfection of this technique has been hampered by the low titer of antibody thus far available, and it is hoped that the use of Freund's adjuvant will remedy the situation. The presence of muramidase or of muramidase-like activity has been detected in normal rabbit serum. This activity is removed

by treatment of the serum with kaolin. With low titer serum, much of the antibody also is removed, so that high titer antibody is made even more necessary.

The use of immunodiffusion with the M. lysodeikticus system has also been examined. Results with whole cells, lysozyme-degraded cells or cell-wall material, as in the agglutination method, indicate too low an antibody titer. However, again, it is expected that the use of high titer antibody will yield useful information.

C. Techniques

1. Labeling

In the progress report dated September, 1962, there was a discussion of some of the complications encountered in labeling diphtheria toxoid with rhodamine. Results have indicated that rhodamine has a pronounced tendency to form aggregates or micelles which, under the proper circumstances, dissolve only very slowly and give rise to anomalous and unstable polarizations.

In the earlier stages of instrument development, it had been advantageous to label with rhodamine rather than fluorescein because of its longer wave length sensitivity and resultant lower fluorescence background. The present instrumentation is excellently suited to the excitation of fluorescein; therefore, its inherent advantage of very high fluorescence yield can now be exploited.

In addition, fluorescein appears to be preferable, chemically. It does not show the tendency that rhodamine does to form micelles, and the polarizations after fluorescein labeling are stable and reproducible.

The use of fluorescein as a label and excitation of its fluorescence by 490 mμ light also avoids an undesirable background fluorescence having its origin either in the water itself or an impurity therein. This fluorescence is excitated at 436 mμ but not at 490 or at 540 mμ. The presence of this fluorescence was one of the reasons for selecting rhodamine as a dye for labeling during the investigations with less sensitive equipment.

2. Immunodiffusion Measurement

Because of the need for a suitable recording of the results of immunodiffusion experiments, an investigation has been initiated to perfect a simple, efficient photographic method for the purpose. While such techniques are at present available, all in current use appear to have serious disadvantages.

Studies on the configuration of the lighting system and of the system for photographic registration have been made. In its present form, as described in Section III-B following, the equipment is capable of taking photographs of good quality. Further improvements are anticipated.

3. Immune Sera Preparation

The value of Freund's adjuvant in the preparation of potent immune sera is well known. However, since the methods of preparation of the necessary stable emulsion leave much to be desired, a study of the emulsification process was initiated. Attempts are being made to improve the mechanics of mixing the component liquids and manipulating them to produce firm, stable emulstions.

As noted above, the potency of rabbit antiserum to diphtheria toxoid was found to be well below that of commercial horse antitoxin. Booster injections of the toxoid have been administered to the same animal in an attempt to increase the antibody titer significantly. In addition, a series of animals has been started on injections of bovine serum albumin with Freund's adjuvant. These antibodies are to be used in conjunction with the ferritin-labeling studies discussed above.

In addition, a new route of multiple-site intradermal immunization, not employed in these investigations previously, has now been put into use. In other antibody systems, this route of injection has proved to be especially effective. The use of Freund's adjuvant is of special interest in obtaining antibodies to the walls of Micrococcus lysodeikticus, or to the cells themselves, since antibody titers in these cases have previously been consistently low.

D. Molecular Size Considerations

In order to investigate the interesting and important relationship between

magnitude of polarization and molecular size, ferritin-labeled antibody to bovine serum albumin (BSA) has been obtained. With this available, a comparison can be made with normal antibody to the same antigen. Since the molecular weight of ferritin greatly increases the mass of the antibody (roughly 1,000,000 compared to 160,000), it would be expected that considerably greater polarization would be obtained in the presence of ferritin label. It is to be expected, on theoretical grounds, that the ultimate polarization obtainable in forming the antigen-antibody complex will be greater the greater the molecular size of the complex. The effect of thus artificially greatly increasing the molecular size of antibody will, in this way, be systematically checked. In addition, antibody to pneumococcus Type II polysaccharide and naturally occurring human antistreptococcal antibodies are available.

Pneumococcus Type II polysaccharide has been obtained and labeled with fluorescein. Although the molecular weight of this polysaccharide is supposed to be in the region of half a million, it may be, because of the elongated character of the molecule, that

there is enough molecular rotation or flexibility to make the fluorescence polarization method of value. Combination of the labeled polysaccharide with its antibody will be followed by fluorescence polarization and other methods, as applicable.

In addition, a streptococcal antigen is available for labeling and study. It is hoped, by the investigation of several new systems like those mentioned, that some unexpected advantages or limitations of the fluorescence polarization method will be uncovered.

Very recent studies by others have indicated that the antibody molecule can be split into 4 peptide chains, the two smallest (mw + 20,000) possessing antibody activity. Antibody fragments of this low molecular size would be ideal for fluorescence polarization measurements if they were the fluorescein-tagged reagent for the reaction. Since considerable information is available regarding the chemistry of the antibody molecule and its labeling with dye, it is planned to explore the possibility of utilizing tagged, small, antibody fragments (diphtheria antitoxin, rabbit

anti-BSA, etc.) and to compare their efficiency to normal 7S tagged antibody molecules in detecting antigens.

E. Data Handling

Recently, under a separate program, a comprehensive theory for the interptetation of fluorescence-polarization measurements on the antigen-antibody reaction has been established. According to this theory, there are three pieces of information which can be obtained from equilibrium measurements of antigen-antibody reactions:

1. The amount of antigen or antibody in the unknown material being titrated.
2. An average equilibrium constant which may be regarded as a measure of avidity.
3. A measure of the heterogenity of the antigen or antibody combining-sites.

Each of these quantities is represented, after involved computation, as a single parameter which can be used to characterize an antigen or antibody preparation.

Some of the problems involved in processing the data obtained have been solved by programming an IBM 7090 digital computer.

Once data, consisting of fluorescence-polarization measurements as a function of the concentration of labeled antigen or antibody have been collected, the computer's task is to define the best values (in the least-squares sense) for five different parameters. Three of these have been defined above and have to do with immunologically important properties of the antigen or antibody. The remaining two are optical parameters which must be determined before the problem can be solved.

The quality of the final result can be estimated by graphically presenting a computed, solid curve using the best values of the five parameters in combination with observed experimental points. In addition, if desired, the standard error of estimate can be tabulated to provide an objective criterion of fit between theory and experiment.

While results are not yet complete, certain significant findings have been made which indicate that this approach is successful. First of all, the present program does lead to values of the five parameters which converge. Secondly, when the final values of the parameters are used to recompute a theoretical curve, the

degree of fit with experimental points is usually excellent. Thirdly, the extended theory for fluorescence polarization, which was mentioned above, is readily seen from the computations to provide a much better fit to the experimental data than did the simple theory which assumed all the antigen-antibody sites to be characterized by a single intrinsic constant.

The heterogenity of antibody sites is well known and has been measured by other means; but the fluorescent-polarization data provides a more rapid and certain means of obtaining this information than any other known method.

The above-described method of processing data by a high speed computer was introduced in order to obtain best least-square values of pertinent parameters. However, now that a suitable program has been set up, large numbers of data such as might be encountered in screening programs or extended research projects can be handled with ease and rapidity.

III. INSTRUMENTATION PROGRESS

A. Polarometer

An improved instrument has been designed and is now under construction. It is shown in a photograph (Fig. 2), taken without baffles in place for a clearer view of the component arrangement. The optical system follows quite closely that described in an earlier report (1), the most important changes being: an increase in the size of the lamp housing to accommodate a more powerful lamp; and introduction of an optical system between the lamp and the Polarometer housing for re-imaging of the arc stream.

Signal-to-Noise ratio. As pointed out in the previous report, an annoying wandering of the output null meter had prevented rapid adjustment of the reference beam to obtain equality with the sample beam. This wandering had been traced to the photomultiplier cell and was attributed to the noise inherent in electronic devices of this nature. This, incidentally, disappears in the absence of light, and is not "dark-current noise" which is orders of magnitude lower in amplitude.

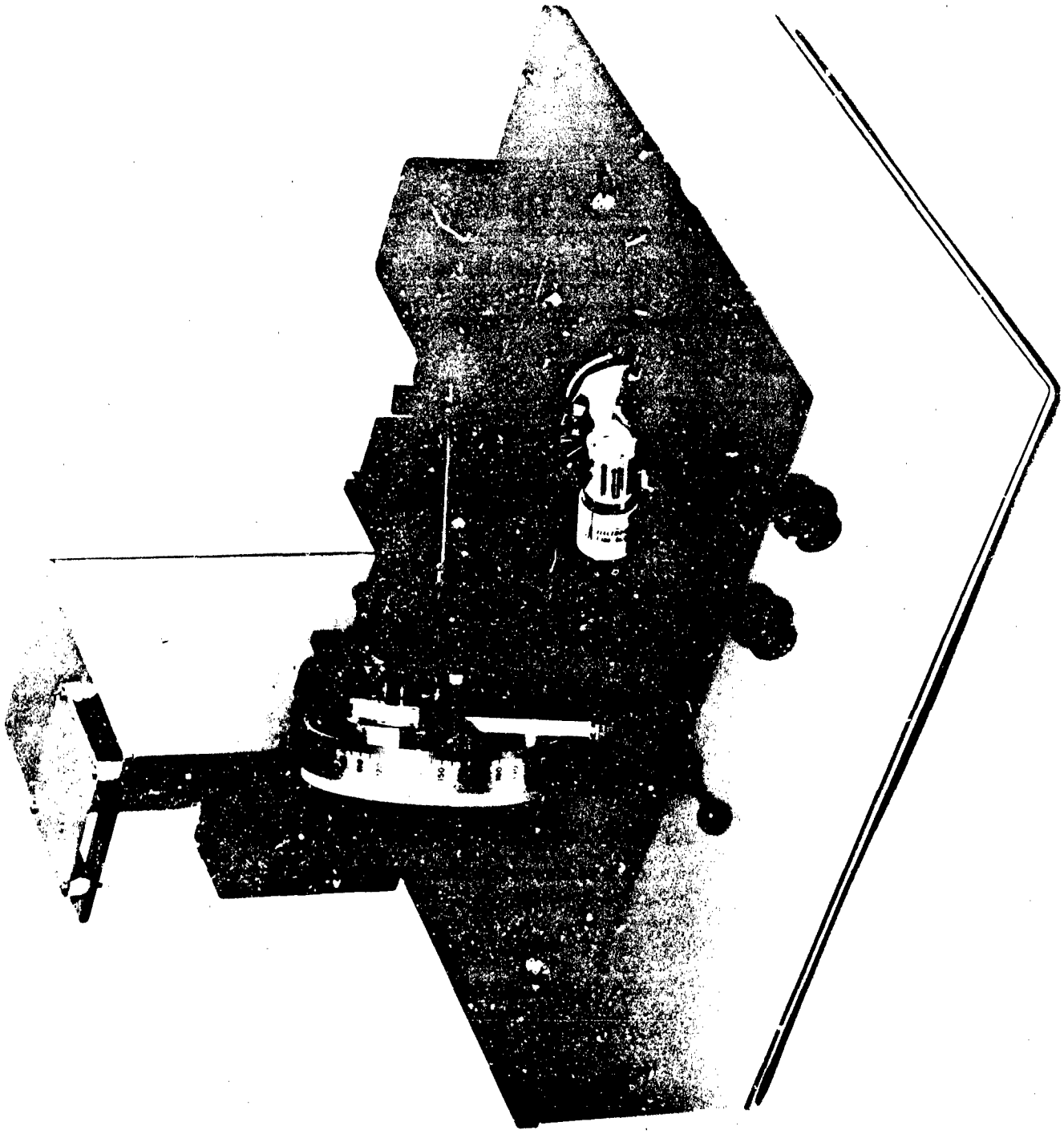

Fig. 2

As soon as the photocell is illuminated with the sample beam, erratic spikes of energy appear at the anode, of a type usually referred to as shot noise. The cell was enclosed in an insulating housing and cooled to dry ice temperature but experiments confirmed previous observations that cooling does not affect this type of noise.

The output reading is affected by noise of this kind because it has a 60-cycle component which may be in any phase with respect to the phase-sensitive detector. It makes a random contribution to the output reading which, after a suitable period of time, averages out. The difficulty stems from the fact that this averaging does not take place quickly and frequently causes the output meter to remain to the right or left of center for several seconds before swinging back. Obviously, if a reading is taken during one of these excursions, it is in error by an amount equal to the noise contribution.

In a series of experiments, a "dc chop" was utilized, in which the 60-cycle chopping motor was eliminated and a shutter installed with which either the sample or

reference beam could be occluded. The output circuit consisted of a vacuum tube voltmeter reading across a resistor placed in series with the anode of the photomultiplier tube. This meter, of course, gave a reading which varied slightly due to the noise; but it was found to be easier to "decide" what the steady-state anode current should be and adjust the reference beam accordingly. Using this technique, it was discovered that the optical system and optical attenuator in the reference beam were performing adequately.

Signal-to-noise has been improved principally by increasing the amount of energy available at the excitation wave length of fluorescein, 4900 Å, as will be discussed below.

Excitation Source. The high pressure mercury lamp previously used has a moderately weak continuum in the 4900 Å region. It has been replaced by a 450 watt Xenon lamp, which produces about eleven times more energy in this region of the spectrum.

Fluorescein can also be excited at 4337 Å (the blue line of mercury), where a

great deal more energy is available from standard mercury sources. Although this wave length was considered, experiments conducted by Doctor Dandliker with the equipment, and later checked independently, reveal that the most pure water available fluoresces at 4337 Å, therefore contributing to the background reading. On the other hand, little or no fluorescence is encountered at 4900 Å. These measurements were made by comparing readings involving, respectively, the empty cell and the cell full of water or diluent, at the two excitation wave lengths mentioned. Although it was taken into consideration that an empty cell does not constitute the same optical entity as a full cell, the results were still conclusive. Fluorescence was measured in all cases at 5250 Å.

The light source currently used is the Osram XBO450W which is a high-pressure, short-arc Xenon lamp of 450-watt-input power. Light from this lamp is directed to the optical system in essentially the same manner as before, except that it is reimaged once before illuminating the Polarometer optics proper. The reason for this reimaging is

two-fold: As the lamps age, their quartz envelope becomes cloudy and scatters light into the optical system, which is, of course, outside of the desired energy beam. Without reimaging, this light tends to illuminate the sidewalls of the cell where spurious effects may occur due to fluorescence of the cell walls. The second reason is that the arc stream of the Xenon lamp is of triangular shape with most of the light coming from one point of the triangle. In order to make the image of convenient size, the reimaging optics contain a short section of cylindrical light-pipe which effectively converts the non-uniform image of the lamp into a uniform circle of light of the required diameter.

Intensity readout. As was mentioned in the previous report, the density of the Inconel neutral density wedge used was logarithmic with angle, requiring additional complicated mathematical operations in order to obtain the relative intensity values required for data reduction. A logarithmic dial could have been utilized. However, since its linearity would be limited by its precision of manufacture and since location of fixed

reference points on such a wedge was not feasible, it was decided to select another measuring device.

Accordingly, there was incorporated a system based on Malus' Law which states that the transmission of a pair of polarizers is proportional to the square of the cosine of the angle between their axes. Good quality polarizers obey this law quite well and have the advantage that a zero point can be determined with great accuracy. Polaroid materials, such as HN22, have been demonstrated to be of adequate optical quality for such a system. In many cases, they exceed the quality of natural crystals which are degraded by cloudiness or inclusions.

In summary, the neutral density wedge was replaced by an attenuator made of Polaroid HN22 in which the movable Polaroid is driven through a worm gear attached to a dial graduated in accordance with the cosine-squared law. The dial is six inches in diameter and an arbitrary scale from 0 to 250 is inscribed around approximately 320° of its circumference. This arbitrary scale measures the rotation of the polarizer over

an angle from the position of extinction to 45°. A 66-fold range of intensity measurements is, therefore, available up to an angle of 85° between the polarizers. Beyond this point, accurate readings cannot be made.

In order to eliminate possible inaccuracies due to the polarization sensitivity of the photomultiplier cell, the polarized light from the attenuator is rendered circular by the application of a quarter wave plate. Since the photomultiplier cell is sensitive only to linearly polarized light, this precaution results in its being unaffected by change in the angular setting of the read-out polarizer.

The optical position formerly occupied by the neutral density wedge was replaced by a circular disc of Polaroid HN22 which, in connection with the old read-out dial, forms a convenient and accurate method for adjusting the range of the instrument for a given experiment.

Polarization Analyzer. The analyzer used in the earlier design was a calcite Rochon prism. Difficulties in

mounting the prism and compensating for the unwanted beam have led to substitution of a Polaroid sheet. In order to maximize transmission, HN38, which has adequate extinction characteristics in the green region, will be applied.

Amplifier. Light levels were found to be too low to render either necessary or possible adjustment of the photomultiplier dynode voltage to maintain constant sensitivity. The amplifier was, therefore, simplified by replacing the APC circuit. A preamplifier is now located at the base of the photomultiplier tube. The filters for passing 60 cycles are L-C filters instead of twin-T for the reason that these filters are not affected by changes in gain of the tubes. The bandpass of the filters is rather wide but drops off rapidly below 60, reaching the half-power point at 52 cycles.

Sensitivity and Reproducibility. These quantities are estimated either by introducing water or diluent into the cell as "zero", or introducing 10^{-9} or 10^{-10} molar solutions of fluorescein. A typical diluent

reading is 15 divisions (out of 250 full scale) with a reproducibility of about $\pm$ 2 divisions. Readings on 10^{-10} molar fluorescein typically run 27 $\pm$ 1. Readings on 10^{-9} molar fluorescein typically run 146 $\pm$ 1 to 2. Measurements made comparing the intensity of fluorescence 10^{-9} and 10^{-10}molar fluorescein give a ratio of intensities of approximately 10 which is within the limit of accuracy of preparing the solutions and making the measurements.

General. The new instrument is being mounted on a steel cart with the optical section on top and the amplifier and its controls directly below. The lamp power-supply is on the bottom shelf where it is unlikely to interfere magnetically with the electronic circuitry.

The operator has little need to manipulate the amplifier controls, being concerned only with insertion and removal of the cell and making a null balance with the optical attenuator. For convenience, the null-balance meter is mounted adjacent to the intensity dial. This is always moved in the same direction as the needle does in returning to null.

A manual shutter for cutting off the incoming beam is provided so that photolysis of the sample will not occur when the cell is left in place for an extended period of time.

The new instrument is being equipped with a thermostatically-controlled sample-holder, using the Peltier effect for cooling and heating. This will, hopefully, provide sample-cell environments ranging from 5 to 37° C.

B. Agar Plate Camera

An improved technique was needed to obtain photographic records of antigen-antibody reactions in an agar medium for subsequent quantitative assay.

Experiments were conducted using both light and dark field illumination. It was found necessary in either case to avoid photographing the small angular deviations due to waviness in the agar-air surface. The Petri dishes used were of plastic and had rather good optical surfaces so that distortion caused by imperfections in the supporting medium were negligible.

At first photographs were made using a standard Polaroid Land Camera with an auxiliary lens so that a close-up of the plate could be obtained. This lens required a focal distance of only 8-7/8 inches. It was quickly discovered that a symmetrical light source was required since orientation of the reaction-precipitate extended around a full circle. A 22-watt "Circline" lamp was substituted, placed at a distance of 7 inches from the specimen. This distance appeared to be optimum for the specimens at hand but was adjustable over a few inches to optimize contrast with the particular medium being used.

The principal defect with this optical system was the proximity of the lens to the specimen. This made it necessary for light rays from the essentially vertical reaction-zones to travel obliquely in order to enter the lens. Thus, if two reaction zones were close together, the thickness of the agar would cause the two to merge and appear as one in the photographic image. For example, it was calculated that when the inside edge of the circle of wells is 15 mm from the center, the angle subtended at the lens is 0.0675 radian. If the agar is 4 mm

thick, this means that the minimum separation that could be resolved is 0.27 mm.

The indicated solution was to use a long-focal-length lens which, although wasteful in space, would significantly improve the amount of fine detail which could be recorded on the photographic plate. This phenomenon is indeed readily demonstrated by observing the plates from different distances. The separation of reaction zones is seen to be more distinct when the eye is more remote.

Another variable in the system was the resolution of the film being used, in this case, Polaroid Type 47 (3000 speed). Since the Bureau of Standards' resolution test pattern gave values between 10 and 14 lines per millimeter, about equal to the resolution of the human eye at ordinary reading distance, it was clearly impossible to improve the situation by resorting merely to enlargement.

Taking all of the above factors into consideration, a camera has been constructed, using a Goerz Double Artastigmat lens, Series III, No. 4, having a focal length of 9-1/2 inches. The lens is mounted in a compound

shutter, having speeds up to 1/150th second. The camera is shown in the photograph (Fig. 3). At the top-left side is a Circline light-source which is adjustable vertically by a rod protruding through the left-rear corner. A dark field is assured by means of a flat-black painted cup which is inserted inside the Circline lamp and which cannot be illuminated except by reflection from the black-painted interior. Alternatively, if it is desired to have a light background, this cup can be turned over, presenting a white-painted side, so that the camera can be used for photographing dye images.

The sample is inserted through a small door which gives access to a platform containing the aperture through which the photograph is taken. Photographs may be taken through the bottom of the Petri dish, provided it has not been scratched or otherwise defaced.

The Polaroid Camera back, seen on the right, accommodates standard 3-1/4 x 4-1/4 film. A later model may contain the new 4 x 5 camera back, which will permit the obtaining of both a positive and a negative, the latter being, hopefully, of higher resolution and therefore available for making enlargements.

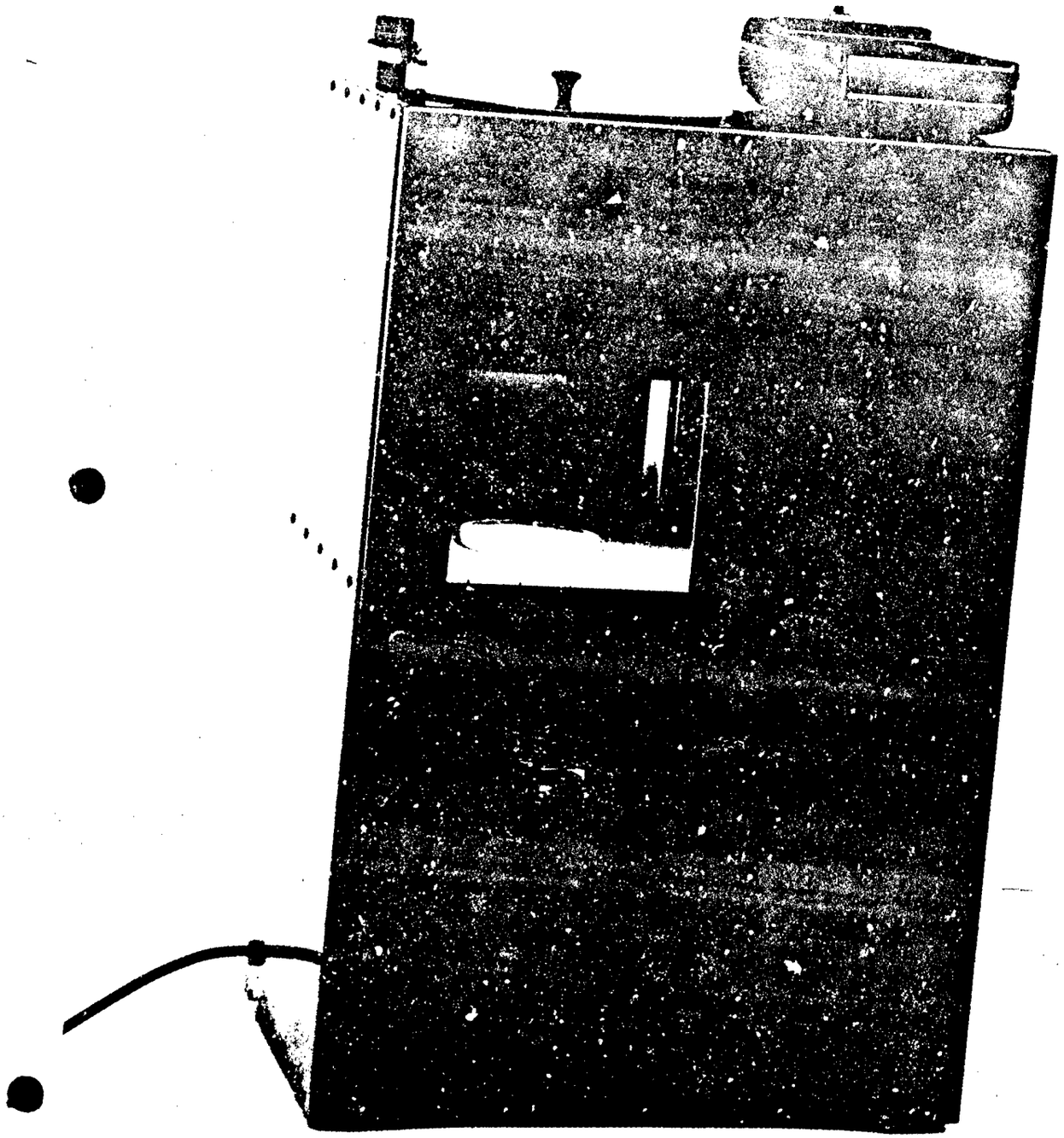

Fig. 3

The lens occupies a horizontal position in a vertical septum which separates the left and the right half of the camera. Two first surface aluminized mirrors for bending the beam, complete the optical system. The standard camera shutter is operated by a simple motor-drive which, with the aid of a microswitch and relay, cocks the shutter and takes the exposure whenever the button on the right is pushed.

The magnification of this camera is 1.7 so that the small dimension of the frame is filled by the standard configuration of six wells. The camera is fixed-focus, adjustable features have been omitted to achieve simplicity and reliability.

IV.

APPENDICES

A. Bibliography

(1) Annual Progress Report, Detection and Measurement of Antigen-Antibody Reactions, dated 30 September 1962.

B. Personnel

Personnel who have made technical contributions to the investigations described in this report are:

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