process is offered as explanation and strongly supported by leading biologists. I consider the high values of the energy of activation reported here as incompatible with this view and as a support for those theories which postulate chemical processes as being responsible for the specific changes in permeability of conducting membranes during activity.

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Circulating Antibody Directed against Penicillin

Detection, by in vitro serological techniques, of circulating antibodies directed against penicillin has not been reported. In the past few months, however, sera from certain individuals have been encountered which appear to react specifically against penicillin. It is the purpose of this report to describe the system in which this reaction is demonstrable and to report studies on the characteristics and specificity of the antibody.

Addition of penicillin to erythrocyte suspensions is frequently a routine procedure in the preparation and preservation of red cells used in specificity panels in blood-bank laboratories. In August 1957, during routine testing, the serum of a prospective transfusion recipient was found to agglutinate all of such a panel of erythrocytes prepared with penicillin; if the same erythrocytes were not exposed to penicillin, this serum caused no agglutination. Furthermore, the exposure of the patient's own erythrocytes to penicillin made them agglutinable by his own serum.

On further study it was found that human erythrocytes of all blood groups, by exposure to appropriate concentrations of penicillin G, O, or K, could be sensitized to react with this serum, and with sera of similar characteristics later discovered in other individuals. Once the erythrocytes were sensitized, they remained sensitized as long as they remained useful for testing—that is, until they began to show marked hemolysis—usually after at least 2 or 3 weeks. The sensitization was not affected by additional exposure of the sensitized cells to penicillinase for periods up to 4 hours, nor was it altered by exposure of the cells to 0.5-percent papain or 0.1-percent ficin.

Thus far, no human red cells have been shown to resist this "penicillinization."

Substitution of penicillinase, papain, or ficin for penicillin in the sensitization procedure gave negative results. Furthermore, in an attempt to see whether other antibiotics would sensitize erythrocytes for this reaction, approximately equal weights (about 10 mg) of the following antibiotics (*1*) were each dissolved in 1 ml of phosphate buffer (final *p*H 7.2 to 7.4) and then incubated wtih erythrocyte suspensions: streptomycin, dihydrostreptomycin, polymyxin B, bacitracin, neomycin B, ristocetin, viomycin, oleandomycin, synnematin B, and the penicillins G, O, and K. The only preparations which sensitized the red cells to react with the particular sera were the penicillins G, O, and K and synnematin B, which is another penicillin derivative.

Penicillin which had been inactivated by the addition of penicillinase (*2*) was no longer able to produce sensitization of erythrocytes.

Studies on the effects of varying the time of the exposure of the erythrocytes to varying concentrations of penicillin G were performed. It was found that the degree of sensitization of the red cells, as measured by their agglutinability by weakly reacting sera, varied directly with the time of exposure of the cells to penicillin and with the concentration of penicillin in the incubation mixture. For example, a 25-percent suspension of erythrocytes could be sensitized to approximately the same degree either by incubation for 24 hours with a concentration of penicillin of 3000 units/ml or by incubation for 10 minutes with a concentration of penicillin of 50,000 units/ml. Concentrations of penicillin of less than 3000 units/ml produced weak and irregular sensitization. Incubation times of more than 24 hours enhanced the sensitization to a slight or negligible degree. As a matter of convenience, therefore, the usual method of preparing "penicillinized" cells for the study of reactive sera has been to add about 8 ml of an equal-part mixture of whole blood and Alsever's solution directly to a vial containing 200,000 units of powdered penicillin G. After incubation at 37°C for 1 hour, an aliquot is withdrawn from the vial, the erythrocytes are thrice washed with isotonic saline and made up to a 4- to 10-percent suspension in saline. Since the sensitization proceeds at all temperatures from 6° to 37°C, the temperature of exposure does not seem to be critical.

Table 1. Inhibition of hemagglutination by prior addition of penicillin to reactive serum. (i) Penicillin + reactive serum = mixture; (ii) mixture + sensitized red blood cells → agglutination.

Concn. of penicillin added to reactive serum (units/ml)	Agglutination
0	2+
100	2+
370	2+
750	2+
1,500	+
3,000	±
6,000	±
12,000	0
25,000	0
50,000	0
100,000	0
200,000	0

Certain sera can be shown to react with erythrocytes prepared in such a fashion. With some sera the reaction can be demonstrated only by the antiglobulin technique. More strongly reacting sera, however, may agglutinate the sensitized erythrocytes directly from a saline suspension in a test tube, or even on a slide.

Sensitized cells exposed to these sera have been heated for 15 minutes at 54°C in saline to elute the antibody. The consequent eluate was demonstrated to react with other penicillinized erythrocytes.

This antibody is stable for at least several weeks at ordinary refrigeration temperatures and resists degradation by a temperature of 56°C for 2 hours.

Additional demonstration of the specificity of these sera was obtained by inhibition tests. In these, an attempt was made to see whether prior incubation of a reactive serum with penicillin would so bind the presumed antibody that the serum would no longer react with sensitized erythrocytes.

Solutions of penicillin G were made up in AB serum in concentrations from 100 to 200,000 units/ml. Equal volumes of each penicillin solution were added to

equal volumes of an appropriate serum on a slide. Penicillinized red cells were then added, and the mixtures were observed for agglutination. As may be seen in Table 1, the solutions containing the higher concentrations of penicillin completely inhibited the agglutination reaction.

Similar inhibition was demonstrated by means of the antiglobulin method. Equal volumes of a buffered solution of penicillin G and of a suitably diluted sample of an appropriate serum were incubated 15 minutes. This mixture was then tested against penicillinized cells by the antiglobulin technique. There was negligible agglutination in this tube, whereas in the tubes in which the buffer or AB serum was substituted for the solution of penicillin G, agglutination was marked.

Among approximately 2000 sera studied thus far, 25 have reacted specifically with "penicillinized" erythrocytes. All individuals from whom reactive sera were obtained have at some time in the past received penicillin therapy. Only a minority have demonstrated any clinical penicillin sensitivity. The significance of the antibody is currently being studied.

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2. Penicillinase was kindly supplied by Dr. Bruno Puetzer, Schenley Laboratories.

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Color Coding of Stroboscopic Multiple-Image Photographs

The advantages of multiple-image photography for analyzing movement have been recognized ever since Marey developed "geometric chronophotography" in 1883 (*1*). The method has remained essentially the same, though in recent years it has been refined by the introduction of stroboscopic recording (*2*). A pattern for study is marked off on the subject in electric lights or reflecting material. The shutter of the camera is left open, and as the subject moves, the light source is interrupted at regular intervals. The movement is recorded as a time-space pattern on a single film. From the record, instantaneous displacements can be read directly, and velocities and accelerations can be obtained by differentiating.

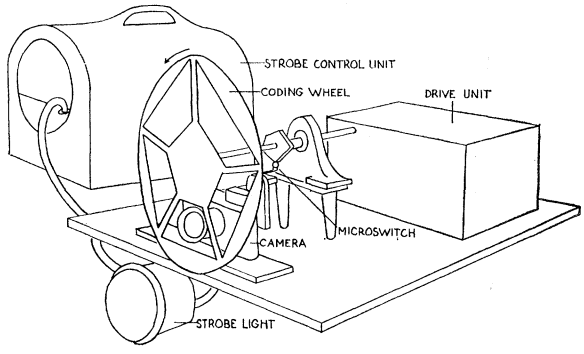

Fig. 1. Apparatus for color coding multiple-image photographs. Each aperture of the coding wheel is covered with a gelatine filter of a different color. As successive apertures come into place in front of the camera the microswitch is actuated, and a synchronized flash is emitted from the strobe.

Black-and-white photography is satisfactory for recording patterns that are relatively simple. As the patterns increase in complexity, however, interpretation becomes uncertain. The direction of movement is not given by black-and-white photography. (The movement pattern for standing-to-sitting, for example, cannot be distinguished by inspection from that for sitting-to-standing.) And when there is more than one trajectory in the pattern, it is sometimes impossible to determine which images are simultaneous. Neither problem is completely solved by the conventional method of omitting one flash from the cycle or altering its intensity.

Much of the confusion in a complex "stick pattern" can be eliminated by taking the photographs on color film and using a coding wheel to record successive images in different colors. If three or more colors are used in constructing the wheel, the direction of movement for each trajectory throughout the pattern will be recorded on the photograph. Simultaneity can readily be determined, since color automatically sorts out the images that belong together. With the help of color, the meaning of a complicated pattern can often be read at a glance. [Another type of coding may be obtained by varying the color of the reflecting material itself in order to distinguish one trajectory from another (*2*).]

We have used color coding as an aid in the analysis of human movement. A pattern for study is marked on the subject in Scotchlite reflecting tape (Silver No. 3270), and pictures are taken by coded flashes from a strobe unit. The apparatus is illustrated in Fig. 1.

The light source is a General Radio Strobolume with its lamp fastened directly below a Robot Star camera, which rests on a steel platform supported by a heavily built tripod. In front of the camera is a light aluminum wheel with a pentagonal center and five apertures, 5.5 cm at the greatest width. Each aperture is covered with a gelatine filter of a different color. A pentagonal cam on the shaft operates a microswitch so that, as successive apertures of the color wheel are centered in front of the camera, the Strobolume is actuated and a flash is emitted from the lamp. The wheel is driven by an 1800 rev/min synchronous motor at speeds of 1, 2, or 4 rev/sec, selected by a system of reduction gears. Pictures are taken on Ektachrome or Anscochrome film with an *f* stop of 4 when the camera is 12 feet from the subject.

The Strobolume operates at two intensities, high beam and low beam. The high beam, which has a flash duration of 40 μsec, cannot be operated for more than a few seconds at a time. The low beam has a flash duration of only 20 μsec and can be operated almost indefinitely at any of the rates we have used. So far, we have been able to record satisfactorily only with the high beam. With the faster color films now on the market it should ultimately be possible to record with the low beam. This would extend the range of movements that can be recorded by the method and add greatly to its usefulness.

Once the apparatus has been set up, a transparency can be made with little trouble or expense. It provides a permanent movement-record that is easy to obtain, easy to interpret, and convenient to file (*3*).

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13 January 1958.