green-off" cells. This raises the intriguing possibility that there may well be a second type of color-vision system represented in these layers, one perhaps having to do with such phenomena as afterimages and contrast.

The majority of cells recorded from in this intermediate pair of layers, however, are pure off-cells. We have not had the opportunity to study these layers or the inhibitory ventral layers to the same extent as we have the dorsal layers, but work on them is continuing.

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14 November 1957

Complex Nature of the Step in Immune Hemolysis Involving Third Component of Complement

The immune hemolytic reaction is complex because complement (C′) consists of four recognized components (C'_1, C'_2, C'_3, and C'_4) (*1*). As a result of the elegant studies of Mayer and his coworkers (*2*), the mechanism of immune hemolysis involving guinea pig C′ and sensitized sheep erythrocytes is considered to comprise the following sequence of steps:

$$E + A \rightarrow EA \quad (1)$$

$$EA + C'_1 + C'_4 \xrightarrow{Ca^{++}} EAC'_{1,4} \quad (2)$$

$$EAC'_{1,4} + C'_2 \xrightarrow{Mg^{++}} EAC'_{1,4,2} \quad (3)$$

$$EAC'_{1,4,2} + C'_3 \rightarrow E^* \quad (4)$$

$$E^* \rightarrow \text{inactive product} \quad (4a)$$

$$E^* \rightarrow \text{ghost} + \text{hemoglobin} \quad (5)$$

where EA represents a sensitized cell, $EAC'_{1,4}$ and $EAC'_{1,4,2}$ represent cells in a state resulting from interaction with C'_1 and C'_4, and C'_1, C'_2, and C'_4, respectively, and E* represents an activated cell which lyses in the absence of C′.

In a comparative study of inhibitors in several sera and of the effect of these inhibitors in the step involving C'_3 (reaction 4), it was found that the titration curve for C'_3 depended on the source of C'_3. This titration was carried out by the addition of different volumes of a given serum to a fixed concentration of $EAC'_{1,4,2}$ in the presence of 0.017*M* disodium ethylenediaminetetraacetate (EDTA). Disodium ethylenediaminetetraacetate was added to prevent further reaction of C'_1, C'_2, and C'_4 with the cells (reactions 2 and 3). Typical titrations for C'_3 of human, pig, and guinea pig sera are given in Fig. 1, which shows that pig serum gives the greatest limiting extent of hemolysis, although at low concentrations it is not as effective as guinea pig serum. Human serum shows only a trace of activity in this system. One would expect, irrespective of the concentration of C'_3, that the final extent of hemolysis would depend only on the fraction of cells in the state $EAC'_{1,4,2}$ which require presumably only C'_3 for lysis. Therefore, the titration curves for all sera would be expected to have a common limiting end point.

In an attempt to account for these differences, the role of inhibitors in this step was investigated (*3*). The addition of sheep serum to guinea pig serum produces inhibition at this step, resulting in a decrease both in the initial slope and in the limiting value of the curve. The differences observed at low concentrations in the curves for pig and guinea pig sera (Fig. 1) might therefore be explained by the presence of inhibitors in pig serum (*4, 5*). However, these same inhibitors cannot be invoked to explain the intersection of these curves and the greater extent of hemolysis produced by high concentrations of pig serum.

Pig serum was heated at 56°C for 15 minutes to destroy C'_1 and C'_2 activity and titrated together with normal pig and guinea pig sera. Heating the pig serum had only a slight effect on its reactivity with $EAC'_{1,4,2}$, and the limiting extent of hemolysis obtained remained greater than that obtained with normal guinea pig serum. Evidently C'_1 and C'_2 are not responsible for the increased reactivity of pig serum.

Classical reagents for the titration of the components of C′, namely R_1, R_2, and R_4, which are deficient in C'_1, C'_2, and C'_4, respectively, were prepared from pig serum (*6*) and titrated. The results are shown in Fig. 2. These reagents, which are nonhemolytic when added to equivalent concentrations of sensitized cells, gave final extents of hemolysis greater than that of normal guinea pig serum. This confirms the observation that C'_1 and C'_2 are not involved and indicates that C'_4 is probably not responsible either.

Pig serum was fractionated by column electrophoresis on powdered cellulose. One fraction, which was found among the β-globulins, did not lyse $EAC'_{1,4,2}$ in the presence of EDTA, but it enhanced the activity of guinea pig serum in this system. Pig serum therefore contains a factor which reacts in the presence of EDTA and is responsible for the increased activity of pig C′.

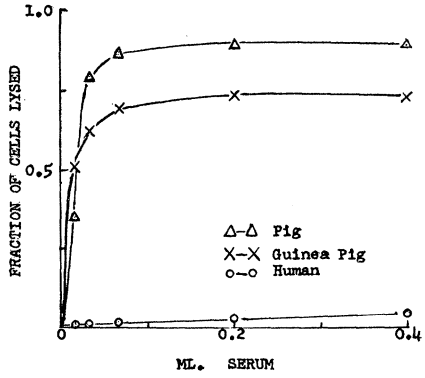

Fig. 1. Lysis of $EAC'_{1,4,2}$ with different sources of C'_3.

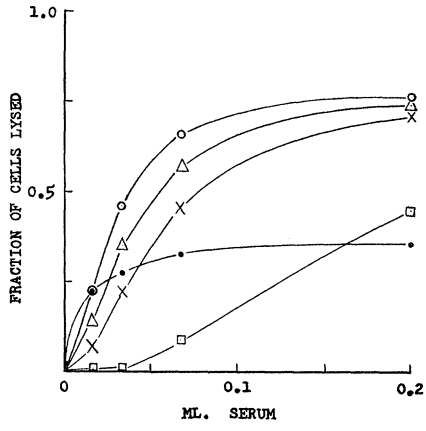

Fig. 2. Lysis of $EAC'_{1,4,2}$ with classical reagents derived from pig serum compared with lysis with guinea pig and pig sera. Open circles, pig serum; triangles, R_4 reagent; crosses, R_1 reagent; squares, R_2 reagent; solid circles, guinea pig serum.

The lysis of $EAC'_{1,4,2}$ appears, therefore, to involve a component, other than C'_3, whose properties do not coincide with those of C'_1, C'_2, or C'_4. This component, necessarily present in guinea pig serum, is not reactive in the presence of 0.017*M* EDTA, while its counterpart in pig serum is active. In this respect it differs from the dual C'_3 factors that Rapp (*7*) found in guinea pig serum, which, when mixed, are active in the presence of EDTA.

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5 August 1957

Multiple Dipole Representation of the Human Heart for Vectorcardiography

Vectorcardiography has progressed to the point where designers of lead systems claim to take into account a multiple dipole representation of the heart. This is stated to be an improvement over considering the heart as a single dipole source, since the heart's volume is indeed appreciable compared with its distances from measuring electrodes, and the electrical activity is spread throughout the heart's wall. The spatial vector loop is then interpreted as a summation of individual dipole contributions, where the perfect lead system is defined as one which gives equal weight to dipoles regardless of their location within the heart volume.

This interpretation leads to pitfalls which bear investigation. Actually, the resulting loop does not contain all the available information concerning a complex heart generator but, indeed, it responds to only the dipole component of such a generator. Only if the heart can be accurately represented by a single dipole of fixed location can a vector loop by itself completely describe its electrical behavior. It is a common misconception among vectorcardiographers that separately located dipole sources can accurately be accounted for by the locus of a single vector loop; for if this were true the next logical (and legitimate) step would be to attribute the loop to a single equivalent dipole.

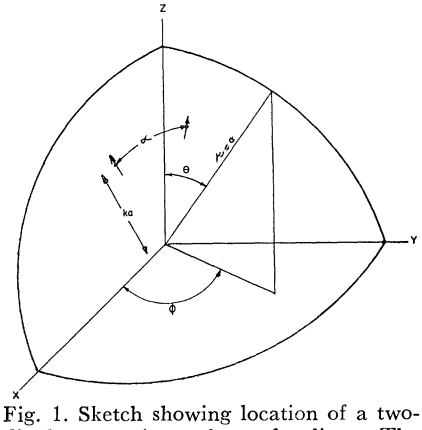

Fig. 1. Sketch showing location of a two-dipole source in a sphere of radius a. The dipoles are located and oriented in the x-z plane. The eccentricity is given by k, which is the percentage of the radius a, and the angular separation is given by α. The positive sense of the dipoles is given by the direction of the arrows, which in this sketch represent radially oriented dipoles; θ is measured from the z axis, and ϕ is measured from the x axis in the xy plane.

The purpose of this study is to show that separately located dipole sources in volume conductors can produce markedly different boundary potentials compared with those produced by a single equivalent dipole source. This single equivalent dipole is assumed to be the dipole component of the complex generator; the difference in boundary potentials represents available information to which the previously defined perfect lead system fails to respond.

Accordingly, boundary potentials produced by two dipole sources in a conducting sphere were calculated and compared with potentials produced by a single equivalent dipole. The equivalence was determined after the Gabor and Nelson (*1*) formulation of equal surface integrals of potential had been applied to a two dimensional disk conductor. The published formulas for two dimensions were found to be in error concerning signs; they should read (*2*):

$$XM_\gamma + YM_x = \gamma \int V(y\mathrm{d}y - x\mathrm{d}x)$$

$$M_x = \gamma \int V\mathrm{d}y\,;\; M_y = -\gamma \int V\mathrm{d}x$$

$$XMy + YM_x = \gamma \int V(y\mathrm{d}y - x\mathrm{d}x)$$

where X and Y are coordinates of equivalent dipole location; M_x and M_y are the component strength of the equivalent dipole; V is boundary potential; and γ is the conductivity.

When these corrected formulas were applied to two dipoles in a circular disk, the equivalent dipole had a strength equal to the vector sum (taken at the same origin) of the individual dipoles and a location based on a "center of gravity" consideration. When these results were extended to the sphere, the strength of the equivalent dipole was still the vector sum of the two dipole sources, and its location was taken as the "center of gravity" of the two sources (Fig. 1). There is, of course, no assurance that this equivalent dipole or one found by actually performing the indicated integrations for location gives the best match as far as actual surface potentials over the entire sphere are concerned. However, the results show that no single dipole can accurately match the two dipole potentials.

The results are shown in Fig. 2 for radial components and in Fig. 3 for tangential components. Two equal strength dipoles located at 40 percent of the sphere's radius and separated by 60 degrees were used since this represents extreme conditions. It is obvious that large errors are inherent in recording only the single dipole component of a two dipole generator. The errors are larger for radially oriented dipoles than for tangentially oriented ones. The comparisons are, of course, worse for increasing eccentricity and angular separation and are perfect for either zero eccentricity or zero angular separation. It should be noted that, if four radial dipoles are symmetrically placed on a cone of revolution, the comparison is vastly improved. Four dipoles, located and oriented thus, simulate a uniform double-layer source, which Frank (*3*) has shown to be very similar to a single dipole.

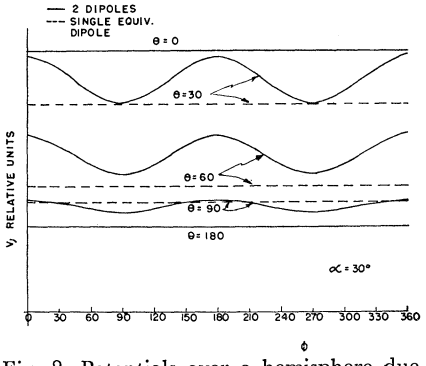

Fig. 2. Potentials over a hemisphere due to two radially oriented dipoles compared with a single equivalent dipole; ϕ and θ are spherical coordinates illustrated in Fig. 1. The dipoles are located and oriented in the planes of $\phi = 0$ degrees and $\phi = 180$ degrees, with an eccentricity of 40 percent of the radius and an angular separation of 60 degrees. The positive sense is away from the origin for both dipoles. Note that $\theta = 0$ and 180 degrees are single points on the sphere surface.

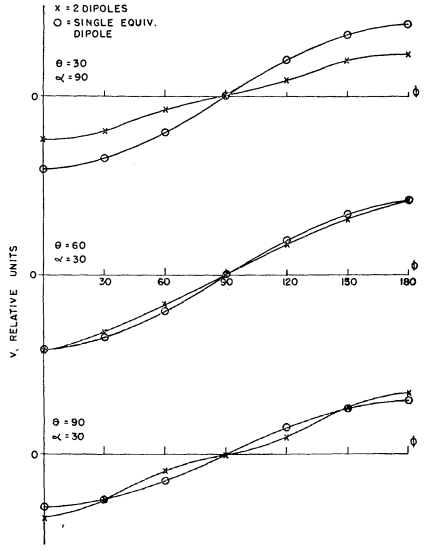

Fig. 3. Same as Fig. 2, except that the dipoles are tangentially oriented with an additive sense.