also reveal certain wholly unexpected phenomena. In those instances where the coagulation time has been delayed by drugs *in vivo* or *in vitro*, there is an inordinate increase in resistance preceding clot formation when the latter is measured by the Lee-White method. Fig. 2 depicts one example where some intravascular defects were anticipated but wherein the extravascular clotting time was not greatly delayed. The blood specimen taken before medication exhibited a very high initial resistance with an accelerated change in resistance before coagulation. The accompanying curves show the alterations in the blood of this patient after administration of heparin.

These data appear to warrant further intensive investigation of the electrical resistance shifts in freshly drawn blood; such an investigation is under way in our laboratories. For the present we offer the speculation that this increased and increasing resistance is a consequence of the orientation or unfolding of certain plasma proteins in such manner as to form electrically neutral and unionized dipoles. The conductance of the proteins themselves might be thus inhibited, or

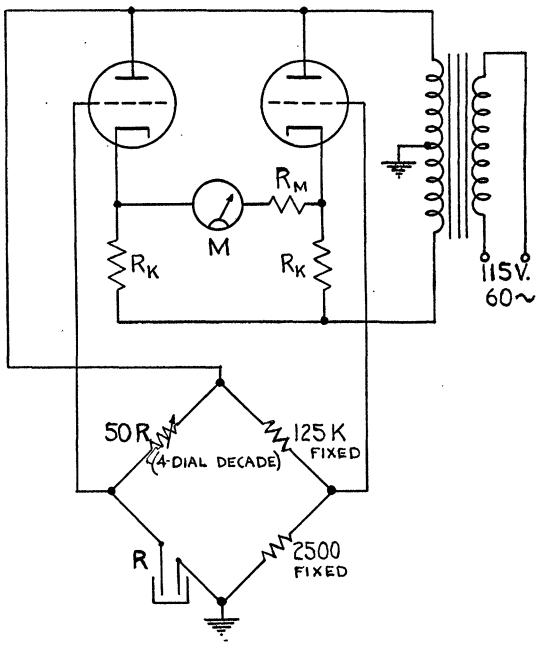

FIG. 3. Alternating-current electrolytic resistance bridge.

the plasma electrolytes might be bound in the hypothetical double layer. The physiological significance of this phenomenon remains to be seen, but it is probable that the latter is affected significantly by the charge of the container—in this instance, pyrex glass. The hypothesis appears to be susceptible to experimental exploration.

The measurements of electrolytic resistance were made with an alternating-current bridge[1] especially designed for this purpose (see Fig. 3). The use of 60-cycle alternating current eliminates polarization in the cell. The voltage across the cell is constant at balance and is equal to about 5 volts r.m.s.; this corresponds to a maximum power dissipation of $\frac{1}{8}$ watt in the cell. In order to minimize the possibility of modification of the reactions in the cell arising from the passage of the measuring current, a switch is provided for closing the bridge energizing circuit only when a reading is taken.

A vacuum tube phase-detector and amplifier is used to amplify the bridge output voltage. A zero-center microammeter connected to the amplifier output is calibrated directly in the percentage by which the cell resistance deviates from the value indicated by the four decade dials of the variable arm of the bridge. The scale of the meter is graduated linearly from −15 to +15 per cent deviation. Cell resistances within the range of 200–10,000 ohms may be measured. The instrument is compact and portable and may be plugged into the house current supply at the bedside.

The conductivity cell constant was approximately 12 reciprocal cm.; this brought the measured resistance into the most accurate range of the bridge. Since the frequency is low and the electrodes small, it is obvious that the inductance and capacitance factors are minimal; certainly, the change with time of these parameters must be negligible except in so far as they might be affected by the speculative double layer.

[1] Manufactured by Matthew Conrad, Rahm Instruments, Inc., 12 West Broadway, New York City.

Tracer Micrography[1]

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In the well-known method of radio autography a radioactive isotope is introduced in a biological or other system, and the distribution of that particular element within the system is determined by bringing the sample in close contact with a photographic emulsion. This method lacks resolving power because, even in the case of perfect contact of the sample with the photographic emulsion, the circle of confusion produced from every point of emission is so great that details less than .1 mm. are very hard or impossible to distinguish.

In order to improve the resolution of this tracer method, electron optical image formation was used for the determination of distribution of a radioactive element within a given sample. This is based on the emission of high-speed electrons by many tracer elements and the use of electron optical lens elements for forming an image on a photographic plate or other suitable recording surfaces. In the absence of any method for correction of the chromatic aberration of electron optical lenses, the first attempts were limited to elements which are emitters of monokinetic β-rays (internal conversion electrons). After some attempts with Cb^{93}, Y^{87}, $Sr^{85,87}$, and Pa^{233}, Ga^{67} was selected for the tests. Gallium chloride was prepared by chemical separation from zinc, bombarded by deuterons in the Department of Terrestrial Magnetism cyclotron, and the solution evaporated drop after drop on a $\frac{1}{4}$-inch tantalum

[1] The early phase of this work was carried out while one of the authors was attached to Stanford University. During that time work was carried out under a Rockefeller grant and with a considerable amount of collaboration on the part of J. Hamilton and E. Segrè. Their cooperation and the help of the Rockefeller Foundation is gratefully acknowledged.

disk. The latter was used as the emitting surface at a distance of about 3½ inches from a photographic film. A magnetic lens consisting of an iron-clad coil with Armco iron pole pieces was used for forming the image. Fig. 1 shows the pole pieces of

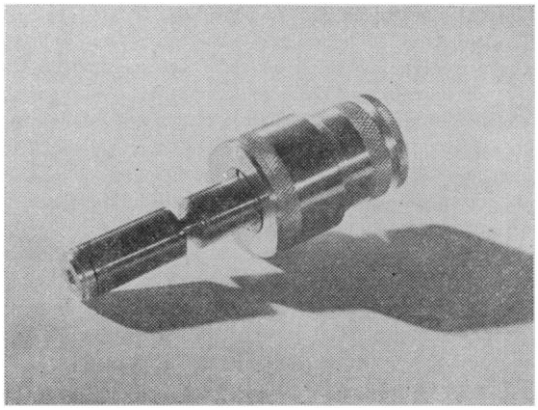

FIG. 1

the magnetic lens, with a specimen inserted at left and a simple film holder attached to the right side. The conditions were selected so that a linear magnification of 1.6× was obtained. Vacuum requirements are very moderate; the mean free path of the electrons is large compared to the apparatus dimensions, even at forevacuum pressure. Preliminary experiments were made with samples of different concentration and thickness of the radioactive layer. Exposure times ranged from 2 to 12 hours, according to the age and concentration of the sample and the numerical aperture of the lens. As a quantitative example, the following may be given: 1 millicurie/mm.^{2} of Ga^{67} at a numerical aperture of 0.04 rad. and a magnification of 1.6× gives a satisfactory photographic density with 1-hour exposure. For the most part, Process-type emulsions were used for the recording of the image. Tracer micrographs can be obtained consistently with good definition if the layer is sufficiently thin to avoid considerable self-absorption. The best resolving power obtained thus far is about 30 μ. Fig. 2 shows comparative exposures of the same speci-

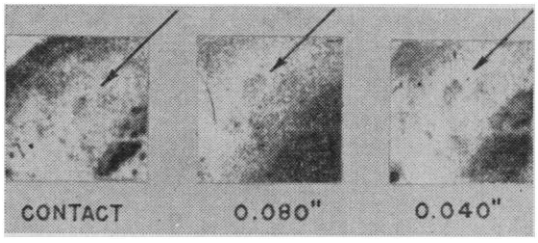

FIG. 2

men both by the radio autograph and by the electron optical method. The electron optical exposures shown are taken with two different limiting apertures having diameters of 0.040 and 0.080 inch, respectively.

Further improvements of the method include an afteracceleration of the β-particles. By such afteracceleration we hope not only to reduce the exposure times but also to achieve a better resolution through a reduction of the spherical aberration. A further reason for afteracceleration is that the chromatic aberration, which is always present (even in the case of monokinetic sources due to self-absorption), can be reduced markedly if the accelerating potential is at least comparable to, or greater than, the energy of the primary emission.

Staining of Nerve Endings in Mouse Epidermis by Feulgen's Nucleal Reaction

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So far as the writer is aware, there is no satisfactory method for the demonstration of nerve endings, especially in the epidermis. Most impregnation methods using metallic salts are tedious, fickle, and expensive. The chief objection is that the end result, even in successful preparations, though sometimes very beautiful, shows not the nerve itself but the black precipitate of silver salts.

During the study of the effect of methylcholanthrene on nerves in mouse skin, as part of a research project organized by E. V. Cowdry, of the Barnard Hospital, the writer found that nerves and nerve endings can be stained by Feulgen's nucleal reaction. Aside from being easy to operate, less time consuming, and quite specific, this method produces no precipitate. The technique is as follows:

Separate mouse epidermis from dermis by the heat method described by Baumberger, Suntzeff, and Cowdry (*1*), fixing it in 1 part formalin and 9 parts absolute alcohol saturated with picric acid (*2*). After carrying the tissue into water, hydrolyze and stain as for thymonucleic acid. The reagents used should be prepared as specified by Cowdry (*3*). It is not necessary to wash out the picric acid completely. Employ graded alcohols and xylols for dehydration and clearing. Mount in balsam for examination *in toto* or embed in paraffin for sectioning. Staining must be carried out before embedding; otherwise, the stainability of the nerves is somehow impaired. No other precautions are necessary.

Epidermis prepared in this way shows nerve fibers together with their endings and cell nuclei. This coloration of nuclei is an advantage rather than a hinderance, for the relation of nerve endings to the surrounding cells is thereby clarified. The cytoplasm and other structures may be counterstained with fast green. Slides first prepared in October 1945 are still in perfectly good condition.

Liang (*4*), also of this laboratory, recently found that nerves can be stained by Schiff's reaction. Consequently, the process of hydrolysis may be omitted. The mechanism of staining is unknown. However, unless nerves and nerve endings are proved to contain thymonucleic acid, the specificity of nucleal reaction for this chemical compound is questionable.

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