The Bioelectric Field Pattern in the Salamander and Its Simulation by an Electronic Analog*

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Summary—**The dc field potential has been determined on the surface of the intact salamander, *Trituris viridescens*. A complex field was found which did not correspond to a simple dipole. The field was found to vary in a dynamic fashion with changes in the level of anesthesia. The spatial organization of the field correlated well with the anatomical organization of the central nervous system of the animal, in that areas of nerve cell aggregations within the central nervous system appeared to serve as both sources and sinks of the potential, with transmission of the steady potential along major nerve trunks. An analog model of the central nervous system was constructed utilizing several bimetallic junctions and a conducting network. This model produced a field in a modified electrolytic tank which corresponded well with the field found in the intact animal. It was concluded that the dc bioelectric potential in organisms may be generated within and conducted by portions of the central nervous system. The possibility that this is a means of data transmission and/or control (in addition to the usual action potential system) is being evaluated.**

THE TERMS "bioelectric field" or "steady state potentials" refer to the dc field gradients which can be found in intact organisms or across portions of living organisms. This concept must be clearly separated from the "action potential" or "spike" which is a transitory electrical disturbance characterizing activity of the nervous system and from the "membrane potentials" which refer to the potential difference between the interior of a cell and its external environment. A great deal of information has been accumulated relating to bioelectric field potentials. The field has been shown to correlate with growth in animals [1], in plants [2], and with the phases of spinal shock [3]. Externally applied fields have been shown to produce tumor regression in mice [4] and to influence the migration of spermatoza [5]. The field has been proposed as the basic factor producing the organization of the organism [6], [7]. However, a fairly extensive review of the literature has not revealed any definite facts pertaining to the sources of the field potentials, and no accurate plots of the field organization in the intact animal have been published. It has been generally assumed that the field resulted in some way from the total aggregate of cells in the organism and that it approximated a simple dipole.

Since dc potentials appeared to correlate well with growth phenomena, a study of the "current of injury" in regenerating forms was planned by the author. It appeared to be desirable first to determine the configuration of the field in the intact organism and then to correlate changes due to the injury potential using the intact field plot as a frame of reference. Methods were worked out to measure the field potentials with sufficient accuracy to draw equipotential plots.

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Method

Silver-silver chloride electrodes were used exclusively in this study. These were relatively low impedance devices (average resistance 50 K) and were equipped with a terminal tuft of saline soaked nylon wicking. (It had been noted early in the study that variations in the pressure with which the electrodes were applied to the skin surface produced great variations in the recorded potential; the terminal wick completely alleviates this difficulty.) After the preparation and aging, paired electrodes can be selected with steady polarization under 100 μv. No attempt was made to null out this polarization since the potentials recorded were greatly in excess of this. The electrodes were clamped in plexiglas rods; stationary electrodes were held in place by a simple sterotaxic type instrument. The remainder of the electronic circuitry was a standard type chopper-stabilized dc amplifier (Hewlett-Packard Model 425A) with an input impedance of 1 megohm. Readout was by means of the center zero meter of the instrument, or the ouput was fed to a calibrated strip chart recorder (Easterline Angus Model AW).

The animals used were adult aquatic-phase *Triturus*, selected from a stable laboratory population. No ill effects were incurred from the procedure and all experimental animals are still alive and well.

Anesthesia was produced by titration with Tricaine (Tricainemethane-sulfonate-Sandoz) in conditioned (oxygenated, dechlorinated) tap water. Titration was found to be superior to a measured dose in view of the variation in size of the animals. This method produces a stable period of anesthesia averaging 30 ± 3 minutes regardless of size of the animal. The animals were removed from solution, rinsed in conditioned tap water, and placed in the prone position on a small pledget of moist cotton. The skin surface was permitted to drain for a minute or two prior to making determinations. It was found that under the experimental conditions the skin surfaces remained slightly moist throughout the procedure, but the shunting effect of a large fluid volume (such as would be present if the animals were totally immersed) is alleviated.

Actual measurements were done in three different ways:

1) The indifferent electrode was placed over the center of the head, and the recording electrode was placed at

various points on the body surface. Readings were taken from the meter for each spot and recorded in the appropriate place on an outlined scale-drawing of the animal. This numerical plot then gives the potential difference for any area measured, with reference to the center of the head. A complete determination required 3-5 minutes.

2) The indifferent electrode was again placed at the center of the head. The recording electrode was placed on the cephalad tip of the animal and slowly moved backward along the midline, at a uniform rate, to the tip of the tail. The output of the amplifier in this case is to a strip chart recorder. Appropriate relays were used to make identifying marks on the record to establish correlation between the recorded potential and surface anatomy. The graph obtained is then equivalent to the potential between any spot on the midline of the animal and the center of the head. Approximately 20-25 "sweeps" of the long axis can be made by this method during one anesthesia.

3) For equipotential plots the indifferent electrode was placed at random on the body surface, and the recording electrode was moved on the skin in the near vicinity, searching for a null point between areas of relative positivity and negativity. This is a time-consuming procedure and generally only one plot of this type can be made during one anesthesia.

All determinations were made within a double-walled Faraday cage and interference effects were not apparent, with the technique used.

Results

1) Numerical field plots were initially done, and three facts were immediately evident. First, the brachial and the pelvic areas were uniformly less negative than the remainder of the organism. In some instances they were at the same potential as the center portion of the head. Second, during the anesthesia, changes would occur in both the magnitude of the potentials and in the polarity of various areas. Generally the basic pattern was adhered to, and changes were in the nature of exaggerations of former potentials. In all instances the polarity changes occurred in anatomical "blocks"; *i.e.*, the pelvic area of the body would become positive in potential. No random changes were noted. Third, in all instances the extremities were negative compared to the head, the negativity increasing toward the distal portions. In many cases the base of the limb or tail would be isopotential with the head center. A typical numerical field plot is reproduced in Fig. 1(a).

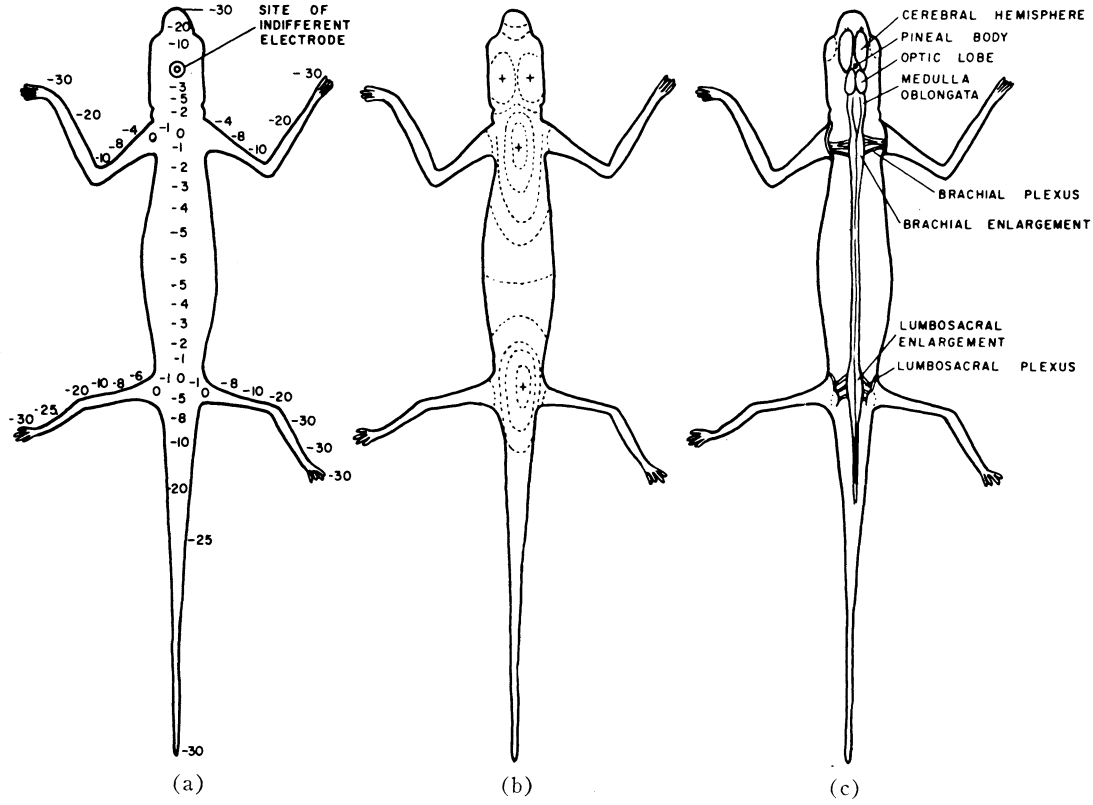

Fig. 1—(a) A representative numerical plot as obtained by method 1). In this case, the pectoral and pelvic areas were equipotential with the head center, and similar "nulls" were found at the bases of three limbs. There was a small but definite negative potential between the limb base null and the midline null. (b) Equipotential field plot as obtained by method 3. The dotted lines are field lines; the "+" within the closed loops indicates polarity referable to local areas outside of the closest field line. (c) Schematic representation of major areas of the neuraxis to general body topography. No great accuracy is claimed for details of the brachial or lumbo-sacral plexi.

2) Midline sweep plots done according to method 2) produced the same type data. Two consecutive sweeps from one experiment are shown in Fig. 2. The drops in the negative potentials at the pectoral and pelvic areas are well illustrated. During the course of an experiment, changes could be seen in the magnitudes of the potential in some areas, although the general over-all pattern was evident. Fig. 3 illustrates the usual changes at intervals of four minutes throughout one experiment. In the majority of the animals studied, there was either a drop in the negative potential of the head or an actual reversal of the polarity of this area just prior to recovery from anesthesia. It is believed that the high potentials initially recorded are due to anesthetic induction, since there was a uniform drop in the potentials prior to anesthetic recovery. In a few individuals which remained passive after recovery from anesthesia (permitting a few midline sweeps to be done) the potentials remained at the same levels as just prior to recovery.

3) An inspection of the numerical and midline recorded plots seemed to indicate that relative sink areas were located over the center of the head, and the centers of the pectoral and pelvic girdles. Equipotential field plots were then done and the results were in accord with this concept. Unfortunately, it was not possible to do this type of determination on the extremities due to their small size. A more detailed equipotential plot can probably be made on amphibia of larger size. Nevertheless, certain aspects of the field are quite clear; the center of the head appears to have two positive sink areas, roughly equipotential. The pectoral girdle is also relatively positive although less than the center of the head and the pelvic girdle is even less positive. Fig. 1(b) illustrates a typical equipotential field plot. It is not noted, but generally nulls were obtained between like areas of the right and left extremities.

4) Several salamanders were dissected to establish the relationship of areas of the neuraxis and body surface areas. (Agreement with the figures and descriptions in Francis [8] were obtained). Fig. 1(c) is a schematic representation, to scale, of the gross aspects of the central nervous system referable to body surface anatomy. It can be seen that the optic lobes and the brachial and pelvic enlargements of the cord correlate quite well with areas of positive sinks as determined by the field plots. The importance of the optic lobes, *per se,* may be exaggerated somewhat in this correlation since they generally have a more dorsal or superficial position relative to the rest of the brain. They are, nevertheless, quite well developed in the salamander. The pectoral and pelvic enlargements are aggregations of neurones producing the nerves going to the fore and hind limbs, respectively. These cells are, however, located primarily on the ventral aspect of the cord in these areas, the dorsal aspects consisting of various fiber tracts. Several attempts were made to locate the negative sources on the ventral aspect of the animals, and in all cases the

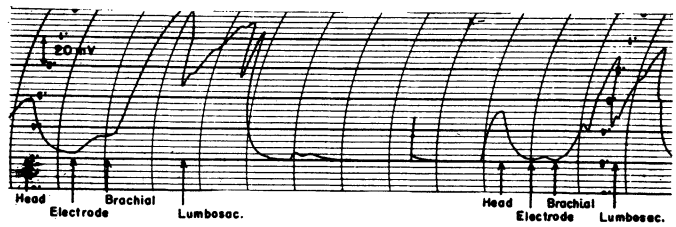

Fig. 2—Two representative, sequential, midline "sweeps" of potential referable to an indifferent electrode at the head center. Line 5 represents zero potential and the area above it in this recording is negative potential. The decrease in negative potential at the brachial and lumbo-sacral areas is evident.

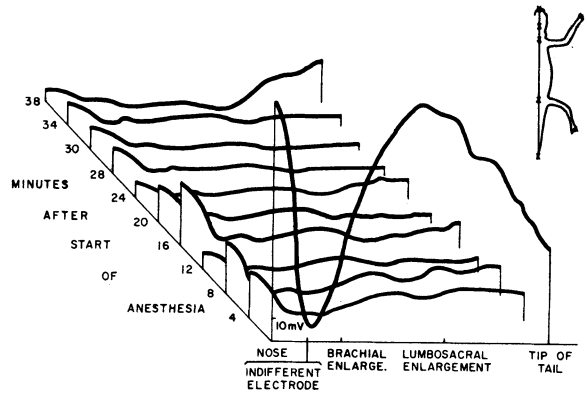

Fig. 3—Representative midline sweep records [method 2] at intervals during anesthesia. The initial curve was taken immediately following full induction. The remainder of the curves are sequential, at 4-minute intervals, until full recovery from anesthesia. At 30 minutes, rhythmic tail movements began, and between 38–39 minutes coordinate ambulation began and recovery was assumed to be complete. The changes in the potential shown (high potientials immediately following induction and lowering of the head potential just prior to recovery) are typical.

midline was generally equipotential, with increasing negativity as one deviated to either side with the recording electrode. Anatomical considerations would indicate difficulty in localizing any deep sources of potential since the visceral cavities would lie between the skin and the neuraxis.

5) In view of the apparent relationship of neuraxial structures and the bioelectric field, the possibility of reproducing the field pattern by an electronic analog was explored. To approximate the spatial relationship of the organism a scale model was constructed of high purity sponge rubber [9]. After several washings in tap water the saturated model was checked with the same electrodes as used for the *in vivo* determinations. The basic polarization voltage of the electrodes was recorded throughout, with only slight diminution in areas distant from the indifferent electrode. The model was then horizontally split lengthwise and the components under test inserted. Since dc excitation was used, polarization effects would occur within 3-5 minutes and a new model was made up for each set of components. The circuits illustrated in Fig. 4 did not produce a pattern resembling that found *in vivo.*

It was then decided to make use of the bimetallic generator principle, and a model was constructed as is shown in

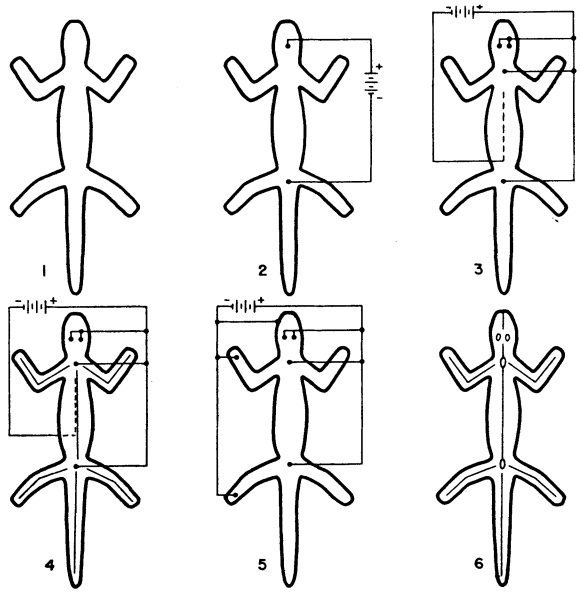

Fig. 4—Representative analog models that did not produce the desired field. Battery voltage in each case 1.34 volts, tap water used as electrolyte.

1) Simple model with no excitation voltages; no changes were noted in electrode potentials regardless of orientation.
2) Simple dipole.
3) Four positive sink areas, negative source supplied by buried copper bus running axially in the center of the model.
4) Same as 3) but with addition of wire "nerve" network.
5) Four positive sinks with negative sources applied at extremity tips.
6) Bimetallic internal sources plus wire "nerve" net with limb "nerve" extensions unconnected.

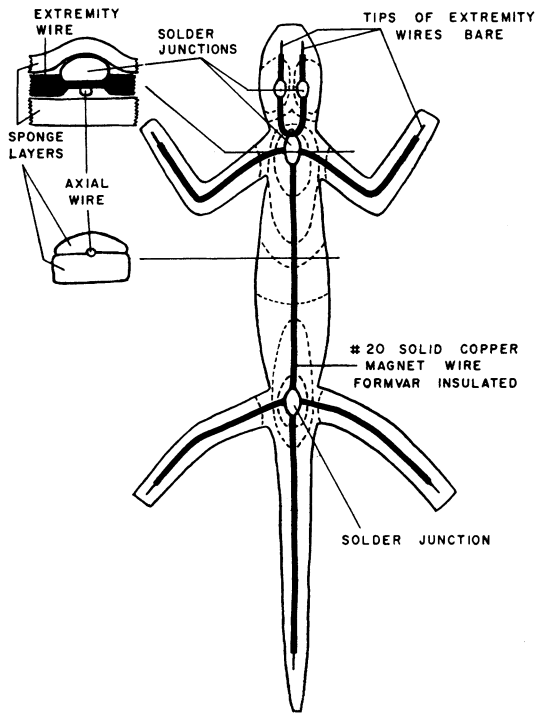

Fig. 5—Phantom view of analog model successfully simulating the field. The lower-most small insert is in error in that the axial wire was insulated throughout its length except at bimetallic junctions and at the tip of the tail. The equipotential field is represented by the dashed lines. The similarity between the bimetallic nodes and the conducting network and the central nervous system is evident.

Fig. 5. The solder junctions were applied in such a manner that the ventral surface of each copper-solder "node" was exposed copper, not solder covered. Each node produced considerable potential when immersed in tap water, the solder being positive and the copper negative. If the copper wire was prolonged out from the node and left insulated except at the tip, a null point was found a few millimeters from the node with increasing negativity towards the tip of the wire. Direct contact was made between all copper wires in the model. While the voltage output of this model was higher than that generally found in the animal, the field organization was entirely similar with positive sinks at each node and with increasing relative negativity extending outward along each extremity axis. The numerical field plots [method 1] in these analogs were quite similar to those obtained in the intact animal except that the limb base null was not always obtained. This was apparently a factor of shape of the extremity since in two models of this type one extremity in each duplicated the field pattern so accurately that a null point was reproduced at the junction of the extremity and the body. The similarity between the analog and the actual anatomical relationships of the central nervous system was striking. The bimetallic junctions coincided with the optic lobes and the brachial and lumbo-sacral ganglionic enlargements. The failure of the other analog models illustrated is considered significant because they had much less similarity to the central nervous system. Application of the negative and positive poles at the appropriate areas with or without the wire "nerve" net was unsuccessful. Only when discrete internal sources were introduced into the field along with the "nerve" net was a comparable field obtained. It is of interest that the circuit was continuous and no discontinuities, which would represent synaptic junctions, were present. If attempts were made to introduce such gaps, considerable distortion of the field occurred with loss of the negative extremity potentials. (Obviously the spatial relationships of the synapse itself cannot be simulated.)

Discussion

Several tentative conclusions appear justified:

1) The bioelectric field in the salamander is a complex field rather than a simple dipole.
2) The spatial organization of the field correlates with the anatomical organization of the central nervous system.
3) An electronic analog of the central nervous system produces a similar field.

Therefore, the hypothesis can be made that the generating sources of the bioelectric field in the salamander are certain ganglionic cell aggregates in the neuraxis and that the potential is carried by at least some of the axons.

If this is true, then one method of checking the point would be to section all of the nerves going to a limb. This has been done in a small series, and in all instances so far there has been a drop in the negativity at the tip of the limb to approximately 10 per cent of its former value in 30 to 60 seconds. If the nerves to both forelimbs are sectioned at the limb base, the negative potentials in both forelimbs will drop but the remainder of the body potentials (including that over the brachial enlargement) will remain the same. If then the animal is "pithed" (the spinal cord sectioned at the lower level of the medulla oblongata) the negative potentials of the midthorax and the tail will fall, the former almost to zero. However, the potentials previously recorded over the brachial and lumbo-sacral neurone centers will remain the same as prior to pithing. Furthermore, the negative potentials in both hind limbs will remain elevated. Section of the nerves to the hind limbs will then produce a prompt drop in their potential. Apparently structures cephalad to the site of cord section have some control over the negative potentials of the thorax and tail, but the extremity potentials arise from within their respective nerve cell aggregates within the cord.

The skin itself is known to produce a potential oriented, however, from the inside to the outside [10]. The skin also is productive of injury potentials, as discussed in the section on methods dealing with pressure effects of the electrodes. It is felt that injury potentials are not a factor in the present study. It would further appear most unlikely that the complex field pattern described, with such close correlation with central nervous system structures, could be produced by skin potentials or variations in skin resistance alone. In the nerve section and cord section experiments, as small a skin incision as possible was utilized, yet marked potential changes were produced in areas supplied by the sectioned nerves at considerable distances from the skin incision.

Burr [1] has reported an increase in the total potential (nose to tail) with increasing growth in embryonic amblystoma (larger salamanders). In attempting to delineate the field pattern in very young embryos, considerable technical difficulties were encountered, due primarily to the small size of the organism. However, the midline sweep technique has been applied to a small series of Amblystoma embryos in the early stages. In the 5-mm stage (the body being roughly ovoid but with a definite cephalo-caudal relationship) the field appears to be a dipole with no discrete features discernable with the present technique. The 8-mm embryo (with a well-formed tail bud and early gill buds) demonstrates a definite positive going notch in the record corresponding to the lumbo-sacral area. Embryos of 8 to 11 mm length show considerable growth in gill and eye structures and further elongation of the tail, but the field patterns appear to show only the lumbo-sacral notch of the 8-mm although more well developed. In the 12-mm embryo definite growth begins in the forelimb buds and the midline potentials now show a notch at the brachial area as well as the pelvic area. On the basis of these preliminary observations, the appearance of complexities in the field (similar to the adult pattern) coincides with the growth of axial appendages. To correlate these field changes with the development of nerve cell aggregates in the embryo will require serial sectioning of various stage embryos and a comparison of the field with the corresponding sections. Such a study is contemplated. Previous studies on the correlation of the bioelectric field and growth have been based upon the assumption that the field is a uniform dipole and is not associated with any definite anatomical structure. It was therefore thought to be concerned with cephalo-caudal "polarity" and growth along this axis. Marsh and Beams [11], were able to reverse the cephalo-caudal relationship in regenerating planaria by exposing the organisms to a dc field with reversal of the normal polarity. A direct relationship between the degree of cephalization of the tail portion and power densities was shown. It is possible that a similar, but correspondingly more complex, relationship may exist in the vertebrates between the field and body organization. Since the results of the present report would indicate that the bioelectric field is closely related to the nerve tissue of the organism, Singer's work [12] on the relationship of the nerves and regeneration ability becomes of considerable interest. He has shown a direct relationship between the number of axons of any type (sensory, motor, autonomic) in the amputation stump and the rate of regeneration. The neural effect would therefore appear to be a factor which is associated with nerve activity in general. In view of the many examples of growth control in lower forms by dc fields, the bioelectric field activity of the central nervous system would seem to be a likely candidate for the factor influencing regeneration. At any rate, it does not appear to be unrealistic to evaluate the postulate that the central nervous system exerts a control over body organization, growth, and regeneration via its dc activity. Such control would mean the transmission of information by this activity, an obvious application of analog methods. Such dc analog activity has been postulated [13] as the most primitive property of nerve tissue (in contradistinction to the digital or pulse coded, all or nothing, spike potential which is well-known). However, the application of such postulates has been limited to local areas within the central nervous system. No such thesis has been advanced for an integrated, total body, steady-state system, subserving such primitive modalities as body organization, growth, orientation, etc. Furthermore, such a system would furnish investigators with a possible mechanism for such puzzling behavior as biological cycles [14], and precise migration over long distances [15].

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A Gas-Hemoglobin Diffusion Photometer*

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Summary—**A double-beam photometer is described which employs a single photocell and motor-driven shutter synchronized with alternately energized screens in a gated amplifier. The equipment is used for indicating and recording monochromatic light absorption changes by a thin layer of hemoglobin-containing media exposed to diffusion of various gases.**

THE uptake, release, and propagation of respiratory and other experimentally and clinically used gases in the organism are basic processes occurring in all living matter. These phenomena belong to the realm of diffusion processes and can be analytically treated according to the theoretical methods developed for such studies. In the case of the presence of substances which react chemically with gases (such as hemoglobin and myoglobin with oxygen and CO), simultaneous diffusion and chemical reaction are involved so that the mathematical treatment becomes more complicated. One of the present authors (Kreuzer) has directed his attention during the past ten years to the study of the oxygen uptake in the lungs. Man and many animals take up oxygen by the respiratory conducting system from the atmospheric air. The oxygen arrives, due to the respiratory movements, in the lungs and forms an essential component of the alveolar air which is the gas phase in contact with the gas exchange tissues of the lungs. From the alveolar air, the oxygen passes, according to its concentration gradient, through the alveolar epithelium, the interstitial tissue, and the pulmonary capillary wall; these three tissue constituents are summarized as pulmonary membrane by the respiratory physiologists. After its arrival in the pulmonary capillary blood, the oxygen diffuses through the blood plasma, crosses the membrane of the red blood cells, and penetrates into the interior of the red cell; this process consists of simultaneous diffusion through the cell and chemical reaction of the gas with hemoglobin. The hemoglobin in the red cells is present as a highly concentrated solution with a concentration of about 30-35 g per cent. From the lungs, the oxygen is carried by the blood to all tissues where it is released, again according to its concentration gradient, and provides the basic fuel for the oxidation processes of the cells.

The study of the oxygen uptake in the lungs is an old and classical field of physiology. Most workers in this field have been studying this process *in vivo* (in man or in animals) by measuring the oxygen uptake in the whole lung. It seemed interesting, however, to attack this problem also in the sense of a step-by-step analysis. The single steps involved were considered separately as relatively simple systems which could be investigated by *in vitro* model experiments.

For this purpose, Kreuzer and Betticher [1] have constructed a suitable apparatus. In this device, the hemoglobin-containing solution or suspension was horizontally spread out in a thin layer (50-1000 μ) in a flat trough on a glass plate with the upper surface uncovered so that the gas could diffuse from the surrounding closed chamber into the medium; the bottom and the top of the chamber were transparent. The changes in light transmission of the hemoglobin-containing medium caused by the uptake of the gases oxygen or CO were recorded photoelectrically and continuously, using appropriate filters or monochromatic light. From such tracings, the time course of the processes could be evaluated. Since the change in light transmission constituted only a small fraction of the total light coming

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