been purified approximately 300 fold by absorption and elution from diethylaminoethyl (DEAE) cellulose (*12*) followed by precipitation with 55-percent saturation of ammonium sulfate at 0°C. Moreover, when DEAE cellulose is developed with a linear NaCl gradient, the corresponding variant and normal LDH isozymes are eluted at similar points. The mobility of normal and variant LDH isozymes from crude hemolyzates is identical in the system (*13*) of varying starch-gel porosity. Such behavior suggests that the molecular weight of normal and variant isozymes is grossly similar (*14*). The enzymic activity of variant LDH from crude hemolyzate approximates that observed in normal American and Nigerian Negroes.

The only blood relative available for testing, a paternal uncle, has a normal LDH pattern. Whatever the origin of the observed LDH variation, it is uncommon (*15*) since no variant LDH patterns were observed among hemolyzates obtained from approximately 50 white subjects, 300 American Negroes, 100 Papuans (*16*), and 200 Nigerians.

Fritz and Jacobson (*17*) examined mouse tissue extracts and observed five LDH-5 components, four LDH-4 components, three LDH-3 components, two LDH-2 components, and one LDH-1 component in gels prepared with 0.005*M* β-mercaptoethanol. These authors suggest that this concentration of β-mercaptoethanol removes bound NAD from a portion of A subunits but not from B subunits. These observations provide a pattern exactly opposite to that in the Nigerian LDH variant. Moreover, the human variant LDH pattern is unaltered in gels containing β-mercaptoethanol in amounts ranging from 0.005 to 0.1*M*. The variant LDH pattern is also unaltered by electrophoresis under conditions where the gel and cathodic chamber contain $10^{-3}M$ NAD. Accordingly, we do not believe that the variant pattern is produced by the mechanism of differential binding of NAD.

The variant electrophoretic pattern can be most easily interpreted as reflecting a mutant allele at the genetic locus producing the LDH B subunit. The observed physical and enzymatic similarities between normal and variant LDH-1, that is, B subunit tetramers, support this interpretation. If the mutant product is designated β, and the normal product B, then a heterozygote at this locus should exhibit the combinations shown in the third column of Table 1. Such combinations will produce the pattern observed in Fig. 1 provided it is assumed that β is more electro-positive than B. Furthermore, if the mutant β and the normal B gene products are produced in equal quantity and thereafter randomly associate as tetramers, then LDH-1 of the heterozygote should contain B_4, $B_3\beta_1$, $B_2\beta_2$, $B_1\beta_3$, and β_4 in the binomial proportions 1:4:6:4:1. The proportions observed during enzyme development on starch gels roughly fit this ratio. Unfortunately diffusion of the localizing formazan dye prohibits satisfactory illustration. Such diffusion, close proximity of components, and the nonlinear relationship between dye intensity and enzyme concentration prevent exact spectrophotometric analysis. The expected fit is more evident among the other variant isozymes. By inspection, variant LDH-2 contains proportions which agree with a predicted 1:3:3:1. The components of variant LDH-3 and LDH-4 approximate the predicted ratios 1:2:1 and 1:1 respectively. LDH-5, if it were detectable, would be expected to be single and indistinguishable from the normal.

The variant LDH electrophoretic pattern is therefore consistent with the hypothesis that individual LDH isozymes are tetramers of two different subunits. The lack of demonstrable inheritance of the variant LDH necessarily limits this interpretation. A recent parallel to our observations is Markert's demonstration (*18*) that LDH-2, LDH-3, and LDH-4 can be generated in vitro by dissociation and subsequent reassociation of a mixture containing equal amounts of LDH-1 and LDH-5. In the reassociated mixture LDH-1, -2, -3, -4, and -5 occur in proportions 1:4:6:4:1. This is the same type of event which we believe has naturally occurred in vivo and produced five LDH-1 components from a mixture of mutant and normal B subunits (*19*).

Note added in proof: C. R. Shaw and E. Barto have described (*20*) inherited variation of LDH in *Peromyscus* which closely parallels the variant described here. Our interpretations are thereby corroborated.

SAMUEL H. BOYER
DAVID C. FAINER
Department of Medicine,
Johns Hopkins Hospital and
University School of Medicine,
Baltimore 5, Maryland
E. J. WATSON-WILLIAMS*
University College Hospital,
Ibadan, Nigeria

References and Notes

1. C. L. Markert and F. Møller, *Proc. Natl. Acad. Sci. U.S.* **45**, 753 (1959).
2. E. Appella and C. L. Markert, *Biochem. Biophys. Res. Commun.* **6**, 171 (1961).
3. C. L. Markert, "Hereditary, developmental, and immunologic aspects of kidney disease," *Proc. 13th Ann. Conf. on Kidney*, J. Metcoff, Ed. (Northwestern Univ. Press, Evanston, 1962); R. D. Cahn, N. O. Kaplan, L. Levine, E. Zwilling, *Science* **136**, 962 (1962).
4. O. Smithies, *Biochem. J.* **71**, 585 (1959).
5. A. Blanco and W. H. Zinkham, *Science* **139**, 601 (1963).
6. M. M. Dewey and J. L. Conklin, *Proc. Soc. Exptl. Biol. Med.* **105**, 492 (1960).
7. E. S. Vesell and A. G. Bearn, *J. Gen. Physiol.* **45**, 553 (1962).
8. S. H. Boyer, D. C. Fainer, M. A. Naughton, *Science* **140**, 1228 (1963).
9. M. D. Poulik, *Nature* **180**, 1477 (1957).
10. Starch granule electrophoresis was performed and K_m determined as described by E. S. Vesell and A. G. Bearn, *J. Clin. Invest.* **40**, 586 (1961).
11. Analog reaction rates were, in general, performed as described by N. O. Kaplan and M. M. Ciotti, *Ann. N.Y. Acad. Sci.* **94**, 701 (1961). Final molar concentration of DL-lactate was 1.3×10^{-2} and of NAD analog was 7.5×10^{-4}. Reaction rates were measured in the interval 20 to 60 sec after beginning incubation.
12. M. A. Hennessey, A. M. Wattersdorph, F. M. Huennekens, B. W. Gabrio, *J. Clin. Invest.* **41**, 1257 (1962).
13. O. Smithies, *Arch. Biochem. Biophys. Supp.* **1**, 125 (1962).
14. Gels were prepared with EBT buffer as described in (*8*). Human hemoglobin and myoglobin were employed as markers of known molecular weight.
15. Vesell and Bearn (*7*) observed no LDH variants among 380 individual human hemolyzates.
16. Samples from Papuans were provided by Dr. B. P. K. Ryan of Port Moresby.
17. P. J. Fritz and K. B. Jacobson, *Science* **140**, 64 (1963). These authors employ a nomenclature for LDH which is opposite to that adopted here. For example, their LDH-1 corresponds to our LDH-5. To avoid confusion, we have modified their designations so as to agree with the nomenclature employed here.
18. C. L. Markert, *Science* **140**, 1329 (1963).
19. Aided by grant B-2058 from the National Institutes of Health.
20. C. R. Shaw and E. Barto, *Proc. Natl. Acad. Sci. U.S.*, in press.

* Present address: Department of Hematology, Manchester Royal Infirmary, Manchester, England.

21 June 1963

Sweating: Its Rapid Response to Muscular Work

Abstract. *Marked increases in sweating rate occurred within 1.5 seconds after the initiation of muscular activity in a warm room. This increase did not seem dependent upon thermal stimuli, but was related to the work rate, and the ambient temperature, or the sweating rate during the pre-exercise period.*

Although many studies have been devoted to the regulation of sweat production in the working human, no completely satisfactory explanation has evolved. Part of the reason for this may be that little attention has been given to reflex mechanisms which could be involved, especially in the

initial activation of the sweat secretory mechanism. Nielsen (*1*) has reported that men working in a cool environment began sweating, as indicated by weight loss, prior to any detectable changes in skin and rectal temperatures. Robinson (*2*) found a fair correlation of sweating rate with skin temperature, but this correlation was altered by a change in work rate. In contrast, the independence of the sweating rate and skin temperature has been emphasized by Benzinger (*3*). It is his conclusion that sweat secretion, in general, is governed almost entirely by the thermal state of the hypothalamus, which is accurately reflected by the measurable "intracranial" or tympanic membrane temperature. However, others have not seen this precise relationship in conditions of both work and rest (*4*).

Some of the above studies (*3*) have been done under steady-state conditions. This is an unsatisfactory approach, as marked changes in sweating in the working man occur prior to the attainment of such a state. In other studies (*1, 2*) the sweating rate has been measured by loss of body weight, a method which is too slow and not sensitive enough to determine small and rapid changes of sweat production. The development of a satisfactory method for recording continuously the rapid changes in sweating rate has opened the way to study the nature of sweating under non-steady-state conditions. In this report we show that, under appropriate conditions, increases in the sweating rate of a working man may be due to a nervous mechanism, reflex or otherwise, not necessarily initiated by changes in skin or hypothalamic temperatures.

The sweating rate was continuously recorded by the method of resistance hygrometry (*5*). Air of preset absolute humidity was passed over two areas in enclosed cups covering 7 cm^2 of skin. Effluent air was led past sensitive elements that decrease resistance with an increase in relative humidity. These resistance changes were continuously recorded on a 1120 visicorder (Honeywell). The instrument was calibrated by comparing the recorded deflection with the weight loss of small, water-filled capsules in conditions very similar to those of the experiment. The time lag required for an increase in humidity in the skin cup to be sensed at the remote elements was 2.0 seconds.

Figure 1 is a tracing of a record, which reads from right to left, from an experiment performed at an ambient temperature of 37.5°C. The control periods, consisting of 1 hour of rest, were characterized by cyclic sweating activity at the rate of 8 to 10 per minute, which is in close agreement with the observations of others (*6*). The coincidence of the cycles in the two different regions is indicative of central nervous system mediation.

In Fig. 1 it may be noted that within 5 seconds after the start of exercise a definite increase in rate of sweating occurred. After correction for the 2-second time lag of the recording system, the actual delay for the increase in the sweating rate was approximately 3 seconds. Upon the initiation of very heavy muscular work, consisting of pedaling a bicycle ergometer at a maximal rate, the sweat production increased after a 3.5-second delay, as shown in

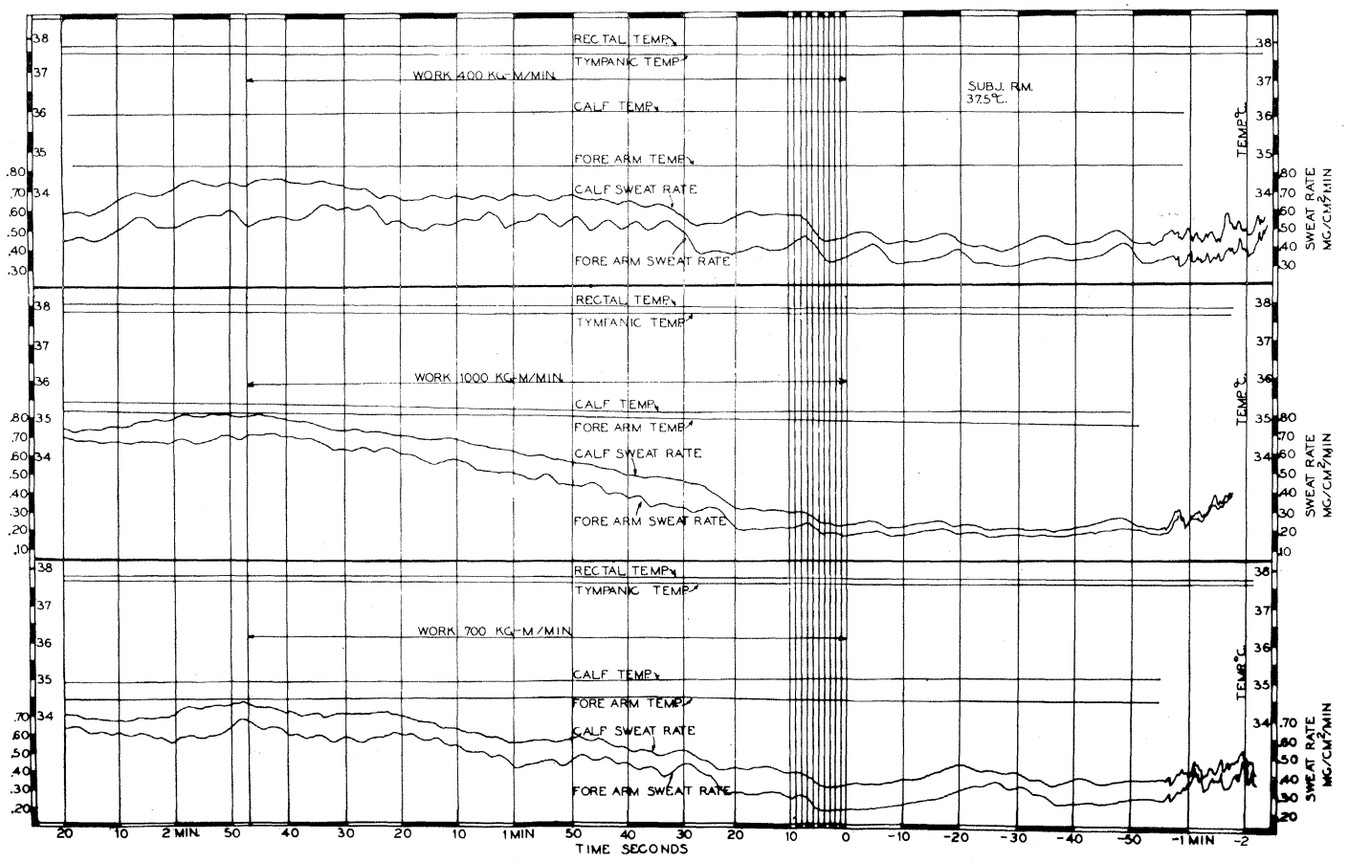

Fig. 1. Continuous recording of sweat production and rectal, tympanic, and skin temperatures, during rest and three different loads of physical work in a warm environment (37.5°C). The graph reads from right to left; zero time indicates the start of the work. One-mintue records of the control periods obtained at a slower recording speed are shown on the right-hand side of the graph.

Fig. 2. Correcting for the instrument time lag gives an actual delay of only 1.5 seconds required for augmentation of sweat production. In all eight experiments performed on four subects, a definite increase was found between 1 and 2 seconds after the start of exercise at this rate and under these thermal conditions. The simultaneous increase in sweat production in both the resting forearm and working leg suggests that the response is mediated through the central nervous system.

Skin temperatures were recorded from 11 points with rapid responding, uncovered, 27-gauge copper-constantan thermocouples. The sweating augmentation occurred before a detectable increase in the calculated mean weighted skin temperature and before any increase in skin temperatures at single points. In many experiments skin temperatures actually decreased early in exercise, probably from shifts of blood flow and evaporate cooling. As Figs. 1 and 2 show, the response also preceded any detectable changes in tympanic membrane and rectal temperature. These were consistent findings in all experiments.

The change in delay time, as shown in Fig. 3, gives an indication of one condition necessary for the response. At 30°C the subject was not sweating in the pre-exercise period and a greater delay for the increase of sweat production was seen at the onset of a work rate of 1000 kg meters per minute. In six experiments at 30°C the time lag ranged between 30 and 80 seconds. Again, sweating occurred prior to detectable increases in body temperature.

The sweating increase early in work appeared to be related to the work rate and the ambient temperature, or, more importantly, the pre-exercise sweating rate. The experiments indicate that the response cannot be caused by a temperature increase at the hypothalamus. The fact that the "intracranial" temperature remained constant is not, in itself, conclusive, as hypothalamic temperature could have increased independently. However, the 1- to 2-second time delay is not long enough, in view of measured circulation times, for the warmed blood of the active areas to reach the brain. Similar reasoning can also preclude any contribution by blood-borne substances.

There is a possibility that heat produced in exercise could activate thermal detectors either in the working muscles or in contact with the venous drainage from these muscles. Robinson and his co-workers (*7*, *8*) have presented strong evidence for this mechanism. The temperature of active muscles increased as much as 1.5°C within one minute after the start of work. The venous drainage from active muscles showed transient temperature decreases with the initiation of work in cooler ambient conditions. The venous drainage temperature increased in the first minute in warmer ambient temperatures and the sweating rate appeared correlated to the temperature of the venous blood. Actual existence of venous or muscle detectors, and whether or not they are sensitive enough to respond to the heat produced in less than two cycles of the ergometer, remain to be tested.

Reflexes originating from mechanoreceptors in muscles and joints could conceivably participate in the sweating increase as well as in respiratory stimulation. Robinson (*7*), however, has shown that vigorous passive exercise of a man's arms and legs does not stimulate sweating out of proportion to the metabolic changes occurring with the passive exercise.

Another mechanism must be considered in view of the rather short response time. The possibility exists that an irradiation of descending motor impulses to centers involved in sweating could produce the activation. Torreli and Brandi (*9*) have recently reviewed the older evidence and have presented a strong case for the participation of such a mechanism in the initial respiratory responses in exercise. Rushmer *et al.* (*10*) have emphasized the role of the central nervous and autonomic systems in cardiovascular control in exercise, although little is known about the afferent limb of the response system. While such a mechanism has not been described for sweating responses, it would be consistent with our data. The evidence suggests that such a mechanism may play an important part in the augmentation of sweating early

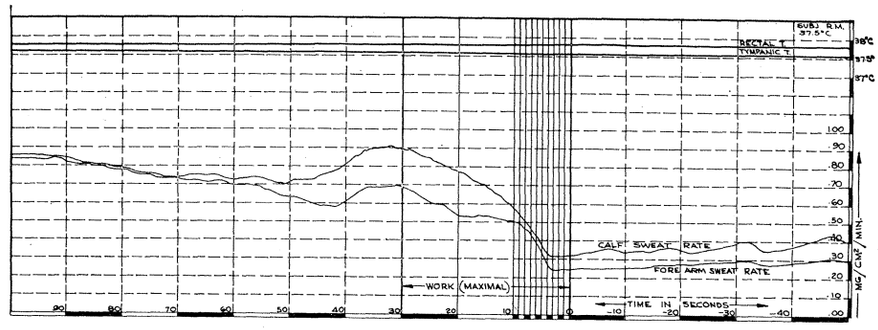

Fig. 2. Increase of sweating with the initiation of extremely hard muscular work at 37.5°C.

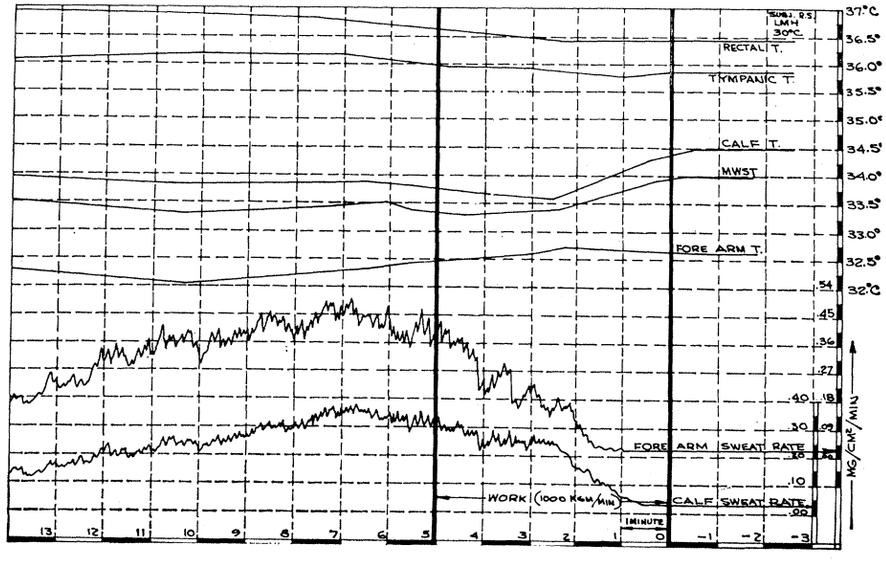

Fig. 3. Sweat production during work in a cool environment. Note the delayed onset of sweating. MWST represents the mean weighted skin temperature calculated from 11 points.

in exercise. As work continues the sweating rate may then be controlled by thermal factors, such as increased core or hypothalamic temperatures. In cooler environments the same nervous stimuli may be present, but ineffective because of inhibition by the cooler temperature (*11*).

W. VAN BEAUMONT
ROBERT W. BULLARD
Department of Anatomy and Physiology, Indiana University, Bloomington

References and Notes

1. M. Nielsen, *Skandinav. Arch. Physiol.* **79**, 193 (1938).
2. S. Robinson, in *The Physiology of Heat Regulation and the Science of Clothing*, L. H. Newburgh, Ed. (Saunders, Philadelphia, 1949), pp. 193–232.
3. T. H. Benzinger, *Proc. Natl. Acad. Sci. U.S.* **45**, 4 (1959).
4. H. S. Belding and B. A. Hertig, *J. Appl. Physiol.* **17**, 103 (1962).
5. R. W. Bullard, *ibid.*, p. 735.
6. R. E. Albert and E. D. Palmes, *ibid.* **4**, 208 (1952); T. Nakayama and K. Tagaki, *Japan. J. Physiol.* **9**, 359 (1959).
7. S. Robinson, *Advances in Biology of Skin* (Pergamon, New York, 1962), vol. 3, pp. 152–162.
8. F. R. Meyer, *Physiologist* **5**, 182 (1962).
9. G. Torelli and G. Brandi, *Intern. Z. Angew. Physiol.* **19**, 134 (1961).
10. R. F. Rushmer, D. A. Smith, Jr., E. P. Lasher, *Physiol. Rev.* **40** (suppl. 4), 27 (1960).
11. Supported by the Office of the Surgeon General, Department of the Army, contract DA-49-0070, MD 947.

1 July 1963

Cerebrally Active Small Moiety from "Taraxein-like" Blood Fractions

Abstract. *A charged, amphoteric, small moiety has been separated from a "taraxein-like" blood fraction by electrodialysis through ion exchange membranes. Cerebral bioassay shows that the activity of the blood extract is contained in the charged small moiety so that, as the activity in the charged compartment rises during electrodialysis, the activity in the feeding (extract) compartment falls.*

In a test of the properties of taraxein (*1*, *2*), the serum fraction reported to be capable of disturbing mental function, we thought it particularly appropriate and relevant to employ a cerebral bioassay. Therefore, we made use of the cerebral effect which we had found in inhibiting cerebral synaptic transmission in the cat brain under light pentobarbital anesthesia (*3*). We measured this action and found it to be qualitatively indentical to the action of serotonin (Fig. 1) and to the action of the exogenous psychotogen LSD-25 and, like these, its action could be attenuated or prevented by a tranquillizer, such as chlorpromazine.

We had, therefore, developed a bioassay system which (i) is specific for the kind of cerebral action we believe to be of prime importance in mental disturbance (*4*), and (ii) is sufficiently sensitive so that a blood sample of 30 ml suffices and there is no need to pool the blood from different subjects. We have obtained similar results with the Heath and Leach (*1*, *5*) extraction (ionic adjustment or salting out) procedures, both long and short, and with the curtain electrophoresis separation method of Frohman and Gottlieb *et al.* (*6*). The albumin and globulin fractions, which are separated by all these procedures, are active cerebral synaptic inhibitors, the albumin fraction being the more potent (see Fig. 1).

In our bioassay, arterial (intracarotid) injection close to the brain produces inhibition of synaptic transmission in a minute or less, as indicated by the reduction of evoked postsynaptic potentials. How the polypetide molecules of taraxein, still of relatively large sizes, can traverse the blood-brain barrier in such a short time is difficult to envisage. On this basis we pointed out in 1959 that "the molecular size of the active constituent is small enough to traverse the blood-brain barrier rapidly (in seconds), but too large to pass through an ultrafilter" and "suggested that small molecules adsorbed to the protein fractions are released to act" (*2*). Independent support came from the report of Bergen *et al.* (*7*), that taraxein, dialyzed through a collodion membrane against a similarly prepared but inactive extract, appeared in the previously inactive compartment.

It occurred to us that separation by dialysis might serve as a method of collecting and identifying the small moiety adsorbed to the protein fraction, if a charged electrode were substituted for the protein in the collecting compartment. We accomplished this by the use of a five-chambered electrodialysis cell or Wood cell (*8*), in which the cathode and anode compartments are separated by selective, ion-exchange membranes arranged to pass anions and cations from the center into their respective compartments, but not into the electrode chambers. Flowing water washes out the small salt molecules that may enter the electrode chambers.

We first experimented with a model

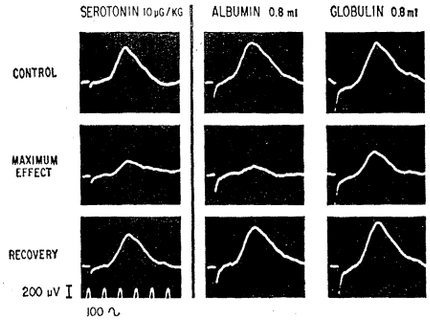

Fig. 1. Inhibition of cerebral synaptic transmission by serum fractions in an intercortical (transcallosal) system. Potentials were evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex at 2-second intervals. Injections were made in the ipsilateral common carotid artery.

made of a mixture of albumin and serotonin. We obtained the expected separation, the serotonin accumulating in the cathode compartment where the cerebral bioassay showed an activity, while activity in the center or feed compartment decreased during the 30-minute period of electrodialysis at 90 volts. Evidently, it is possible to electrodialyze serotonin in this way without appreciable loss of activity.

Proceeding to the "taraxein-like" material itself, we started with the albumin, which in our assay system is the more active of the two fractions and is also the more readily solubilized. In cats in which the sensitivity was such that 10 to 20 μg of serotonin produced an approximately 50-percent inhibition of cerebral synaptic transmission, bioassay indicated successful electrodialysis in ten out of ten trials with the activity appearing each time in the cathode compartment. Essentially similar results were obtained when the *p*H of the center compartment ranged

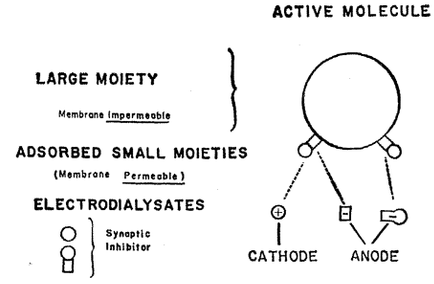

Fig. 2. A scheme to explain the appearance of activity in both the cathode and anode compartments of the electrodialysis cell. The electrodialyzates are charged moieties. Typical of those that are potentially available are RNH_2H^+, $RCOO^-$, and ϕOH^-.