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

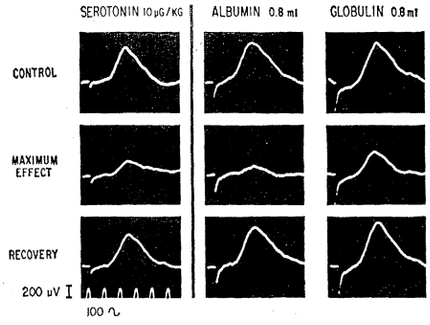

Fig. 1. Inhibition of cerebral synaptic transmission by serum fractions in an intercortical (transcallosal) system. Potentials were evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex at 2-second intervals. Injections were made in the ipsilateral common carotid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

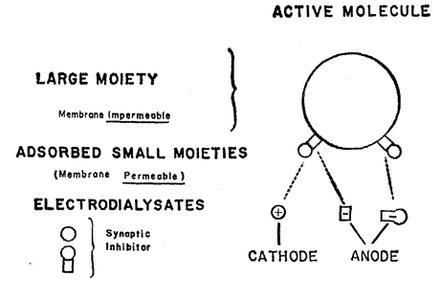

Fig. 2. A scheme to explain the appearance of activity in both the cathode and anode compartments of the electrodialysis cell. The electrodialyzates are charged moieties. Typical of those that are potentially available are RNH_2H^+, $RCOO^-$, and ϕOH^-.

from 5 to 8. Saline controls were negative and served to assure us that the ion-exchange membranes were not releasing any active contaminant.

There is an important additional and unexpected result. Activity appeared in both the cathode and the anode compartments (in seven out of seven trials), while activity in the center compartment decreased during the 30-minute period of electrodialysis in the four which were assayed at the end as well as at the beginning of dialysis. Figure 2 offers a tentative explanation of this result and constitutes an important formulation for further testing. As indicated, it is assumed that the smaller active moieties, that are absorbed onto the larger ones of the active molecules extracted from serum (especially schizophrenic serum), are composed of two species. Initially, these species are united into moieties with resulting negative charges, but they break up further into positively charged and negatively charged fragments. These migrate toward their appropriate electrodes. Assuming that the positively charged moieties, whether in isolation or combined with the negatively charged ones, are the synaptic inhibitors, then one would expect higher activity at the cathode, as was usual, than at the anode, where dilution results from the presence of the inert portion of the negative moieties. Our findings support this hypothesis. Some charged fragments which are potentially available are indicated at the bottom of Fig. 2.

Our data indicate that successful separation of the postulated smaller active moiety was achieved and that this moiety has migration characteristics that call for amphoteric properties along the lines suggested in Fig. 2. We appear to have found another instance of a carrier function for an "albumin fraction" of blood. Similar testing is being carried on with the "globulin fraction" with which taraxein activity has usually been associated, although it has not always been found in the same protein fraction (*5*). The electrodialyzates contain nitrogen and further chemical characterization of these substances is in progress.

THOMAS F. REDICK
ALICE G. RENFREW
LORENZO PIERI
AMEDEO S. MARRAZZI
Veterans Administration Research Laboratories in Neuropsychiarty
and *Mellon Institute,*
Pittsburgh, Pennsylvania

16 AUGUST 1963

References

1. R. G. Heath, S. Martens, B. E. Leach, M. Cohen, C. Angel, *Am. J. Psychiat.* **114**, 14 (1957).
2. A. S. Marrazzi, A. G. Renfrew, E. R. Hart, J. E. Wilson, *Federation Proc.* **18**, 419 (1959).
3. A. S. Marrazzi and E. R. Hart, *J. Nervous Mental Disease* **122**, 453 (1955).
4. A. S. Marrazzi, *Am. J. Psychiat.* **116**, 911 (1960).
5. R. G. Heath, B. E. Leach, L. W. Byers, *Proceedings of the Third World Congress of Psychiatry* (Univ. of Toronto Press and McGill Univ. Press, Montreal, Canada, 1961), vol. 1, p. 619.
6. C. Frohman, E. D. Lechy, G. Tourney, P. S. Beckett, J. S. Gottlieb, *Am. J. Psychiat.* **117**, 401 (1960).
7. J. R. Bergen, R. B. Pennel, H. Hoagland, H. F. Freeman, *Federation Proc.* **18**, 10 (1959).
8. T. Wood, *Biochem. J.* **62**, 611 (1956).

18 June 1963

Sensitivity of Cultured Mammalian Cells to Streptomycin and Dihydrostreptomycin

Abstract. *Cultured mammalian cells killed by streptomycin were essentially unaffected by an identical concentration of dihydrostreptomycin.*

It was recently reported that cultured mammalian cells are sensitive to streptomycin in relatively high concentration (3 to 5 mg/ml) and that variants resistant to this amount of streptomycin had been obtained (*1*). We have observed that strains of cells sensitive to streptomycin are essentially unaffected by dihydrostreptomycin.

The strains of cells, media, and general conditions for conducting the tests have been reported previously (*1*). Inasmuch as the physical environment of the cells is a factor in determining their response to streptomycin (*1*) the tests were carried out with identical results in plastic petri dishes, glass petri dishes, and glass tubes. Medium containing 100,000 cells per milliliter of a sensitive strain was added to the culture vessel, the cultures were observed daily, and cell counts were made at the end of 7 days on a Coulter electronic particle counter.

The amount of streptomycin necessary to produce toxic effects on mammalian cells is so large in relation to antibacterial concentrations that our initial observations were thought possibly to be the result of sufficient impurity in the streptomycin to be toxic for the cells. Commercial streptomycin and dihydrostreptomycin preparations, as well as two highly purified samples of each (*2*), were tested for differences in the response of the cells (Fig. 1). Although the dihydrostreptomycin affected the growth response of the culture slightly, the cells attached themselves to the glass and appeared healthy during the course of the experiment. The differences in numbers recorded for the different dihydrostreptomycin preparations are not significant. The cultures containing streptomycin appeared ill within 48 hours; the cells became detached from the glass and appeared to be dead when observed at 72 hours. Thus, the counts recorded for the cultures containing streptomycin represent dead cells.

These experiments were repeated with six strains of cells with identical results. With two of these strains, determinations of dose response were made and the effect of streptomycin persisted undiminished down to a concentration of 2 mg per milliliter and tapered off with lower concentrations.

The reason for the difference in the action of streptomycin and dihydrostreptomycin is not known. In most work, but not all (*3*), on the effect of these substances on bacteria differences have not usually been noted. Indeed, many workers use the term streptomycin in discussing their work although they actually used dihydrostreptomycin (*4*).

Studies on the mode of action of streptomycin on bacteria suggest that the primary lesion occurs in the membrane (*4*, *5*), or on the ribosomes (*6*), but the former interpretation has recently been questioned (*7*). The ability of streptomycin to combine with the nucleic acids (*8*) would lend support to a hypothesis that involves their action on cell components containing nucleic acids. That relatively large amounts of streptomycin are required to destroy mammalian cells, in contrast to the small amounts required for bacteria suggests the pos-

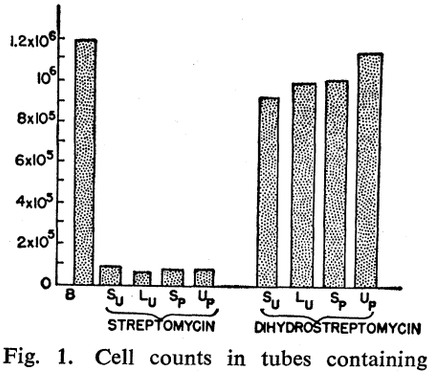

Fig. 1. Cell counts in tubes containing 1 ml of medium alone and with 5 mg of streptomycin or dihydrostreptomycin added. S_U, S_P, Squibb USP and purified; U_P, Upjohn purified; L_U, L_P, Lilly USP and purified.