lute temperature, a is the degree of dissociation of the monomeric group, ψ is the electric field, k is Boltzmann's constant, and ε is the elementary electronic charge. On the assumption that half-dissociation of the monomeric group in Fig. 1 occurs at *p*H 6.3, the electric field (ψ) was calculated to be approximately 100 mv. The surface-charge density necessary to generate a field of this strength was calculated from the following expression derived by Overbeek (*8*) (the Gouy-Chapman model of an electric double layer was used, and it was assumed that the molecular surface roughly approximates a plane surface):

$$\sigma_0 = \sqrt{\frac{DcRT}{2\pi}}\, 2 \sinh\left(\frac{F\psi_0}{2RT}\right) \qquad (2)$$

where σ_0 is the surface-charge density, c is the concentration of the counterions in the medium at infinite distance from the face of the molecule, D is the dielectric constant of the medium, R is the gas constant, and F is Faraday's constant; the other terms are the same as those appearing in Eq. 1. The value for σ_0 was found to be 1.5×10^{14} charges/cm^2. This corresponds to 2.4 charges per 5.1-A length of a cylinder having a diameter of 9.8 A. On the assumption that the polypeptide chains within the paramyosin have the form of an a-helix (*9*), a polyglutamic acid segment could have a maximum of 3.8 charges per 5.1-A length.

The possibility that the high charge density is due to the presence of polyglutamic acid segments along the molecule can be tested by partial digestion of the paramyosin and examination of the fragments formed for the presence of highly acidic polypeptides. Preliminary studies with paper electrophoresis, in which tryptic digests of paramyosin were used, have indicated the presence of a fast-moving negative component which could correspond to a polyanion (*10*).

Note added in proof. Kensal Van Holde of the chemistry department, University of Illinois, has calculated by an independent method the charge density that could give rise to the suggested shift in pK' of the glutamic acid residues in paramyosin. He has used the model proposed by T. Hill [*Arch. Biochem. Biophys.* **57**, 229 (1955)] which consists of a charged cylinder of infinite length, radius b, and closest center-to-center approach for small external ions of distance a. The titration curve should, in the region of the pK' of the above groups, be expressed by

$$pH = pK' + 1n\,[\alpha/(1-\alpha)] - \frac{}{0.868w(c - \alpha n)}$$

where α is the fraction of groups ionized, n is the total number of acid groups, c is the number of positive groups in the neighborhood, and $w = F_e/Z^2KT$ [Z is the actual charge and F_e is the electrostatic free energy for charging the model in question, the expression for which is given by Hill (see above)]. For a shift of pK' of 2.0 *p*H units, assuming that cationic groups are sufficiently remote to be neglected in this calculation and that the cylinder has the radius of an α-helix ($a = 7$ A; $b = 5$ A), the number of charges in a length of 5.1 A is 3.7.

WILLIAM H. JOHNSON
JOSEPH S. KAHN*

Physiology Department,
University of Illinois, Urbana

References and Notes

1. W. H. Johnson, J. S. Kahn, A. G. Szent-Györgyi, *Science* **130**, 160 (1959).
2. D. R. Kominz, F. Saad, K. Laki, *Proceedings of the Conference on the Chemistry of Muscular Contraction, Tokyo, Japan* (1956), p. 66.
3. A. Katchalsky, in *Progress in Biophysics and Biophysical Chemistry*, J. A. V. Butler and J. T. Randall, Eds. (Academic Press, New York, 1954), vol. 4, p. 1.
4. M. Laskowsky, Jr., and H. A. Sheraga, *J. Am. Chem. Soc.* **76**, 6305 (1954).
5. I. M. Klotz, *Science* **128**, 815 (1958).
6. G. S. Hartley and J. W. Roe, *Trans. Faraday Soc.* **36**, 101 (1940).
7. A. Katchalsky, *J. Polymer Sci.* **12**, 393 (1951).
8. J. T. G. Overbeek, in *Colloid Science*, H. R. Kruyt, Ed. (Elsevier, Amsterdam, Netherlands, 1952), p. 115.
9. A. G. Szent-Györgyi and C. Cohn, *Science* **126**, 697 (1957).
10. We thank George Karreman of the Eastern Pennsylvania Psychiatric Institute for help in the theoretical aspects of this work. We are also deeply grateful to Andrew G. Szent-Györgyi for a generous supply of crystalline paramyosin.
* This work was done during the tenure of a postdoctoral fellowship supported in part by a grant from the National Science Foundation and the Graduate College of the University of Illinois.

30 July 1959

On the Mechanism of Action of Chlorpromazine

Abstract. Chlorpromazine is shown to be a powerful electron donor. Observations are described supporting the assumption that the therapeutic action of this drug is connected with this property.

Redox reactions between organic substances as a rule involve a change between two forms differing by two H atoms (two electrons plus two protons). Michaelis (*1*) pointed out that in this process the electrons may transfer one by one, and he demonstrated the intermediate state. Accordingly, substances regarded as oxidants or reductants are expected to have two stable configurations differing from each other by two hydrogen atoms.

J. Weiss (*2*) discovered that complexes may have properties which indicate an electron transfer from one molecule to the other within the complex. Mulliken (*3*) and his associates further developed this theory of "charge transfer." In such charge-transfer complexes an electron is supposed to go from the highest filled orbital of the donor to the lowest empty orbital of the acceptor; this transfer involves no change in configuration in classical chemical terms. In most cases studied by Mulliken the electron donated spent only a small fraction of its time with the acceptor and needed additional energy, supplied by the absorption of light, to be almost completely transferred to the acceptor.

The findings obtained in one of our laboratories indicated that complexes may be formed by biologically important substances in which an electron is practically wholly transferred from the donor to the acceptor, even in the ground state. There was reason to believe that such charge-transfer reactions are a frequent occurrence and so may play a role in reactions connected with a transfer of energy. The question arose whether charge transfer might play a role in the mechanism of drug action. Preliminary experiments indicated that this may be the case. For instance, various alkaloids were found to act readily as donors in complexes with iodine dissolved in chloroform.

Chlorpromazine has striking biological activity. It is, therefore, of great interest that, as indicated in this report, chlorpromazine also has striking properties as an electron donor, and the question arises whether the tranquilizing action of this drug may not be due to its charge-transfer properties. It is not possible to predict from its biological activity whether a pharmacologically active substance is an electron acceptor or an electron donor. If the drug acted, for example, on the electric double layer on the cell surface, where there is a positive charge on the outside and a negative charge on the inside, then an electron transfer on the outside would lead to a depolarization and thus to excitation, while a donation on the inside could be expected to lead to a hyperpolarization and, with it, to inhibitions. So, the final action depends on the affinities and location. What can be said, in a general way, is that one would expect pharmacological activity from a good donor rather than from a good acceptor, since the charge transfer depends to a great extent on the overlap of wave functions and a strong donor property involves a rather extended wave function. Whatever the case may be, one would expect pharmacological agents to show exceptionally strong donor or acceptor properties if their biological activity is actually due to a charge transfer.

With these thoughts in mind, the electron levels of chlorpromazine have been calculated by means of the LCAO method. The K values obtained were -0.217 for the highest filled, and -1.000 for the lowest empty orbital (*4*). This finding is rather striking and unexpected. To our knowledge, chlorpromazine is the first substance ever found in which, in its ordinary stable state, the highest filled level corresponds to an antibonding orbital, as indicated by the minus sign of K. This makes chlorpromazine a quite extraordinarily strong electron donor. Negative values for highest orbitals were hitherto found only in substances on which H atoms were forced by reduction of the molecule in question, as in leucomethylene blue (MBLH) and reduced flavin mononucleotide ($FMNH_2$) (*5*).

In phenothiazine the highest filled molecular orbital also has a negative value and is antibonding (K of the highest filled orbital is -0.210 and K of the lowest empty orbital is -1.000). So, evidently, the strong donor properties of chlorpromazine are linked to the phenothiazine part of the chlorpromazine.

The assumption that charge transfer may be involved in pharmacological reactions of chlorpromazine is supported by the fact that we found *d*-lysergic acid diethylamide, another drug with strong action on the central nervous functions, to be a very good donor. The K value of the highest filled orbital was found to be 0.218; of the lowest empty orbital, -0.726. Also, serotonin is a very good donor, more so than indole or tryptophan (K of the highest filled electronic orbital, 0.461; of the lowest empty orbital, -0.870), and it also has a strong action on the central nervous system. (This agreed with absorption measurements of the complexes (*6*) of these substances, which also indicate that serotonin is a good donor, better than tryptophan.)

The calculations are supported by the chemical behavior of the substances in question. The negative values for MBLH and $FMNH_2$, as pointed out by B. Pullman and A. Pullman, is supported by the great instability of these substances, which readily undergo autoxidation in the presence of O_2. Chlorpromazine, which is not readily autoxidizable, at least not in the dark, is thus unique also in being relatively stable in spite of its energy values. However, the strong donating ability of this drug can be demonstrated by freezing an aqueous solution of chlorpromazine in Dry Ice in the presence of an equimolar riboflavin solution ($10^{-3}M$). On freezing, the solution turns greenish brown and loses all fluorescence—an expression of a complex formation.

Similarly, 1-methyl Medmain is a very good donor (highest filled electronic orbital, 0.348; lowest empty orbital, -0.869). The same is true for 1-benzyl-2-methyl-5-methoxy-(N, N-dimethyl)-tryptamine (highest filled level, 0.427; lowest empty level, -0.866,), which gives strongly colored complexes when frozen with flavin mononucleotide. The assumption that this color is actually due to the formation of a charge-transfer complex is supported by the spectrum. As has been shown earlier (*6*), the spectrum of the serotonin-flavin mononucleotide or of the tryptophan-flavin mononucleotide complex has a peak at 490 mμ, the wavelength characterizing the free radical of flavin mononucleotide at acid *p*H. Also, the chlorpromazine complex, when measured at -78°C (*7*), shows a strong absorption at this wavelength; this indicates that the absorption is actually due to the flavin mononucleotide, which has accepted one electron. In addition, there is a broad absorption at 570 mμ. This peak may be tentatively identified as the absorption of the free radical of flavin mononucleotide at neutral *p*H (*8*). However, other possibilities suggest themselves. The peak may be due to the reverse of the usual charge transfer absorption, in which an electron moves from riboflavin back to chlorpromazine upon the absorption of a light quantum. A positive identification of the 570 mμ absorption must await future work.

If further experience bears out the assumption that the action of the drugs studied, as well as the action of other drugs with related effects, is due to charge transfer, then this may contribute to understanding of the mechanism of normal and abnormal psychic functions (*9*).

G. KARREMAN
Eastern Pennsylvania Psychiatric Institute, Philadelphia, Pennsylvania
I. ISENBERG
A. SZENT-GYÖRGYI
Institute for Muscle Research, Marine Biological Laboratory, Woods Hole, Massachusetts

References and Notes

1. L. Michaelis, "Fundamentals of oxidation and reduction," in *Currents in Biochemical Research* (Interscience, New York, 1946).
2. J. Weiss, *J. Chem. Soc.* **1942**, 245 (1942).
3. R. S. Mulliken, *J. Am. Chem. Soc.* **74**, 811 (1952).
4. For the meaning of K, B. Pullman and A. Pullman, *Proc. Natl. Acad. Sci. U.S.* **44**, 1197 (1958).
5. B. Pullman and A. Pullman, *Biochim. et Biophys. Acta.*, in press.
6. I. Isenberg and A. Szent-Györgyi, *Proc. Natl. Acad. Sci. U.S.* **44**, 857 (1958).
7. For details of the measurements see I. Isenberg and A. Szent-Györgyi, *ibid.*, in press.
8. H. Beinert, *J. Am. Chem. Soc.* **78**, 5323 (1956).
9. This work was supported by grant No. H-2042 (C3) of the National Heart Institute and by grants from the Commonwealth Fund, the National Science Foundation, and the United Cerebral Palsy Associations. The energy levels were calculated with the aid of the Univac of the University of Pennsylvania, supported by the National Science Foundation. We are greatly indebted to B. Pullman and G. Berthier, both of the Sorbonne, University of Paris, France, for generously supplying their effective parameters. We wish to thank Smith, Kline and French Laboratories, Philadelphia, Pa., for samples of chlorpromazine-HCl, and Sandoz Chemical Works, Inc., of Hanover, N.J., for providing a supply of *d*-lysergic acid diethylamide.

22 June 1959

Microbial Fermentation in Certain Mammals

Abstract. The fermentation in the caecum and large intestine of ruminants is negligible compared with that in the rumen. In small ruminants the rate per unit contents is faster than in large ones, due to faster turnover. The cellulolytic bacteria of several ruminants are similar but differ in nutritional requirements.

A sojourn in Kenya to study microbial fermentation rates in the rumen of zebu and European cattle (*1*) also afforded an opportunity (*2*) to examine other mammals. Anaerobic culture tubes containing rumen fluid (or elephant caecal or colonic fluid) and cellulose agar (*3*) were prepared in the field, the contents of the appropriate organ of the freshly killed animal being used as part of the medium. The sterilized tubes were inoculated with serial dilutions of the fresh contents, and the medium was solidified and returned to the laboratory for incubation at 39°C. Characteristic clearing of the cellulose resembling that due to *Bacteroides succinogenes* from cattle (*3*) was observed in cultures from the eland, kongoni, zebu, and camel. A few colonies resembling *Butyrivibrio* (*4*) were also seen. In some culture series, noncellulolytic colonies were sufficiently visible that both total and cellulolytic colony counts could be made. The counts were similar in magnitude to those obtained in cattle by comparable methods (*5*). Data of the experiments are shown in Table 1.

Bacteroides succinogenes-like colonies from the kongoni and the eland, respectively, were inoculated into parallel culture tubes containing media prepared with sterile rumen fluid from the host or from a zebu. Difference in the diameter of the clearings developing in the thin cellulose agar showed that both bacterial strains grew faster with homologous than with zebu rumen fluid, though the difference for the kongoni was slight.

The contribution of each part of the