because of the involvement of a cation rather than an anion, can serve to describe the maximum P_{Na} as a function of E. In Fig. 2 a curve so calculated (designated by $w = 0$) is compared with experimental curves obtained for the squid giant axon in media at different calcium concentrations (*20*). The latter curves have been put on an absolute potential scale by assigning a resting potential of 50 mv; this low resting potential is employed because in the calcium studies the fibers were short and the change in membrane potential for 50 percent inactivation, at normal calcium levels, was about 10 mv higher than in preparations which had a resting potential of 60 mv and gave the experimental curves in Fig. 1 (*20, 21*). The calculated curve was obtained with bC_I assigned 25 dyne/cm. It can be seen in Fig. 2 to deviate from the experimental curves in a manner to be expected from the use of a medium very low in calcium.

One way in which calcium might act in the living membrane is by introducing a threshold for C_M; below this concentration the lipophilic anion fails to penetrate M. This could come about through the conversion of the state of the sodium sites from a liquid condensed type to the solid condensed

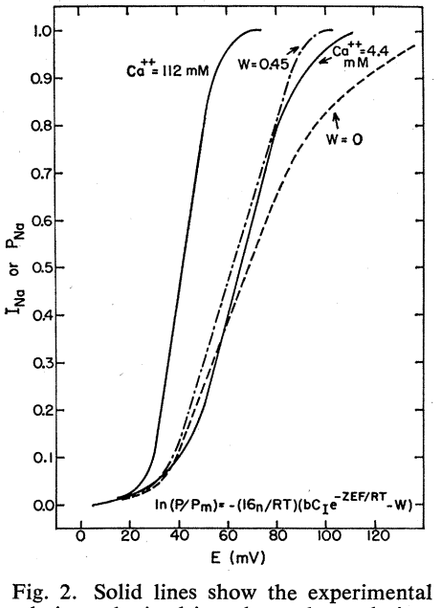

Fig. 2. Solid lines show the experimental relations obtained in voltage-clamped giant axons between the maximum inward (sodium) current, I_{Na}, obtained after prolonged polarizations at the various transmembrane potentials, E, at two different concentrations of calcium in the medium (*20*). Resting potential of these preparations is taken as 50 mv (see text). Broken lines are the corresponding curves in terms of P_{Na}, calculated from the equation shown, for $w = 0$ and $w = 0.45$ dyne/cm.

form, as occurs with stearic acid monolayers on a calcium-containing substrate (*22*). In any case, the introduction of this factor—as the term w in the equation given in Fig. 2—can be seen to lead to an inactivation curve very similar to the experimental one obtained in low calcium when w is taken as less than 0.5 dyne/cm. Increase in w will shift the theoretical curve further to the left, as obtained experimentally by increasing the calcium concentration of the medium.

The present proposal has the advantage that it is in keeping with known phenomena observed in simple physicochemical systems as well as in excitable systems; moreover, the current actually transferred by the postulated lipophilic ions can be negligible compared to that transferred by the inorganic cations they control. The latter situation, as well as the steepness of the permeability-potential relationships, have been pointed out to be critical requirements of a satisfactory molecular hypothesis.

The agreement so far obtained for hypothetical and experimental curves by no means establishes the details of the model. The functional relationships that have been employed are still arbitrary in the sense that they had to be taken from the few monolayer studies available; as such studies are extended to other lipoidal species or mixtures, these relationships may prove less unique. An actual working model is also desirable, especially to explore the possibilities with respect to the kinetics of the permeability changes which have had to be neglected for lack of physicochemical data to approach this problem. Moreover, besides model studies which can of themselves be misleading, research should be carried out on living membranes to test for mechanisms such as have been proposed. One approach would be to compare the effect of different concentrations and types of stabilizers and labilizers on the resting and active permeabilities of the squid giant axon with their effect on, say, gas permeation of monolayers prepared from lipid extracts of axon sheaths. Still another approach would be to add different types of lipoidal molecules to solutions used to perfuse the interiors of giant axons (*23*) in a search for synergistic effects on the permeability changes normally occurring during voltage clamp (*24*).

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References and Notes

1. A. M. Shanes and N. L. Gershfeld, *J. Gen. Physiol.* **44**, 345 (1960).
2. A. M. Shanes, *Pharmacol. Rev.* **10**, 59 (1958).
3. J. C. Skou, *Acta Pharmacol. Toxicol.* **10**, 325 (1954).
4. R. J. Archer and V. K. La Mer, *J. Phys. Chem.* **59**, 200 (1955).
5. V. K. La Mer and G. T. Barnes, *Proc. Natl. Acad. Sci. U.S.* **45**, 1274 (1959).
6. A. M. Shanes, W. H. Freygang, H. Grundfest, E. Amatniek, *J. Gen. Physiol.* **42**, 793 (1959).
7. H. L. Rosano and V. K. La Mer, *J. Phys. Chem.* **60**, 348 (1956); M. Blank and F. J. W. Roughton, *Trans. Faraday Soc.* **56**, 1832 (1960).
8. M. Blank, *J. Phys. Chem.* **65**, 1698 (1961).
9. J. Guastalla, in *Mem. Serv. Chim. Etat Paris* **41**, 316 (1959).
10. A. M. Shanes, *Nature* **188**, 1209 (1960).
11. ———, in *Proc. Intern. Union Physiol. Sci., XXII Intern. Congr.*, vol. 1, part 1 (Lectures), p. 93 (1962).
12. J. C. Skou, *Biochim. Biophys. Acta* **30**, 625 (1958).
13. G. T. Barnes and V. K. La Mer, in *Retardation of Evaporation by Monolayers*, V. K. La Mer, Ed. (Academic Press, New York, 1962), p. 9.
14. M. Blank and V. K. La Mer, *ibid.*, p. 59.
15. A. L. Hodgkin and A. F. Huxley, *J. Physiol. London* **116**, 449 (1952).
16. J. Posternak and E. Arnold, *J. Physiol. Paris* **46**, 502 (1954); G. M. Schoepfle, *Federation Proc.* **16**, 114 (1957).
17. J. C. Skou, *Acta Pharmacol. Toxicol.* **10**, 317 (1954).
18. A. M. Shanes, *J. Gen. Physiol.* **33**, 57 (1949).
19. I. Tasaki, in *Nervous Transmission* (Thomas, Springfield, Ill., 1953), p. 103.
20. B. Frankenhaeuser and A. L. Hodgkin, *J. Physiol.* **137**, 217 (1957).
21. A. L. Hodgkin and A. F. Huxley, *ibid.* **116**, 497 (1952).
22. W. D. Harkins, *The Physical Chemistry of Surface Films* (Reinhold, New York, 1952).
23. T. Oikawa, C. S. Spyropoulos, I. Tasaki, T. Teorell, *Acta Physiol. Scand.* **52**, 195 (1961); P. F. Baker, A. L. Hodgkin, T. I. Shaw, *Nature* **190**, 885 (1961).
24. Supported by U.S. Public Health Service grant B 3321(C1), NB 03322-02 from the National Institute of Neurological Diseases and Blindness, and G 22235 from the National Science Foundation.

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Inherited Variant of Erythrocyte Carbonic Anhydrase in Micronesians from Guam and Saipan

Abstract. *A variant of one form of red cell carbonic anhydrase was discovered in "Chamorro" inhabitants from the islands of Guam and Saipan. Segregation of this trait in four pedigrees indicates that it is under the control of a single autosomal gene.*

Human erythrocyte carbonic anhydrase has been shown, by the use of electrophoretic and chromatographic separation procedures, to be present in at least two distinct molecular forms (*1, 2*). Because these forms, after separation by starch gel electrophoresis, were first detected in our laboratory by their esterase activity, they were designated as *D* esterases (*3*). When it was demonstrated, however, that these *D* esterases behaved enzymatically as carbonic anhydrase (*4*), they were re-

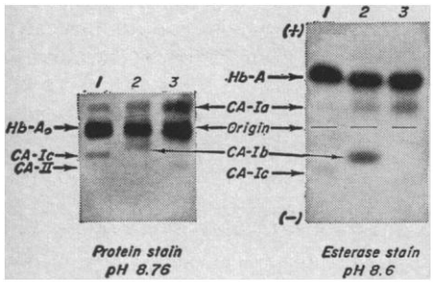

Fig. 1. Starch gel electrophoresis patterns of protein stain and esterase activity stain of normal and variant forms of erythrocyte carbonic anhydrase: *1*, CA-Ic variant; *2*, CA-Ib variant, and *3*, normal pattern. Esterase activity of CA-II not evident in photograph. Electrophoresis for esterase pattern carried out in borate buffer (*p*H 8.6, 0.02*M*). Enzyme activity visualized with Blue RR salt as dye-coupler and β-naphthyl acetate as substrate. In protein stain (nigrosin), electrophoresis carried out at *p*H 8.76 to prevent overlapping of variant forms by Hb-A_2.

ferred to in a later report (*2*) as CA-I and CA-II. Thus, the normal carbonic anhydrase form which migrates anodally at *p*H 8.6 in our starch gel electrophoresis system (*2*) is now designated as CA-Ia (previously Da_1), and the normal cathodally migrating form as CA-II (previously *Db*). A variant of one of these forms, apparently under the control of a single autosomal gene, has been described from a Caucasian family residing in Michigan (*3*) and is now referred to as CA-Ib (previously Da_2). We now describe another variant of CA-Ia, CA-Ic.

The new variant (CA-Ic) was discovered in four "Chamorros" (*5*) during a survey of hemolysate esterase patterns from 490 adult, native inhabitants of Guam, Saipan, and Tinian in the Mariana Islands of western Micronesia. Two of the propositi (one male and one female) were residents of Guam and the other two (one male and one female) were from the island of Saipan. The new variant can be readily distinguished from the CA-Ib variant by both its increased cathodal migration and differential esterase activity (Fig. 1). In hemolysates from individuals with the CA-Ic variant, the esterase activity of the CA-Ic band is moderately increased over that of the normal CA-Ia form; whereas in hemolysates with the CA-Ib variant, the esterase activity of the CA-Ib band is markedly increased over the normal form. The characteristic decrease in protein stain intensity and esterase activity of the CA-Ia band in hemolysates containing the variant forms suggests that CA-Ia, CA-Ib, and CA-Ic are allelic products. As can be seen in the protein stain pattern in Fig. 1, the quantity of CA-II is not noticeably altered in the variant hemolysates.

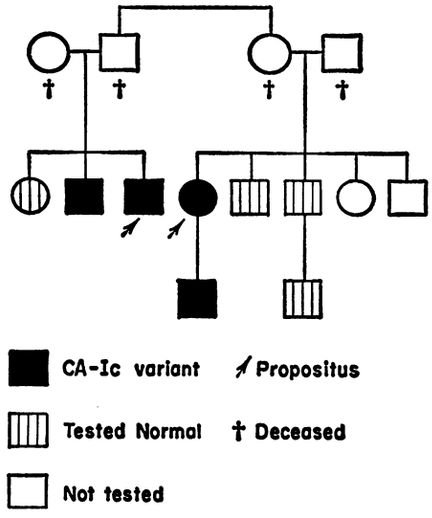

Fig. 2. Pedigree showing segregation of CA-Ic variant.

Preliminary studies of four pedigrees with the CA-Ic trait revealed the following: the first pedigree showed a father to daughter inheritance of the variant (his brother was normal); in the second pedigree three of four female sibs and their paternal uncle possessed the variant (the mother was normal and the father was dead); the third pedigree showed only one female with the trait (her two sons and two maternal half brothers were normal); and in the fourth pedigree, which is shown in Fig. 2, the male and female propositi were found to be first cousins and it appears that the variant was inherited through their respective father and mother. After correcting for ascertainment, the ratio of variant to normal in all segregating sibships tested was 7 : 7. These findings are consistent with single autosomal gene control of the CA-Ic variant.

The starch gel electrophoresis and dye-coupling procedures used in this study have been described elsewhere (*2*). Carbonic anhydrase activity of the electrophoretically separated forms on starch gel was demonstrated by the use of a modification of the Kurata histochemical technique (*6*). Both the dehydrase activity (Kurata stain) and the esterase activity (coupling azo dye stain) of the carbonic anhydrase forms were selectively inhibited when acetazolamide ($10^{-5}M$) was present in the incubation mixtures. In addition, colorimetric assays for hydrase activity (*7*) and esterase activity, and their inhibition by acetazolamide, have been demonstrated on human erythrocyte carbonic anhydrase-Ia after purification on diethylaminoethyl cellulose (*4; 8*).

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References and Notes

1. P. O. Nyman, *Biochim. Biophys. Acta* **52**, 1 (1961); E. E. Rickli and J. T. Edsall, *J. Biol. Chem.* **237**, PC258 (1962).
2. R. E. Tashian and M. W. Shaw, *Am. J. Human Genet.* **14**, 295 (1962).
3. C. R. Shaw, F. N. Syner, R. E. Tashian, *Science* **138**, 31 (1962).
4. R. E. Tashian and D. D. Douglas, in preparation.
5. The "Chamorros" and "Carolinians" are the principal Micronesian people populating the Mariana Islands. The survey included 305 "Chamorros" and 147 "Carolinians" as well as 38 individuals of "Chamorro-Carolinian" admixture. Propositi were classified as "Chamorro" or "Carolinian" if they indicated that all four grandparents belonged to the same ethnic group.
6. Y. Kurata, *Stain Technol.* **28**, 231 (1953).
7. F. J. W. Roughton and V. H. Booth, *Biochem. J.* **40**, 309 (1946).
8. Supported in part by research grant 9252 from the U.S. Public Health Service. We thank Drs. David Dunn, Jose Torres, and Manuel Cruz for collecting the blood specimens from Saipan, and Drs. Charles R. Shaw and Zwi Hart for the blood sample of the CA-Ib variant.

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Floral Induction and the Stimulation of Cell Division in Xanthium

Abstract. *The mitotic index in the apical region of the stem of* Xanthium *seedlings doubled within the 24 hours immediately following a single inductive dark period and an increase occurred within 16 hours in one case. The significance of this finding in relation to the onset of reproductive development is discussed.*

Vegetative plants of the short-day species *Xanthium pennsylvanicum* can be induced to flower by exposure of their leaves to a single long dark period (*1*). The flowering stimulus so formed begins to move into the stem