50 to 100 μM leads to accumulation of globin mRNA (*6*), increased activity of heme synthetic enzymes (*13*), and increased expression of cell surface and cytoplasmic markers of embryonic and fetal erythroid cells (*6*). We found that the embryonic and fetal hemoglobin accumulation that results from induction of K562 cells with low concentrations of hemin is reversible and is not accompanied by terminal differentiation.

Hemin, therefore, caused accumulation of hemoglobin in K562 cells without apparent disturbance of cell growth or loss of proliferative capacity. This clearly dissociates increases in intracellular hemoglobin content from other events considered central to erythroid differentiation. Whether this indicates some specific effect of hemin as an inducer, or some attribute of K562 cells which prevents them from undergoing terminal differentiation, is not known. The results suggest that mechanisms controlling hemoglobin metabolism may be found that are independent of irreversible erythroid maturation.

ANN DEAN
FRANCOIS ERARD
ARTHUR B. SCHNEIDER*
ALAN N. SCHECHTER
Laboratory of Chemical Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205

References and Notes

1. Reviewed in *Cellular and Molecular Regulation of Hemoglobin Switching*, G. Stamatoyannopoulos and A. W. Nienhuis, Eds. (Grune and Stratton, New York, 1978); E. J. Benz and A. W. Nienhuis, *N. Engl. J. Med.* **297**, 1371 (1977); D. J. Weatherall and J. B. Clegg, *Cell* **16**, 467 (1979).
2. P. A. Marks and R. A. Rifkind, *Annu. Rev. Biochem.* **47**, 419 (1978).
3. C. B. Lozzio and B. B. Lozzio, *Blood* **45**, 321 (1975).
4. T. R. Rutherford, J. B. Clegg, D. J. Weatherall, *Nature (London)* **280**, 164 (1979).
5. B. P. Alter and S. C. Goff, *Biochem. Biophys. Res. Commun.* **94**, 843 (1980).
6. E. J. Benz, M. J. Murnane, B. L. Tonkonow, B. W. Berman, E. M. Mazur, C. Cavallesco, T. Jenko, E. L. Snyder, B. F. Forget, R. Hoffman, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3509 (1980).
7. T. R. Rutherford and D. J. Weatherall, *Cell* **16**, 415 (1979).
8. J. Gusella, R. Geller, B. Clarke, V. Weeks, D. Housman, *ibid.* **9**, 221 (1976).
9. B. P. Alter and S. C. Goff, *Blood* **50**, 867 (1977); G. Rovera, J. Abramczuk, S. Surrey, *FEBS Lett.* **81**, 366 (1977); J. Ross and D. Sautner, *Cell* **8**, 513 (1976); K. Lowenhaupt and J. Lingrel, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5173 (1979).
10. J. Gusella, S. C. Weil, A. S. Tsiftsoglou, V. Volloch, J. R. Neumann, C. Kays, D. Housman, *Blood* **56**, 481 (1980).
11. Although we (data not shown) and others (*5*, *6*) have observed incorporation of increased amounts of radioactive label into globin upon hemin induction of K562 cells, this does not rigorously prove that an increased rate of synthesis rather than a diminished rate of degradation has caused the accumulation of hemoglobin in these cells.
12. L. C. Anderson, M. Jokinen, C. G. Gahmberg, *Nature (London)* **278**, 364 (1979); R. Hoffman, M. J. Murnane, E. J. Benz, R. Prohaska, V. Floyd, F. Dainiak, B. G. Forget, H. Furthmayr, *Blood* **54**, 1182 (1979); T. Rutherford, J. B. Clegg, D. R. Higgs, R. W. Jones, J. Thompson, D. J. Weatherall, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
13. R. Hoffman, N. Ibrahim, M. J. Murnane, A. Diamond, B. G. Forget, R. D. Levere, *Blood* **56**, 567 (1980).
14. P. Basset, Y. Beuzard, M. C. Gavel, J. Rosa, *ibid.* **51**, 971 (1978).
15. We thank T. Rutherford, J. Ortaldo, and S. Orkin for providing K562 cells; T. Rutherford for sharing experimental results before publication; and D. Stewart for preparation of the manuscript. A.B.S. is the recipient of an NIH research career development award AM 00103.
* Permanent address: Michael Reese Hospital, University of Chicago, Chicago, Ill. 60616.

27 October 1980; revised 22 December 1980

Outward Currents in Developing *Drosophila* Flight Muscle

Abstract. *The development of two different voltage-sensitive potassium channels was studied in* Drosophila *flight muscle by voltage clamp techniques. Early in development active channels are not present in the membrane. The first channels to appear are the A current channels, which carry a fast, rapidly inactivating potassium current. The channels for delayed rectification appear later. Channels carrying inward current also appear only after the A current channels. During development, the A current may be easily studied in isolation from other currents and thus provides a desirable system for studying the genetic determinants of this current.*

Investigation of adult *Drosophila* flight muscle (*1*) has revealed two distinct outward currents, a fast transient current, which we refer to as the A current, because of its similarity to the molluscan A current (*2*), and a slower current similar to the well-known delayed rectifier (*3*). We now report that the two currents mature at different times during pupal development.

After the observation was made in *Mollusca* that a single cell can have two or more different types of potassium channels (*2*), this phenomenon was reported in many different animal taxa, including mammals (*4*). Little is known,

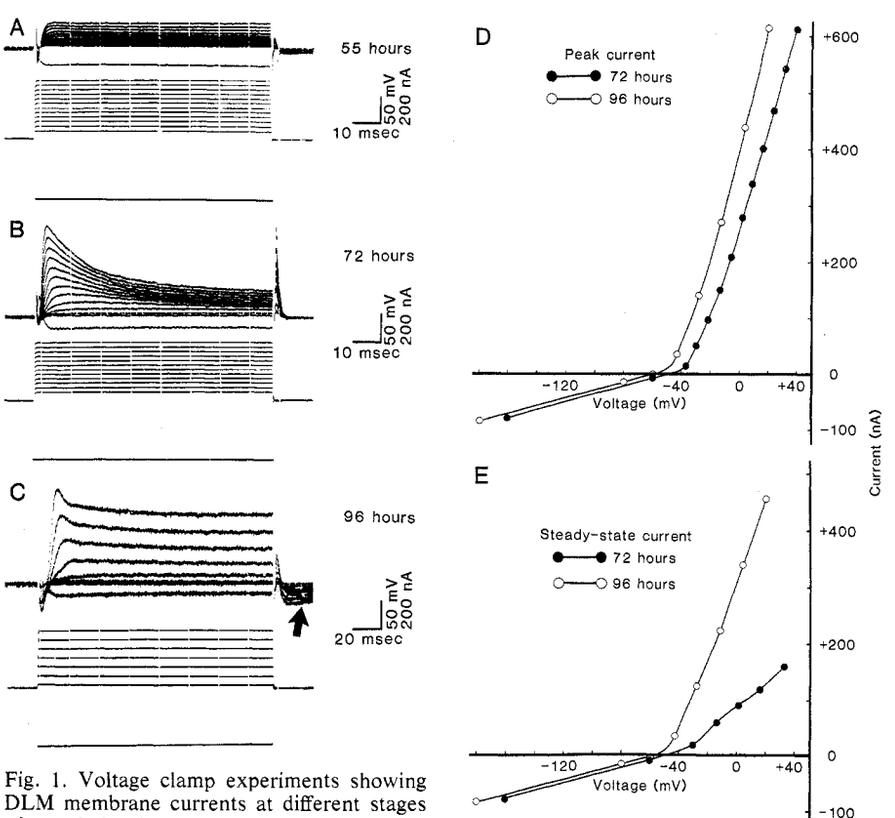

Fig. 1. Voltage clamp experiments showing DLM membrane currents at different stages of pupal development. (A to C) Hours are timed from puparium formation; current is shown in the upper traces, voltage in the lower traces. Outward current is in the upward direction. Holding potentials in (A) and (B), −60 mV; in (C), −80 mV. Because of the high capacitance of these fibers (approximately 0.02 μF) and limitations of the voltage clamp equipment, accurate current readings cannot be made until approximately 3 msec after the command pulse is initiated. The initial rising slope of current is therefore due to charging time rather than to the opening of a current channel. See text for explanation of arrow. (D and E) Current-voltage relationships for DLM fibers; the early peak current component at 72 and 96 hours is shown in (D), and the steady-state current at 72 and 96 hours is shown in (E). Current in (E) is measured 200 msec after command pulses are applied.

0036-8075/81/0424-0461$00.50/0 Copyright © 1981 AAAS

however, about the development of these distinct outward current mechanisms. Developmental studies of electrical properties of membranes have frequently focused on the generation of action potentials and thus on inward current mechanisms (*5*). One reason for this is that the presence of a regenerative action potential mechanism is easy to detect by the simple technique of constant current injection. Although this technique is satisfactory for detecting an inward current, it offers very little information about the kinetics of the outward current; furthermore, the technique is not at all suitable for distinguishing between different outward currents. In contrast, voltage clamping is an ideal technique for studying outward currents (*2, 3*) but is often not applicable to developing systems. The large dorsal longitudinal flight muscles (DLM) of *Drosophila* are well suited to both developmental studies (*6*) and voltage clamp techniques (*1*). In *Drosophila*, genetic techniques are also available that can be used for developmental studies of membrane electrogenesis and in studies of membrane biophysics.

To follow the development of the two separate *Drosophila* DLM currents, we subjected immature DLM fibers in situ to voltage clamp analysis at various times during the pupal period. The electrophysiological techniques used for these experiments have been described (*1*). Minor modifications were necessary to apply voltage clamping techniques to pupae rather than to adult *Drosophila* (*7*).

In *D. melanogaster*, the period of metamorphosis from puparium formation to the emergence of the adult lasts approximately 96 hours (*6*). The DLM develops from a group of presumptive muscle cells that appear in the thoracic region shortly after the formation of the puparium. Differentiation of this muscle begins 21 hours after puparium formation. At 50 hours, cross striation is visible, and the fibers are more than two-thirds of their final length. Differentiation is almost complete at 84 hours (*6*).

Neither the A current nor the delayed current is present in fibers approximately 55 hours after puparium formation. These very young fibers have membrane properties that can best be described as having a small, instantaneously rectifying leak (Fig. 1A). A similar rectifying instantaneous current was also observed in larval muscle fibers of *Drosophila* and is probably present in DLM fibers at all stages. Membrane electrical differentiation of the DLM proceeds rapidly after 55 hours, and by 72 hours the A current

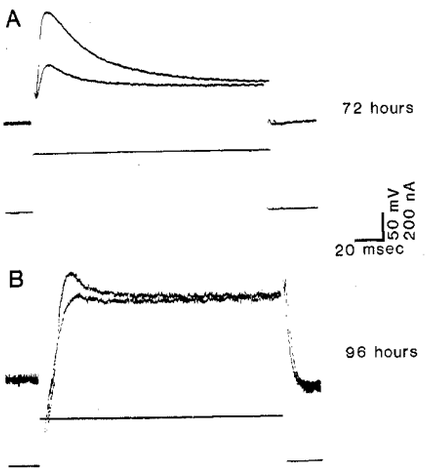

Fig. 2. Voltage clamp experiments showing the suppression of the A current by prior activation of the current. In both (A) and (B), a 100-mV depolarizing command pulse was applied first and then reapplied after 250 msec. In each case, the second of the two command pulses evoked a smaller A current response than the first. In (B) the turn-off of the A current is largely masked by the turn-on of delayed rectification. In (A) and (B), the holding potential is −80 mV. Membrane current is shown in the upper traces and voltage is shown in the lower traces. Outward current is in the upward direction.

is prominent, with an amplitude similar to that of mature fibers (Fig. 1B). At this stage the membrane current-voltage relationship at hyperpolarized levels is also similar to that of mature fibers (Fig. 1D). Delayed rectification is not obvious at this stage. During the next 24 hours, delayed rectification develops until the amplitude of the delayed current is similar to that of adult fibers (Fig. 1C) (*1*). The maturation of the delayed current is marked by the appearance of large, slow current tails that are characteristic of this current (Fig. 1C, arrow). The slow current tails are absent at the 72-hour stage, even when the membrane voltage is stepped down to −100 mV. Because the potassium reversal potential is more positive than the holding potential of −80 mV (*7*) (Fig. 1C), the current tails are in the inward (downward) current direction. At the 96-hour stage, the potassium-blocking agent tetraethylammonium ion (50 m*M*) greatly reduces the amplitude of both outward currents. Membrane resistance measured at hyperpolarized voltages is greatly increased by this treatment. As previously reported for adult animals (*1*), the A current has a long recovery period; thus, a portion of the A current may not be reactivated immediately after a prior activation of the current (Fig. 2, A and B). In each of these experiments, two equal 100-mV command pulses were applied 250 msec apart. In each case the second of the two command pulses evoked a much smaller A current response than the first pulse. Delayed rectification is not suppressed by prior activation; thus, Fig. 2, A and B, effectively illustrates the maturation of the delayed current with respect to the A current during the period of 72 to 96 hours of pupal development. In Fig. 2B, a large outward current (delayed rectification) is maintained for the duration of the command pulse, whereas in Fig. 2A, most of the current is the transient A current, which largely decays during the duration of the pulse.

Since the resting potentials of muscle fibers during development were lower than those in adults, the effects of different holding potentials on current-voltage relationships were investigated at different stages of development. At all stages of development, the current-voltage relationships were not changed by shifting the holding potentials between −60 and −90 mV. Adult muscle fibers have resting potentials within this range (*1*), but developing muscle fibers have resting potentials lower than this (*7*). Shifting the holding potential to more positive values results in partially inactivating a portion of the A current. This is true at any stage of development when the A current is present. At a holding potential of −30 mV the A current can no longer be activated by depolarizing command pulses, but it reappears if the holding potential is shifted to more negative values.

Thus, in a 72-hour pupa with DLM fibers having resting potentials of approximately −48 mV, the A current is partially refractory. Since resting potentials of developing cells may vary and thus currents may be refractory to different extents, current-voltage relationships measured by constant current injection differ greatly among cells having different resting potentials. However, current-voltage relationships determined by the voltage clamp technique from these same cells may be almost identical. For this reason data obtained by constant current injection was not considered reliable.

Inward current was not noted in DLM fibers until after approximately 84 hours. In these older pupae, active depolarizing responses could sometimes be evoked by injection of constant current into the muscle fiber (not shown). Application of 50 m*M* tetraethylammonium ion to 72-hour pupae did not result in active depolarizing responses to constant current injection. Adult cells treated in this manner produce prolonged all-or-none action potentials.

A study of the development of sepa-

rate outward current mechanisms in starfish oocytes showed that total potassium conductance also changed from a rapidly inactivating form to a slowly inactivating or noninactivating form during egg maturation (*8*). The interpretation in that study was that potassium inactivation disappears at maturation and that the transient outward current is modified to become the delayed rectifier. In the *Drosophila* DLM, however, there appear to be two distinct currents with different kinetics of activation as well as inactivation. In voltage clamp experiments in mature fibers, shifting the holding potential to more positive values than −60 mV not only eliminates the peaking component of outward current, but also reveals the much slower activation time of the delayed rectifier.

LAWRENCE SALKOFF*
ROBERT WYMAN
Department of Biology, Yale University, Post Office Box 6666, New Haven, Connecticut 06511

References and Notes

1. L. Salkoff and R. Wyman, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6216 (1980).
2. J. A. Connor and C. F. Stevens, *J. Physiol. (London)* **213**, 21 (1971); *ibid.*, p. 31; E. Neher, *J. Gen. Physiol.* **58**, 36 (1971); S. Hagiwara, K. Kusano, N. Saito, *J. Physiol. (London)* **155**, 470 (1961).
3. R. W. Aldrich, Jr., P. A. Getting, S. H. Thompson, *J. Physiol. (London)* **291**, 507 and 531 (1979); S. H. Thompson, *ibid.* **265**, 465 (1977).
4. P. C. Schwindt and W. E. Crill, *Neurosci. Abstr.* **5**, 295 (1979); H. Brown, D. DiFrancesca, S. Noble, *J. Exp. Biol.* **81**, 175 (1979); E. F. Barrett and J. N. Barrett, *J. Physiol. (London)* **255**, 737 (1976).
5. K. Takahashi, S. Miyazaki, Y. Kidokoro, *Science* **171**, 415 (1971); Y. Kodokoro, *Nature (London) New Biol.* **241**, 158 (1973); N. C. Spitzer and P. I. Baccaglini, *Brain Res.* **107**, 610 (1976); N. C. Spitzer and J. Lamborghini, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1641 (1976); I. Spector and J. M. Prives, *ibid.* **74**, 5166 (1977); P. H. O'Lague, D. D. Potter, E. J. Furshpan, *Dev. Biol.* **67**, 384 (1978); C. Goodman and N. Spitzer, *Nature (London)* **280**, 208 (1979).
6. D. Bodenstein, in *The Biology of Drosophila*, M. Demerec, Ed. (Hafner, New York, 1965), pp. 275–367; S. A. Shafiq, *J. Cell Biol.* **17**, 351 (1963).
7. Experiments were performed at temperatures between 10° and 14°C. The preparation was cooled with a Cambion Peltier plate. To expose the muscles to saline, we removed a small portion of the dorsal pupal case and opened the scutellum with microdissection needles. To prevent infusion of the saline throughout the pupal case, we sealed off the exposed area with petroleum jelly. To respirate the preparations, we opened the ventral part of the pupal case and exposed it to an airstream. The resting potentials of early (72-hour) muscle fibers averaged −48 mV with or without respiration. In mature (96-hour) pupae, resting potentials of muscle fibers averaged −60 mV and required respiration to maintain polarization at that level. Resting potentials are commonly more negative than potassium reversal potentials in insect muscle fibers. This is probably because membrane potentials are maintained at a more negative level by active metabolic processes [H. Huddart and D. W. Wood, *Comp. Biochem. Physiol.* **18**, 681(1966); M. B. Rheuben, *J. Physiol. (London)* **225**, 529 (1972)]. The composition of the saline used in these experiments was NaCl, 128 m*M*; KCl, 4.7 m*M*; $CaCl_2$, 1.8 m*M*; and phosphate, 1.0 m*M*; *p*H 6.9. In experiments with 96-hour pupae, calcium was removed from the saline to block the inward current, and 1.4 m*M* [ethylenebis(oxyethylenenitrilo)]tetraacetic acid was added. Magnesium chloride (14 m*M*) was added for membrane stability. The DLM fibers are nearly isopotential cylinders, measuring approximately 0.1 by 0.8 mm. Approximate passive membrane properties: R_m = 2 kilohm/cm^2; C_m = 9 μF/cm^2. Stocks of *Drosophila melanogaster* used were of the wild-type Canton-S strain.
8. S. Miyazaki, H. Ohmori, S. Sasaki, *J. Physiol. (London)* **246**, 55 (1975).
9. We thank R. Aldrich for reading the manuscript and making helpful suggestions and C. Stevens for invaluable advice and the loan of equipment. Supported by a Muscular Dystrophy research fellowship grant to L.S. and by Public Health Service grant NS 14887.
* To whom correspondence and reprint requests should be sent.

14 October 1980; revised 23 December 1980

Carbamoyl Phosphate Synthetase (Glutamine-Hydrolyzing): Increased Activity in Cancer Cells

Abstract. *The specific activity of carbamoyl phosphate synthetase (glutamine-hydrolyzing), the first and rate-limiting enzyme of de novo uridine 5′-triphosphate biosynthesis, was increased in 13 transplantable hepatomas, particularly in the rapidly growing tumors (5.7- to 9.5-fold), and the rise was correlated with tumor growth rates. Thus, synthetase activity was linked with both hepatic neoplastic transformation and progression. Synthetase specific activity was also elevated in a transplantable sarcoma (18-fold) and a kidney adenocarcinoma (5-fold). The increased activity should enhance the capacity of the pathway and should confer selective advantages to cancer cells.*

Neoplastic cells express a program that in pyrimidine metabolism requires increased activities of the key enzymes of biosynthesis and decreased activities of those of degradation (*1–3*). We now report that the specific activity of carbamoyl phosphate synthetase (glutamine-hydrolyzing), E.C. 6.3.5.5 (CPS II), the first and rate-limiting enzyme of de novo biosynthesis of uridine 5′-triphosphate (UTP) (*4, 5*), was increased in 13 rat hepatomas, and the rise was positively correlated with the increase in the growth rates of the tumors. These observations were made in experiments designed to resolve an apparent contradiction in that the concentration of cytidine 5′-triphosphate (CTP) in the rapidly growing hepatoma 3924A was increased fivefold over that in normal liver, but the UTP pool was unchanged (*6*). Since UTP is a feedback inhibitor of CPS II (*5*), the lack of increase in UTP concentration in the hepatoma might be advantageous to the cancer cells by not blocking the activity of CPS II. A selective metabolic advantage might also be conferred on cancer cells if there was an increase in CPS II activity. We tested this hypothesis by investigating the behavior of the activity of CPS II in chemically induced, transplantable solid hepatomas carried in inbred rats and in the normal, differentiating, and regenerating liver.

Methods for the maintenance of normal and tumor-bearing animals, for killing the animals and for excising liver and tumors, and studies on differentiating and regenerating liver have been reported (*1–3*). The growth rates of the hepatoma lines ranged from 2 to 52 weeks, measured as the time required for the tumors, which had been transplanted subcutaneously, to attain a diameter of 1.5 cm. Rat liver contains a mitochondrial enzyme, the ammonia- and *N*-acetyl-L-glutamate–dependent carbamoyl phosphate synthetase, E.C. 6.3.4.16 (CPS I), which provides carbamoyl phosphate for urea synthesis, as well as CPS II, a cytosolic enzyme which is solely responsible for pyrimidine biosynthesis (*5*). The activity of liver CPS I is 1000 times greater than that of CPS II. In the preparation of homogenates, the mitochondrial CPS I might be liberated, and it could interfere with accurate measurement of CPS II activity in crude extracts. By modifying a purification procedure for the rat liver enzyme (*7*), we developed a simple and reproducible technique for preparing an enzyme fraction for the CPS II assay; this method was applied to normal livers and hepatomas (legend to Fig. 1). That the partially purified CPS II obtained was not contaminated with CPS I was verified by absence of activation with acetylglutamate plus ammonium chloride. To calculate the CPS II activity of the soluble supernatant, we used the recovery of the activity of aspartate carbamoyltransferase, E.C. 2.1.3.2, in the same samples in which CPS II activity was measured, since aspartate carbamoyltransferase exists as a multienzyme complex with CPS II (*7, 8*). The recovery of CPS II activity was in good agreement with that observed for the highly purified enzyme added at the step of tissue homogenization (*9*). Typically, CPS II was purified about 11- and 33-fold from the 105,000*g* supernatant fractions of hepatoma 3924A and the control liver, respectively. Specific activities for both purified enzymes were 809 and 302 nmole/hour per milligram of protein, and

0036-8075/81/0424-0463$00.50/0 Copyright © 1981 AAAS