stances. Thus, the assumption on which the bicondylar angle measurement is based—that a tangent to the condyles is always horizontal—is invalid for the chimpanzee. Even in modern man the condylar tangent in a normal stance may deviate from horizontal (*7*). Whereas the apparently high bicondylar angle in *A. africanus* (Transvaal Museum 1513 and Sts 34) appears to be best interpreted in terms of an adducted femoral posture, the possibility remains that the functional significance of this feature is not the same as for modern man. In evaluating the locomotor ability of fossil forms, evidence of skeletal excursion is more meaningful than single measurements which may not be of comparable functional significance among differently adapted species.

The configuration of the margin of the femoral head in chimpanzee and man reflects some basic differences in femoral excursion. On the human femur, the articular surface is prolonged onto the femoral neck along the superior margin (arrow, Fig. 2C). In the chimpanzee, similar prolongation occurs along the posterior margin (arrow, Fig. 2A). Inasmuch as the femoral head and acetabulum are approximately congruent, the configuration of the margin of the femoral head relates to the depth and orientation of the acetabulum and the excursion of the femur. The superior margin in man is related to a relatively deep and ventrally facing acetabulum, and also to the fact that the femur normally moves in an adducted (valgus) position (Fig. 2, G and H). In the chimpanzee, the prolonged posterior margin is related to an excursion pattern of an abducted (varus) and flexed femur (Fig. 2, D and K) and an acetabulum that is relatively shallower and faces laterad. The intermediate configuration of the femoral head margin in *A. robustus* (for example, Transvaal Museum SK 82; Fig. 2B), together with the relatively shallow acetabulum (Transvaal Museum SK 50), is evidence that femoral excursion was of an intermediate pattern (Fig. 2, E and J). If the *A. robustus* femur were adducted as much as in man, a disproportionate area of articular surface would lie outside the bony acetabulum (Fig. 2, F and I). Although a very broad glenoid labrum may have made this possible, such an arrangement appears unlikely in view of the usual congruency of articular surfaces at the hip.

Major structural similarities of the australopithecine pelvis to that in modern man are convincing evidence of advanced adaptation to bipedality (*8*). However, certain obvious dissimilarities, such as the greater flaring of the iliac blades, may be explicable in terms of a more abducted femoral excursion. Furthermore, the present interpretation of a lack of valgus posture in some early Pleistocene hominids is in agreement with the biomechanical analyses of Napier and Preuschoft (*9*).

FARISH A. JENKINS, JR.
Department of Biology and
Museum of Comparative Zoology,
Harvard University,
Cambridge, Massachusetts 02138

References and Notes

1. W. L. Straus, *Clin. Orthop. Related Res.* **25**, 9 (1962); B. G. Campbell, *Human Evolution* (Aldine, Chicago, 1966); B. A. Sigmon, *Amer. J. Phys. Anthropol.* **34**, 55 (1971).
2. H. Elftman and J. Manter, *Amer. J. Phys. Anthropol.* **20**, 69 (1935); H. Elftman, *J. Mammal.* **25**, 67 (1944).
3. J. B. de C. M. Saunders, V. T. Inman, H. D. Eberhart, *J. Bone Joint Surg.* **35A**, 543 (1953); H. D. Eberhart, V. T. Inman, B. Bresler, in *Human Limbs and Their Substitutes*, P. E. Klopsteg and P. D. Wilson, Eds. (McGraw-Hill, New York, 1954), p. 437.
4. W. E. Le Gros Clark, *J. Anat.* **81**, 300 (1947); W. L. Straus, *Clin. Orthop. Related Res.* **25**, 9 (1962).
5. K. G. Heiple and C. O. Lovejoy, *Amer. J. Phys. Anthropol.* **35**, 75 (1971). The bicondylar angle is variously defined, but as used by Heiple and Lovejoy it is the angle between the femoral shaft and a perpendicular drawn from a line tangential to both femoral condyles as seen in anterior view. See Heiple and Lovejoy for details.
6. C. O. Lovejoy and K. G. Heiple, *ibid.* **32**, 33 (1970).
7. Anteroposterior radiographs of normal adult human knees demonstrate that, in at least a stationary stance, the condylar tangent frequently deviates from horizontal. Eighteen subjects were asked to assume a "natural standing position," and a wire plumb line was included for vertical reference. Maximum varus and valgus deviation observed was 5.5° and 11.7°, respectively, with a standard deviation for the total sample of 2.9°.
8. R. A. Dart, *Amer. J. Phys. Anthropol.* **7**, 301 (1949); W. E. Le Gros Clark, *ibid.* **13**, 19 (1955); *Man-Apes or Ape-Men?* (Holt, Rinehart & Winston, New York, 1967).
9. J. R. Napier, *Arch. Biol.* **75** (Suppl.), 673 (1964); H. Preuschoft, *Folia Primatol.* **14**, 209 (1971).
10. Work supported by NSF GB-30724 and NIH 5 S05 RR 07046-06. I thank B. Patterson and J. G. Fleagle for helpful discussions, C. R. Taylor for procuring the chimpanzees, J. Klopping and V. Rowntree for technical assistance, and Drs. H. J. Griffiths and E. L. Radin for radiographs of human knees. L. Meszoly prepared the illustrations.

5 September 1972

Phosphorylase: A New Isozyme in Rat Hepatic Tumors and Fetal Liver

Abstract. *A third set of phosphorylase a and b isozymes, distinguishable kinetically and immunologically from liver and muscle forms, is present in various rat hepatomas, and is also present, together with the adult liver form, in fetal rat liver. This is one of several striking examples of suppression of isozymes of adult liver coupled with the appearance of fetal isozymes in hepatomas.*

Malignant neoplasms are characterized by anomalies of genetic expression, manifested by ectopic production of polypeptide hormones (*1*), and by the synthesis of tumor-specific antigens (*2*), while organ-specific antigens are suppressed (*3*). This phenomenon has a counterpart in enzyme alterations of experimental rat hepatomas; also involving the suppression of isozymes which play a functional role in the adult, differentiated liver cell, and their replacement by other isozymes which are low or absent in adult liver (*4*, *5*). It is of further significance that in some instances both antigens and isozymes associated with tumors are found in normal fetal tissue, suggesting that genes coding for proteins synthesized in the fetal state and which are repressed during normal embryonic development are reexpressed in tumors (*5–7*).

We report recent results from our laboratory, which provide a striking new illustration of this pattern of isozyme alteration in demonstrating the presence in certain rat hepatomas of a third form of glycogen phosphorylase, which differs kinetically from the rat liver type and is distinguishable immunologically from the rat muscle type. It exists in both a and b forms, which are interconvertible by a phosphatase and kinase, also present in these tumors. Its presence in other normal tissues remains to be investigated, but it is undetectable in adult rat liver and skeletal muscle. However, it is present at relatively low activity in fetal rat liver.

Evidence for the existence of this set of phosphorylase isozymes is presented in Fig. 1, A–E. By isoelectric focusing of partially purified supernatant fractions of homogenized and centrifuged tissue, both the muscle and liver phosphorylases appear as single peaks with respective isoelectric points of 6.2 and 5.9 (Fig. 1, A and B). The

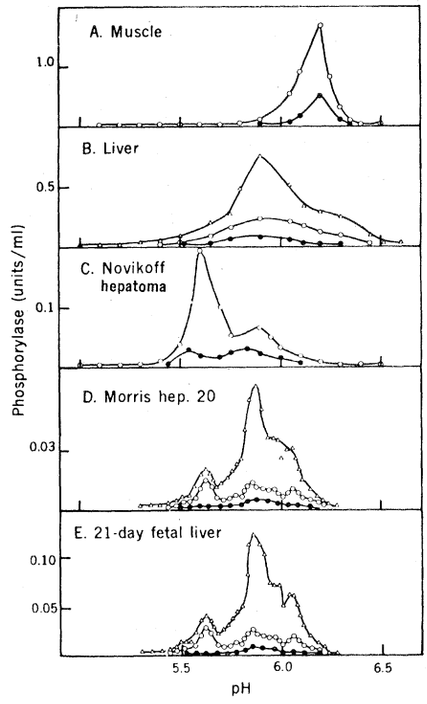

Fig. 1. Isoelectric focusing of phosphorylase of (A) rat skeletal muscle, (B) rat liver, (C) Novikoff ascites hepatoma, (D) Morris hepatoma 20, and (E) 21-day rat fetal liver. Isoelectric focusing was conducted as described in the instructions of LKB Instruments, with the 1801 column, and either the postmitochondrial glycogen pellet or a 25 to 50 percent saturated ammonium sulfate fraction of the 105,000*g* supernatant for hepatoma 20 (which contained virtually no glycogen) as the enzyme source. A 1 percent solution of ampholytes in 1 m*M* dithiothreitol was used to give the *p*H gradient with a sucrose gradient of 0 to 45 percent wt/vol. The initial voltage was maintained at 150 to 200 volts; after 12 hours it was increased to 300 to 350 volts and maintained there for 48 hours at 2°C. One-milliliter fractions were then collected for *p*H determination and phosphorylase assay according to Sutherland (*13*), with glucose-1-P-^{14}C as substrate, either without AMP (●), with 1 m*M* AMP (○), or with 1 m*M* AMP and 0.5*M* Na_2SO_4 (△). Isoelectric focusing completely separated the phosphorylases from the synthetases, which had *p*I values below 5 (not shown). Data for A, B, and C were obtained with a broad *p*H gradient of 3 to 10; D and E over a narrow range of 5 to 7.

Novikoff hepatoma similarly treated yields a phosphorylase peak with an isoelectric point of 5.6 (Fig. 1C). All three phosphorylases are predominantly in the b form, as indicated by their low activity without adenosine monophosphate (AMP). The tumor form is further distinguished from the liver form in that it does not require sulfate ion for activation (*8*). Other poorly differentiated, rapid-growing hepatomas such as the Morris 3924A hepatoma give a pattern similar to that of the Novikoff hepatoma, whereas the well-differentiated, slow-growing Morris hepatoma 20 possesses both the tumor form as a minor, and the liver isozyme as a major, component (Fig. 1D). As shown in Fig. 1E, 21-day fetal liver has a minor peak at *p*H 5.6 coinciding with the tumor form, together with a major peak at the *p*H of the adult liver isozyme.

Immunologic data in Fig. 2 further distinguish the tumor form from the muscle isozyme. An antibody to the crystalline rat muscle isozyme incubated with the tissue preparation inactivated the rat muscle isozyme essentially completely, but had little or no effect on either the liver isozyme or the Novikoff or hepatoma 20 isozyme. To characterize these isozymes further, the assays, after incubation with the antibody, were conducted with and without sulfate ion. The liver isozyme exhibited the usual SO_4^{2-} requirement, and there was a partial SO_4^{2-} requirement for hepatoma 20 phosphorylase, in keeping with the data in Fig. 1D, showing the presence of both liver and hepatoma isozymes. Hepatoma 3924A was partially inhibited by the muscle phosphorylase antibody, indicating that it may possess some muscle phosphorylase; but the major portion of the activity was unaffected and it is similar to the other hepatoma phosphorylases in lacking a SO_4^{2-} requirement. No inhibition was observed with a normal rabbit γ-globulin fraction.

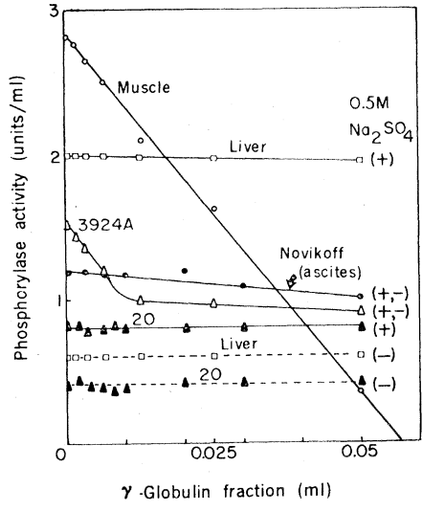

Fig. 2. Inactivation of phosphorylases by antiserum to rat muscle phosphorylase b. An antiserum was prepared as a rabbit serum globulin fraction after serial injections of a crystalline preparation of rat skeletal muscle phosphorylase prepared as described by Sevilla and Fischer (*14*), together with complete Freund's adjuvant. The glycogen pellets containing the enzyme were hydrolyzed with human salivary amylase, and a fixed amount, containing approximately 0.1 to 0.3 unit, was treated with antiserum essentially according to Schliselfeld *et al.* (*15*). After 20 minutes' incubation at 30°C, assays were conducted with 1 m*M* AMP; with 0.5*M* Na_2SO_4 (solid lines); and without Na_2SO_4 (broken lines). Plus and minus signs indicate the presence or absence of 0.5*M* sulfate.

Previous work from our laboratory and others (*4, 5, 7, 9*), has shown that this disorder of gene expression is so manifested in hepatomas that with loss of differentiation and increased growth rate, hepatomas lose glucokinase, aldolase B, and liver type pyruvate kinase. These are isozymes that are hormonally responsive and that play important functional roles in liver carbohydrate metabolism, and their loss in all likelihood is responsible for some of the metabolic deviations of these tumors from their cell of origin.

It has been suggested (*10*) that these and similar isozyme alterations may represent the molecular basis for the lack of control that is characteristic of the neoplastic cell type. The phosphorylases represent not only a further documentation and extension of these findings, but provide a particularly appropriate system for studies of control mechanisms and their loss in cancer, since probably no other regulatory enzyme system has been so intensively studied, and the interconversion of its active and inactive (a and b) forms represents one of the most fruitful models for understanding the molecular action of such hormones as cyclic AMP, glucagon, epinephrine, and insulin (*11*). Moreover, there is now a long history of peculiarities of glycogen storage in tumors, which may be better understood with the recognition of these new isozymes (*12*). The

chemical and kinetic properties and the enzymatic interconversion of the a and b forms of this hepatoma phosphorylase require further study.

KIYOMI SATO
HAROLD P. MORRIS
SIDNEY WEINHOUSE
Fels Research Institute and
Department of Biochemistry,
Temple University School of Medicine,
Philadelphia, Pennsylvania 19140, and
Department of Biochemistry,
Howard University, Washington, D.C.

References and Notes

1. C. M. Goodall, *Int. J. Cancer* **4**, 1 (1969); M. B. Lipsett, *Ann. Int. Med.* **61**, 733 (1964).
2. R. T. Prehn, *Fed. Proc.* **24**, 1018 (1965).
3. L. Lengerova, *Advan. Cancer Res.* **16**, 235 (1972).
4. T. Ono and S. Weinhouse, Eds., *Isozymes and Enzyme Regulation in Cancer* (Gann Monogr. 13, Japanese Foundation for Cancer Research, Tokyo, in press).
5. S. Weinhouse, *Cancer Res.* **32**, 2007 (1972).
6. G. I. Abelev, *Advan. Cancer Res.* **14**, 295 (1971); P. Gold, *Progr. Exp. Tumor Res.* **14**, 43 (1971).
7. W. E. Knox, *Enzyme Patterns in Fetal, Adult and Neoplastic Rat Tissues* (Karger, Basel, 1972).
8. M. M. Appleman, E. G. Krebs, E. H. Fisher, *Biochemistry* **5**, 2101 (1966).
9. F. Schapira, *Pathol. Biol.* **18**, 209 (1970); ——— and C. Gregori, *C. R. Acad. Sci.* **272**, 1167 (1971); T. Matsushima, S. Kawake, M. Shibuya, T. Sugimura, *Biochem. Biophys. Res. Commun.* **30**, 565 (1968).
10. F. A. Farina, R. C. Adelman, C. H. Lo, H. P. Morris, S. Weinhouse, *Cancer Res.* **28**, 1897 (1968); V. R. Potter, *Exploitable Molecular Mechanisms and Neoplasia* (an Annual Symposium on Fundamental Cancer Research) (Williams & Wilkins, Baltimore, 1969); G. Weber, *Naturwissenschaften* **55**, 418 (1968).
11. E. M. Fischer, M. G. Heilmeyer, Jr., R. H. Haschke, in *Current Topics of Cellular Regulation*, B. L. Horecker and E. R. Stadtman, Eds. (Academic Press, New York, 1971), vol. 4, pp. 211–265; H. DeWulf, *The Control of Glycogen Metabolism in the Liver* (Vancer, Louvain, 1971).
12. V. N. Nigam and A. Cantero, *Advan. Cancer Res.* **16**, 1 (1972).
13. E. Sutherland, *Methods Enzymol.* **1**, 215 (1955).
14. C. L. Sevilla and E. H. Fischer, *Biochemistry* **8**, 2161 (1969).
15. L. H. Schliselfeld, C. H. Davis, E. G. Krebs, *ibid.* **9**, 4959 (1970).
16. This work was supported by grants from the National Cancer Institute, CA-12227, CA-10916, and CA-10729; and BC-74M from the American Cancer Society.

25 July 1972; revised 14 September 1972

Synaptic Transmission at Single Glomeruli in the Turtle Cerebellum

Abstract. *We have recorded from the granular layer of the turtle cerebellum extracellular unitary potentials that appear to reflect pre- and postsynaptic events at the synapse between a single swelling of a mossy fiber and the dendritic tips of several granule cells. The presynaptic component is an all-or-none potential. It can be directly activated by spinal stimulation and is unaltered by repetitive activity or by high concentrations of magnesium. The postsynaptic component is a graded potential. It follows the presynaptic component by approximately 1 millisecond and is depressed by repetitive activity and by high concentrations of magnesium. The recording of large potentials produced by the flow of postsynaptic current within a single glomerulus suggests powerful transmission. Electron micrographs demonstrate large cerebellar glomeruli in the turtle and a substantial accumulation of mitochondria in the dendritic tips of granule cells.*

We report here the electrical activity and structure of single glomeruli in the turtle cerebellum. Specimens of *Pseudemys scripta elegans* were sedated with sodium pentobarbital (3 mg/kg) and immobilized with gallamine (1 to 2 mg/kg), both injected intraperitoneally, and were respirated artificially. Tungsten or steel microelectrodes (*1*) having impedances of 1 to 3 megohms (1 khz) were advanced through a slit in the dura. The unconvoluted, layered structure of the turtle cerebellum ensures that the microelectrode records sequentially from the molecular, Purkinje cell, and granular layers. The characteristic "simple" and "complex" spikes of Purkinje cells (*2*) provided one physiological landmark and the abrupt quiescence on entering the fourth ventricle another. This routine method for locating the granular layer was confirmed in 18 cases by histological marking techniques (*1*).

We have recorded a variety of unitary potentials from this granular layer. The most striking potential consisted of an all-or-none, positive-negative biphasic spike followed, after a brief delay (0.8 to 1.6 msec), by a slower negative wave of variable amplitude (Fig. 1). The initial portion is designated the B (biphasic) potential and the subsequent negative deflection, the negative afterwave (NAW). Together they are referred to as a B complex (*3*). We have isolated 34 B complexes in the course of 87 experiments. Although encountered less frequently than other wave shapes, B complexes have some unusual features which make them the subject of this report.

The B complexes shown in Fig. 1A were elicited by a maintained extension of the left hind limb. This produced a train of B potentials, each of which was followed by a NAW. The two superimposed traces (Fig. 1A) show only three discharges from the train; the B potentials are marked by dots and the first, second, and *n*th NAW's are labeled. The amplitude of the NAW decreased after the first discharge, whereas the B potential was unchanged. Another unit (Fig. 1B) responded with a train of B complexes when the right hind limb was touched. The superimposed traces, triggered on the rising phase of the B potentials, show each NAW in the train. The amplitude of the NAW declined progressively, the first in the sequence being the largest and the last the smallest.

Responses to electrical stimulation of the spinal cord allowed a more controlled investigation of the depression of the NAW produced by prior activity. The dura overlying the spinal cord was exposed by boring a hole through the carapace and vertebrae. After slitting the dura, we placed a monopolar electrode over the cord in contact with the cerebrospinal fluid. Shocks of 1 to 10 volts (0.1-msec duration) activated many of the units recorded in the granular layer. The B complexes which were studied discharged once at a fixed latency in response to a single shock. The time course of the depression of the NAW was studied by observing the amplitude of the second of the two NAW's elicited by paired shocks delivered to the spinal cord. The depression was maximum for intershock intervals of 5 to 10 msec (Fig. 1E). The NAW progressively increased with longer intervals (Fig. 1, F to H). Complete recovery required up to 1 second.

The all-or-none nature of the B potential indicates that it is a single action potential. The delay between the B potential and the NAW, as well as the graded nature of the latter, suggests that the NAW represents current at postsynaptic structures. A waveform similar to the B complex has been observed with extracellular microelectrode recordings at the giant synapse of *Loligo*, the neuromuscular junction of the rat and crayfish, and in the auditory system (*4*, *5*). In all these cases an