References and Notes

1. B. L. Baker, S. Dek, A. R. Midgby, B. D. Gersten, *Anat. Rec.* **166**, 557 (1970); B. L. Baker, *ibid.* **172**, 264 (1972); R. Hess, D. Barratt, J. Gelger, *Experientia* **24**, 584 (1968); R. F. Phifer and S. S. Spicer, *Lab. Invest.* **23**, 543 (1970).
2. D. DeWied, in *The Neurosciences, Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), pp. 653–666.
3. L. Terenius, W. H. Gispen, D. DeWied, *Eur. J. Pharmacol.* **44**, 395 (1975).
4. D. T. Krieger, A. Liotta, M. J. Brownstein, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 648 (1977).
5. S. J. Watson, H. Akil, S. Sullivan, J. D. Barchas, *Life Sci.* **21**, 733 (1977); R. Elde, T. Hökfelt, O. Johannson, L. Terenius, *Neuroscience* **1**, 349 (1976); A. H. Coons, in *General Cytochemical Methods*, J. F. Danielli, Ed. (Academic Press, New York, 1958).
6. B. A. Eipper, R. E. Mains, D. Guenzi, *J. Biol. Chem.* **251**, 4121 (1976).
7. The swine antiserum to immunoglobulin G (IgG) was from Accurate Chemical and Scientific Corp., Hicksville, N.Y.
8. J. C. Adams, *Neuroscience* **2**, 141 (1977).
9. The ACTH 1-24 was from Ciba-Geigy Corp., Ardsley, N.Y.
10. H. D. Moon, C. H. Li, B. M. Jennings, *Anat. Rec.* **175**, 529 (1973).
11. G. Pelletier, R. Leclerc, F. Labrie, J. Cote, M. Chretien, M. Lis, *Endocrinology* **100**, 770 (1977).
12. C. H. Li and D. Chung, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1145 (1976); J. Hughes, T. Smith, H. W. Kosterlitz, L. A. Fothergill, B. Morgan, H. R. Morris, *Nature (London)* **258**, 577 (1975); L. H. Lazarus, N. Ling, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2156 (1976); P. Crine, S. Benjannet, N. G. Seidah, M. Lis, M. Chrétien, *ibid.* **74**, 1403 (1977).
13. R. E. Mains, B. A. Eipper, N. Ling, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3014 (1977).
14. D. T. Krieger *et al.*, *Biochem. Biophys. Res. Commun.* **73**, 930 (1977); H. Akil, C. H. Li, J. D. Barchas, in preparation.
15. S. J. Watson, J. D. Barchas, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
16. R. E. Mains and B. A. Eipper, in preparation.

17. We thank B. Eipper and R. Mains for their gift of ACTH antiserum and for encouragement; C. H. Li, University of California, San Francisco, for the human β-LPH; and D. Blacker for technical assistance. This work was supported by NIDA grants DA 01522 and DA 01207, and NIMH program-project grant MH 23861 (to J.D.B.). S.J.W. is supported by NIMH training fellowship MH 11028, and by a Bank of America-Giannini Foundation postdoctoral fellowship. J.D.B. holds research scientist development award MH 24161.

11 November 1977

Do Albumin Clocks Run on Time?

About 10 years ago, Sarich and Wilson (*1*) presented evidence from modern primates that serum albumin proteins change at a regular rate, and proposed that observed differences in albumins between species could be used to estimate times of divergence and help reconstruct phylogenies. Despite some criticisms (*2*), the approach has become widely accepted, and current workers cite an impressive body of evidence from a variety of vertebrates [for example, ranid and hylid frogs (*3, 4*), iguanid lizards and crocodylians (*5*), marsupials (*4*), placental carnivores (*6*), and primates (*7, 8*)] that serum albumins change at a regular rate, calculated to be 0.6 million years (m.y.) per immunological distance unit (IDU) (*9*). Demonstration of regularity in albumin evolution and calibration of rate of change ultimately rest on interpretation of paleontological evidence. Contrary to published assertions, the fossil record provides little evidence for the accepted calibration rate, or even for the hypothesis that serum albumins evolve at a regular rate.

The currently accepted albumin clock rate is from Sarich (*7*), based on comparisons between prosimian and anthropoid primates. A mean immunological distance (ID) of about 100 (*10*) and an estimated time of divergence between modern prosimians and anthropoids of about 60 m.y. yielded a rate of 1 IDU per 0.6 m.y. (or 1.67 IDU per m.y.). Two years later, Sarich [table 3 in (*8*)] presented data that increased the prosimian versus anthropoid mean ID to 125, but he retained the original rate of 1 IDU/0.6 m.y., and that is the rate that all later papers cite. I have summarized Sarich's (*7, 8*) primate ID data in Table 1, along with ranges of estimated times of divergence based on current interpretations of the fossil record (*11*), and the resulting estimated rates of albumin change calculated from the mean ID's. The minimum likely rate from the latest prosimian versus anthropoid comparison (1 IDU/0.48 m.y.) is higher than the maximum likely rates suggested by various higher primate comparisons (around 1 IDU/0.60) m.y.). A date older than 60 m.y. for the prosimian-anthropoid split is highly unlikely since at that time (middle Paleocene), the first primate radiation had just gotten under way and modern primates arose from a second, later radiation (*12*). Dates younger than the minimum estimated times of divergence for the various anthropoid comparisons listed in Table 1 are highly unlikely, since by those times (or very shortly thereafter), we have undoubted fossils of the relevant groups involved. Thus the prosimian versus anthropoid data suggest a rate of albumin change that is in contradiction with rates suggested by data from higher primate comparisons. Despite this internal inconsistency, those data remain the cornerstone for the albumin clock calibration.

The placental carnivore data cited as evidence for a rate of serum albumin change of 1 IDU/0.6 m.y. are canoid versus feloid comparisons (*6*) that showed a mean ID of 89. Given the range of possible times of divergence suggested by the fossil record (see Table 1), one can say only that those data are not in contradiction with a rate of 1 IDU/0.6 m.y. However, a full analysis of the data from that study shows within the canoids a *Canis* versus arctoids mean ID of 46. That yields a maximum rate (1 IDU/0.80 m.y.) that is lower than the minimum likely rate of the canoid versus feloid split (1 IDU/0.67 m.y.). It is highly unlikely that canoids and feloids diverged earlier than 60 m.y., since at that time the miacid radiation that later gave rise to modern carnivores was just beginning (*12*), and it is also unlikely that *Canis* and arctoid stocks diverged later than 37 m.y., since about that time we have the earliest members of those groups. Thus,

Table 1. Immunological distances and the fossil record.

Pair	ID		Estimates from	
	Mean	Range	Fossil record divergence dates (m.y.)	Albumin clock rates (m.y./IDU)
Three prosimians versus three anthropoids (*7*)	103	93 to 112	45 to 60	0.44 to 0.58
Six prosimians versus five anthropoids (*8*)	125	115 to 131	45 to 60	0.36 to 0.48
Eleven ceboids versus five catarrhines (*7*)	59	43 to 70	35 to 55	0.59 to 0.93
Six cercopithecoids versus five hominoids (*7*)	35	28 to 42	20 to 30	0.57 to 0.86
Two gibbons versus four other hominoids (*8*)	13	12 to 15	No good evidence	
Homo versus *Pan* + *Gorilla* (*8*)	8	7 to 9	5 to 20	0.62 to 2.5
Four canoids versus three feloids (*6*)	89	69 to 105	37 to 60	0.42 to 0.67
Canis versus six arctoids (*6*)	46	31 to 56	37 to 60	0.80 to 1.30

0036-8075/78/0609-1182$00.50/0

within the carnivore data, as within the primate data, there is a contradiction.

The evidence from marsupials and hylid frogs for regularity in albumin change at a rate of 1 IDU/0.6 m.y. is based on comparisons of Australian versus New World species that yielded mean ID's of 103 for the marsupials and 129 for the frogs (*4*). The estimated time of divergence was taken from plate tectonic evidence of a South America–Antarctica separation dated at about 70 m.y., which yields rates of albumin change of 1 IDU per 0.68 and 0.54 m.y., respectively. However, a geological date for time of separation of continents is poor evidence for times of divergence of species, since speciation may occur in the absence of oceanic barriers (that is, before time of continent separation), and colonization by rafting or island hopping is possible for many groups of animals long after continents begin to separate. The poor fossil record of early marsupials suggest no better bracketing of the divergence time of South American versus Australian species than about 60 to 100 m.y. (*13*), a range too broad to provide good evidence for a given albumin change rate. The fossil record of hylid frogs is even poorer than that of the marsupials, and therefore also useless for independent evidence of albumin clock calibration or for concluding, as has been done (*4*), that hylid albumins evolved at approximately the same rate as those of marsupials.

The reptile data for albumin rates are based on comparisons within iguanids and crocodylids (*5*). The authors noted that the maximum ID's observed within each of those two families (97 and 96) were close to the maximum ID's observed within mammalian carnivores and primates, further noted that crocodylids and many lizard families are known from the Cretaceous (approximately 135 to 65 m.y.), and concluded that those reptile families were comparable in age to orders of placental mammals, and therefore that the rate of albumin evolution in reptiles was comparable to that of mammals. Those conclusions do not follow from the evidence presented: the Cretaceous is a long period, most mammalian orders arose near the end of that period, and the fossil record of iguanids and crocodylids is not good enough to indicate even roughly when various genera diverged and thus allow testing of the accepted rate of albumin evolution. The same problem exists for the data from ranid frogs (*3*), for which the fossil record is equally poor and inadequate to test hypotheses about albumin rates.

Thus, of the various groups of vertebrates cited as providing evidence for a regular rate of albumin change at 1 IDU 0.6 m.y., only some primates and carnivores have a fossil record that is good enough to allow rough calibration of the albumin clock, and for both of those groups there are internal inconsistencies within the data. Further, the hypothesis that albumins change at a regular rate (in a stochastic sense) is contradicted by the existence of "slow" versus "fast" species assemblages in most groups examined—for example, prosimians versus anthropoids (*8*), bears versus other carnivores (*6*), and *Caluromys* and *Marmosa* versus other didelphid marsupials (*4*). Such deviations from regularity are acknowledged by the same investigators who argue for a regular rate of albumin evolution (*4, 14*). In some recent papers (*14, 15*) data from transferrin comparisons are combined with those from albumins to infer phylogeny and times of divergence, but the transferrin clock is calibrated by comparison with the albumin clock, which means it is not an independent check.

Comparisons of serum albumins are a promising approach to test hypotheses of phylogenetic relationships generated from comparative anatomical and paleontological evidence. Even without good absolute rate calibrations, such comparisons can provide evidence on phylogenetic relationships if it has first been demonstrated that albumins have evolved at a regular rate in the groups compared. This comment should not be construed as a criticism of the approach, or a haggling over details of rate calibrations, but rather I mean it as a plea for more scientific rigor in the development and use of the approach so that its full potential may be realized.

LEONARD RADINSKY
Anatomy Department,
University of Chicago,
Chicago, Illinois 60637

References and Notes

1. V. Sarich and A. Wilson, *Science* **154**, 1563 (1966); *Proc. Natl. Acad. Sci. U.S.A.* **58**, 142 (1967).
2. For example, D. Read and P. Lestrel, *Science* **168**, 578 (1970); T. Uzzell and D. Pilbeam, *Evolution* **25**, 615 (1971); O. Lovejoy, A. Burstein, K. Heiple, *Science* **176**, 803 (1972).
3. D. Wallace, M. King, A. Wilson. *Syst. Zool.* **22**, 1 (1973).
4. L. Maxson, V. Sarich, A. Wilson, *Nature (London)* **255**, 397 (1975).
5. G. Gorman, A. Wilson, M. Nakanishi, *Syst. Zool.* **20**, 167 (1971).
6. V. Sarich, *ibid.* **18**, 386 (1969).
7. _____, in *Perspectives on Human Evolution*, S. Washburn and P. Jay, Eds. (Holt, Rinehart and Winston, New York, 1968), p. 94.
8. V. Sarich, in *Old World Monkeys*, J. Napier and P. Napier, Eds. (Academic Press, New York, 1970), p. 175.
9. Immunological distance was defined by Sarich (*6, 8*) as 100 log index of dissimilarity, the latter being the factor by which the antiserum concentration must be raised in order to produce a heterologous curve, of complement fixation equal in height to that produced by the homologous antigen. Reactions of immunological identity will have an ID of 0. The use of a log factor is an important assumption in the model.
10. Although Sarich still used the index of dissimilarity in (*7, 8*), I have converted those data into IDU's for this discussion and in Table 1.
11. I have chosen generous minimum and maximum estimates of times of divergence based on paleontological evidence, so that new evidence will narrow rather than broaden these ranges.
12. A. Romer, *Vertebrate Paleontology* (Univ. of Chicago Press, Chicago, 1966), pp. 217 and 232.
13. Marsupials and placentals diverged around 110 m.y. which suggests 100 m.y. as a reasonable upper limit for the Australian–South American marsupial divergence; South American faunas appear to have been isolated by about 60 m.y., but in the absence of knowledge of Australian faunas of that time, the 60-m.y. minimum limit is based on plate-tectonic evidence.
14. J. Cronin and V. Sarich, *J. Hum. Evol.* **4**, 357 (1975).
15. V. Sarich, *Nature (London)* **245**, 218 (1973); *Trans. Zool. Soc. London* **33**, 165 (1976).
16. I thank P. Gingerich, R. Kay, and M. McKenna for their opinions on primate divergence times, but take sole responsibility for the estimates given in Table 1. Supported by NSF grant DEB76-17746 A01.

26 September 1977

That the albumin clock generally runs on time is evident from relative rate tests (*1, 2*), which do not depend on the fossil record. These tests involve analysis of the relative amounts of change along the diverging branches of a molecular phylogenetic tree (*1, 2*). Therefore, we are unwilling to agree with Radinsky that the demonstration of regularity in albumin evolution rests ultimately on the interpretation of fossil evidence (*3*). The fossil record is needed only to calibrate the albumin clock.

It is unfortunate that to calibrate evolutionary clocks one must compare the quantitative biochemical measurements with paleontological estimates of divergence times that are only semiquantitative. In order to understand the uncertainties of divergence time estimates, it is essential to consider how these times are arrived at from fossil evidence.

The problem of inferring divergence times from fossil evidence is illustrated in Fig. 1. Consider the very simple case of two present-day species, A and B. We wish to use fossil evidence to find out their divergence time. Assume that the last common ancestor of A and B is the hypothetical species C. Consider also the hypothetical species D, the common ancestor of all present-day species. These four species are connected by three hypothetical lineages: the common ancestral lineage leading from D to C, the lineage leading from C to A, and the lineage leading from C to B. Now consider a fossil. What can the fossil tell us about the time when species C split into two noninterbreeding species, that is, the progenitors of A and B? The first step

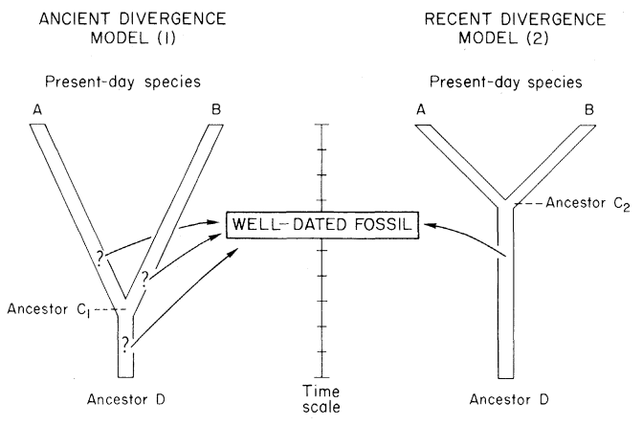

Fig. 1 (left). The problem of inferring divergence times from fossil evidence.

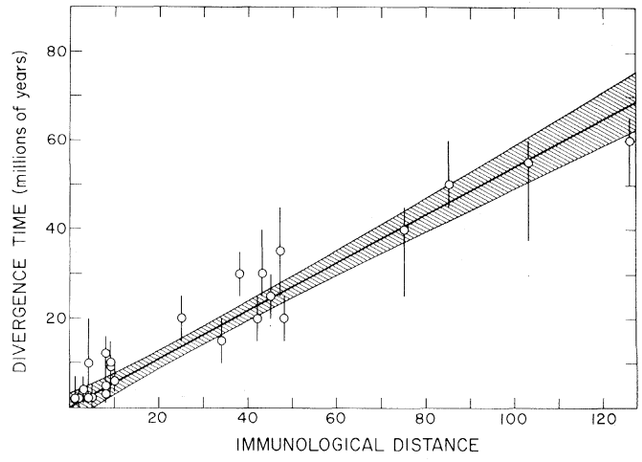

Fig. 2 (right). Calibration of the albumin evolutionary clock. Paleontologically estimated divergence times for 25 pairs of carnivore and ungulate taxa are plotted against the amount of sequence difference, measured immunologically, that has accumulated between the albumins of these same creatures. The immunological distance values were obtained by comparing the albumins by the microcomplement fixation test (*5*). The best estimate of divergence times is indicated by the empty circles and the approximate extremes of these estimates by the vertical lines. The data for this figure are taken from figure 2 in (*1*). The least squared line is $t = 0.54\ y$, where t is divergence time and y is immunological distance. The hatched area indicates the 95 percent confidence limits for the predicted values of t.

in solving this problem is to determine the time t when this fossil lived. A reliable date can often be obtained by radiometric methods, which are quantitative and objective. The second step is to determine the genealogical relationship of the well-dated fossil to the lineages DC, CA, and CB. This is not a straightforward task (*4*). The paleontologist decides intuitively which of the alternative phylogenetic models (models 1 or 2) to adopt and, in the case of model 1, which of the three lineages gave rise to the fossil.

Suppose one decides that model 2 is correct and thus that the fossil descended from the DC lineage; it follows that the time since the CA and CB lineages separated is probably less than t. Alternatively, one may choose model 1 and assign the fossil to any one of the three lineages. If one assigns the fossil to either the CB or CA lineages, the AB divergence time is inferred to be greater than t, whereas if assignment is made to the DC lineage, the AB divergence time could be greater or less than t. Thus paleontological estimates of divergence times are of questionable precision.

Despite the semiquantitative nature of the current paleontological estimates of divergence times, it is possible to calibrate the albumin clock approximately. This is best done with young taxonomic groups having an abundant and well-studied fossil record because, in such cases, the error in estimating divergence times is probably minimized. The mammals are such a group; and within this group the carnivores and ungulates are especially appropriate. Their albumins have evolved at a rather steady rate during the past 70 million years (Fig. 2). The paleontologic estimates of divergence times for 25 pairs of carnivore and ungulate taxa are plotted against the amount of sequence difference, measured immunologically, that has accumulated between the albumins of these same creatures. The immunological distance scale is based on comparison of the albumins with the microcomplement fixation method, each unit of immunological distance corresponding to about one amino acid substitution in albumin (*1, 2, 5*). If the best estimates (Fig. 2, circles) and the two extremes are considered as three separate estimates of the divergence time, a correlation coefficient of 0.92 is obtained between time and immunological distance. When only the best estimates of divergence times are considered, a correlation coefficient of 0.97 is obtained and the equation of the least squared line is

$$t = 0.54\ y$$

where t is divergence time, and y is immunological distance. This calibration line is shown in Fig. 2, and its slope (0.54) is an estimate of the rate constant for albumin evolution.

The approximately linear relation between time and sequence evolution (Fig. 2) is not unique to mammalian albumin. This kind of relation has also been found with other mammalian proteins, including hemoglobins, myoglobins, cytochromes c, and fibrinopeptides (*1, 6*), as well as with amphibian lactate dehydrogenases (*7*) and reptilian transferrins (*8*).

Having illustrated the time dependence of albumin evolution in carnivores and ungulates (Fig. 2), we turn to the problem of albumin evolution in primates. In the case of primates there are wide differences of opinion about the times of divergence, wider than Radinsky indicates [table 1 in (*3*)]. We do not regard the time ranges he gives as outer bounds (*1, 2*) and, for this reason, we do not accept his conclusion that primate albumin evolution has been inconsistent with the clock hypothesis. We consider that all of the primate albumin data are consistent with the mean evolutionary rate observed for carnivores and ungulates, namely, approximately one immunological distance unit per 0.54 million years. In addition, relative rate tests have been applied to primate albumins. These tests suggest that albumin evolution has been as steady in primates as in other mammals (*1, 2*).

It is harder to calibrate protein evolution and time of divergence for taxonomic groups whose fossil record is poorer than that of mammals. Compared to mammals, the modern families of reptiles and amphibians have a poor fossil record (*9*). Yet it was particularly important to examine albumin evolution in reptiles and amphibians. During the past 75 million years, these lower vertebrates have been evolving about ten times more slowly at the organismal level than mammals have (*1, 9, 10*). Reptiles and amphibians therefore provided an excellent opportunity to disprove the clock hypothesis. The results of extensive comparative studies have given no indication that lower vertebrate albumins have

been significantly retarded in their evolution (*1*). These results were more consistent with the clock hypothesis than with prevailing evolutionary theory, which assumed a simple relation between protein evolution and organismal evolution.

We have calculated an approximate standard error for the slope of the calibration line in Fig. 2 and constructed 95 percent confidence limits for the divergence times predicted from this equation. These limits must be regarded only as an approximate minimum estimate. To do this analysis, we assumed that the magnitude of the errors associated with each divergence time is the same for all values of immunological distance and that errors are normally distributed.

The error to which the calibration line in Fig. 2 is subject has several components: a paleontological error, an experimental error, and a stochastic or probabilistic error. The paleontological error is the result of the uncertainties in divergence time estimates, while the other two errors are independent of these estimates. The experimental error is the error in estimating how different the protein sequences are (by either direct sequencing or immunological procedures). The probabilistic error is an intrinsic feature of the clock. The clock is not metronomic (*1, 6*). The number of sequence changes that have occurred in a given protein, such as cytochrome c, in different species during a particular period of time follows a frequency distribution (*1, 11*). Several workers have attempted to measure this intrinsic error of the evolutionary clock for sequenced proteins (*6*) and for immunological comparisons of serum albumin (*12*). For sequenced proteins this error appears to be about twice as great as that expected for a probabilistic process like radioactive decay (*6*).

Even though the precision of protein clocks requires further definition, they appear precise enough to be extremely useful as tools for analyzing the dynamics of evolutionary processes during the past 100 million years or so. Protein clocks appear to have the potential for giving us a temporal view of evolutionary relations among all living species. By contrast, the fossil record is too poor to give us such a complete view. The vast majority of species do not have good fossil records. For this reason, it seems unlikely that paleontology will ever be able to estimate divergence times for more than a small fraction of living species that had common ancestors within the past 100 million years. With the approximate time depth between species provided by protein clocks, we can view the properties of those species from a time perspective. That is, we can calculate rates of evolutionary change for any property of the species being compared. Protein clocks are thus giving new perspectives on evolution at various levels of biological organization and generating new hypotheses concerning the biochemical basis of evolution at the supramolecular level (*1, 13*).

STEVEN S. CARLSON
Department of Biochemistry and Biophysics, University of California, San Francisco 94143
ALLAN C. WILSON
Department of Biochemistry, University of California, Berkeley 94720
RICHARD D. MAXSON
Department of Genetics and Development, University of Illinois, Urbana 61801

References and Notes

1. A. C. Wilson, S. S. Carlson, T. J. White, *Annu. Rev. Biochem.* **46**, 573 (1977).
2. V. M. Sarich and J. E. Cronin, in *Molecular Anthropology*, M. Goodman and R. E. Tashian, Eds. (Plenum, New York, 1977), p. 141.
3. L. Radinsky, *Science* **200**, 1182 (1978).
4. It is unlikely that the fossil represents an organism which was an actual member of lineages DC, CA, or CB. It is more likely that the fossil represents a species that evolved as an offshoot of one of these lineages. This is because extinction rates are generally high, especially for mammals [G. L. Bush, S. M. Case, A. C. Wilson, J. L. Patton, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3942 (1977); D. M. Raup, *Paleobiology* **4**, 1 (1978)]. If we were to consider all the mammalian species existing at time t, then, for $t > 20$ million years, only a small fraction of those species were members of lineages that survive today. For this reason the thin arrows leading to the fossil in Fig. 2 are shown as branching from the lineages DC, CA, and CB before time t.
5. A. B. Champion, E. M. Prager, D. Wachter, A. C. Wilson, in *Biochemical and Immunological Taxonomy of Animals*, C. A. Wright, Ed. (Academic Press, New York, 1974), p. 397.
6. W. M. Fitch, in *Molecular Evolution*, F. J. Ayala, Ed. (Sinauer, Sunderland, Mass., 1976), p. 160.
7. S. N. Salthe and N. O. Kaplan, *Evolution* **20**, 603 (1966).
8. S.-H. Mao and H. C. Dessauer, *Comp. Biochem. Physiol.* **40A**, 669 (1971).
9. A. S. Romer, *Vertebrate Paleontology* (Univ. of Chicago Press, Chicago, ed. 3, 1966).
10. L. M. Cherry, S. M. Case, A. C. Wilson, *Am. Zool.* **17**, 910 (19 → *Science* **200**, 209 (1978).
11. Radinsky has commented on the occurrence of albumins which seem to have evolved more slowly or more quickly than the average. Some investigators call these albumins "slow" or "fast" evolving, and the existence of this phenomenon is taken as evidence contradictory to the evolutionary clock. However, this variation in rate is within the range that could reasonably be explained by a probabilistic clock.
12. In the case of mammalian albumin evolution, measured immunologically, Nei estimated an error that is roughly four times bigger than is expected for a Poisson process [M. Nei, *J. Mol. Evol.* **9**, 203 (1977)]. His estimate includes both the experimental error and the intrinsic error of the albumin clock.
13. A. C. Wilson, *Stadler Genet. Symp.* **7**, 117 (1975); ———, T. J. White, S. S. Carlson, L. M. Cherry, in *Molecular Human Cytogenetics*, R. S. Sparkes, D. E. Comings, C. F. Fox, Eds. (Academic Press, New York, 1977), p. 373.
14. We thank E. A. Zimmer and L. R. Maxson for helpful comments.

27 March 1978

Autonomous Timer in Malpighian Tubules

Friedman and Johnson (*1*) have suggested the existence of an autonomous biological timer in *Drosophila* Malpighian tubules which "assures synchrony between the appearance of urate oxidase activity in the tubules and emergence of the adult from the puparium." This conclusion is based on the demonstration that, in constant light, tubules transplanted from single, developmentally aged pupae expressed urate oxidase activity (in day-old hosts) at about the expected time of emergence for each pupa. The authors have also indicated that the circadian clock regulating emergence may have components in common with this Malpighian timer.

It is tempting to ascribe control of all manner of developmental events to a circadian oscillator. In fact, it would be pleasing if the temporal organization of the whole of development could be described in terms of circadian input. However, this viewpoint is not supported by the circadian literature. Pittendrigh and Skopik (*2*), working from earlier observations of Harker (*3*), have shown that at least two defined points in the development of three species of *Drosophila* (bristle pigmentation, yellow eye pigmentation) are not coupled to circadian oscillations. In addition, development in *Drosophila* clock mutants which display altered eclosion and activity profiles is seemingly normal (*4*).

It is also important to distinguish between two very different kinds of mechanisms which might both be called timers. A series of biochemical reactions in a defined developmental pathway could conceivably be called a developmental clock. This "clock" would probably not be temperature-compensated and, hence, rather inaccurate over a normal environmental range of temperatures. Eclosion and activity in *Drosophila*, on the other hand, are regulated on a circadian basis by a temperature-compensated multioscillator system (*5*). This second type of clock is inherently more accurate, since it is buffered against temperature changes.

The observation that Malpighian tubules autonomously express urate oxidase activity in a host supports the contention that the rate of development of

0036-8075/78/0609-1185$00.50/0