

Hauser, P. MacNeillage, K. E. Ware, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1514 (1996)], and chimpanzees [S. T. Boysen and G. G. Berntson, *J. Comp. Psychol.* **103**, 23 (1989); T. Matsuzawa, *Nature* **315**, 57 (1985)].

2. C. R. Gallistel and R. Gelman, *Cognition* **44**, 43 (1992); S. Dehaene, G. Dehaene-Lambertz, L. Cohen, *Trends Neurosci.* **21**, 355 (1998).

3. W. H. Meck and R. M. Church, *J. Exp. Psychol. Anim. Behav. Processes* **9**, 320 (1983).

4. H. Davis and R. Perusse, *Behav. Brain Sci.* **11**, 602 (1988).

5. R. K. Thomas and R. B. Lorden, in *The Development of Numerical Competence: Animal and Human Models*, S. T. Boysen and E. J. Capaldi, Eds. (Erlbaum, Hillsdale, NJ, 1993), pp. 127–147.

6. Previous studies (3) [J. L. Emmerton and J. Niemann, *Animal Learn. Behav.* **25**, 234 (1997); J. W. C. Breukelaar and J. C. Dalrymple-Alford, *J. Exp. Psychol. Anim. Behav. Processes* **24**, 84 (1998)] assessed an animal's ability to represent ordinal relations among numerosities. Our experiment differs from those studies in that we obtained first-trial data. More important, these studies were not designed to assess an animal's ability to differentiate adjacent numerosities reliably or an animal's ability to extrapolate an ordinal relation to numerosities outside the training range.

7. Before this experiment began, both subjects were trained to order 11 four-item lists of photographs using a simultaneous chaining paradigm [H. S. Terrace, in *Quantitative Analyses of Behavior: Discrimination Processes*, M. L. Commons, R. J. Herrnstein, A. R. Wagner, Eds. (Ballinger, Cambridge, MA, 1984), pp. 115–138; K. S. Swartz, S. Chen, H. S. Terrace, *J. Exp. Psychol. Anim. Behav. Processes* **17**, 396 (1991)]. Animals were maintained in accordance with NIH guidelines.

8. There were 16 stimulus locations on the video monitor (arrayed in a 4 × 4 matrix). The configuration of the exemplars was selected randomly on each trial from a set of 43,680 spatial configurations. This ensured that subjects could not learn the correct sequence as a series of rote motor movements. Reinforcers were 190-mg banana-flavored Noyes pellets. Errors terminated the trial without reinforcement and resulted in an 8-s blackout of the video monitor. Subjects were trained to a low criterion on successive stimulus sets: correct completion of 20% of trials within a single session (under the conservative assumption of no backward errors, the expected chance level is 4% = 25% × 33% × 50% × 100%) or until three sessions on a particular stimulus set were completed. A low criterion was used to emphasize rule learning (as opposed to memorization of the correct order for a specific set of stimuli) and to establish a "learning set" for mastering new lists [H. F. Harlow, *Psychol. Rev.* **56**, 51 (1949)].

9. Rhesus monkeys learned the ordinal position of list items during training by the simultaneous chaining paradigm [S. Chen, K. B. Swartz, H. S. Terrace, *Psychol. Sci.* **8**, 80 (1997)].

10. Before the test sessions, multi-set training sessions were conducted. During multi-set training, the 35 training sets were presented randomly within each session to prepare subjects for the test sessions in which a different stimulus set was presented on each trial.

11. The decrease in the accuracy of both subjects on the second day of testing was not significant [$t(1) = 3$, $P > 0.2$].

12. Categorical discrimination of numerosity is defined as behavioral control by the number of elements contained by a particular stimulus that is independent of other discriminable dimensions such as size, color, shape, and total area.

13. Other studies of serial learning [B. McGonigle and M. Chalmers, in *Reasoning and Discourse Processes*, T. Myers, K. Brown, B. McGonigle, Eds. (Academic Press, London, 1986), pp. 141–164; N. Ohshima, *Jpn. Psychol. Res.* **39**, 236 (1997); S. H. Hulse and N. P. Dorsky, *Learn. Motiv.* **8**, 488 (1977)] suggest that children, monkeys, and rats learn monotonic sequences more easily than nonmonotonic sequences.

14. D. Washburn and D. Rumbaugh [*Psychol. Sci.* **2**, 190 (1991)] trained rhesus monkeys on a forced-choice task on which they were required to select one of two arabic numerals, each associated with a number of discrete

food items. Subjects reliably chose the symbol associated with the larger number of food pellets, but hedonic value was confounded with numerosity [see also A. Olthof, C. M. Iden, W. A. Roberts, *J. Exp. Psychol. Anim. Behav. Processes* **23**, 325 (1997); L. Hiestand and H. Davis, *Math. Cognit.* **2**, 171 (1996)]. Another study [R. K. Thomas, D. Fowlkes, D. Vickery, *Am. J. Psychol.* **93**, 247 (1980)] showed that squirrel monkeys can discriminate random dot displays of adjacent numerosities; however, this study did not obtain first-trial data. Also, because the numerosity pairs were trained successively, in separate blocks, it could not be determined whether the subjects learned a series of pairwise numerical discriminations (that is, numerical categorization) or whether they used an ordinal rule.

15. We used a two-item rather than a four-item test to isolate the difficulty of particular numerosity comparisons. As in the four-item test, the two stimuli were presented in random spatial locations on each trial. Familiar-familiar pairs were presented twice as frequently (60 trials per session) as the unreinforced familiar-novel and novel-novel pairs (30 trials per session) to maintain a high level of responding throughout the 20 test sessions. On unreinforced trials, subjects were allowed to respond in any order and received neither positive nor negative feedback.

16. The binomial probability of obtaining the observed number of correct responses on each of the 10 novel-novel numerosity combinations was below 0.05 for all pairs, with the exception of 6:8, 7:8, and 8:9. There was no effect of the area of the elements on accuracy [Rosencrantz, $t(19) = -0.35$, $P > 0.7$; Macduff, $t(19) = -1.2$, $P > 0.2$]. However, performance on novel-novel pairs was lower than performance on familiar-familiar pairs [Rosencrantz, $t(19) = 7.76$, $P < 0.001$; Macduff, $t(19) = 4.26$, $P < 0.001$]. Although this pattern resembles a generalization decrement, the reduced performance could also be attributed to the observed distance effect or to Weber's law (or both). The discriminability of novel-novel pairs should be poorer than that of

familiar-familiar pairs because the novel-novel pairs tend to have smaller Weber fractions and numerical distances than the pairs that contain familiar numerosities (Fig. 4).

17. B. O. McGonigle and M. Chalmers, *Nature* **267**, 694 (1977).

18. To apply an ordinal rule to novel numerosities, monkeys must be able to use a mechanism that can perform relative magnitude operations on sets of discrete items. The accumulator model (3) of the numerical ability of animals posits that each discrete (to be counted) event results in the closing of a neural gate, allowing a constant amount of flow into an accumulator. A similarity ratio is then computed to determine which value in reference memory is closer to the test stimulus. The resulting analog representation is well suited for judgments of relative magnitude. However, the accumulator model does not explain how an organism would create a serial input from a simultaneous display.

19. R. Gelman and C. R. Gallistel, *The Child's Understanding of Number* (Harvard Univ. Press, Cambridge, MA, 1978).

20. See R. S. Moyer and T. K. Landauer [*Nature* **215**, 1519 (1967)] for a demonstration that human subjects, required to indicate the larger of two arabic numerals, show an accuracy and latency distance effect. See P. B. Buckley and C. B. Gillman [*J. Exp. Psych.* **103**, 1131 (1974)] for a similar demonstration with random dot patterns.

21. T. Dantzig, *Number: The Language of Science* (Macmillan, New York, 1930).

22. Supported by National Institute of Mental Health grant MH40462 (H.S.T.) and NSF and NRSA graduate fellowships (E.M.B.). We thank P. Balsam, S. Carey, J. Gibbon, E. Hearst, J. Horvitz, A. Markman, J. Metcalfe, M. Platt, and R. Remez for valuable comments. We also thank E. Anderson and S. Chen for help in conducting the research.

1 July 1998; accepted 11 September 1998

Smart Engineering in the Mid-Carboniferous: How Well Could Palaeozoic Dragonflies Fly?

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The wings of archaic Odonatoidea from the mid-Carboniferous of Argentina show features analogous to "smart" mechanisms in modern dragonflies that are associated with the agile, versatile flight necessary to catch prey in flight. These mechanisms act automatically in flight to depress the trailing edge and to facilitate wing twisting, in response to aerodynamic loading. The presence of similar features suggests that the earliest known odonatoids were already becoming adapted for high-performance flight in association with a predatory habit.

Insect wings are the principal adult locomotory structures of the largest group of animals. They are proving to be spectacular examples of microengineering. They lack in-

ternal musculature, and their three-dimensional shape during the flapping cycle is largely determined by their elastic response to aerodynamic and inertial forces, moderated by thoracic muscles inserted at or near the base (*1*).

The dragonflies (order Odonata) are supremely versatile, maneuverable fliers, and this is reflected in their wing morphology. High-speed cinefilm, videotape, and still photographs show that the wings twist extensively along their span, allowing the insects to

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develop weight support on both up- and downstrokes and giving fine instantaneous control of aerodynamic forces over a wide speed range (2–5). This twisting is facilitated by a flexible, relatively unsupported posterior wing margin. Useless flaglike fluttering is prevented by a series of automatic devices within the wings (2, 4, 5); the latter are in effect “smart” aerofoils, passively maintaining appropriate profiles and attitudes throughout the stroke in direct response to the external forces that they experience.

Odonata show several such devices. Two examples are the “nodus,” and the basal complex comprising the “supratriangle” and “triangle” of entomologists, characteristic of the suborder Anisoptera (Fig. 1A).

The nodus (Fig. 1B) is a combined brace and shock absorber, at the junction of two regions of the leading edge spar with very different mechanical properties. The proximal region consists of three veins linked by angle bracket-like cross-veins into a rigid girder. At the nodus the concave subcostal vein (ScP in Fig. 1B) ends, and the spar continues distally as a shallower girder with weaker cross-veins and an inverted V-shaped section. This latter conformation typically allows supinatory twisting while restricting pronatory twisting (5, 6) and permits the passive upstroke torsion described above. This differentiation of the supporting spar, basally rigid and distally compliant, is hence an adaptation for versatile, maneuverable, multispeed flight.

The basal complex of Anisoptera (Fig. 1C)

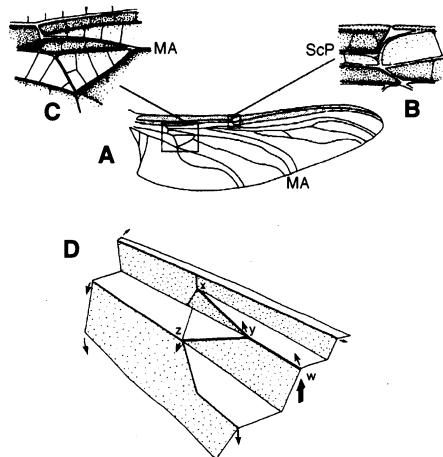


Fig. 1. (A to C) Hind wing of *Aeshna cyanea* (Müller) (Odonata, suborder Anisoptera), showing the main longitudinal veins and the detailed structure of the nodus and the triangle-supratriangle complex. (A) The whole wing, with the leading-edge spar shaded; (B) the nodus; (C) the basal complex. MA, anterior median vein; ScP, posterior subcostal vein. (D) Diagrammatic representation of the operation of the triangle-supratriangle complex of anisopterous Odonata in lowering the trailing edge in response to an upward force applied in the distal median area of the wing.

is an angular, strongly three-dimensional conformation of veins. A force manually applied more distally to the underside of the wing of a living insect, so simulating the lift, levers the trailing edge down about this basal complex, cambering the wing and increasing its angle of pitch (2, 5). Figure 1D shows diagrammatically the operation of this mechanism, which is easily modeled in thin card. A force applied at W, representing the anterior median vein (MA in Fig. 1, A and C), lowers the regions anterior and posterior to the modeled basal complex XYZ. In the actual wing the anterior area is relatively rigid, so that downward deflection is concentrated posteriorly. In flight this holds down the trailing edge, improving the wing's camber and attitude in direct response to aerodynamic loading (2, 5).

The Odonatoidea have a long and excellent fossil record. Apparent automatic mechanisms familiar in modern forms can be identified in many Mesozoic fossils, and their evolution would reward detailed study. They are less evident in most Palaeozoic members of the odonatoid stem group. The familiar giant meganeurid protodonates of the Carboniferous and Lower Permian, though strikingly convergent in wing planform with modern Anisoptera, lack both nodus and basal complex. However, the most archaic known odonatoids are the far smaller Eugeopteridae from the mid-Carboniferous of La Rioja, Argentina (7). They had wingspans between 80 and 100 mm, well within the size range of modern dragonflies. Until recently only wings were known, but new material, currently being studied, shows Eugeopteridae to have been proportioned rather like modern Libellulidae, but with relatively smaller bodies, and with prothoracic winglets—an archaic feature familiar in some other Carboniferous insects, but previously unknown in odonatoids (Fig. 2A).

Despite their many plesiomorphic characters, these insects show specializations that parallel those just described in modern drag-

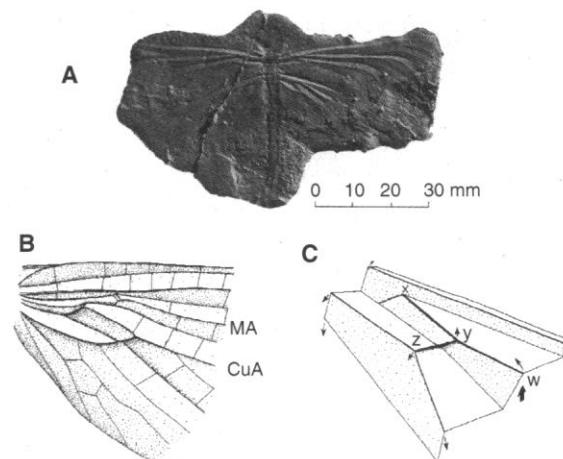
onflies and provide good evidence for early development of versatile flight techniques. There is no nodus, but the stiffening posterior subcostal vein meets the fore-margin well before the wing tip, so that the distal part of the wing would have been relatively compliant to supinatory twisting.

Both fore and hind wings show a basal, three-dimensional vein complex that superficially resembles that of Anisoptera, and when modeled in card responds to manual loading in a similar manner. These are certainly analogous, not homologous, adaptations, because different veins are involved in the two groups, and the Eugeopteridae are unlikely to be directly ancestral to modern Odonata. It seems clear that smart, trailing edge-lowering mechanisms arose independently in these early forms, associated as now with a flexible posterior margin in torsionally compliant wings.

Figure 2B shows the form of the basal part of the wing, and Fig. 2C a schematized version of the mechanism as we interpret it, with the complex shown as a double, three-dimensional parallelogram. An upward force applied at W—here representing the anterior cubital vein (CuA)—flexes the parallelogram about its diagonal and applies depressing torques to the wing both anteriorly and posteriorly. In the actual wing the diagonal flexion of the complex would slightly bend three veins, and their elastic recovery may have aided the reversal of the mechanism when the aerodynamic load was removed. The mechanism in Eugeopteron parallels that in modern Anisoptera in that both involve raising the apex of an L-shaped vein formation—XYZ in both Figs. 1D and 2C—so tending to twist downward the regions in front and behind. Again, the greater rigidity of the anterior part of the wing would resist this torsion, and to compensate, the posterior area would be depressed further.

How, and how well, did these insects fly? Certainly not as skillfully as modern Anisoptera, which coevolved in the Mesozoic and Tertiary with the many groups of agile insects

Fig. 2. (A) Undescribed eugeopterid from La Rioja, Argentina (Carboniferous, Namurian/Westphalian). Photograph of a latex cast. (B) The hind-wing base of *Eugeopteron lunatum* Riek. (C) Diagrammatic representation of the operation of the basal mechanism in *Eugeopteron*, as here interpreted. CuA, anterior cubital vein; MA, anterior median vein.



on which they feed. Carboniferous odonatooids were already predatory (8), but no contemporary insects would have approached the maneuverability of many extant species. The relatively smaller, less sturdy bodies and consequent lower wing-loadings of Eugeopteridae indicate lower maximum speeds than in Anisoptera, and the absence of a nodus suggests a poorer capability for wing twisting, truncating the lower end of their speed range: It is unlikely that they could hover like modern dragonflies. Nonetheless, the flexible trailing edge and the shortened subcostal vein indicate that some supinatory twisting was possible, and the group appears to

be following a trend, paralleled in many other insect groups, toward improving flight versatility by recruiting upstroke forces to supplement those of the far more effective downstroke, and varying their magnitude and direction at need. The "smart" wing-base mechanism is best interpreted as an elegant means of maintaining downstroke efficiency in the presence of these adaptations to improve upstroke usefulness.

References

1. R. J. Wootton, *J. Zool. London* **193**, 447 (1981); *Sci. Am.* **263**, 114 (November 1990); *Annu. Rev. Entomol.* **37**, 113 (1992).

2. D. J. S. Newman, thesis, University of Exeter (1982); A. R. Ennos, *J. Exp. Biol.* **142**, 87 (1989).
3. S. Dalton, *Borne on the Wind* (Chatto & Windus, London, 1975); G. Ruppel, *J. Exp. Biol.* **144**, 13 (1989); J. H. Brackenbury, *Insects in Flight* (Blandford, London, 1992); J. M. Wakeling and C. P. Ellington, *J. Exp. Biol.* **200**, 557 (1997); *ibid.*, p. 583.
4. R. Å. Norberg, *J. Comp. Physiol.* **81**, 9 (1972).
5. R. J. Wootton, *Adv. Odonatol.* **5**, 153 (1991).
6. ———, *J. Exp. Biol.* **180**, 105 (1993).
7. E. F. Riek and J. Kukalová-Peck, *Can. J. Zool.* **62**, 1150 (1984).
8. C. Brauckmann and W. Zessin, *Dtsch. Entomol. Z.* **36**, 177 (1989).

1 June 1998; accepted 22 September 1998

An MTP Inhibitor That Normalizes Atherogenic Lipoprotein Levels in WHHL Rabbits

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Patients with abetalipoproteinemia, a disease caused by defects in the microsomal triglyceride transfer protein (MTP), do not produce apolipoprotein B-containing lipoproteins. It was hypothesized that small molecule inhibitors of MTP would prevent the assembly and secretion of these atherogenic lipoproteins. To test this hypothesis, two compounds identified in a high-throughput screen for MTP inhibitors were used to direct the synthesis of a highly potent MTP inhibitor. This molecule (compound 9) inhibited the production of lipoprotein particles in rodent models and normalized plasma lipoprotein levels in Watanabe-heritable hyperlipidemic (WHHL) rabbits, which are a model for human homozygous familial hypercholesterolemia. These results suggest that compound 9, or derivatives thereof, has potential applications for the therapeutic lowering of atherogenic lipoprotein levels in humans.

Apolipoprotein B (apoB)-containing lipoproteins [chylomicrons, very low density lipoproteins (VLDL) and their respective metabolic products, chylomicron remnants, and low density lipoproteins (LDL)] promote coronary artery atherosclerosis, which is a leading cause of death in industrialized nations. MTP is a heterodimeric lipid transfer protein consisting of protein disulfide isomerase and a unique 97-kD subunit that is localized in the endoplasmic reticulum of hepatocytes and enterocytes (1–3). Defects in MTP cause abetalipoproteinemia (3–5), a disorder in which the production of VLDL and chylomicrons is

disrupted. Patients with abetalipoproteinemia have plasma cholesterol levels of ~40 mg/dl and plasma triglyceride levels of <10 mg/dl (6), whereas normal adults have levels of 180 to 220 and 100 to 150 mg/dl, respectively. These findings suggest that inhibitors of MTP might be therapeutically useful for inhibiting the production of VLDL and chylomicrons, thereby reducing the levels of atherogenic lipoprotein particles.

Abetalipoproteinemia is an extreme example of MTP inhibition and would not be the intended clinical end point for a drug. A related genetic disease, hypobetalipoprotein-

emia, is caused by mutations in apoB (6, 7). Heterozygous people with this disease have half the normal levels of apoB-containing lipoproteins and lack the clinical signs and symptoms of patients with abetalipoproteinemia. Family studies have shown that individuals with hypobetalipoproteinemia have a prolonged life span (8).

We performed a high-throughput screen of a large chemical library to identify inhibitors of MTP-mediated triglyceride transfer. This resulted in the discovery of BMS-200150 (compound 1 in Fig. 1), which inhibits the MTP-mediated transport of triglycerides between membranes in vitro (9) and inhibits the secretion of apoB-containing lipoproteins from HepG2 cells, a human liver-derived cell line. However, the compound was not active in animal models. Compound 2, an analog of BMS-200150 with an extended alkyl linker, was of comparable potency in both the lipid transfer and HepG2 apoB secretion assays (Fig. 1 and Table 1). A fluorenyl amide (compound 3), which is a much less potent inhibitor, was also identified in the high-throughput screening. Subsequently, nitrogen substitution of 3 with a short-chain alkyl group (for example, 4) was

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