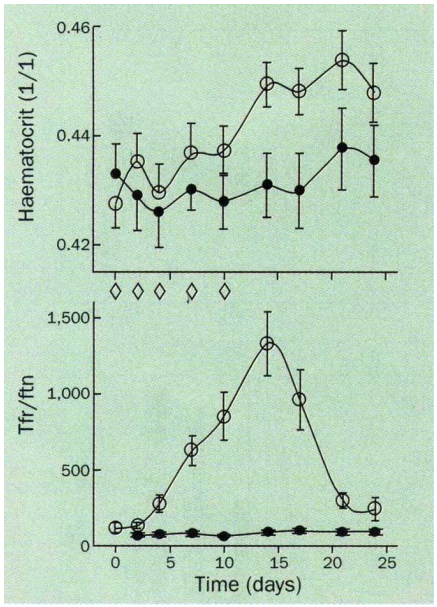
Erythropoietin abuse in athletes

SIR — The availability of recombinant human erythropoietin (rHuEpo) has made it a drug of choice for athletes looking for an artificial performance enhancer. The lay press still represents the main reporting vehicle for such illicit use of erythropoietin, the most dramatic picture having been portrayed in the German weekly magazine *Der Spiegel*: 18 deaths related to erythropoietin administration among racing cyclists[1].

Although it is on the list of banned substances issued by the medical commission of the International Olympic Committee, the non-medical use of erythropoietin remains uncontrollable. No reliable analytical technique[2,3] is available to detect its use as an ergogenic agent. The kinetic constraints (for example, short half-life, delayed erythropoietic effects) of an approach based on the electrophoretic mobility measurement of erythropoietin[4] prompted us to investigate a new marker of erythroid activity, the soluble transferrin receptor, released predominantly from haematopoietic progenitors, in healthy athletes receiving either placebo or rHuEpo administration. Our data indicate that erythropoietin induces striking changes in the serum soluble transferrin receptor (Tfr) content. These observations could eventually be considered in the design of a probe to detect erythropoietin misuse.



Haematocrit (upper panel) and soluble transferrin receptor/ferritin[7] (Tfr/ftn; lower panel) values in blood samples collected repeatedly (days 0, 2, 4, 7, 10, 14, 17, 21, 24) in healthy, trained, adult males (age ± s.d. 21.4 ± 0.3 yr) given 5 subcutaneous injections (◊) of placebo (●; $n=10$) or 200 U kg^{-1} commercial (Eprex, France) recombinant human erythropoietin (○; $n=19$).

Because rHuEpo administration stimulates erythropoiesis and induces the redistribution of storage iron into erythroid elements, Tfr as an index of both tissue iron deficiency and expanded erythroid progenitor mass has been expressed in relation to serum ferritin (ftn), a measure of body iron store, thus giving the serum Tfr/ftn index. This approach is particularly appealing, as the expression of such a ratio obviates problems related to the variable effects of hydration, as is the case with haematocrit readings. Moreover, changes in this Tfr/ftn index could reflect rHuEpo abuse, as well as any other manoeuvres that accelerate erythropoiesis[5].

Analysis of variance performed on the data in the figure indicates that no significant change ($P=0.53$) in serum Tfr/ftn occurred over the entire observation period in the placebo-treated subjects, while striking increases were induced by the rHuEpo treatment. The rHuEpo-induced increases were statistically different ($P<0.05$) from basal values (pooled placebo group Tfr/ftn values) for serum Tfr/ftn values measured on days 4, 7, 10, 14, 17 and 21. This relatively low-dose rHuEpo treatment yielded no significant ($P>0.05$) increase in haematocrit values. One can speculate that the magnitude of a Tfr/ftn increase observable with a rHuEpo dose sufficient to yield an ergogenic haematocrit increase would be even more dramatic.

Notwithstanding the discriminative power of low haematocrit values observed in anaemia, patients presenting an increased serum Tfr concentration associated with primary or non-pharmacological secondary polycythaemia, or with iron-deficient or megaloblastic anaemia, are not likely to yield false-positive results, as they generally do not achieve élite-level physical performances. Physical exercise *per se* does not seem to be associated with increased Tfr/ftn serum values[6], thus precluding a false-positive identification of erythropoietin from blood sampled at the competition site.

Hence, observation of concomitant changes in haematocrit and Tfr/ftn values could permit the discrimination of pathological from physiological conditions, and thus distinguish between rHuEpo abusers (or even athletes who had undergone blood transfusions) and those competing fairly. The most recent technological developments already allow measurement of these discriminating variables from a few microlitres of capillary blood sampled from the fingertip or ear lobe. This first breach in athletes' immunity to detection of their use of engineered hormones as performance enhancers is a pledge in favour of the blood matrix to detect and deter sophisticated abusers.

1. Le Monde cycliste **23**, 10–12 (1991) (transl. from *Der Spiegel* 10 June 1991).
2. Berglund, B. *et al. Int. J. Sports Med.* **8**, 66–70 (1987).
3. Casoni, I. *et al. Int. J. Sports Med.* **14**, 307–311 (1993).
4. Wide, L. *et al. Med. Sci. Sports Exerc.* **27**, 1569–1576 (1995).
5. Brugger, W. *et al. Blood* **81**, 2579–2584 (1993).
6. Gareau, R. *et al.* in *Blood Samples in Doping Control* (Pensumtjeneste, Oslo, 1994).
7. Flowers, C. H. *et al. J. Lab. clin. Med.* **114**, 368–377 (1989).

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Derivative of the hyperbolic cotangent

SIR — The derivative of the hyperbolic cotangent is a standard result which appears in essentially every compilation of mathematical formulas[1]. Here we point out that this result is incomplete; there is, in fact, an additional term which is proportional to the Dirac delta function. We present the correct formula, outline its proof and give an example of its importance in the analysis of a physical problem.

The correct formula is

$$\frac{\mathrm{d}}{\mathrm{d}y}\coth y = -\operatorname{csch}^2 y + 2\delta(y) \qquad (1)$$

where $\delta(y)$ is the Dirac delta function. The usual derivation of the first term does not properly handle the fact that $\coth y$ has, in addition to the obvious $1/y$ singularity, a discontinuity at $y=0$. A better approach is to write

$$\coth y = \pm\left\{1 + \frac{2}{\mathrm{e}^{2|y|}-1}\right\} \qquad (2)$$

where the + and – signs refer to $y>0$ and $y<0$, respectively. Regardless of the sign

of y, the derivative of the second term in equation (2) gives the conventional result: the first term in equation (1). However, the first term in equation (2) can be replaced by $\theta(y) - \theta(-y)$, where $\theta(y)$ is the Heaviside function ($=1$ for $y > 0$ and 0 for $y < 0$). Since the derivative of the latter is the Dirac delta function, the second term in equation (1) follows.

As an example of the relevance of this new result in a physical problem, we refer to our analysis of a quantum particle coupled to a quantum-mechanical heat bath (which is fundamental to many fields of physics, including statistical mechanics, condensed matter and quantum optics)[2]. We derived exact results for a very general class of models but, in the particular case of constant friction ζ, we found that the autocorrelation of the quantum-mechanical random force $F(t)$ could be written in the form

$$\frac{1}{2}\langle F(t)F(t')+F(t')F(t)\rangle = \frac{\zeta}{\pi}\int_0^\infty d\omega\ \hbar\omega\coth(\hbar\omega/2kT)\cos[\omega(t-t')] = kT\zeta\frac{d}{dt}\coth\left[\pi kT(t-t')/\hbar\right] \quad (3)$$

where T is the temperature. In the classical limit ($\hbar \to 0$), use of the correct formula (1) leads to the familiar white-noise result

$$\frac{1}{2}\langle F(t)F(t')+F(t')\rangle \underset{\hbar\to 0}{\longrightarrow} 2kT\zeta\delta(t-t') \quad (4)$$

However, we would get zero if we used the conventional result without the delta function; it is only by using equation (1) that we achieve agreement with equation (4).

Naturally, we were motivated to re-examine the derivatives of the other hyperbolic functions (especially the csch since, like the coth, it is also a discontinuous function), but we found that the derivative of the coth is unique in requiring modification.

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1. Abramowitz, M. & Stegun, I. A. (eds) *Handbook of Mathematical Functions* (Dover, New York, 1970).
2. Ford, G. W., Lewis, J. T. & O'Connell, R. F. *Phys. Rev.* A**37**, 4419–4428 (1988).

Scientific Correspondence

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Cheaters in yucca/moth mutualism

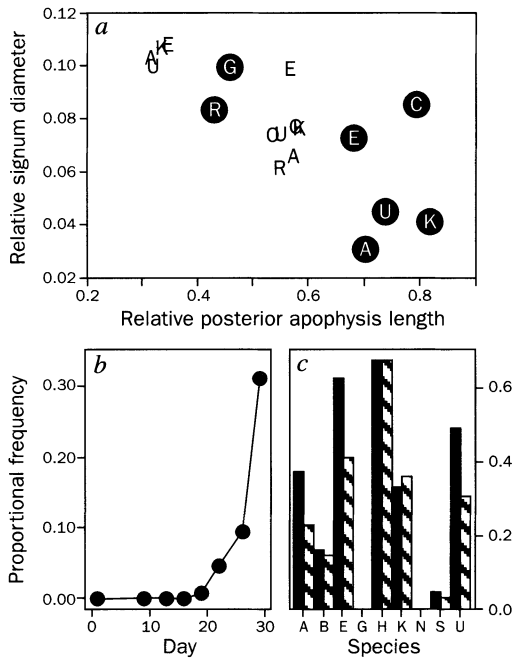
SIR — A long-standing puzzle in the obligate pollination/seed predation mutualism between yuccas and yucca moths is that many adult female yucca moths (*Tegeticula yuccasella* (Riley)) lack functional maxillary tentacles[1,2], the structures used to transfer pollen. Here I report that the yucca moths without maxillary tentacles are non-pollinators, and are morphologically, behaviourally and phenologically distinct from normal yucca moths. By ovipositing in fruit rather than flowers, non-pollinators experience relatively low larval mortality, and consequently can have a great impact on seed production by yuccas.

Discriminant function analysis of characters of the female genitalia shows that adult female yucca moths without maxillary tentacles are morphologically distinct from normal yucca moths collected from the same yucca hosts (*a* in the figure; $P = 0.007$ to <0.0001), and moths lacking maxillary tentacles, but collected from different yucca hosts, are morphologically distinct ($P<0.0001$). This provides additional support for *T. yuccasella* being a group of closely related species[1,3].

On *Yucca kanabensis* in southern Utah and on *Y. elata* in central Arizona, yucca moths without maxillary tentacles do not function as pollinators. They never attempt to collect pollen, carry pollen, approach stigmas of fresh flowers, or transfer pollen. Although they rest in fresh flowers with the pollinators during the day, at night non-pollinators oviposit only in 10–30-day-old fruit, inserting their eggs through the carpal wall directly into developing seeds. The flight season of non-pollinators is later than that of normal yucca moths (*b* in the figure), which explains the high variation in the occurrence and relative abundance of non-pollinators from single collections of yucca moths[1].

Non-pollinators are widespread and abundant in the southwestern United States, from Texas to California. Based on collections of moths and fruit, non-pollinators occur on at least 10 species of yuccas (*a, c* in the figure), and in those fruit attacked by non-pollinators, their larvae were 2.13 times more abundant than pollinator larvae. Individual fruit contained up to 50 larvae of the non-pollinators, well above the 15 larvae required to damage all seeds in a fruit[4]. Considering all fruit collected, non-pollinators occurred in more than 30% of the fruit from four yuccas, and the larvae of non-pollinators constituted more than 30% of all yucca moth larvae in five species (*c* in the figure).

Two aspects of these observations deserve emphasis. First, non-pollinators are more closely related to other members of the *T. yuccasella* complex than they are to any other yucca moths or false yucca moths[1]. This contrasts with the pollination/seed predation mutualism between figs and fig wasps, where pollinators and non-pollinators belong to different fam-



a, Morphology of adult female yucca moths scaled relative to wing length. Each letter represents the mean for moths of a given type and host. Symbols enclosed in solid circles represent moths that lack maxillary tentacles. From 7 yuccas with moths lacking maxillary tentacles, I selected for analysis 52 females without maxillary tentacles and 133 normal females. Letter codes for the taxa are: A, *angustissima;* C, *constricta;* E, *elata;* G, *glauca;* K, *kanabensis;* R, *reverchoni;* U, *utahensis*. *b*, Proportion of adult female yucca moths that lacked maxillary tentacles as a function of days within the flowering season for *Y. kanabensis*. Sample sizes are shown in parentheses. *c*, Proportion of fruit containing at least one larva of non-pollinators (hatched bars), and the proportion of yucca moth larvae from non-pollinators (solid bars). Data from 77 collections of fruit from 9 species of yucca. In the 2,520 fruit there were 7,388 larvae from pollinators and 3,961 larvae from non-pollinators. Grouped by species from left to right in the figure, the total number of fruit examined were 620, 536, 440, 40, 80, 520, 20, 160 and 104, respectively, and the total number of larvae examined were 1,786, 496, 2,097, 71, 228, 3,225, 16, 269 and 592, respectively. Letter codes for yucca taxa: A, *angustissima;* B, *baileyi;* E, *elata;* G, *gilbertiana;* H, *harrimaniae;* K, *kanabensis;* N, *neomexicana ;* S, *schidigera;* U, *utahensis*.