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LETTERS

Malaria Vaccine

As one who, in 1976 with J. B. Jensen, provided methods for the continuous culture of *Plasmodium falciparum* (*1*), methods which have led to and greatly facilitated not only the development of SPf66 but also other work on an erythrocytic-stage vaccine for malaria, I would like to comment on the "dilemma" raised by the malaria vaccine (John Maurice, News & Comment, 20 Jan., p. 320). I see no serious dilemma. It is clear that SPf66 is sufficiently effective to warrant additional and more extensive trials. It is good to note that such a large trial is already in the planning stage for 1995 in Tanzania. Meanwhile, results from several trials now in progress are expected to be in during this year. If they are disappointing, the clamor for a million doses will decrease. If they are good, there will be little reason to restrict development as long as the funds for it can be provided. Of course, there is a lot we do not know, but we will be learning much in the next few years, with careful follow-up of those already vaccinated. Of special interest might be trials combining SPf66 with the use of insecticide-impregnated bed nets for the protection of young children, as suggested in a recent review by M. Tanner *et al.* (*2*).

If Manuel Patarroyo's work had been received with less criticism and more cooperation, perhaps we would be 5 years ahead of where we are now. It was, it seemed to me, an ideal example of how basic science should be applied to problems in developing countries. The basic work—culture of the parasites and use of the methods of Bruce Merrifield for peptide synthesis—was done at the Rockefeller University. A creative young scientist then applied these methods to a major medical problem in his country. He had support from his government, excellent laboratory facilities, and lots of drive and motivation. He furthermore had the special advantage of the availability of large numbers of *Aotus* monkeys in which to do his preliminary testing of some of the many antigens of *P. falciparum*. He came up with a combination of three peptides that together gave protection in monkeys. He then did something both clever and original—he took small fragments of these peptides and polymerized them into a synthetic polypeptide with a molecular weight of about 20,000. There was accordingly no need for him to use a carrier protein (the method others were using). In his first trials in humans, he showed great courage. He chose a cutoff point for treatment that turned out to be safe, yet provided for a significant result.

Very likely there will be a better malaria vaccine than SPf66. Meanwhile, we ought to be using what we have. I am reminded of a conversation with Tom Rivers many years ago. He was relating how he had to decide whether to go ahead with large-scale use of the Salk-killed vaccine for poliomyelitis or wait for the live vaccine, which might be better. He did not wait.

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Detecting Alzheimer's Disease

The report "A potential noninvasive neurobiological test for Alzheimer's disease" by Leonard F. M. Scinto *et al.* (11 Nov., p. 1051) describes patients with Alzheimer's disease who exhibited marked hypersensitivity in pupil dilation to a dilute solution of tropicamide. As ophthalmologists who routinely dilate patients' eyes with tropicamide, we have observed extremely variable pupil response to dilating agents. We repeated the protocol described in the report with 13 healthy subjects with a mean age of 32 years, and with no family history of Alzheimer's disease. The pupils of these subjects dilated (paired t test, $P < 0.0005$, data not shown) in a fashion similar to that of patients with Alzheimer's disease, as reported by Scinto *et al.*

While dilute tropicamide solution may be investigated as a screening test for Alzheimer's disease in elderly patients, we urge caution if it is used for this purpose in young, healthy adults.

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We recently studied 20 elderly persons from a community sample of psychiatric patients in London. Ten were diagnosed as having probable Alzheimer's disease and 10 as having multi-infarct dementia (MID) by clinical criteria and by computerized axial tomography. Patients who were using any medication (such as anticholinergic or opioid drugs) that might interfere with pupillary response were excluded. We used a methodology (*1*) similar to that used by Scinto *et al.* There were no significant differences between the groups for age, sex, or cognitive score. While the group with Alzheimer's disease reacted almost identically to that in the study by Scinto *et al.*, the reaction of the group with MID was indistinguishable from that of the Alzheimer's disease group ($P = 0.72$, data not shown). Therefore, it seems that anticholinergic drops may not enable one to distinguish between different forms of dementia. Although the posterior part of the eye is a central nervous system organ, pupillary control is a function of the peripheral nervous system. It seems possible that concurrent administration of systemic drugs to some of the patients in the study by Scinto *et al.* affected those results.

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1. B. Sacks and S. Smith, *J. Neurol. Neurosurg. Psychiatr.* **52**, 1294 (1989).

We studied 20 patients with Alzheimer's disease for pupillary response to topically administered dilute pilocarpine (0.125%), a cholinergic agonist (*1*). We analyzed changes in pupillary size using three methods. The average median and mean pupillary size differences in the group with Alzheimer's disease were compared to those of corresponding eyes in a control group matched for age and iris pigmentation (a relevant variable, as lightly pigmented irides often respond more to topically administered agents). To take into account the initial pupil size, we also calculated the ratio of the postdrug pupil size to predrug pupil size; mean ratios in the group with Alzheimer's disease were compared to those of corresponding eyes in the control group. Last, a 13-point scoring system was developed to combine analyses of changes in pupillary response and take into account the inherent imprecision in pupillary eye measurements.

Our patients with Alzheimer's disease demonstrated hypersensitivity in pupillary miosis induced by a cholinergic agonist, dilute pilocarpine. Induced miosis was more than twofold greater in the group with Alzheimer's disease than in the control group. Our pretesting evaluation of patients and normal subjects excluded Adie's pupils as a possible source of confounding error, and demonstrated normal tear lakes, normal tear break-up time, and normal corneal sensitivity in both groups, minimizing (although not eliminating) the likelihood that underlying corneal pathology might account for increased permeability of topically administered medication in patients with Alzheimer's disease.

Our results complement those of Scinto *et al.*, and yet we see two possible interpretations of both studies. One is that the two studies seem to support each other and offer evidence for upregulation of cholinergic receptors within the iris of the patient with Alzheimer's disease; there was exaggerated mydriasis to a cholinergic antagonist (tropicamide) and exaggerated miosis to a cholinergic agonist (pilocarpine). Although the pharmacology of the agents was different, the relevant receptor involved would be the same. Another interpretation, however, is that both

studies might merely illustrate increased corneal penetration of any topical agent in the presumed Alzheimer's patient.

There may be a range of induced pupillary mydriasis, or miosis, or both, that could be used as a marker for individuals with Alzheimer's disease. Such pharmacologic investigations need to be pursued, but they should take into account matching control populations for iris pigmentation, scoring the change in anisocoria after topical administration of an agent to one eye as the true drug-induced effect, and using the second eye as a control to take into account elements such as fatigue before concluding that upregulation of cholinergic receptors in the iris allows for recognition of the effects of plaques and tangles.

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1. B. Katz, S. Rimmer, M. R. Klauber, *Ophthalmology* **95** (suppl.), 134 (September 1988).

In a 1981 pupillometric study, we found a supersensitive miotic response to ocular administration of the muscarinic agonist pilocarpine (0.125%) in patients with Alzheimer's dementia as compared with normal or mildly impaired subjects matched for age, sex, race, and iris pigmentation (*1*). The instrument used was identical and the methodology comparable to those in the report by Scinto *et al.* We chose not to follow up on the pilocarpine results because of difficulties associated with controlling for potentially variable corneal penetration, aqueous fluid turnover, and iris pigmentation across different subject groups. Furthermore, we did find a statistically significant difference (Student's t test, $P < 0.05$) in pilocarpine miosis between brown-eyed and nonbrown-eyed (hazel, blue, or green) subjects independent of the above patient-control difference.

Data from a study by Sacks and Smith (*2*) suggest that eye color may also modulate the mydriatic response to tropicamide, the muscarinic antagonist studied by Scinto *et al.* Sacks and Smith found that the mydriatic responses to tropicamide appeared to be greater in Down syndrome subjects with blue and hazel eyes than in those with brown eyes. In the study of Scinto *et al.* the sizes of some of the subgroups tested were relatively small (for example, four patients with non-Alzheimer's type dementia were studied), so suggestions about the specificity of their findings to Alzheimer's disease seem premature in the absence of data from subject groups matched for eye color.

We would also like to point to an apparent pharmacological anomaly, namely that Alzheimer's disease is associated with iris cholinergic receptor supersensitivity to both an agonist (pilocarpine) and an antagonist (tropicamide). One would expect to find agonist supersensitivity with antagonist subsensitivity or vice versa. At this point, we suggest that the most parsimonious explanation of our data as well as the findings of Scinto *et al.* is that of a nonspecific corneal epithelial tissue degeneration that permits abnormal penetration of drugs across the cornea in Alzheimer's disease. Even if the reported difference between patients with Alzheimer's disease and normal subjects proves to be a result of nonpharmacological factors, it may still represent a potentially valuable diagnostic tool.

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1. N. Sitaram and N. Pomara, *IRCS Med. Sci.* **9**, 409 (1981).
2. B. Sacks and S. Smith, *J. Neurol. Neurosurg. Psychiatr.* **52**, 1294 (1989).

Response: We thank our colleagues for their comments about our report of an enhanced pupillary response to dilute tropicamide on the part of patients with probable Alzheimer's disease. Several letters suggest that our finding may have been a result of an increased permeability of the cornea in patients with probable Alzheimer's disease. We agree that a change in permeability is one possible mechanism.

Marx *et al.* discuss the potential variability in dilation response. They calculated the change in pupil diameter by subtracting the values for the untreated eyes from the treated eyes. In contrast, our measurements were calculated as the increase in diameter over the pretreatment baseline of the same eye. When our method of data analysis was applied to the data of Marx *et al.*, the response of their middle-aged, healthy control group was more similar to that of our elderly, healthy control group, allowing age differences, than to that of our patients with probable Alz- heimer's disease. Apparent differences in findings might be a result of the type and resolution of the measurement system used, the preparation of the agent used to dilate, or the conditions under which measurements are made. Our experiments suggest that young subjects (20 to 40 years of age) show a minimal response (less than or equal to 5% over baseline measurement of the treated eye) to tropicamide in concentrations of 0.25% and 0.01% (data not shown).

Treloar *et al.* report that, using methods similar to ours, they were unable to distinguish patients with a clinical diagnosis of MID from patients with a diagnosis of probable Alzheimer's disease. Patients clinically diagnosed with vascular dementia, when autopsied, are often (40 to 50%) found to have sufficient plaques and tangles to meet the pathological criteria for Alzheimer's disease (*1*). Katz and Pomara and Sitaram report finding a hypersensitivity to cholinergic agents in the pupil response of patients with Alzheimer's disease. Although some of these studies used pilocarpine, a cholinergic agonist, they underscore the potential benefits of the pupil dilation response as an assay for degenerative dementia.

Some investigators have suggested that screening for drugs that affect pupillary response could explain the differences between their results and ours. We screened our subjects for use of medications that have known pupillary effects, and other medication use in all of our study groups was similar.

In light of comments about the methodology in our report, we offer the following clarifications. (i) We measured baseline pupil diameter in both eyes before any intervention with a control or drug agent. (ii) Fatigue, anxiety, or hyper-arousal were carefully monitored during the time of testing and no differences were noted between patients and control subjects. (iii) Both patients and control subjects were tested at different times of the day with no systematic bias in time of test for any group. (iv) No group had a systematic bias to a particular iris color. (v) Over the course of measurement intervals, there was no significant difference in the pupil diameter of the untreated eye for patients with Alzheimer's disease as opposed to normal control subjects (Kruskal-Wallis pairwise multisample test, $P = 0.15$). (vi) A random examination of the raw data revealed no difference in blink rate for patients or controls during measurement periods. (vii) Although our results were reported in terms of percentage change over baseline, the absolute differences over baseline yield the same curves as appear in the figures in our report.

We did not report a mechanism to ex-

plain the finding. The intent of our study was to determine if patients with a clinical diagnosis of probable Alzheimer's disease could be distinguished from healthy controls on the basis of changes in pupil diameter to the topical administration of a dilute solution of tropicamide.

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Corrections and Clarifications

A correction (25 Nov., p. 1308) for the caption that accompanied photographs in the article (Research News, 11 Nov., p. 973) about the report by Leonard F. M. Scinto *et al.* "A potential noninvasive neurobiological test for Alzheimer's disease" (11 Nov., p. 1051), was itself incorrect. The photos showed the undilated and dilated eye of a normal 36-year-old member of the Scinto group, not the undilated and dilated eye of an Alzheimer's patient, as the correction stated. The error was the result of a misunderstanding between *Science* and the Brigham and Women's Hospital news office, which took the photos. A patient photo was not supplied, Scinto says, because subjects were not then being studied and hospital policy does not permit treatment of a patient, even with dilute eyedrops, for the purpose of taking a photo. As the photo subject was a normal individual, Scinto also notes that the dilation was achieved by a 1% solution of tropicamide (a standard dose), rather than the 0.01% used in the trial. *Science* regrets the error.

The News & Comment article "Brookhaven prepares for boron trials" (17 Feb., p. 956) by Andrew Lawler omitted the information that terminally ill patients who have undergone conventional treatments, including radiation and chemotherapy, are not eligible for the boron trials expected to begin this month at Brookhaven National Laboratory.

In a correction that appeared in the Book Reviews section of 13 January (p. 267), Solomon W. Golomb's name was misspelled.

In figure 5 (p. 1372) of the report "Activation and regeneration of rhodopsin in the insect visual cycle" by A. Kiselev and S. Subramaniam (25 Nov. 1994, p. 1369), the labels "thermally unstable" and "thermally stable" were inadvertently interchanged.

In the report "A three-dimensional model for the hammerhead ribozyme based on fluorescence measurements" by T. Tuschl *et al.* (4 Nov. 1994, p. 785), the text of lines 28 through 30 in column 3 on page 785 should have read, "... we located 5-carboxyfluorescein at (d, $-29.5°$, $L-3.75$ Å) and 5-carboxytetramethylrhodamine at (a, $-29.5°$ $-\Delta$, -3.75 Å)." In the same report, the second line of equation 1 in the legend to figure 3 was incorrectly printed. The correct equation appears below.

$$E = \left\{1 + \left[\left[(2.81\text{Å}\cdot(N-1)+L)^2 + a^2 + d^2 - 2\cdot a\cdot d\cdot\cos(32.7°\cdot(N-1)+\Delta)\right]^{1/2}\Big/R_0\right]^6\right\}^{-1}$$

Equation 1 in note 17 of the same report was also incorrectly printed. The correct equation appears below.

$$E = 1/[1 + (R/R_0)^6]$$