active brain areas." But James Pritchard and Robert Shulman were unable to explain this increase in anaerobic glucose breakdown by an increase in lactate.

The main purpose of glycolysis is to provide pyruvate for the trichloroacetic acid (TCA) cycle, not to make adenosine 5′-triphosphate. The glycolytic production of pyruvate reduces the cytosol by increasing the ratio of NADH [a reduced form of NAD^+ (nicotinamide adenine dinucleotide)] to NAD^+. Thus, glycolysis cannot continue without "something" returning the cytosolic redox potential to normal.

Two distinct pathways exist for normalizing the cytosolic redox potential. The first is the malate-aspartate and glycerophosphate shuttles. These are used almost exclusively during low-to-medium rates of TCA cycling. During high rates of respiration, it is thermodynamically unfavorable for these reducing-equivalent-carrying TCA-cycle intermediates to leave the mitochondria. During these high rates of respiration, the shuttles cannot increase their contribution to cytosolic redox stability, and the cytosolic pyruvate-lactate reaction begins producing net lactate.

Pierre Magistretti interprets Raichle's argument (oxygen use lags behind glucose consumption and blood flow) as "astroglia metabolize the excess glucose to lactate, which they then pass on to neurons as an energy source." This does not explain the problem of low oxygen use (as Peter Fox points out), because this lactate shunt would require the same amount of oxygen as if the neurons oxidized the glucose themselves.

So, where does this lactate go? If we remember that lactate is formed mainly to oxidize the cytosol of highly metabolically active cells, then we can narrow our search. I think the search should begin with the real metabolic markers of nonaerobic glucose use, including lactate, pyruvate, and alanine. Because the lactate-pyruvate reaction occurs in red cells, both lactate and pyruvate should be considered equivalent and calculated collectively. Alanine should be measured because of the importance of the cytidine 5′-triphosphate cytosolic reaction in fueling the malate-aspartate shuttle; alanine production also increases like crazy during metabolic acidosis.

Raichle's last question (as phrased by Barinaga) is, "what need [is] the excess blood flow . . . serving if it's not answering a call for oxygen"? Blood flow probably increases in response to the decrease in pH or the increase in carbon dioxide caused by glycolysis. Thus, the products of the increase in glycolytic metabolic acidosis cause an increase in glucose presentation to the cell—a beautiful example of control mechanisms regulating cytosolic redox potential.

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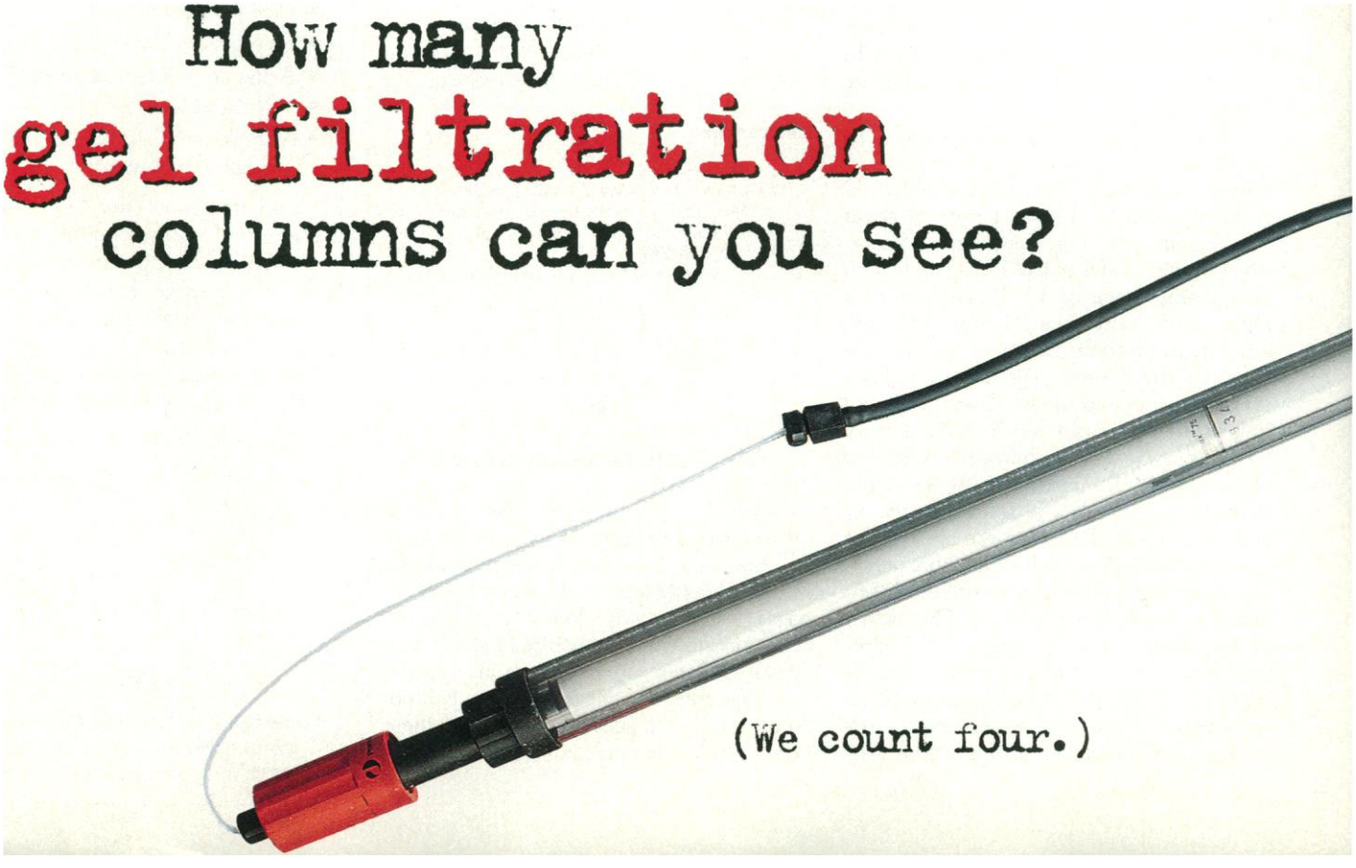
Sickle Cell Anemia Research and a Recombinant DNA Technique

The development of any new technology that could increase the frequency of homologous recombination by three to six orders of magnitude over that seen in normal mammalian cells would represent a major breakthrough. The report by Cole-Strauss *et al.* (6 Sept. 1996, p. 1386), in which an RNA-DNA hybrid oligonucleotide carrying the wild-type β^A sequence was used to correct 50 to 80% of B cells that carry a mutant β^S-globin locus, therefore gained much attention from the scientific community.

The possibility that this unexpected result could be a consequence of a potential artifact was previously raised in a letter by Thomas and Cappechi (7 Mar., p. 1404). Careful examination of the data presented



in figure 4 (p. 1388) of the report by Cole-Strauss suggests that such an explanation is likely. As noted by Cole-Strauss *et al.*, wild-type cells are polymorphic at the third base of codon 2, with a mixture of cytosine (C) and thymidine (T) at this position. The sequence analysis of β^A (first row) shows this clearly. The mutant β^S gene does not have this polymorphism, and only C is present at this position (third row). Because the "correcting" oligonucleotides SC1 and SC2 also contain C at this position, recombination should not introduce any changes at this site of the β^S allele. After incubation with SC1 (fourth row), however, where the dramatic correction of nearly 50% A in the mutants that previously only had T at base 2 of codon 6 is in evidence, one can also see that there may be 25% T at base 3 of codon 2. There should be 100% C. Such a result might be expected to occur if one had a population containing equal quantities of the mutant and wild-type cells, because there does not appear to be any simpler explanation for the change at the third base of codon 2. Thus, this rate of recombination appears to be a result of contamination of mutant cells by wild-type cells, and the perceived frequency of recombination is equal to the percentage of contamination.

Consistent with this explanation, experiments with the SC2 oligonucleotide (figure 4A, row 5 of the report) also show that the proportion of C is greatly increased at the third base in codon 2. Because the mutant cells contain only C at this position, while the wild-type cells are polymorphic, we think it is likely that, in this case, contamination of the wild-type cells by mutant cells led to the observed high frequency conversion of the β^A to the β^S allele at base 2 of codon 6. We think that this alternative argument of significant contamination explains the unexpected effects seen at the third position of codon 2, away from the sickle-cell mutation of interest at codon 6.

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Response: Our work has been aimed at demonstrating the feasibility of using small synthetic oligonucleotides to effect an alteration in a selected gene in vivo. Recognizing that our results represented an improvement in the ability to cause desired changes in genes without the need for selection of the modified cells, we have taken a cautious approach in our presentation, in performing controls, replicating each experiment, and avoiding statements of specific conversion frequency. This work represented a logical extension of our previous publication (*1*), in which we demonstrated highly efficient targeting of an episomal target in Chinese hamster ovary (CHO) cells with oligonucleotides of similar design. In all of our efforts, we have considered three types of potential artifacts.

1) PCR. There is a potential for polymerase chain reaction (PCR)–mediated artifacts and mutations when an analysis is based solely on the results of PCR amplification. We based our detection and quantitation in (*1*) on an assay that did not require PCR (we used oligonucleotide hybridization of unamplified episomal DNA). In later work (*2*), the assays included a Southern blot (DNA) analysis, where no DNA amplification was performed. Subsequently, we found that the presence of a vast excess of the mutagenic oligonucleotides in the PCR reaction cannot introduce the desired mutation (*3*).

2) Contamination. Our awareness of

the limits of the conventional homologous recombination methodology (frequency of conversion ranging from 1 to 1000 to 1 in 10^6) led us to select methods of detection effective over a range of frequencies. Apparent conversion can always be argued as contamination or random reversion of a mutation, so we used multiple methods of detection. The automated sequence analysis methodology that uses fluorescent dyes is approximately equal in sensitivity to a properly controlled restriction polymorphism analysis. In response to this criticism, we tested whether a mixture of the β^S and β^A cells would generate the expected results and pattern. We established that when a mixed sequence is detectable by sequencing, it is also evident in the polymorphism assay. Consequently, the lack of Bsu 36 1 digestion (*2*) in the sickle homozygous cells indicates that the cells are not, nor did they become, polymorphic. The conversion experiment has been carried out many times, with parallel detection by polymorphism and sequence assays, and although there is variability in the frequency of conversion, we can detect the conversion event in untreated cells by both assays, which indicates that the methodology does represent an advance over existing technologies. The appearance of double and even triple peaks in automated sequence chromatograms is a common occurrence and is rarely indicative of contamination. We have sequenced both strands in several samples to rule out this possibility.

3) Cloning. It is imperative that the stable inheritance of the introduced mutation needs to be demonstrated to rule out trivial explanations. Because we encountered difficulty in cloning the cells used in this study, we have relied on demonstrating conversion of the beta allele in two different cell types, human HeLA and HUH7, using the SC2 oligonucleotides described in the report (*2*). Both cells are amenable to cloning by limiting dilution, and we have completed experiments that confirm stable genetic changes at the specific base of the β-globin gene. Other workers have recently, independently completed a study showing the targeted conversion of both the alkaline phosphatase and the β globin genes in the genome of HUH7 cells (*4*).

We are aware that our two papers (*1*, *2*) have led many (including ourselves) to test the method on multiple targets and in different cells. We mentioned in our report (*4*) the potential variability of different cell types and frequency of conversion by highlighting our lack of success in the TK6 cell line. Future studies should reveal how this technology is applicable to different cell types and genes.

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Synergistic Effect of Environmental Estrogens: Report Withdrawn

I write to formally withdraw the report "Synergistic activation of estrogen receptor with combinations of environmental chemicals" (7 June 1996, p. 1489) (*1*), for which I was corresponding author.

We have conducted experiments duplicating the conditions of our earlier work, but have not been able to replicate our initial results.

Also, since our publication in *Science* (*1*),