Correspondence

The Ethics of Research in Developing Countries

To the Editor: The editorial by Dr. Angell (March 30 issue)[1] that accompanied our article[2] conflated two Rakai Project studies. We conducted a community-based, randomized trial of sexually transmitted disease (STD) control for the prevention of human immunodeficiency virus (HIV) infection in the Rakai district of Uganda,[3] involving 15,127 persons. We subsequently performed a secondary analysis of HIV viral load in 415 retrospectively identified couples in which one partner was HIV-positive and one was initially HIV-negative.[2]

In her editorial, Dr. Angell suggests that the HIV-positive study participants could have easily been treated with antiretroviral drugs. This is incorrect. The STD trial conducted surveys at 10-month intervals in 56 dispersed rural communities between 1994 and 1998. Antiretroviral monotherapy is of limited value,[4] combination therapy was not described until 1996,[5] and the results of definitive trials were reported only in 1998.[6] Most important, neither we nor the Ugandan government had, or currently have, the clinical capacity to manage antiretroviral treatment, including side effects and compliance. The study was approved by four institutional review boards (IRBs) in Uganda and in the United States and was monitored by a data safety and monitoring board of the National Institutes of Health, which included Ugandan representatives. None of these boards recommended the use of antiretroviral agents in this rural setting.

Dr. Angell notes that "in most states it would be expected that caregivers would see that seronegative partners were informed of their special risk," even if the HIV-seropositive partners had not agreed to the disclosure. Participants in the study in Uganda consented to enrollment as individuals, not as couples, and involuntary disclosure of the results of HIV tests would have breached the confidentiality guaranteed as part of the informed-consent process. Ugandan policy states, "It is the right of the patient to decide who else to inform about their results,"[7] because of concern about stigma, discrimination, and violence resulting from involuntary disclosure.[7-9] Involuntary disclosure would also have undermined trust in the national program of confidential HIV testing and counseling, a cornerstone of Uganda's successful HIV-prevention policy. We promoted and provided voluntary, confidential, free HIV testing and counseling; strongly encouraged persons who underwent testing to share the results with their partners; offered counseling for couples on request; and provided free condoms and health education. Dr. Angell cites U.S. guidelines recommending involuntary disclosure,[10] which were published after the trial ended and which are not uniformly accepted in this country.

Dr. Angell implies that we offered substandard care to the members of the control group in the STD trial. This is incorrect. For example, the results of tests for syphilis were made available in both study groups, with home treatment offered to members of the intervention group and referrals to government clinics, which were stocked with free penicillin by the Rakai Project, offered to members of the control group. Syphilis in pregnant women declined by 70 percent in both groups.[2] At the time of each survey, we provided free treatment for symptomatic subjects in both groups. At the end of the trial, members of the control group were offered home-based antibiotic therapy identical to that provided in the intervention group. We agree with Dr. Angell that investigators should "provide better care for human subjects than is generally available in the community." In both study groups, the care provided far exceeded that available in rural Uganda and in many states in this country.

Dr. Angell questions the relevance of our studies to Uganda. Evaluating the control of STDs for the prevention of HIV infection was directly relevant to Ugandan policy. The secondary finding of reduced rates of HIV transmission with lower viral loads provides an impetus for the development of safe, effective, simple, and affordable strate-

gies (use of antiretroviral agents or vaccines) to control the spread of HIV by reducing viremia. The relevance and ethics of research performed in developing countries need to be addressed in well-informed international forums.

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10. HIV partner counseling and referral services — guidance. Bethesda, Md.: Department of Health and Human Services, December 30, 1998.

To the Editor: In research involving human subjects, ethical principles and standards are for the protection of the subjects. If the intention is to study the subjects for their benefit individually, collectively, and in the community, then the main objective must be to benefit the subjects in their own setting. The intention must be to help the population find a way to cope with the disease burden, not to offer new options that are just as out of reach as those that already exist. Otherwise, it would be difficult to claim that the study was conducted for the benefit of the local population, not for that of other populations.

In the study by Quinn et al., is there any hope that the information gleaned will benefit the population studied? Will members of this population be able to afford viral-load testing? Will adult circumcision be of any benefit? Will this information lead to a reduction in the cost of antiretroviral drugs, making them more affordable in developing countries? These and other questions about the ethical nature of such studies must be answered if the study subjects are not to be seen as being exploited.

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To the Editor: As an AIDS researcher in a "developing" country, I know that new drugs and effective vaccines for HIV infection are urgently needed. However, this urgency is being used to lower the standards established by the Declaration of Helsinki,[1,2] which states that the "best proven diagnostic and therapeutic methods" must be provided to all study subjects. A 1999 draft of a document intended as a substitute for the declaration proposes the wording "best proven methods that would otherwise be available to the subject of research." Thus, if nothing were available, the "best proven" method would be to make nothing available. The researchers' rationale is that poor countries do not provide antiretroviral agents anyway and that their high costs would make the trials too expensive to conduct. The lack of availability of antiretroviral agents is used to justify the performance of placebo-controlled trials even though effective drugs exist. The argument is that such trials are more efficient and less expensive to perform than non–placebo-controlled trials.

Clinical trials should be performed when use of the "best proven" methods can be assured. This approach may delay access to trials for some countries but will be safer and ethical. If at the end of the trial the drug, vaccine, or procedure is found to be effective, it should be made available wherever it is needed. The plan for this provision should be discussed at the outset among all parties. The justification for different ethical standards for poor countries is based on economic grounds, not on ethical or scientific grounds. Such trials should not be permitted.[3]

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1. Brennan TA. Proposed revisions to the Declaration of Helsinki — will they weaken the ethical principles underlying human research? N Engl J Med 1999;341:527-31.
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To the Editor: I am perplexed by the *Journal*'s publication of the article on HIV transmission by Quinn et al. and Dr. Angell's accompanying editorial. She expresses grave doubts about the study. The statements that "ethical standards should not depend on where the research is performed" and "such a study could not have been performed in the United States" strongly indicate that she considers the study unethical. If so, why did she publish the article?

It seems that the *Journal* is trying to have it both ways: championing the rights of poor Africans in the editorial while endorsing the study by publishing the report on it.

True progress in protecting human subjects in developing countries will require tougher choices.

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To the Editor: Although ongoing public discussion about the ethics of international research is crucial, we believe that Dr. Angell's editorial did not advance the discussion. The editorial detracted from the policy and prevention implications of the article by Quinn et al. We are concerned that much-needed ethical research on interventions to prevent the further spread of HIV infection in countries where it has a high prevalence will be derailed. Moreover, the ensuing confusion will delay the implementation of effective strategies to prevent HIV infection. Clearly, no one wants this to happen.

We strongly agree that all research conducted in international settings should have relevance to the study participants themselves, as well as to the country in which the research is taking place. In the case of HIV-prevention trials, highly ethical research has led to findings that have helped reduce both sexual and perinatal HIV transmission in such countries as Thailand and Uganda, thus saving many thousands of lives. The facts in the Rakai study are clear. The investigators went to great lengths to adhere to basic principles of ethical research. Furthermore, the study was deemed a high priority by health officials in Uganda.

The finding that HIV transmission was directly related to the viral load serves as a foundation for further research on low-cost, easily administered HIV therapies. This information will help prevent the spread of the virus in countries with the greatest problems in controlling the epidemic.

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To the Editor: Dr. Angell's editorial is an articulate summary of the ethical dilemmas posed by the study of HIV transmission in Uganda reported by Quinn et al. However, I am disturbed by her tacit assumption that the *Journal,* or any other peer-reviewed journal, should be the final arbiter in conflicts over the publication of ethically questionable research.

Undoubtedly, the ethical issues were not considered lightly by the two national agencies and two universities whose IRBs approved the research. For most reports on research involving human subjects, journals accept the authors' assurance that the study was approved by a suitable IRB, with the assumption that the IRB was given complete information. When investigators acknowledge that there are special ethical issues but document reviews by two, three, or more IRBs, journals should not dispute the decision to conduct the research or publish the results. Such a policy would not suggest a cavalier attitude toward the ethical treatment of human subjects but would reflect the recognition that ethical dilemmas should be resolved not by individuals or small groups but by bodies that are more broadly representative of society — the essential role of IRBs.

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Dr. Angell replies:

To the Editor: The two parts of the Rakai Project addressed different questions. The first part asked whether the transmission of HIV could be reduced by treating other STDs in a population. To answer that question, the investigators had to treat the two groups of villages differently, so that the experimental villages would have a lower rate of STDs than the control villages. That is exactly what they achieved. Otherwise, they could not have answered the question they asked. I do not question the relevance of that part of the project to Uganda (a point on which I agree with Dr. Mullings), only whether the design of the trial was ethical.

The second part of the project asked whether the heterosexual transmission of HIV is a function of the viral load. The authors retrospectively measured HIV type 1 RNA in 415 couples discordant for HIV. However, as the authors make clear, the results of HIV serologic testing were available to the investigators and the subjects throughout the 40-month study. Since both partners were tested for HIV at 10-month intervals, seronegative partners of seropositive persons could easily have been identified and informed of their special risk. The fact that Ugandan policy advises against this practice does not constitute an ethical argument. Instead, it might suggest a review of the justifications for undertaking the study in a country with such a policy. Furthermore, it is difficult to see the relevance to present-day Uganda of this part of the Rakai Project, unlike the STD part.

I did not specify any particular form of treatment in saying that "the investigators could easily provide the drugs" to HIV-positive study participants, but I meant whatever treatment was offered in developed countries at that time. My point was that I was not persuaded by the argument that participants could be offered less simply because they lived in an underdeveloped country where the best current treatment was not generally available outside a research study. Furthermore, I do not agree with the implication by Gray et al. that a study, once launched, cannot be altered or even halted when other work identifies important new treatments.

In answer to Dr. Handsfield, I believe that an ethical review of a study is similar to a scientific review. No one involved should fail to call attention to scientific flaws on the grounds that it is someone else's business to do so. An editor should not publish a paper if he or she believes it is fatally flawed, either scientifically or ethically. In answer to Dr. Hearst, although I did have important reservations about the study, I did not believe the issues were so clear-cut that I was willing to override the judgments of the peer reviewers and other editors on my staff. These issues need further debate, and that is happening here.

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A Study in Rural Uganda of Heterosexual Transmission of Human Immunodeficiency Virus

To the Editor: Largely ignored in the report by Quinn et al. of their study of the heterosexual transmission of human immunodeficiency virus type 1 (HIV-1) in Uganda (March 30 issue)[1] is the finding that of 137 uncircumcised men who were negative for HIV-1, 40 seroconverted, whereas 0 of 50 circumcised men seroconverted. This finding suggests that male circumcision is at least as protective against female-to-male transmission of HIV-1 as low viral load in the female partner. Yet the authors do not consider male circumcision among their list of possible strategies for the prevention of HIV-1 infection.

There are now more than 30 epidemiologic studies from sub-Saharan Africa dating back to 1987 that report a significant protective effect of male circumcision against HIV-1 infection.[2] Is it not time for those in Rakai, Uganda (where Quinn et al. conducted their study), as well as others, to benefit from these studies? The feasibility of offering information on voluntary male circumcision and circumcision services to this community with a high prevalence of HIV-1 infection could at least be investigated. Justice and scientific evidence demand it.

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1. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. N Engl J Med 2000;342:921-9.
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To the Editor: Quinn et al. state that 51 of the 415 untreated HIV-1–positive subjects in their study had undetectable serum levels of HIV-1 RNA (<400 copies per milliliter). Such a large number of untreated subjects with viral loads of less than 400 copies per milliliter (12 percent of untreated subjects) is surprising. In the large Multicenter AIDS Cohort Study, use of the same methods indicated that only about 3 percent of untreated HIV-positive persons had plasma levels of less than 400 copies per milliliter.[1] According to the editorial accompanying the article by Quinn et al.,[2] plasma HIV-1 RNA levels tend to be higher, not lower, in sub-Saharan Africa than in more developed countries, and the incidence of host mutations that might lead to low viral levels is lower. It is possible, therefore, that the serum specimens from these 51 subjects had degraded or become denatured during the one to three years of storage before laboratory measurement. Are the "undetectable" values erroneous? If so, this may cast doubt on the other viral RNA measurements and thus on the conclusion that the plasma HIV-1 RNA level is the chief predictor of heterosexual transmission of HIV-1.

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1. Lyles RH, Munoz A, Yamashita TE, et al. Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. J Infect Dis 2000;181:872-80.
2. Cohen MS. Preventing sexual transmission of HIV: new ideas from sub-Saharan Africa. N Engl J Med 2000;342:970-2.

The authors reply:

To the Editor: Bailey comments on the association between circumcision and reduced rate of acquisition of HIV-1 in male subjects in our study of couples discordant for HIV-1 status. Although circumcision was strongly associated with reduced acquisition of HIV-1 in these highly exposed couples, additional analyses suggest that generalization to the whole population is complicated by confounding.[1] In our representative population in Rakai, we found that circumcision was associated with a reduced rate of HIV-1 acquisition; this was particularly true for circumcision performed before puberty. However, this effect was mainly due to the lower incidence of HIV-1 among Muslims, who constitute the largest group of circumcised males. Circumcision was not significantly protective among non-Muslim men or in couples in which both partners were HIV-1–negative.[1] The 30 African epidemiologic studies mentioned by Bailey are mainly cross-sectional investigations with inconsistent findings and inadequate control for potential confounding. These observational data are difficult to interpret, and clinical trials are needed before circumcision can be promoted as a means of preventing HIV infection.

Fremont-Smith questions the proportion of subjects with undetectable viral loads in our study on the basis of a comparison with a subgroup analysis from the Multicenter AIDS Cohort Study[2] and a previous report that plasma HIV-1 RNA levels tend to be higher in sub-Saharan Africa.[3] Unfortunately, these studies cannot be directly compared with ours. Both studies referred to by Fremont-Smith used plasma, whereas we used serum. HIV-1 RNA levels in plasma are 30 to 80 percent higher than those in serum,[4] so specimens with low levels of HIV-1 RNA in plasma may have undetectable levels in serum. Prolonged periods between collection, processing, and storage in our study may also have lowered viral detection, since the greatest decrease in RNA levels occurs within the first six hours after collection. However, the overall results remain internally valid, since the methods of sample preparation and assay were consistent throughout the study. Our estimate of the risk of transmission per log (base 10) increment in viral load is nearly identical to the risk observed in a study of mother-to-infant transmission[5] and in a study of heterosexual transmission in Zambia[6]; this consistency further supports the validity of our measurements of viral levels.

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1. Gray RH, Kiwanuka N, Quinn TC, et al. Male circumcision and HIV acquisition and transmission: cohort studies in Rakai, Uganda. AIDS (in press).

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Liver Damage Due to Alendronate

To the Editor: Alendronate is indicated for the treatment of postmenopausal women with osteoporosis and patients with glucocorticoid-induced osteoporosis.[1] We report liver dysfunction due to alendronate in a postmenopausal woman given the drug for osteoporosis.

A 71-year-old woman was referred to our clinic for an evaluation of osteoporosis. She had been thought to have primary biliary cirrhosis four years earlier, was receiving ursodeoxycholic acid (ursodiol), and had normal liver function. The diagnosis of primary biliary cirrhosis had been based on high serum alkaline phosphatase and antimitochondrial antibody concentrations, but liver biopsy had not been performed.

Three years later, on evaluation at our clinic, the patient had back pain and severe osteoporosis, with fractures in L4 and L5. The bone mineral density of the lumbar spine was low (T score, −4.21). The T score was −4.66 one year later, at which time she was treated with alendronate (10 mg per day). Two months later, routine biochemical studies revealed high serum liver enzyme concentrations (Table 1). The alendronate was withdrawn but not the ursodiol, and the serum liver enzyme concentrations slowly returned to the normal range.

Serologic tests for hepatitis A, B, and C viruses, Epstein–Barr virus, and cytomegalovirus were negative. Liver biopsy showed lobular inflammation, fatty changes, and portal infiltration of lymphocytes, granulocytes, and eosinophils. Some hepatocytes had necroinflammatory lesions, with piecemeal necrosis. Masson staining revealed no fibrosis. No signs of primary biliary cirrhosis were found.

The main side effects of alendronate are gastric and esophageal inflammation,[2] but renal failure, ocular damage, skin reactions, and hypocalcemia have also been reported. A case of hepatitis that developed after treatment with alendronate was recently reported in a 77-year-old woman.[3]

The mechanism by which alendronate may cause liver damage is not known. Reactivation of the primary biliary cirrhosis might have caused the high serum liver enzyme concentrations in our patient, but there was no evidence of such reactivation. Another possibility is that alendronate, like other aminobisphosphonates, inhibits the synthesis of cholesterol in the liver,[4] which may alter liver function, since this pathway is essential in the maturation of Ras-related G proteins.[5]

Regardless of the mechanism, physicians treating patients with a bisphosphonate should be alert to the possibility of hepatic dysfunction during therapy.

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1. Saag KJ, Emkey R, Schnitzer TJ, et al. Alendronate for the prevention and treatment of glucocorticoid-induced osteoporosis. N Engl J Med 1998;337:292-9.
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3. Lieverse RJ. Hepatitis after alendronate. Neth J Med 1998;53:271-2.
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The above letter was referred to Merck & Co., the manufacturer of alendronate, which offers the following reply:

To the Editor: Halabe and colleagues describe a woman who was found to have high serum liver enzyme concen-

TABLE 1. RESULTS OF LIVER-FUNCTION TESTS BEFORE, DURING, AND AFTER TREATMENT WITH ALENDRONATE.*

SERUM TEST	NORMAL RANGE	SEPT. 1995	NOV. 1996	MARCH 1997	APRIL 1997†	APRIL 1997	MAY 1997	JUNE 1997	JULY 1997	AUG. 1997
Aspartate aminotransferase (U/liter)	0–37	18	27	118	106	176	278	168	52	43
Alanine aminotransferase (U/liter)	0–40	15	32	163	158	233	447	245	64	45
γ-Glutamyltransferase (U/liter)	7–50	63	28	118	100	140	232	167	38	40
Alkaline phosphatase (U/liter)	82–290	252	97	151	140	172	224	185	109	115
Bilirubin (mg/dl)	0.2–1.0	0.7	0.9	0.7	1.1	1.2	1.0	1.1	0.9	1.1

*Treatment with alendronate (10 mg per day) was started in January 1997. The treatment was discontinued in April 1997, and a liver biopsy was performed in May 1997.

†Treatment with alendronate was discontinued.

trations and hepatitis while receiving alendronate. The authors attribute the hepatitis to alendronate therapy because it was diagnosed two months after alendronate was started. Yet the patient had had a similar episode of unexplained hepatic dysfunction four years earlier. That episode had been attributed to primary biliary cirrhosis, but the later liver biopsy revealed no evidence of primary biliary cirrhosis. Also, the patient's serum liver enzyme concentrations continued to increase for a month after the discontinuation of alendronate. Therefore, the recurrence of an underlying liver disease seems a likely explanation for the findings in this patient.

Alendronate, an inhibitor of osteoclast-mediated bone resorption, rapidly attaches to the bone surface. The drug is not taken up by cells or metabolized, and that which is not deposited in bone is rapidly excreted by the kidneys. Therefore, the potential for systemic toxicity is low.[1] Alendronate therapy has been extensively evaluated at high doses (25 to 40 times the 10-mg daily dose) in multiple animal studies without any evidence of hepatotoxicity.[2] In randomized, double-blind trials involving a total of more than 17,000 patients treated for up to eight years, with repeated assessments of liver function, there were no increases either in the number of reports of hepatitis or in the mean serum concentrations of aminotransferases, alkaline phosphatase, or bilirubin during treatment with alendronate, as compared with placebo.

The authors speculate that alendronate may affect liver function by inhibiting hepatic synthesis of cholesterol. This seems unlikely. After an oral 10-mg dose, plasma concentrations of alendronate are undetectable (<5 ng per milliliter, or approximately 20 nM)[3] and are well below the extracellular concentrations (10 to 30 μM) required to inhibit farnesyl diphosphate synthase in cells that do not actively take up alendronate, as compared with osteoclasts that take up alendronate as bone is resorbed.[4,5] In any event, the histologic findings reported by Halabe and colleagues suggest that their patient had inflammatory, rather than toxic, liver injury.

The pharmacologic characteristics of alendronate, extensive data from studies in animals and humans, and five years of experience with the use of the drug in more than 3.5 million patients all support the conclusion that alendronate is very unlikely to be hepatotoxic. We do not believe that the case presented by the authors, with incompletely defined prior liver disease, alters this conclusion or supports their recommendation of periodic measurements of serum aminotransferases in patients receiving a bisphosphonate.

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1. Watts N, Freedholm D, Daifotis A. Alendronate: from the laboratory to the patient: the clinical tolerability profile of alendronate. Int J Clin Pract 1999;Suppl 101:51-61.
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Ehlers–Danlos Syndrome Type IV

To the Editor: As one of the medical advisers to the Ehlers–Danlos Support Group in Britain, I have examined and advised more than a dozen families with Ehlers–Danlos syndrome type IV. I would like to draw attention to a few clinical features that did not appear to be present in the large series of patients studied by Pepin et al. (March 9 issue).[1] In my experience, teenage boys are at high risk for arterial rupture, which is often fatal. This may be because during the prepubertal growth spurt, the defective collagen is further weakened. In addition, patients who undergo surgery are prone to have arterial rupture in the postoperative period. This may be because of the increase in collagenase activity after surgical trauma.

Pepin et al. comment that preexisting aneurysms are only occasionally documented among those who have arterial ruptures. The reason, I believe, is that patients with Ehlers–Danlos syndrome type IV do not have true aneurysms. Aneurysms, if present, follow arterial tears and are walled-in hematomas or pseudoaneurysms.[2] Finally, I would like to draw attention to the danger of varicose-vein surgery in unrecognized cases of Ehlers–Danlos syndrome type IV, since the extreme fragility of all blood vessels can lead to a loss of a limb or even loss of life.[3] These tragic cases support Pepin and colleagues' suggestion that knowledge of the diagnosis prevents complications.

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1. Pepin M, Schwarze U, Superti-Furga A, Byers PH. Clinical and genetic features of Ehlers–Danlos syndrome type IV, the vascular type. N Engl J Med 2000;342:673-80.
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To the Editor: Pepin et al. describe a group of patients with Ehlers–Danlos syndrome type IV. In the accompanying editorial, Pyeritz states that "detecting the biochemical defect in Ehlers–Danlos syndrome type IV is simpler, more sensitive, and cheaper than finding a specific mutation in *COL3A1*."[1] We report a case in which the standard biochemical assay failed to detect abnormal type III collagen or procollagen but in which molecular testing revealed a mutation in the *COL3A1* gene.

A 40-year-old man with a history of bilateral renal-artery stenosis and spontaneous hematothorax was admitted with possible appendicitis. Laparotomy revealed a pulseless ileocolic artery and an ischemic colon. Despite resection of the ischemic parts, the patient needed a second laparotomy, performed the next day, which revealed new intestinal ischemia, again necessitating resection. On the third day, an-

other laparotomy was needed and revealed a ruptured abdominal aorta. A final laparotomy, which was performed on day 9, showed a ruptured gallbladder. Further surgical intervention was deemed futile, and the patient died on day 28 after admission.

The patient's family history was negative for the Ehlers–Danlos syndrome, and he had no skin or joint abnormalities. Reevaluation of intestinal and renal specimens obtained earlier revealed necrotic degeneration of the vascular wall, aneurysms, and medial degeneration. A fibroblast culture was performed, but the electrophoretic mobility of type III collagen and the ratio of type III to type I collagen were normal (Fig. 1A). Analysis of complementary DNA (cDNA) revealed that the eighth exon of the *COL3A1* gene was missing in 50 percent of the messenger RNA (mRNA) (Fig. 1B) as a result of a base substitution in intron 8 (+5G→A). The normal appearance of type III collagen may be due to the fact that skipping exon 8 results in a shorter protein and not a shift in the reading frame. The formation of the triple helix is hampered, but the mutation is close to the N-terminal end and does not result in overmodification.

This case demonstrates that the standard biochemical as-

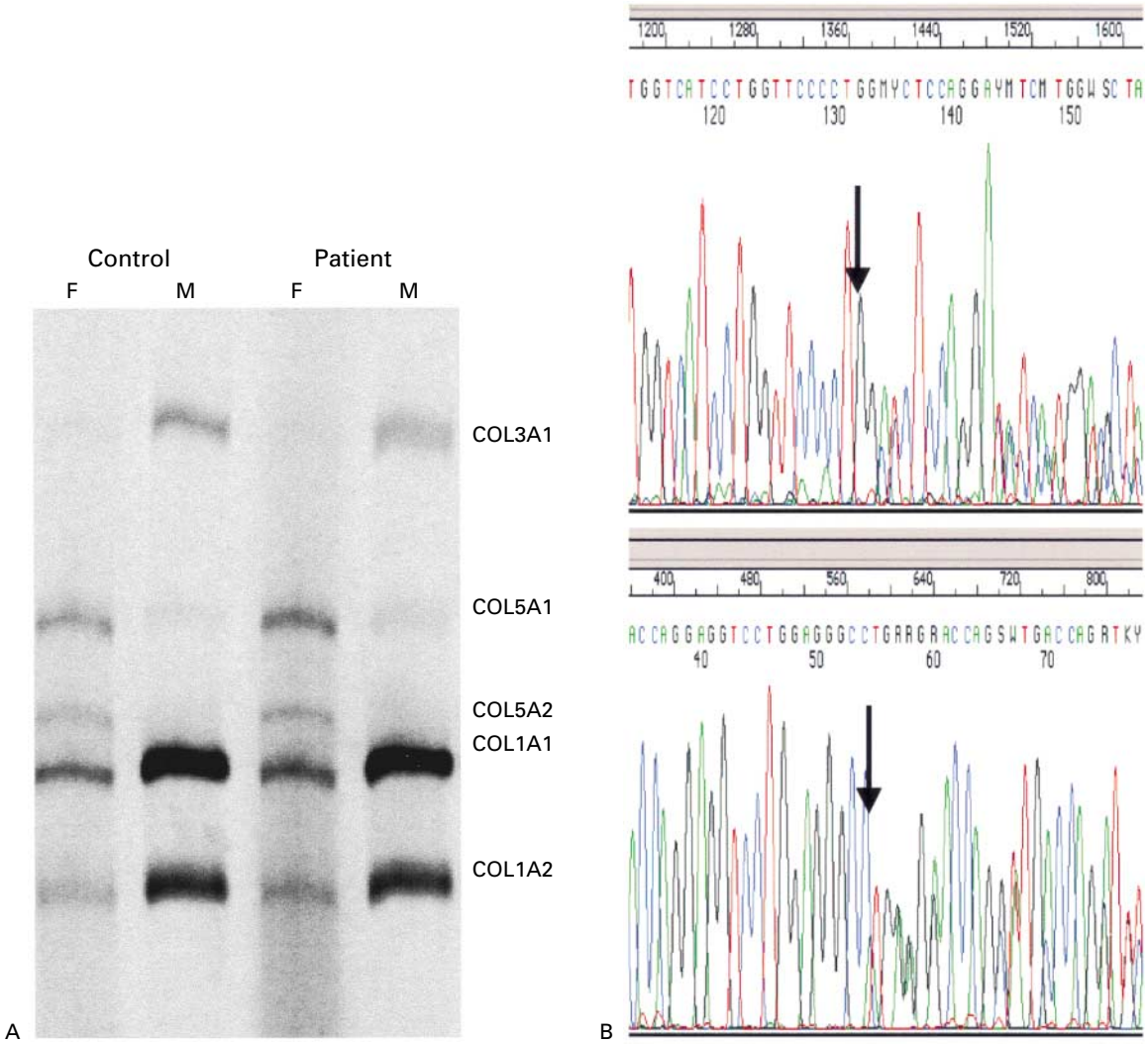

Figure 1. Analysis of Collagen and Sequencing of Complementary DNA.

Panel A shows an autoradiogram of sodium dodecyl sulfate–polyacrylamide gel electrophoresis of ^{14}C-labeled collagen extracted from cultured fibroblasts (F) and culture medium (M) from a control and the patient. The collagen fractions with the type and chain numbers are indicated on the right-hand side of Panel A. There is no sign of overmodification of COL3A1. The fraction of type III collagen is 6 percent of the total collagen, which is not below the lower limit of normal (5 to 18 percent in 40 controls). Panel B shows the results of sequencing (with an ABI 310 sequencer, Perkin–Elmer) of the patient's *COL3A1* complementary DNA. The sense sequence (top panel) at the start of exon 8 (arrow) is mixed with the sequence of exon 9. The antisense sequence (bottom panel) at the end of exon 8 (arrow) is mixed with the exon 7 sequence. This result indicates the presence of messenger RNA that lacks exon 8.

say for Ehlers–Danlos syndrome type IV may produce false negative results and that a search for a *COL3A1* mutation can be diagnostic. Therefore, we would like to caution against the suggestion that molecular testing is of limited value in the diagnosis of Ehlers–Danlos syndrome type IV, since it can identify additional cases that may be missed by the biochemical assay.

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The authors reply:

To the Editor: The vascular type of the Ehlers–Danlos syndrome, also known as Ehlers–Danlos syndrome type IV and the Sack–Barabas variety, does affect young people adversely. In our study, male index patients were more likely than female index patients to have vascular rupture as their first complication (as shown in Table 1 of our article). Among those with vascular first complications for whom ages were known, 21 male subjects (35 percent) and 10 female subjects (34 percent) were 20 years of age or younger. Three male subjects and one female subject 20 years of age or younger died as a result of the initial arterial complication. In the entire cohort we studied, vascular events were the most common cause of death in subjects of both sexes (Table 2 of our article). We agree with Barabas that complications often come in rapid succession and that surgery in patients not known to have the disorder can be fraught with difficulty.

We sympathize with the difficulties encountered by Pinto et al. in identifying persons with splice-site mutations that affect introns near the 5′ end of the triple-helix coding region. We found that the subtle shift in mobility of α1 (III) chains in gels is more readily apparent when the pro-α1 (III) chains in molecules secreted into the medium of cultured cells are examined under reducing conditions. If the clinical findings are compatible, this shift is sufficient to confirm the diagnosis.

We agree that molecular analysis, either with the use of cDNA synthesized from RNA or by analysis of individual exons amplified from genomic DNA, occasionally is a valuable adjunct to diagnosis. Two of the patients in our series had the same mutation that Pinto et al. describe (IVS8+5G→A). In addition, three had mutations that resulted in the skipping of exon 7 (IVS7+1G→C in two and a complex rearrangement of the same donor site in the third), and three had mutations in which exon 9 was skipped (IVS9+1G→A, IVS9+2T→G, and IVS9+2T→A). All had subtle but detectable shifts in the electrophoretic mobilities of the pro-α1 (III) chains or in the efficiency of secretion of the molecules. More difficult to detect at the protein level are mutations that result in premature-termination codons in the *COL3A1* transcript and that destabilize the mRNA. These mutations reduce the amount of type III procollagen produced to about half the normal level, a difference that may be difficult to appreciate. In such cases, the analysis of heterozygosity for expressed polymorphic sequences in genomic DNA and in RNA is a valuable adjunct to diagnosis.

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To the Editor: My experience with patients affected by the vascular form of the Ehlers–Danlos syndrome confirms several of the clinical issues raised by Barabas and by Pinto et al. First, rupture of a large artery or a viscus and major surgery seemingly unleash factors that contribute to further disruption of connective tissue. This process requires investigation so that measures in addition to surgery can be undertaken to reduce morbidity and mortality. Second, arteries usually rupture without antecedent dilatation or dissection, and except for trauma, the precipitants are unknown. Finally, arterial rupture may be stabilized by the formation of a pseudoaneurysm, and in such cases careful observation rather than aggressive treatment may be the better part of valor.[1]

Pinto and colleagues suggest that not all defects in type III procollagen may be detectable in all biochemical genetics laboratories. This is true in theory, but in practice most failures to detect alterations at the level of the protein in patients with the vascular form of the Ehlers–Danlos syndrome probably occur for a variety of technical reasons. Analysis at the level of the mRNA or of the gene sequence may be of additional value, for example, to detect a mutation, to establish linkage, or to determine whether both alleles are being expressed. Unfortunately, at this time, no laboratory in the United States performs analyses of the genes of interest as a clinical service.

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Hepatic Hemangioma

To the Editor: We disagree with the decision of Drs. Landor and Petrozzo (March 16 issue)[1] to perform two additional radionuclide imaging studies "to confirm a presumptive diagnosis of hemangioma." Hepatic hemangioma is typically an incidental finding, being present in 2 percent of the population. Computed tomography (CT) results in a specific diagnosis in nearly all cases, except those involving very small lesions.[2] Large, cavernous hemangiomas often have a central scar or necrosis and have progressive,

nodular or cloudlike enhancement, with the same degree of attenuation as is present in blood vessels.[3] In our view, the CT features in the patient evaluated by Drs. Landor and Petrozzo were diagnostic and required no confirmatory tests. In some cases of atypical hemangioma, single-photon-emission CT technetium-99m–labeled red-cell scanning (or magnetic resonance imaging) can provide a valuable confirmation of the diagnosis. We do not believe that the single-photon-emission CT technetium-99m sulfur colloid scan provides useful information in this clinical setting.

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Editor's note: The above letter was referred to Dr. Landor, who declined to reply.

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Ulcerative Colitis in a Sigmoid Neovagina

To the Editor: The cause of ulcerative colitis remains unknown. Direct exposure to alimentary antigens or intestinal bacterial flora may trigger the mucosal inflammation in persons who are predisposed to ulcerative colitis.[1] We describe a case of ulcerative colitis involving the patient's sigmoid neovagina, in which the findings are evidence against either of these hypotheses.

A 60-year-old woman presented with a four-month history of abdominal pain in the left lower quadrant and bloody diarrhea. She had undergone vaginal reconstruction with a sigmoid-colon autotransplant at the age of 25 years because of congenital vaginal agenesis. She had never smoked and was not taking any drugs. Colonoscopic examination demonstrated diffuse inflammation and hemorrhagic ulcers of the rectosigmoid mucosa, suggestive of the presence of ulcerative colitis. Biopsy disclosed findings consistent with this diagnosis: epithelial necrosis and ulcerations, a chorionic infiltrate, decreased secretion of mucus, distortion of crypts, and crypt abscesses. The upper digestive tract, the terminal ileum, the remainder of the colon, and the neovagina were macroscopically and histologically normal. Microbiologic and serologic studies were negative. Barium bowel studies were normal. The outcome was favorable after treatment with oral prednisolone, and maintenance therapy with oral mesalamine was started.

Three months later, the patient had leukorrhea and vaginal bleeding. Examination and biopsy of the neovagina disclosed findings identical to those previously seen in the rectocolic mucosa. After treatment with mesalamine suppositories, the symptoms and histologic abnormalities resolved promptly. Two years later, a similar involvement of the neovagina occurred and rapidly resolved after treatment with intravaginal mesalamine suppositories. A few days later, there was a new flare-up of distal ulcerative colitis, which resolved after treatment with mesalamine enemas.

The findings in this case report provide evidence against direct exposure to alimentary antigens as a cause of ulcerative colitis.[1] Depletion of short-chain fatty acids, the main luminal nutrients for colonocytes, or changes in colonic bacterial flora are also unlikely to be the cause.[1] Diversion colitis, an iatrogenic inflammatory process affecting portions of the colon or rectum that are excluded from the fecal stream, may trigger ulcerative colitis in the in-stream colon and has been described in the sigmoid neovagina of patients without preexisting bowel disease.[2,3] This diagnosis is unlikely in our patient because of the temporal relation between colitis in the neovagina and colitis in the remainder of the colon.

Inflammation at one site in the large intestine may trigger ulcerative colitis at different sites, as is suggested by the frequent association of periappendiceal inflammation with left-sided colitis in some patients.[4] We hypothesize that, in our patient, locally activated leukocytes, or anticolonic autoantibodies generated in the inflamed colon, circulated and were recruited to the neovaginal mucosa.[1,2,5]

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