that the group as a whole did not show any progression in a two-year period is surprising and was not commented on by the authors. It would also perhaps have been better to have measured thickening of the capillary basement membrane in the nondiabetic subjects after two years to assess the natural evolution of this structure.

With these exceptions, this work, if confirmed by others, will offer even further support to the view that tight control of diabetes is important even many years after diagnosis.

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The above letter was referred to the authors of the article in question, who offer the following reply:

To the Editor: Recalculation of our data as suggested by Dr. Bergman makes no difference in the overall results of our study. The elimination of Patient 6 from our experimental-treatment group does result in a decrease in the average base-line thickness of the capillary basement membrane for the entire group. The new value (for the 12 remaining patients) is 1570±118 Å (mean ±S.E.M.), which is not significantly different from 1870±95 Å, the base-line value for the conventional-treatment group ($t = 1.723$, $P>0.10$).

I agree that it would have been more satisfying to repeat the measurement of the capillary basement membrane in the nondiabetic control subjects; however, that was not possible.

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RESPONSE TO HEPATITIS B VIRUS VACCINE IN SUBJECTS WITH LOW LEVELS OF ANTIBODY TO HEPATITIS B SURFACE ANTIGEN

To the Editor: Recently, we undertook a cross-sectional survey of 813 medical and dental students in order to estimate the prevalence of antibody to hepatitis B surface antigen (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc).[1] Seventeen students (2 per cent) were found to be reproducibly reactive for low levels of anti-HBs (defined as a sample [S] to negative control [N] ratio of 10 or less), accompanied by a negative test for anti-HBc. The specificity of this finding has been questioned.[1,2] In order to examine it further, we asked students with low levels of anti-HBs and a matched group of 24 seronegative classmates to submit to a repeat blood test one year later. Nine of the original 17 anti-HBs–reactive students were available for a repeat test. All were again positive for low levels of anti-HBs (mean, 6.5±5) and negative for anti-HBc. One of the previously seronegative students was found to have a low level of anti-HBs (S/N, 4.0) and a negative test for anti-HBc. The 33 students (10 with low levels of anti-HBs and 23 who were seronegative) were then vaccinated with a single lot of hepatitis B vaccine (Heptavax B), and a blood sample was analyzed for anti-HBs one month after the initial dose.

All the students with low levels of anti-HBs but only 18 of the seronegative controls (78 per cent) were anti-HBs–positive at the one-month interval. Anti-HBs S/N ratios were significantly higher in the former group. In addition, whereas 4 (40 per cent) of the subjects with low levels of anti-HBs had S/N values above 100 after a single dose of vaccine, only 1 of 23 seronegative students (4 per cent) had similar values ($\chi^2 = 4.4$, $P<0.03$).

High levels of anti-HBs (S/N>100) in response to an initial dose of vaccine have been observed in less than 5 per cent of susceptible vaccinated adults[3] and were noted in only 4 per cent of seronegative controls in the present study. Thus, we believe the finding that 40 per cent of students with low S/N ratios for anti-HBs had high antibody levels after a single dose of vaccine is more consistent with an anamnestic response than with a primary antibody response in these subjects. Since the response to the vaccine in the other subjects with low levels of anti-HBs did not differ from that observed in the majority of seronegative students, it may be concluded that the low anti-HBs ratios before vaccination were frequently not a reliable indicator of past infection with hepatitis B virus. Taken collectively, however, our data indicate that it may be inappropriate to assume that a low level of anti-HBs accompanied by a negative test for anti-HBc generally indicates nonspecific reactivity and susceptibility to infection with hepatitis B virus. The demonstration of IgM and IgG anti-HBs after vaccination of such persons may allow a better appreciation of the frequency with which they have primary and anamnestic responses, respectively.

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NEUTRAL-RED UPTAKE BY AMNIOTIC-FLUID MACROPHAGES IN NEURAL-TUBE DEFECTS: A RAPID TEST

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To the Editor: Determination of alpha-fetoprotein and acetylcholinesterase levels and ultrasonic methods have been used in the diagnosis of fetal neural-tube defects. In addition, macrophages are present in cultures of amniotic-fluid samples obtained from cases of fetal neural-tube defects.[1] These macrophages appear as rapidly adhering cells in culture vessels within 12 to 24 hours after cultivation. Other workers have determined the prevalence of these cells and their morphologic properties instead of enumerating macrophage populations.[2-4] More recently, direct microscopical examination of cytologic smears, instead of culture techniques, has been developed as a method of diagnosis.[5-7]

We report on a simple method for detecting viable amniotic-fluid macrophages in suspension, on the basis of the pinocytic activity of these cells.[8] We found that amniotic-fluid macrophages ingest neutral red, a cationic dye, and are readily identified as "red cells" by microscopical examination. We studied 78 amniotic-fluid preparations obtained by transabdominal amniocentesis. These specimens were stored at room temperature in plastic syringes for no longer than one hour in order to prevent adherence of the cells to the solid surfaces. Using the trypan-blue exclusion test, we found that 67 to 92 per cent of the cells were viable. Five-milliliter aliquots of amniotic fluid were centrifuged at $110\times g$ for 10 minutes in plastic tubes. The supernatant fluid was removed, and each pellet was resuspended in 0.5 ml of HAM F-10 (GIBCO) culture medium containing 0.5 mg of neutral red per milliliter (BDH Chemicals, Poole, England). After the neutral red had been added, the cell suspensions were incubated at 37°C for 15 minutes. The cells were centrifuged and resuspended in 0.25 ml of HAM F-10 medium. One drop of this suspension was examined with a light microscope. Epithelial cells appeared as unstained pale forms, whereas the macrophages took on an intense red color. Cell populations were quantified with the aid of a hemacytometer, and the neutral-red pinocytotic cells were counted. In 31 cases, no colored cells were found (Group A). In the 16 cases, there were 0 to 1200 macrophages per milliliter of amniotic fluid (Group B). In 30 cases, 2700 to 65,000 cells per milliliter had taken up neutral red (Group C).

All pregnancies were allowed to proceed to term or were terminated by induced abortion. Every fetus from Groups A and B was free of neural-tube defects. Fetuses from Group C had abnormalities

or open lesions: 13 cases of anencephaly (5 of these fetuses also had spina bifida), 7 cases of spina bifida, 3 cases of craniorachischisis, and 2 cases of exencephaly. However, in one case, although the level of neutral-red pinocytotic cells was 900 per milliliter, the fetus had iniencephaly.

This simple and rapid test, based on the pinocytosis of neutral red by amniotic-fluid macrophages, provides both qualitative and quantitative information for prenatal diagnosis of neural-tube defects.

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SELECTIVE ALOPECIA WITH MITOXANTRONE

To the Editor: The ongoing examination of new drugs for the treatment of malignant disorders is a consequence of the need to develop more effective as well as less toxic therapy. One of the new drugs currently under investigation is the anthracenedione mitoxantrone. Recent studies have shown that it is effective in breast cancer,[1] lymphoma,[2] and leukemia.[3] The drug may be less cardiotoxic than conventional anthracyclines[4] and may also be less likely to induce nausea, vomiting, or stomatitis. Hair loss is less commonly seen than with doxorubicin, and this makes the drug more acceptable for patients who are worried about alopecia. We have recently seen selective loss of white hair in two patients receiving mitoxantrone and wonder whether the loss is unique to this drug. It suggests that patients with mixtures of white and black hair may acquire a more "youthful" appearance after receiving this form of chemotherapy.

Case 1 was a 41-year-old man with a diagnosis of chronic-phase chronic myelogenous leukemia, established in 1980. He was treated with conventional chemotherapy until April 1983, when increased blast forms were noted in a blood smear. He then received high-dose cytarabine, without restoration of the chronic phase. He was admitted to our hospital, where he received mitoxantrone (12 mg per square meter of body-surface area) daily for five days, beginning on November 17, 1983. Three to four weeks after initiation of therapy he began to notice hair loss. However, the loss was less than 50 per cent and consisted almost entirely of white hair. His bone marrow recovered with a partial remission, and he was discharged from the hospital. Over the next three months his hair remained dark, with some regrowth of the white hair.

Case 2 was a 72-year-old woman in whom chronic myelogenous leukemia developed in 1982. She did well with busulfan until December 1983, when thrombocytopenia developed. A bone-marrow aspiration revealed the presence of numerous blasts, and she was admitted to the hospital. She received mitoxantrone (12 mg per square meter) daily for five days, beginning on January 12, 1984. She tolerated the chemotherapy and had minimal nausea and vomiting. Approximately three weeks after chemotherapy she noted some hair loss. Over the course of the next several days, the hair loss continued but was almost completely restricted to the white hairs. She had a remission and was discharged from the hospital.

Selective loss of white hair may not be unique to mitoxantrone, and further information on other drugs would be welcome. The more youthful appearance that results from treatment with mitoxantrone may be a useful side effect of therapy.

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ESTIMATIONS OF QUANTITATIVE PROTEINURIA

To the Editor: Ginsberg et al. (Dec. 22 issue)* emphasize the value of determining protein/creatinine ratios in random urine samples, as a substitute for 24-hour collections for analysis of protein excretion.

In a random urine sample, the upper limit of protein concentrations in normal persons may vary from 1 mg per deciliter at a urinary flow rate of 10 ml per minute to 30 mg per deciliter at a flow rate of 0.3 ml per minute. Use of the creatinine concentration is a method of compensating for this large variability in the rate of urinary flow.

I should like to draw attention to the simple technique of using the specific gravity of urine as an index of the rate of urine formation. With an average Western diet, the specific gravity varies from about 1.001 at 10 ml of urine formation per minute to 1.035 or so at 0.3 ml per minute. Thus, it is reasonable to regard the upper limit of the normal protein concentration in milligrams per deciliter as equal to the last two figures of the specific gravity. Although this calculation is perhaps less accurate than the protein/creatinine ratio, it is readily performed in a doctor's office or clinic, using a refractometer at the time of the urine examination, and has advantages in a routine screening examination. At our company we use this method in routine urinalysis for insurance medical examinations and find it satisfactory.

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*Ginsberg JM, Chang BS, Matarese RA, Garella S. Use of single voided urine samples to estimate quantitative proteinuria. N Engl J Med 1983; 309:1543-6.

UPDATE FOR THE CANADIAN MULTICENTRE TRIAL OF CYCLOSPORINE IN RENAL ALLOGRAFTS

To the Editor: Our previous report (Oct. 6 issue)* gave estimated one-year actuarial graft survivals, with 33 patients having been followed for one year and with a mean follow-up of 8.1 months. All patients in centers that compared cyclosporine with azathioprine (Imuran) have been followed for one year (as of August 17, 1983). The final one-year actuarial figures are given below.

Among 142 patients receiving cyclosporine/prednisone, the one-year actuarial graft survival was 77.5 per cent (previous estimate, 80.4 per cent), and among 149 patients receiving standard treatment, including 41 receiving antilymphocyte globulin, the one-year actuarial graft survival was 69.8 per cent ($P = 0.038$, one-tailed test; [previous actuarial estimate, 64 per cent; $P = 0.003$, two-tailed test]). In this latter group, patients treated with azathioprine and

*Canadian Multicentre Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. N Engl J Med 1983; 309:809-15.