T4 lymphocytes. The present finding that T4 lymphocytes may be productively infected in the absence of cell death suggests that a range of biologic effects may occur after infection. This fact may have relevance both to the prolonged latent period between virus exposure and disease and to possible abnormalities in T4 cell function (*11*) that may contribute to immune deficiency. Moreover, persistance of viable infected cells would permit continual spread of virus in the host. The mechanisms that regulate viral production and produce either cell death or alterations in cellular function after ARV infection remain essential to our understanding of the pathogenesis of AIDS.

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15. Peripheral blood lymphocytes were depleted of monocytes by adherence to plastic, and the T cells were isolated by rosetting with neuraminidase-treated sheep erythrocytes and subsequent separation over lymphocyte-separation medium (Litton Bionetics). Cells (65×10^6) were concentrated in 0.5 ml of phosphate-buffered saline with 10 percent fetal bovine serum (FBS) and incubated for 30 minutes at 4°C with 50 μl of OKT8 (Ortho Diagnostic Systems) and Leu-M3 (Becton-Dickinson) monoclonal antibodies. Cells were then washed and passed over Sepharose 6-MB (Pharmacia, 10-ml column) conjugated with $F(ab)'_2$ goat antibody to mouse immunoglobulin G (Cappel). The resulting OKT8/Leu-M3–depleted cells, more than 90 percent of which were T4 positive, were incubated for 3 days with PHA (Gibco, 10 μg/ml) in RPMI 1640 with penicillin (100 U/ml) and streptomycin (100 μg/ml) with 20 percent FBS. Cells were then washed and placed in 1 ml of filtered (0.45 μm) supernatant from an HUT-78 cell line producing ARV-2 (10^4 infectious particles per milliliter) (*3*). After incubation for 4 hours at 37°C, cells were washed and maintained in medium supplemented with lectin-free IL-2 (Electronucleonics). At designated times, RT activity was determined on 3-ml samples of filtered culture supernatants (*3*). Viral antigens on methanol:acetone–fixed cells from a patient with AIDS-related complex were detected by IFA as described in the legend of Fig. 2. The serum used had anti-ARV activity as determined by Western blot analysis of viral antigens and no detectable reactivity with normal T lymphocytes. No reactivity of this serum was seen in control cultures of T4 cells not infected with ARV. Cell viability was determined by trypan-blue exclusion.

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Characterization of gp41 As the Transmembrane Protein Coded by the HTLV-III/LAV Envelope Gene

Abstract. *Radiolabeled amino acid sequencing was used to characterize gp41, an antigen of HTLV-III/LAV, the virus believed to be the etiological agent of the acquired immune deficiency syndrome. This antigen is the one most commonly detected in immunoblot assays by sera of patients with AIDS or AIDS-related complex (ARC) and other individuals infected with HTLV-III/LAV. A mouse monoclonal antibody that was reactive with gp41 precipitated a 160-kilodalton protein (gp160) in addition to gp41, but did not precipitate a 120-kilodalton protein (gp120) from extracts of metabolically labeled cells producing HTLV-III. Extracts of infected cells that had been labeled with tritiated leucine or isoleucine were immunoprecipitated with the monoclonal antibody. The immunoprecipitates were fractionated by polyacrylamide gel electrophoresis and the p41 was eluted from the gel bands and subjected to amino-terminal radiolabeled amino acid sequencing by the semiautomated Edman degradation. Leucine residues occurred in cycles 7, 9, 12, 26, 33, and 34 among 40 cycles and isoleucine occurred in cycle 4 among 24 cycles analyzed. Comparison of the data with the deduced amino acid sequence of the* env *gene product of HTLV-III precisely placed gp41 in the COOH-terminal region of the* env *gene product. Gp160 is thus the primary* env *gene product and it is processed into gp120 and gp41.*

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Human T-cell lymphotropic (leukemia) virus type-III (HTLV-III) has been isolated from several patients with acquired immune deficiency syndrome (AIDS), AIDS-related complex (ARC), and from asymptomatic carriers of infection (*1*). The lymphadenopathy associated virus (LAV) (*2*) and other isolates (*3*) from similar sources appear to be closely similar to HTLV-III (*4–6*). Many data indicate that HTLV-III/LAV is the etiological agent of AIDS (*1, 7–11*).

By use of the Western blot technique (*12*) investigators have identified several proteins in HTLV-III preparations that appear to be major targets of antibody reaction with sera from patients with AIDS and from individuals infected with HTLV-III (*7, 13*). Most of the serum samples that have high titers of antibodies react against HTLV-III proteins of molecular weights 120,000 (120K), 66K, 51K, 41K, 31K, 24K, and 17K (*7, 13*). However, the antigen most consistently correlated with seropositivity to HTLV-III is the 41K protein (p41) (Fig. 1A) (*7*). Less frequently, p24, the major HTLV-III core protein, is the only antigen recognized. This difference may have a bearing on the level of intracellular virus replication or the degree of exposure of virus antigens as a result of the lytic activity of the virus on the target cell. P41 is not only the most consistently detected antigen, but reactivity to it is also the most persistent, being present even at late stages of the disease. Whether p41 is a product of an HTLV-III gene or a cellular protein induced by viral infection is an important question.

We developed mouse hybridomas secreting monoclonal antibodies to p41 and used these antibodies to isolate and characterize p41. BALB/c mice were immunized with successive intraperitoneal inoculations of HTLV-III (100 μg) that had been purified on density gradients and lysed by detergent treatment. The lysates were emulsified in complete Freund's adjuvant for the first inoculation and in incomplete adjuvant for the following four booster doses given 1 week apart. Three days after a final intraperitoneal inoculation with disrupted virus in phosphate-buffered saline, splenic lymphocytes were fused with the NS-1 mouse myeloma line. The cell fusion, cell culturing, and cloning of hybridoma lines were essentially as described (*14*). Cellular fluids from growing hybrids were screened for antibody to HTLV-III proteins by an enzyme-linked immunosorbent assay (ELISA) (*7*). Lysates of HTLV-III were coated on 96-well microtiter plates and allowed to react with the antibody overnight. The resulting antigen-antibody complexes were then revealed by reaction with peroxidase-conjugated goat antibody to

mouse immunoglobulin G. Supernatants from hybrids that scored positive in this test were then further assayed by the immunoblot technique to define the specificity to HTLV-III p41. Of the several hybridomas tested, one, designated M25, was secreting antibodies specific for p41 in HTLV-III preparations (Fig. 1B). The antibody did not react with cells not infected with HTLV-III.

To characterize the protein reactive with the monoclonal antibody M25, we analyzed lysates of HTLV-III–producing cells by immunoprecipitation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Growing cultures of H9/HTLV-III were labeled with [^{3}H]leucine, [^{3}H]isoleucine, or [^{35}S]cysteine in separate experiments. The washed cells were lysed with a mixture of detergents and the clarified extracts were treated overnight with ascitic fluids from M25 or an antibody-positive human serum. The precipitates were collected and after extensive washing were boiled with SDS–β-mercaptoethanol and analyzed by SDS-PAGE. Results obtained with extracts of cells labeled with [^{3}H]leucine and [^{35}S]cysteine are shown in Fig. 2. The human serum precipitated a large number of viral proteins, including the *gag* proteins p17 and p24 and the *gag* precursor p53 (*15*), in addition to p41 and two large molecular weight proteins (p160 and p120) that we and others speculated earlier to be related to the HTLV-III envelope (*15, 16*). With [^{3}H]leucine-labeled extract the reactivity of M25 was mainly directed against p41 with additional reaction to p160 (Fig. 2B). The pattern with [^{3}H]isoleucine-labeled cells was identical to that with [^{3}H]leucine-labeled cells. When the proteins were labeled with [^{35}S]cysteine, p160 was the predominant antigen detected (Fig. 2A). P41 was only weakly detected with this label and required long exposure of the autoradiogram. However, in none of the experiments was p120 detected by this monoclonal antibody. This pattern is consistent with p160 being a precursor of p41 and the epitope specified by M25 being located in the p41 region of the precursor.

The monoclonal antibody reacted with antigens on the surface of H9/HTLV-III cells in live cell membrane immunofluorescence assays (*17*), suggesting that p41 might be a component of the envelope of HTLV-III. To further characterize p41, we immunoprecipitated it from H9/HTLV-III cell extracts labeled with [^{3}H]leucine and [^{3}H]isoleucine and subjected it to radiolabel sequencing by the Edman degradation. The [^{3}H]leucine-labeled p41 band (Fig. 2B) and [^{3}H]isoleucine-labeled p41 band (not shown) as identified by autoradiography were sliced out of the polyacrylamide gel and eluted with distilled water containing 10 nmol of sequence grade sperm whale apomyoglobin (Beckman). Each sample

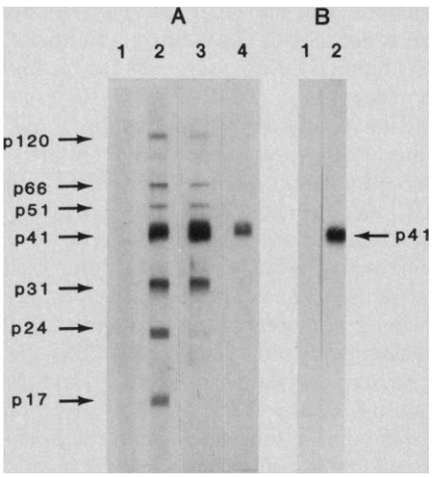

Fig. 1. Detection of HTLV-III p41 by human sera and a mouse monoclonal antibody in the immunoblot assay. Lysates of HTLV-III were fractionated by SDS-PAGE. The proteins were electrophoretically transferred to nitrocellulose sheets according to Towbin *et al.* (*12*). These sheets were then incubated for 8 hours at 37°C in a 5 percent solution of nonfat dry milk containing 0.01 percent Antifoam (Sigma) and 0.0001 percent Merthiolate (blocking medium) to block nonspecific protein binding sites. Strips were cut from these sheets containing a representative profile of viral antigens and incubated in individual test tubes with 2.5 ml of blocking medium containing 4 percent normal goat serum and 25 μl of the human test serum, or mouse monoclonal antibody in ascitic form. The strips are washed three times with 0.5 percent sodium deoxycholate, 0.1*M* NaCl, 0.5 percent Triton X-100, 1 m*M* phenylmethylsulfonyl fluoride, and 10 m*M* sodium phosphate (wash medium). The washed strips are incubated for 30 minutes in 2.5 ml of blocking medium containing 4 percent normal goat serum with 2.5×10^6 count/min of ^{125}I-labeled goat IgG reactive against human IgG, or mouse IgG, as appropriate. The strips were washed again as before, dried, mounted, and autoradiographed. (A) Lane 1, serum from a negative human control; lanes 2, 3, and 4, sera from AIDS patients. (B) Lane 1, ascitic fluid from NS-1 myeloma as negative control (*14*); lane 2, ascitic fluid from M25.

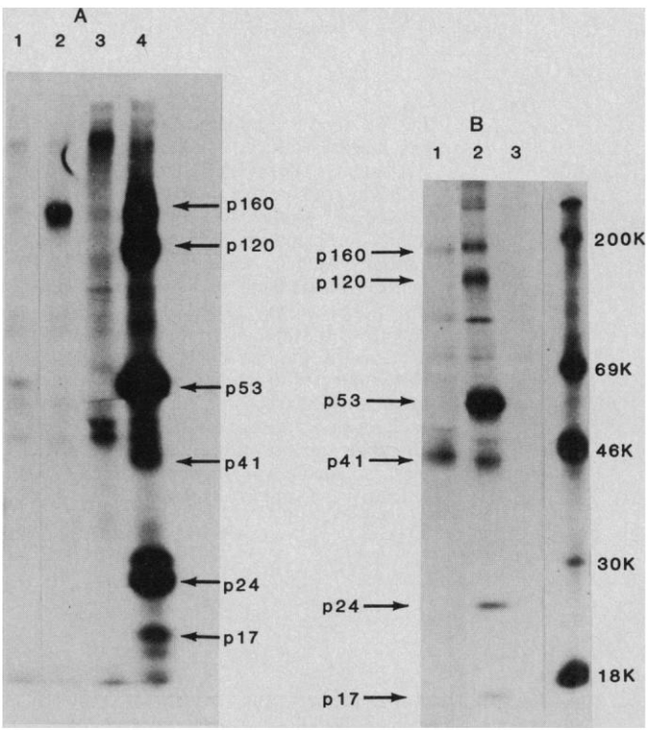

Fig. 2. Immunoprecipitation of metabolically labeled HTLV-III proteins by AIDS sera and monoclonal antibody to HTLV-III p41 (M25). Tissue culture cells were radioactively labeled by incubation for 8 hours ([^{35}S]cysteine) or 18 hours ([^{3}H]leucine and [^{3}H] isoleucine) in medium containing [^{35}S]-cysteine, [^{3}H]leucine, or [^{3}H]isoleucine (100 μCi/ml). Labeled cells were washed twice in serum-free RPMI medium and disrupted at 4°C by repeated aspiration through a 25-gauge needle in 10 m*M* sodium phosphate (*p*H 7.2), containing 0.5 percent NaCl, 1 percent Triton X-100, 0.5 percent sodium deoxycholate, and 0.1 percent SDS (PBS-TDS). The lysates were absorbed for 3 hours at room temperature with protein A-Sepharose and equal portions of normal sera. Immunoprecipitation analysis was performed by the addition, to 1 ml of labeled and clarified extract, of either 1.0 μl of ascites fluid or 10 μl of human sera, 0.2 ml of rabbit antiserum to mouse κ light chain only in the sample containing mouse monoclonal antibody, and 0.2 ml of a 10 percent suspension of protein A-Sepharose. The samples were incubated for 18 hours at 4°C. Immunoprecipitates were collected by centrifugation at 2000*g* for 10 minutes, washed repeatedly in PBS-TDS, suspended in 50 μl of 0.65*M* tris-hydrochloride (*p*H 6.7), 1 percent SDS, 10 percent glycerol, 2.5 percent 2-mercaptoethanol, and 0.1 percent bromophenol blue, heated for 2 minutes at 90°C, and analyzed by SDS-PAGE. (A) H9/HTLV-III cells labeled with [^{35}S]cysteine. Lane 1, ascitic fluid from parental NS-1 myeloma as negative control (*14*); lane 2, ascitic fluid from M25; lane 3, negative human control serum; lane 4, serum from an AIDS patient. Panel (B) H9/HTLV-III cells labeled with [^{3}H]leucine. Lane 1, ascitic fluid from M25; lane 2, serum from AIDS patient; lane 3, negative human control serum.

was transferred to a prewashed dialysis bag and dialysed at 4°C against three changes of 5 percent acetic acid (2 liters of each). A portion was removed for counting and the remainder was used for NH_2-terminal sequence analysis. Protocols for radiolabeled semiautomated Edman degradation in a Beckman spinning cup sequenator have been described (*18*). Ten percent of cycles 2 and 9 were removed, processed, and analyzed for the phenylthiohydantoin derivative of leucine found in myoglobin at these two positions. The anilinethiazolinone amino acids in *n*-chlorobutane were transferred to scintillation vials, dried with N_2 and a heat lamp, and redissolved in 10 ml of Aquasol (New England Nuclear). Each fraction was counted for 20 minutes.

Isoleucine was unambiguously assigned at position 4 of the 24 cycles examined (Fig. 3A). Leucine occurred in cycles 7, 9, 12, 26, 33, and 34 out of 40 cycles analyzed (Fig. 3B). The amino acid sequence determined is a perfect match with the predicted sequence (*4*) and it precisely locates p41 in the *env* gene of HTLV-III provirus clones BH-10 and BH-8 (*4*). The probability of having the observed identities occur by chance is 1.5×10^{-10}. The absence of additional radioactive residues from sequencing analysis indicates that p41 isolated by immunoprecipitation with the monoclonal antibody M25 was radiochemically homogeneous.

Use of this monoclonal antibody has thus enabled us to identify definitively the primary *env*-gene product of HTLV-III and to understand its processive cleavage pattern. That the antibody precipitated a p160 and a p41 from cell extracts whereas only p41 was recognized in viral extracts by the immunoblot technique demonstrates that p160 is the primary cellular translational product and p41 is one of the products of processing during viral maturation. Radiolabeled amino acid sequencing has identified p41 as the COOH-terminal fragment generated by cleavage of p160 between Arg^{518} and Ala^{519} as in the deduced amino acid sequence of the *env* gene product (Fig. 4) (*4*). Thus, p41 is the transmembrane protein of HTLV-III. Examination of the nucleotide sequence identifies hydrophobic domains in p41 as also seen in other retroviral homologs (*19*). The cleavage site identified by the amino acid sequence analysis is similar to those in other retroviral *env*-genome products (*19*). The predicted size of a single polypeptide starting with Ala^{519} and extending to Leu^{863} and containing six potential glycosylation sites would be much larger than 41 kD, provided that all potential sites were glycosylated. Since p41 is experimentally identified as a glycoprotein (*13*), at least some of the sites are glycosylated. This implies that some further processing occurs at the COOH-terminal end of the primary *env* product to generate p41. Further sequence analysis of p41 will be necessary to precisely locate such processing sites.

The NH_2-terminal fragment of the primary cleavage of p160 should be the gp120, which is not recognized by M25 but is readily identified in both the cellular extracts and in viral lysates by sera of individuals positive for HTLV-III antibody (Figs. 1 and 2) (*13, 15, 16*). This conclusion is in keeping with the finding of common amino acid sequences between p120 and p160 by peptide mapping (*15*) and identical NH_2-terminal amino acid sequences between p120 and p160 by Edman degradation (*20*). The gp100 described by Hunsmann *et al.* (*21*) has the same NH_2-terminal sequence as p120 and p160 (*22*) and may be the same as p120 described by others. The proposed scheme for the processing of the primary *env* product is depicted in Fig. 4.

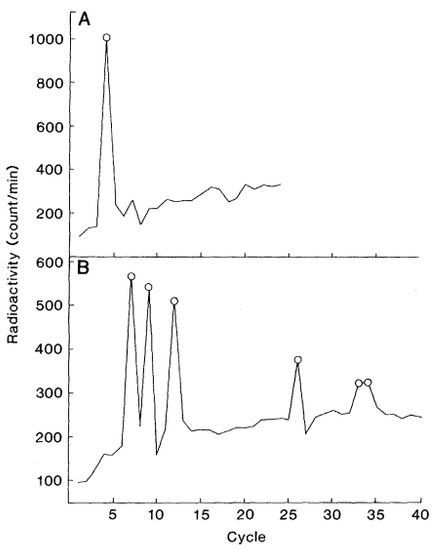

Fig. 3. Amino terminal sequence analysis of p41 labeled with (A) [^{3}H]isoleucine and (B) [^{3}H]leucine. H9/HTLV-III cells were labeled with the radioactive amino acids and the labeled p41 was isolated from cell extract by immunoprecipitation with the monoclonal antibody M25 as described in Fig. 2. The radioactive p41 band was identified by autoradiography and was sliced out of the gel and eluted with water (see text). The dialyzed proteins in the presence of apomyoglobin were subjected to semiautomated Edman degradation. The recovery of radioactivity in each cycle is given in the figure. Positive identifications are indicated by the open circles. Isoleucine was found in position 4 while leucine was found in positions 7, 9, 12, 26, 33, and 34.

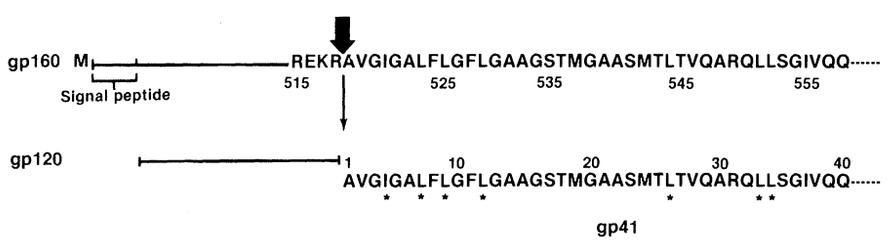

Fig. 4. Diagram representing the processing of the primary *env* gene product into gp120 and gp41. Numbers below the amino acid sequence of gp160 denote the positions in the deduced amino acid sequence for the primary gene product. Bold arrow indicates the identified cleavage site. Amino acid residues identified by asterisks are those determined by radiolabel sequence analysis (see Fig. 3). Numbers above the amino acids in gp41 denote the degradation cycle of the sequencing procedure.

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Atrial Natriuretic Factor Ameliorates Chronic Metabolic Alkalosis by Increasing Glomerular Filtration

Abstract. *The kidney maintains the elevated plasma concentration of bicarbonate that occurs in chronic metabolic alkalosis. A reduction in the glomerular filtration rate (GFR) can maintain the filtered bicarbonate load at a normal level so that a normal rate of bicarbonate reabsorption suffices to prevent urinary excretion of this anion. It is also possible that bicarbonate reabsorption might increase so as to maintain the alkalosis if GFR were not reduced. To examine this latter possibility, atrial natriuretic factor was used in alkalotic rats to restore a more normal GFR and to increase the amount of bicarbonate filtered by the glomerulus. Proximal bicarbonate reabsorption remained relatively static. Higher than normal amounts of bicarbonate were then delivered out of the proximal tubule, bicarbonate appeared in the urine, and the plasma concentration of bicarbonate fell. A reduction in GFR is thus necessary for the maintenance of chronic metabolic alkalosis. Normalizing GFR induces bicarbonaturia and initiates repair of the alkalosis.*

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Metabolic alkalosis, one of the four major acid-base disorders, is characterized by an elevated concentration of the principal blood buffer, bicarbonate. The kidney is responsible for sustaining this disorder because it does not allow the excess bicarbonate to be excreted in the urine. Traditionally, the renal mechanism thought to prevent bicarbonate excretion in the setting of a high filtered load of bicarbonate was the stimulation of proximal tubular bicarbonate reabsorption. Such augmentation of proximal bicarbonate transport was ascribed to simultaneous extracellular volume contraction and potassium depletion.

Alternatively, recent evidence suggests that the high plasma concentration of bicarbonate found in chronic metabolic alkalosis is sustained by a reduction in the glomerular filtration rate (GFR) (*1*, *2*). This depression in GFR prevents an increase in the filtered bicarbonate load, such that a normal rate of proximal bicarbonate reabsorption is sufficient to prevent bicarbonaturia. However, it can be argued that a decrease in GFR is coincidental with, but not necessary for, the maintenance of alkalosis; enhanced bicarbonate reabsorption might maintain the alkalosis if GFR did not fall. To address this possibility, extracellular volume expansion has been used to restore more normal levels of GFR. Proximal bicarbonate reabsorption under these conditions did not rise, so that bicarbonaturia developed and the alkalosis began to diminish (*1*). This observation supported the view that a reduction in GFR was critical for the maintenance of the alkalosis. However, the method for normalizing GFR (extracellular volume expansion) might have exerted effects on proximal acidification independent of changes in GFR and filtered bicarbonate load (*3*, *4*).

A means of increasing GFR without altering extracellular volume recently has been afforded by the purification, sequencing, and synthesis of atrial natriuretic factor (ANF), a cardiac-derived vasoactive hormone (*5*). ANF increases single nephron and whole kidney GFR (*6*, *7*). ANF has been shown by micropuncture and in vitro microperfusion techniques to have no inhibitory effect on proximal tubular transport (*7*, *8*). Specifically, it does not have a direct effect on proximal or whole kidney acidification, independent of the changes in tubular flow rate it induces (*7*). The purpose of the present study was to assess whether proximal bicarbonate reabsorption can increase to supernormal levels during metabolic alkalosis after restoration of nearly normal rates of single nephron glomerular filtration with ANF.

Ten Munich-Wistar rats were rendered alkalotic as described (*1*). For 11 to 14 days, they were fed an electrolyte-deficient diet supplemented with Na_2SO_4 (2.6 meq/day), injected with deoxycorticosterone acetate (0.5 mg/day, intramuscularly), and allowed free access to drinking water containing sodium bicarbonate (80 m*M*). Rats prepared in this way sustain marked chloride and potassium deficiencies (decrease in plasma volume of 28 percent and reduction in the potassium content of muscle of 46 percent (*1*). Food was withdrawn 24 hours before anesthetization with Inactin (100 mg/kg, intraperitoneal). During the surgical preparation for micropuncture, plasma volume losses were replenished (0.9 percent body weight) with plasma obtained from donor rats maintained on the same diet (*1*). An inulin infusion (*1*) was then started, and, after a stabilization period of 1 hour, free-flow micropuncture samples were obtained from Bowman's space and the end-proximal tubule. Simultaneous urine collections were also made. ANF (25-amino-acid synthetic rat auriculin, California Biotechnology, Inc., Palo Alto) was then administered as a bolus (10 μg/kg) and sustaining infusion (1 μg/kg per minute) in a modified bicarbonate Ringer's solution (40 m*M* $NaHCO_3$, 88 m*M* NaCl) at 30 μl/min. This rate is sufficient to replace urinary volume and electrolyte losses (*7*), and the measured sodium and chloride balances in the present study were not significantly different from zero. After a stabilization period of 15 minutes, the second period of micropuncture and clearance measurements commenced. Total CO_2 concentrations in urine and tubular fluid were measured by microcalorimetry. For convenience, the results were expressed as bicarbonate, the major component of the measured total CO_2. Means ± standard error of the mean are given, and significance was assessed by means of the paired *t* test with $n = 10$ in all cases.

Chronic metabolic alkalosis predictably developed on this regimen: the bicarbonate concentration of the glomerular ultrafiltrate increased to 46.9 ± 1.0 meq/liter (about 75 percent above normal), with associated hypochloremia (98 ± 2 meq/liter), and an arterial *p*H of 7.57 ± 0.01 and arterial pCO_2 of 47 ± 1 mmHg. As in earlier work (*1*, *2*), a reciprocal reduction from normal values (*9*) occurred in the single nephron (32.2 ± 1.3 nl/min) and whole kidney (0.66 ± 0.03 ml/min) GFR. As a result, the animals had normal levels (*9*) of filtered (1502 ± 52 peq/min), reabsorbed (1189 ± 42 peq/min), and distally deliv-