# Kinetic Parameters and Renal Clearances

# of Plasma Adenosine 3',5'-Monophosphate and

# Guanosine 3',5'-Monophosphate in Man

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A B S T R A C T Kinetic parameters and the renal clearances of plasma adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) were evaluated in normal subjects using tritium-labeled cyclic nucleotides. Each tracer was administered both by single, rapid intravenous injection and by constant intravenous infusion, and the specific activities of the cyclic nucleotides in plasma and urine were determined.

Both cyclic AMP and cyclic GMP were cleared from plasma by glomerular filtration. The kidney was found to add a variable quantity of endogenous cyclic AMP to the tubular urine, amounting to an average of approximately one-third of the total level of cyclic AMP excreted. Plasma was the source of virtually all of the cyclic GMP excreted.

Plasma levels of the cyclic nucleotides appeared to be in dynamic steady state. The apparent volumes of distribution of both nucleotides exceeded extracellular fluid volume, averaging 27 and 38% of body weight for cyclic AMP and cyclic GMP, respectively. Plasma production rates ranged from 9 to 17 nmoles/min for cyclic AMP and from 7 to 13 nmoles/min for cyclic GMP. Plasma clearance rates averaged 668 ml/min for cyclic AMP and 855 ml/min for cyclic GMP. Approximately 85% of the elimination of the cyclic nucleotides from the circulation was due to extrarenal clearance.

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## INTRODUCTION

Adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) were identified in urine several years ago (1-3), but definitive studies concerning the sources of the excreted cyclic nucleotides have not been reported. Cyclic AMP has been found in dog plasma (1), and we have identified both cyclic nucleotides in human plasma, raising the possibility that renal plasma clearance could account for at least a portion of the cyclic nucleotides excreted into the urine. Two hormones known to stimulate renal adenyl cyclase systems (4) have been reported to increase cyclic AMP excretion (5, 6) suggesting the kidney as a source of urinary cyclic AMP. Similarly, the kidney has been suggested as a source of urinary cyclic GMP (7). In the absence of definitive clearance studies, it has not been possible to know whether the cyclic nucleotides in urine are derived solely from the kidney, solely from plasma, or both from plasma and from the kidney.

The present studies were designed to evaluate the renal clearances as well as other kinetic parameters of the extracellular cyclic nucleotides. Tracer doses of tritium-labeled cyclic nucleotides were administered to human subjects by rapid intravenous injection and by constant intravenous infusion. Specific radioactivity determinations of the cyclic nucleotides in plasma and urine provided information regarding the sources of cyclic AMP and cyclic GMP excreted into the urine. The effects of certain hormones on plasma and urinary cyclic nucleotides are reported in two accompanying publications (8, 9).

## METHODS

### Materials

Cyclic AMP, cyclic AMP-³H (2.35 Ci/mmole), and cyclic AMP-¹⁴C (40.9 mCi/mmole) were purchased from

Schwarz Bio Research Inc. (Orangeburg, N. Y.). Cyclic GMP was synthesized by Professor T. Posternak, and cyclic GMP-$^3$H (2.6 Ci/mmole) was obtained from Calbiochem (Los Angeles, Calif.). Creatinine purchased from Technicon Co., Inc. (Tarrytown, N. Y.; lot 3447D) and inulin obtained from Fisher Scientific Company (Pittsburgh, Pa.; lot 771070) were used as reference standards. Inulin for injection was purchased from Warner-Chilcott Laboratories (Morris Plains, N. J.). The Diaflo apparatus and membranes used in protein binding studies were obtained from Amicon Corp. (Lexington, Mass.).

## Preparation of tritium-labeled cyclic nucleotides for human use

The tritium-labeled cyclic nucleotides were purified by column chromatography as described below. The eluates were neutralized; ethanol was added to a final concentration of 50%, and the solutions were sterilized by passage through a 0.45 $\mu$ Millipore filter. With sterile technique, aliquots of the stock solution were transferred to glass ampules, sealed, and stored at $-20°C$ until used. The purified radioactivity was shown to be virtually free of radioimpurities by treatment with cyclic 3',5'-nucleotide phosphodiesterase (1) in similar fashion to that described below for chromatographed samples of urine and plasma. Aliquots of the stock solutions were tested by Analytica Corp. (Westbury, N. Y.) and found to be pyrogen-free.

## Subjects and procedures

Healthy adult volunteers were selected for study. No subject received more than a total of 55 $\mu$Ci of cyclic AMP-$^3$H and/or GMP-$^3$H.

Subjects took nothing other than water by mouth for 4–15 hr before study. Experiments were conducted with the subjects in a basal, reclining state in quiet surroundings. Oral hydration was supplemented by the intravenous infusion of 0.45% NaCl solution. Cleanly voided urine samples were collected at 15-, 30-, or 60-min intervals and were immediately frozen and stored at $-20°C$ until analysis. Urine flow rarely fell below 5 ml/min and usually exceeded 10 ml/min. Two indwelling intravenous catheters were employed, one of large gauge in an antecubital vein for sampling and another of smaller gauge in a vein of the contralateral forearm for infusions and injections. Blood samples were collected at 5–15-min intervals in heparinized syringes and handled as described below.

In experiments involving inulin clearance and inulin space determinations, subjects received a priming dose of 50 mg/kg followed by an infusion designed to maintain a constant plasma inulin level of 0.25 mg/ml. After 60–90 min had been allowed for inulin equilibration, a urine sample was obtained, signaling the beginning of timed clearance periods. The inulin infusion was abruptly discontinued at the same time as the tracer infusion was terminated (see below). A plasma sample and a timed urine specimen were obtained before the injection of inulin for inulinoid blank determinations.

Immediately before each experiment, an ampule of cyclic AMP-$^3$H or cyclic GMP-$^3$H was warmed to room temperature, agitated, and opened, and the contents were diluted appropriately with 0.9% NaCl solution. The isotope solution was administered either by a single, rapid intravenous injection or by a constant flow infusion pump. Inulin was administered by gravity flow using a microdrip apparatus.

## Preparation of urine and plasma samples for determination of tritium-labeled cyclic nucleotides and endogenous cyclic nucleotides

*Urinary cyclic AMP and cyclic AMP-$^3$H.* Separate 5-ml aliquots of urine were analyzed for endogenous cyclic AMP and cyclic AMP-$^3$H. As a means of correcting for losses incurred during the purification procedures, 0.0015 $\mu$Ci of cyclic AMP-$^{14}$C was added to the former, and 20 nmoles of nonradioactive cyclic AMP was added to the latter. The aliquots were adjusted to 0.11 N HCl and applied to 30 × 0.60 cm columns of Dowex-50-X8 resin (100–200 mesh, in the hydrogen form). After sample addition, 32 ml of 0.1 N HCl were added to the column, followed by 40 ml of 0.03 N HCl. Cyclic AMP was collected in the 42nd–72nd ml. The nucleotide fraction was lyophilized, taken up in 0.5–1.0 ml of 0.05 M Tris-Cl buffer (pH 7.5), and the pH was adjusted to approximately 7 using bromthymol blue as an external indicator. The reduction in acid strength during chromatography did not alter the elution pattern but diminished the acidity of the lyophilized cyclic AMP fraction, which contained an unknown nonvolatile substance which was acidic in nature and contributed difficulty in neutralization in more concentrated urine samples. The precautionary reduction in acid strength may have been unnecessary in the present studies, which dealt almost exclusively with extremely dilute urine specimens.

Aliquots of the samples used for cyclic AMP-$^3$H determination were counted in modified Bray's solution (10) in a Packard Tri-Carb liquid scintillation spectrometer yielding a counting efficiency of 35% for tritium. Samples were routinely counted for 30 min; the standard deviation was no greater than 3%. An aliquot of a solution of cyclic AMP-$^3$H of known disintegrations per minute (calibrated with tritiated H$_2$O) was then added to each sample, and the samples were recounted in order to convert counts per minute to disintegrations per minute. The recovery of added nonradioactive cyclic AMP in each sample was determined by enzymatic assay (see below), and the appropriate correction was applied in the computation of the cyclic AMP-$^3$H content in the urine specimens. The slight contributions of endogenous cyclic AMP to the assayed recoveries were subtracted.

The endogenous cyclic AMP content was determined by enzymatic assay, and the figures were corrected for cyclic AMP-$^{14}$C recovery (which was determined by channels-ratio counting in a liquid scintillation system). The slight contributions of the isotopic forms of cyclic AMP to the assayed levels were subtracted.

The recovery of cyclic AMP from urine was highly reproducible, averaging 82.8 ±1.1% (SEM) for the 81 samples processed in the present studies.

*Urinary cyclic GMP and cyclic GMP-$^3$H.* Separate aliquots of urine were analyzed for cyclic GMP-$^3$H and endogenous cyclic GMP. 30 nmoles of nonradioactive cyclic GMP were added to 5-ml samples of urine for cyclic GMP-$^3$H determination. When endogenous cyclic GMP determinations were desired on extremely dilute urines, a lyophilization step before column chromatography provided a sufficient concentration factor to insure an adequate quantity of the nucleotide for satisfactory assay. 5 ml of sample (urine or lyophilized urine dissolved in water) were adjusted to 0.11 N HCl, applied to 30 × 0.60 cm columns of Dowex-50-X8 resin, and followed by 0.1 N hydrochloric acid. Cyclic GMP was collected in the 15th–32nd ml. The nucleotide fractions were handled as described above for

cyclic AMP samples; little difficulty was encountered in neutralizing the cyclic GMP samples.

The cyclic GMP-³H content was determined as described above for cyclic AMP-³H, the figures being corrected for the recovery of the nonradioactive cyclic GMP additions as determined by enzymatic assay (see below). The contributions of endogenous cyclic GMP to the assayed levels were negligible.

Endogenous cyclic GMP was determined by enzymatic assay. Separate aliquots of these chromatographed samples were counted, and the cyclic GMP-³H content (in disintegrations per minute) was obtained. Recoveries were computed from a knowledge of quantity of cyclic GMP-³H present in the urine specimens, which had been established by the determinations just described. These recoveries were applied in the calculation of endogenous cyclic GMP excretion, and the slight contributions of cyclic GMP-³H to the assayed levels were subtracted.

The recovery of cyclic GMP from the urine averaged 75.9 ±0.9% (SEM) for the 105 samples processed in the present studies.

*Plasma cyclic AMP, cyclic AMP-³H, cyclic GMP, and cyclic GMP-³H.* Pilot studies showed that whole human blood incubated in vitro (37°C) was capable of slowly metabolizing added tracer quantities of tritiated-cyclic nucleotides. The t₁ of cyclic nucleotide-³H disappearance from the system ranged from 30 to 150 min at 37°C. This process can be slowed by reduction in temperature, centrifugation, or addition of methylxanthines and is presumed to be due to cyclic 3',5'-nucleotide phosphodiesterase activity. The potential source of error presented by this enzymatic activity was minimized by the procedures described below, which provided a considerable margin of safety due to the slowness of the process in human blood. An additional method (used in nonisotopic studies) for avoiding this potential error is described in an accompanying publication (8).

Freshly drawn, heparinized blood samples were well mixed, added to chilled tubes, and immediately centrifuged at 6000–8000 *g* for 3–6 min at 4°C or room temperature. The plasma was removed, and aliquots were taken as follows: 5-ml samples for cyclic AMP-³H or cyclic GMP-³H analysis were pipetted into vials containing 15 nmoles of nonradioactive cyclic AMP or cyclic GMP, respectively; 15-ml samples for endogenous cyclic AMP analysis were pipetted into vials containing 0.0007 μCi of cyclic AMP-¹⁴C; 15- or 20-ml samples for endogenous cyclic GMP analysis were pipetted into vials without additions. All vials were well mixed and rapidly frozen in a dry-ice chest; the total elapsed time between withdrawal of a blood sample and placing the aliquots on dry ice never exceeded 10 min. The samples were stored at −70°C until analysis. Before column chromatography, the plasma samples were rapidly thawed and deproteinized by the addition of perchloric acid to a final concentration of 0.3 N, such that 15 ml of protein-free extract were obtained. The extracts were applied to 60 × 0.60 cm columns of Dowex-50-X8 resin (100–200 mesh, in the hydrogen form) and followed with 0.1 N HCl. The fractions collected were the 28th–62nd ml for cyclic GMP and the 86th–135th ml for cyclic AMP. The handling of the nucleotide fractions and the determinations of endogenous and tritiated cyclic nucleotide content were performed as described above for samples from urine. No difficulties in neutralizing the samples were encountered.

The recoveries of cyclic AMP and cyclic GMP averaged 70.5 ±0.5% (SEM) and 61.8 ±0.7% (SEM), respectively,

for the 134 cyclic AMP and 142 cyclic GMP samples processed in the present studies. The recoveries of the cyclic nucleotides from the larger (15 or 20 ml) plasma samples were approximately 7% lower than the above figures because of the relatively greater losses incurred during plasma deproteinization.

Tracer doses of the tritium-labeled cyclic nucleotides were employed in the present studies; the maximum chemical contributions of cyclic AMP-³H or cyclic GMP-³H to the endogenous plasma level of cyclic AMP or cyclic GMP in any single sample were 0.8 and 2.0%, respectively, in the infusion studies and 3.2 and 7.0%, respectively, in the rapid injection studies.

Pilot studies established that the choice of heparin or EDTA as anticoagulant did not influence plasma levels of the cyclic nucleotides. Heparin was routinely used.

## Determination of total radioactivity in plasma and urine

Aliquots (1 ml) of urine and plasma with and without the addition of internal standards (cyclic AMP-³H or cyclic GMP-³H) were counted in a liquid scintillation system, and the total tritium content was expressed as disintegrations per minute.

## The identification of the purified radioactivity from plasma and urine as tritiated cyclic nucleotide

The chromatographically purified radioactivity in samples from urine and plasma drawn from the early, mid, and late portions of studies was identified as tritiated cyclic nucleotide. Two equal aliquots of the samples containing cyclic AMP-³H or cyclic GMP-³H were taken, MgCl₂ (1.0 M) was added to a final concentration of 4mM, and 10 U of cyclic 3',5'-nucleotide phosphodiesterase (1) was added to one aliquot, the other serving as a control. The aliquots were incubated at 37°C for 2 hr with intermittent mixing, adjusted to 0.11 N HCl, and rechromatographed over a short Dowex-50 column. The theoretical amount of radioactivity (allowing a 15% loss due to chromatography) was recovered from the control aliquots; no detectable radioactivity was recovered from the phosphodiesterase-treated aliquots.

## Plasma protein binding experiments

The possibility of binding of the cyclic nucleotides to plasma proteins was evaluated using the diafiltration technique in a manner similar to that described by other investigators (11), except that a reservoir cell was not employed. The Diaflo UM-1 membrane, which restricts the passage of molecules in excess of 10,000 mol wt, was used; and prepurified N₂ at a pressure of 30 psi was employed. The characteristics of the filtration cell and membrane ("washout" or "dead space" volume and "background" binding) were initially studied using 0.05 M Tris-Cl buffer (pH 7.4) containing 0.02 μCi of cyclic AMP-³H or cyclic GMP-³H. In the plasma studies, the filtration cell was charged with 20 ml of fresh human plasma containing 0.02 μCi of cyclic AMP-³H or cyclic GMP-³H (constituting approximately 3 and 10% increases in plasma cyclic nucleotide content, respectively), and 1.5 ml of the post-washout ultrafiltrate were collected. The reduction in the plasma volume in the filtration cell did not exceed 20% of the original volume. Aliquots of the ultrafiltrate and residual plasma were

counted in a liquid scintillation system with and without internal standards (cyclic AMP-$^3$H or cyclic GMP-$^3$H), and the tritium content was expressed in disintegrations per minute.

## Enzymatic assays of cyclic nucleotides

Both cyclic nucleotides were assayed by the method of Hardman, Davis, and Sutherland (12, 13) as modified by Ishikawa, Ishikawa, Davis, and Sutherland (14). This general method involves the initial conversion of the cyclic nucleotide to its nucleoside triphosphate, followed by a magnification step in which orthophosphate is generated by a recycling system. All samples were assayed in duplicate. Each cyclic nucleotide determination involved the following: (a) a pair of tubes (with phosphodiesterase) containing sample and a known quantity of cyclic nucleotide internal standard for detection of possible inhibition (recovery of less than 100% of the internal standard) of the assay, (b) a pair of tubes (with phosphodiesterase) for sample cyclic nucleotide measurement, and (c) a pair of tubes (lacking phosphodiesterase) as a sample blank. The cyclic AMP assay doubled the reaction blank at 5 pmoles and could reliably measure as little as 1–2 pmoles; the cyclic GMP assay doubled the reaction blank at 10 pmoles and could reliably measure as little as 2–4 pmoles. An aliquot of a standard urine run through all procedures with each batch of samples for 14 months yielded mean values of 3.27 ±0.04 $\mu$M (SEM) for cyclic AMP (33 determinations) and 0.39 ±0.016 $\mu$M (SEM) for cyclic GMP (19 determinations). The generally low plasma levels of cyclic GMP and the less sensitive assay employed for cyclic GMP determination combine to make plasma cyclic GMP analysis more difficult and inherently less precise than the urinary measurements.

Partial inhibition of the assay was consistently encountered during cyclic GMP determinations in samples from urine specimens; this was corrected for by the internal standard. The inhibitory material has not been identified, and the degree of inhibition has varied from one subject to another. This problem was significant only when substantial inhibition occurred in samples of low cyclic GMP content.

Occasional chromatographically purified samples from plasma have yielded high sample blanks in the cyclic AMP assay. The contaminants presented a problem only when high in relation to the amount of cyclic AMP present in the samples. Refractionation of these samples over an additional short Dowex-50 column reduced the blank to low levels.

## Ancillary analyses

Urinary creatinine was determined by the Jaffe reaction (15). Inulin was measured by the method of Heyrovsky (16).

## CALCULATIONS

*Analysis of curves following single, rapid intravenous injection of tracer.* Two exponential components were resolvable from the curves by the peeling technique or residuals method (17), and these components were fitted graphically by the method of least squares using a digital computer. The plasma concentration of cyclic nucleotide-$^3$H as a function of time was expressed as the sum of two exponentials:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad (1)$$

where Cp is the concentration of cyclic nucleotide-$^3$H in the plasma at any instant in time; A and B are the intercepts on the ordinate of the "fast" and "slow" components, respectively; $\alpha$ and $\beta$ are the fractional rate constants (taken to be positive); and t is time.

*Apparent volumes and spaces.* In the single injection experiments, the initial volume of distribution ($V_1$) of the injected cyclic nucleotide-$^3$H was calculated (18, 19). In the constant infusion studies, the "final" apparent volume of distribution ($Vd_{inf\ eq}$) of the infused cyclic nucleotide-$^3$H was calculated by the infusion equilibrium method (18, 20). Estimates of extracellular fluid volume as measured by inulin space ($Vd_{in}$) were obtained by the calibrated infusion technique (21–23). Since urine flow exceeded 5 ml/min in our studies, no correction for delay time was made (24). The calibrated infusion technique for inulin space estimation and the infusion equilibrium method for determining cyclic nucleotide-$^3$H distribution are formally equivalent.

*Plasma clearance rate, production rate, and nephrogenous cyclic nucleotide.* The plasma clearance rate (PCR), frequently referred to as the metabolic clearance rate, represents the volume of plasma completely and irreversibly cleared of cyclic nucleotide-$^3$H in unit time by all processes of metabolism and excretion. The PCR was calculated for both the injection and infusion studies by standard equations (18, 25, 26) and was subdivided into renal ($C_r$) and extrarenal or metabolic ($C_m$) clearances. The corresponding rates (nmoles per minute) of total ($r_{e1}$), renal ($r_e$), and extrarenal or metabolic ($r_m$) elimination of the cyclic nucleotides from plasma were given by the product of the appropriate clearance term and the plasma level of endogenous cyclic nucleotide.

The rate of cyclic nucleotide input into the miscible pool ("production rate," PR) was calculated for both the injection and infusion studies by the plasma isotope dilution method ($PR_p$) using standard equations (25, 26). The production rate determined by the plasma method measures only the rate of cyclic nucleotide input into the pool being sampled and does not detect cyclic nucleotide input into other noncommunicating pools (e.g., nonmiscible intracellular pools and the urine, see below).

When tracer amounts of cyclic nucleotides-$^3$H are administered by injection or infusion and steady-state conditions exist, proportionately the same quantities of cyclic nucleotide-$^3$H and endogenous cyclic nucleotide are excreted from the miscible pool. Thus, the cyclic nucleotide production rate may be estimated by (26, 27):

$$PR_u = \frac{dose}{\Sigma S_u \cdot t} \qquad (2)$$

where $PR_u$ is the production rate as determined by urinary isotope dilution (which may or may not equal $PR_p$, see below); $\Sigma S_u$ is the specific radioactivity of the urinary cyclic nucleotide-$^3$H after all the tritiated-cyclic nucleotide to be excreted is collected, and t is the time from administering the isotope to the termination of urine collection. It is essential to note that if nephrogenous cyclic nucleotide (formed *de novo* in the kidney) is added directly to the tubular urine, $\Sigma S_u$ is not an accurate reflection of the cumulative integrated excretion of isotopically labeled and endogenous cyclic nucleotide from the plasma. Consequently, in this situation, $PR_u$ will be in error in proportion to the degree of dilution of $\Sigma S_u$ by unlabeled cyclic nucleotide from the kidney. Clearly, if cyclic nucleotide produced by the kidney reaches the circulating miscible pool, it is reflected in $PR_p$.

Nephrogenous cyclic nucleotide can be detected by dilution of the urine cyclic nucleotide-$^3$H specific radioactivity relative to that in plasma at infusion equilibrium and may be quantified as follows:

$$r_k = \left(1 - \frac{(S_u)_{eq}}{(S_p)_{eq}}\right) \cdot R_e \qquad (3)$$

where $r_k$ is the rate of nephrogenous cyclic nucleotide addition to the tubular urine; $(S_u)_{eq}$ and $(S_p)_{eq}$ are the cyclic nucleotide-$^3$H specific radioactivities in urine and plasma at infusion equilibrium, and $R_e$ is the total rate of cyclic nucleotide excretion. In the infusion studies, where $r_k$ can be quantified, equation 2 may be corrected so that $PR_u$ yields an accurate estimate of cyclic nucleotide production rate in the miscible pool:

$$PR_u = \frac{dose}{\Sigma S_u \cdot \dfrac{1}{\dfrac{(S_u)_{eq}}{(S_p)_{eq}}} \cdot t} = \frac{dose}{\Sigma S_u \cdot \dfrac{(S_p)_{eq}}{(S_u)_{eq}} \cdot t} \qquad (4)$$

When nephrogenous cyclic nucleotide is added to the urine, $PR_u$ may not be estimated for the single injection studies, since no direct quantification of $r_k$ is possible by isotopic means.

*Fractional irreversible and reversible tracer loss.* In the single injection experiments, the fractional rate constants for irreversible and total (irreversible and reversible) loss of cyclic nucleotide-$^3$H from the initial volume of distribution ($V_1$) were calculated (28), and the fractions of tracer irreversibly (f) and reversibly (1-f) lost were computed (28). The quantity of tracer undergoing bidirectional flux from $V_1$ is expressed as per cent, that is, $(1-f)100 = \%$.

*Rate of renal excretion of the cyclic nucleotides.* The total rate of urinary excretion of cyclic nucleotide is equal to the rate of excretion from the plasma plus the rate of any nephrogenous addition:

$$R_e = r_e + r_k \qquad (5)$$

where $R_e$ is the total rate, $r_e$ is the rate due to renal plasma clearance, and $r_k$ is the rate of tubular addition of nephrogenous cyclic nucleotide. The rate of cyclic nucleotide excretion is actually a complex function of the production rate in the miscible pool, the relative magnitudes of renal and extrarenal elimination, and the magnitude of any nephrogenous contribution. Thus, the relation of urinary cyclic nucleotide to the system is depicted quite clearly by the expression:

$$R_e = PR_p - r_m + r_k \qquad (6)$$

## RESULTS

### Cyclic nucleotide content in human plasma and urine

The subjects in our series, principally normal young male adults, excreted between 75 and 375 nmoles of cyclic AMP and between 15 and 125 nmoles of cyclic GMP per hour. Expressing these levels in relation to the quantity of creatinine that was concomitantly excreted gave a somewhat narrower range; cyclic AMP excretion ranged from 1.5 to 5.0 $\mu$moles/g of creatinine and cyclic GMP from 0.3 to 1.8 $\mu$moles/g of creatinine.
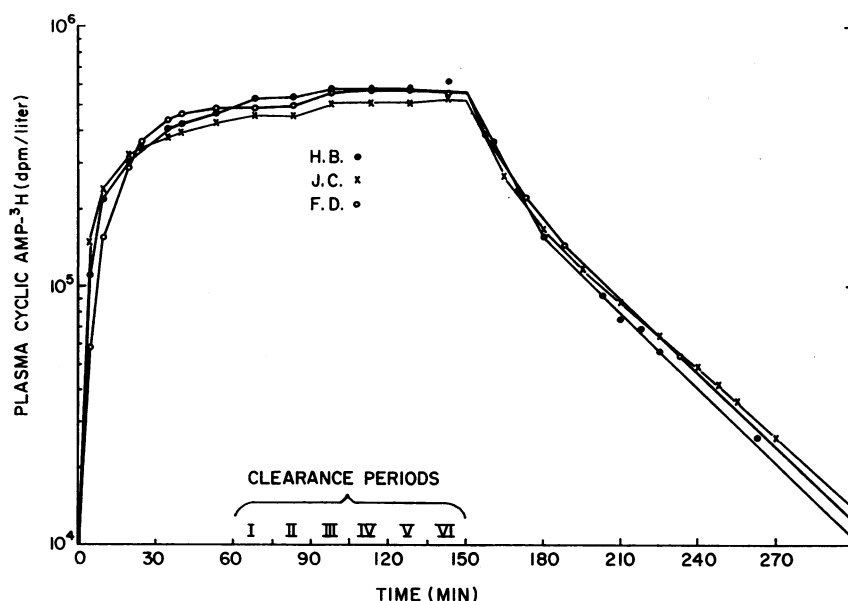


FIGURE 1 Plasma cyclic AMP-$^3$H appearance and disappearance curves during and following constant intravenous infusion of the tracer in man. Note the semilogarithmic plot. Time is expressed in relation to infusion initiation (t = 0); the infusions were terminated at 150 min. The roman numerals I–VI along the abscissa signify the six successive 15-min clearance periods obtained in each study. Plasma samples for endogenous cyclic AMP determination were drawn at 98, 113, 128, and 143 min.

TABLE I

Renal Clearances of Inulin, Cyclic AMP-³H, and Endogenous Cyclic AMP

| Subject | Clearance period | Urinary cyclic AMP-³H | Plasma cyclic AMP-³H* | Clearance cyclic AMP-³H | Clearance inulin | Urinary cyclic AMP | Plasma cyclic AMP* | Clearance cyclic AMP | Clearance ratio: cyclic AMP / inulin | $\frac{S_u}{S_p}$‡ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | dpm × 10⁻³/min | dpm/ml | ml/min | ml/min | nmoles/min | nM | ml/min | | |
| J. C. | I§ | 67.7 | 458 | 148 | 142 | 4.40 | | | | |
| | II | 62.6 | 453 | 138 | 137 | 4.07 | | | | |
| | III | 69.0 | 508 | 136 | 133 | 4.54 | 15.0 | 303 | 2.28 | 0.45 |
| | IV | 69.1 | 508 | 136 | 137 | 4.22 | 15.5 | 272 | 1.99 | 0.50 |
| | V | 64.3 | 502 | 128 | 128 | 4.04 | 15.0 | 269 | 2.10 | 0.48 |
| | VI | 69.3 | 546 | 127 | 133 | 4.43 | 15.9 | 279 | 2.09 | 0.46 |
| | Mean ±SEM | 67.0 ±1.1 | 496 ±14 | 136 ±3 | 135 ±2 | 4.28 ±0.08 | 15.4 ±0.2 | 281 ±8 | 2.12 ±0.06 | 0.47 ±0.01 |
| H. B.‖ | Mean ±SEM | 71.1 ±2.8 | 560 ±12 | 127 ±5 | 132 ±4 | 4.32 ±0.15 | 24.8 ±0.6 | 179 ±10 | 1.34 ±0.04 | 0.72 ±0.02 |
| F. D.‖ | Mean ±SEM | 58.2 ±1.4 | 542 ±16 | 108 ±3 | 109 ±3 | 2.70 ±0.10 | 13.8 ±0.1 | 192 ±7 | 1.79 ±0.02 | 0.55 ±0.01 |

\* Plasma samples were obtained at the midpoints of the clearance periods.
‡ Urinary cyclic AMP-³H specific activity ($S_u$) divided by plasma cyclic AMP-³H specific activity ($S_p$).
§ The clearance periods correspond to the six successive 15-min periods depicted in Fig. 1.
‖ Experimental protocols were exactly as with subject J. C., but only the mean data are shown.

Plasma concentrations of cyclic AMP ranged from 10 to 25 nM, and cyclic GMP ranged from 2 to 16 nM.

### Renal clearance determinations

*Cyclic AMP.* Fig. 1 illustrates the plasma levels of cyclic AMP-³H attained in three subjects during and after a 2½ hr constant intravenous infusion of the tritium-labeled nucleotide. Infusion equilibrium was achieved at approximately 90 min in all three subjects.

Table I contains data on the renal clearances of inulin, cyclic AMP-³H, and endogenous cyclic AMP which were obtained during the infusion studies depicted in Fig. 1. The clearance of cyclic AMP-³H was almost identical with the clearance of inulin in every clearance period; the mean ratio of cyclic AMP-³H clearance to inulin clearance was 0.98 for the 18 clearance periods obtained. The binding studies demonstrated that cyclic AMP-³H was not detectably bound to plasma proteins. Thus, the mechanism of renal clearance of plasma cyclic AMP appears to be glomerular filtration.

The renal clearance of endogenous cyclic AMP always exceeded the clearances of inulin and cyclic AMP-³H, the ratios of endogenous cyclic AMP clearance to inulin clearance averaging 1.34, 2.12, and 1.79 for the three subjects studied. The plasma content and rates of excretion of cyclic AMP were stable during each of these experiments. An alternative means of expressing the data is by use of the plasma and urinary cyclic AMP-³H specific activities. The specific activity of cyclic AMP-³H in the urine was lower than that in plasma, the ratios of urinary to plasma values averaging 0.72, 0.47, and 0.55 for the studies shown in Table I. Thus, unlabeled cyclic AMP must have been added directly to the urine by the kidney, thereby reducing the specific activity of urinary cyclic

TABLE II
Renal Clearances of Inulin and Endogenous Cyclic AMP

| Subject | Urinary cyclic AMP | Urinary cyclic AMP ($R_e$) | Plasma cyclic AMP* | Clearance cyclic AMP | Clearance inulin | Clearance ratio: cyclic AMP / inulin | Renal excretory elimination ($r_e$) | Nephrogenous component ($n_e$) |
|---|---|---|---|---|---|---|---|---|
| | μmoles/g of creatinine | nmoles/min | nM | ml/min | ml/min | | nmoles/min | nmoles/min |
| W. C. (2)‡ | 2.44 | 3.11 | 22.4 | 140 | 115 | 1.22 | 2.58 | 0.53 |
| T. C. (3) | 2.89 | 3.01 | 14.6 | 206 | 110 | 1.88 | 1.61 | 1.40 |
| J. G. C. (3) | 3.11 | 5.01 | 22.2 | 226 | 133 | 1.70 | 2.95 | 2.06 |
| J. N. (2) | 2.29 | 2.92 | 12.5 | 233 | 112 | 2.08 | 1.40 | 1.52 |
| H. H. (3) | 1.62 | 2.63 | 15.4 | 171 | 121 | 1.41 | 1.86 | 0.77 |

\* The plasma samples were drawn at the midpoints of the clearance periods.
‡ Number in parentheses indicates the number of successive 15-min clearance periods obtained. All values in the table are mean values of the observations.
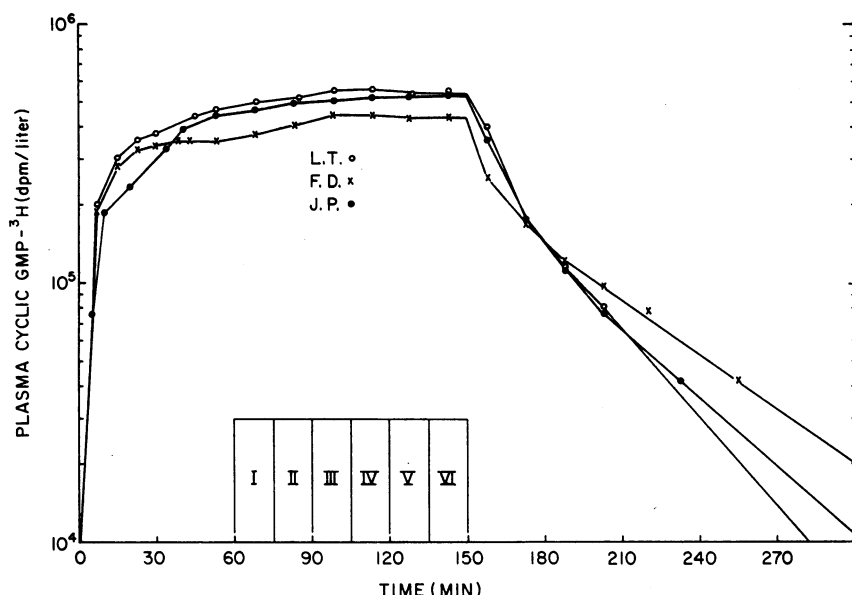
FIGURE 2 Plasma cyclic GMP-³H appearance and disappearance curves during and following constant intravenous infusion of the tracer in man. Note the semilogarithmic plot. Time is expressed in relation to infusion initiation (t = 0); the infusions were terminated at 150 min. The roman numerals I–VI along the abscissa signify the six successive 15-min clearance periods obtained in each study. Plasma samples for endogenous cyclic GPM determination were obtained at 98, 113, 128, and 143 min.

AMP-³H and yielding clearances of endogenous cyclic AMP which exceeded inulin and cyclic AMP-³H clearance. Based on the specific activity calculations, 28, 53, and 45% of the total endogenous cyclic AMP excreted by these subjects was due to the release of nephrogenous cyclic AMP into the urine.

Table II contains data on the clearances of inulin and endogenous cyclic AMP in five additional studies in which isotopic cyclic AMP was not employed. The mean ratios of endogenous cyclic AMP clearance to inulin clearance ranged from 1.22 to 2.08; thus, the nephrogenous component of urinary cyclic AMP varied from 18 to 52% of the total in these subjects. For the eight subjects in Tables I and II, the average nephrogenous contribution was 39% of the endogenous cyclic AMP excreted.

The quantities of cyclic AMP contributed to the urine by plasma and by the kidney in these eight subjects are shown in Tables II and IV. Urinary cyclic AMP ranged from 2.6 to 5.0 nmoles/min, of which 1.4–3.3 nmoles/min were derived from plasma and 0.5–2.2 nmoles/min were nephrogenous. Clearly, the total rate of cyclic AMP excretion in different subjects may be a consequence of variation in either plasma or nephrogenous contribution, or both, so that in a given individual a determination of urinary cyclic AMP alone provides rather limited information.

*Cyclic GMP.* Fig. 2 illustrates the plasma levels of cyclic GMP-³H in three subjects during and after a 2½ hr intravenous infusion of the tritium-labeled nucleotide. As in the cyclic AMP-³H studies, infusion equilibrium was achieved within 90 min.

Table III contains data on the renal clearances of inulin, cyclic GMP-³H, and endogenous cyclic GMP which were obtained during the infusion studies depicted in Fig. 2. Cyclic GMP-³H clearance was virtually equal to inulin clearance during each clearance period in all three subjects; the mean ratio of cyclic GMP-³H clearance to inulin clearance was 1.0 for the 18 clearance periods obtained. The binding studies demonstrated no detectable binding of cyclic GMP-³H to plasma proteins. Thus, as with cyclic AMP, cyclic GMP appears to be cleared from the plasma by simple glomerular filtration.

The rates of excretion and the plasma levels of endogenous cyclic GMP were quite stable during the infusions. The mean ratios of endogenous cyclic GMP clearance to inulin clearance were 1.16, 0.87, and 0.97 for the three subjects, with an average of 1.0 for the total of 12 clearance periods. The mean ratios of urine to plasma cyclic GMP-³H specific activity were 0.84, 1.17, and 1.01, respectively, for the three subjects. Thus, there was no consistent indication of dilution of the cyclic GMP-³H specific activity in urine relative to that in plasma. Under basal conditions, therefore, virtually all

| Subject | Clearance period | Urinary cyclic GMP-³H | Plasma cyclic GMP-³H* | Clearance cyclic GMP-³H | Clearance inulin | Urinary cyclic GMP | Plasma cyclic GMP* | Clearance cyclic GMP | Clearance ratio: cyclic AMP / inulin | $\frac{S_u\ddagger}{S_p}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | dpm × 10⁻³/ min | dpm/ml | ml/min | ml/min | nmoles/min | nM | ml/min | | |
| L. T. | I§ | 53.1 | 501 | 106 | 101 | 1.41 | | | | |
| | II | 60.1 | 515 | 117 | 115 | 1.71 | | | | |
| | III | 55.6 | 553 | 101 | 106 | 1.66 | 16.1 | 103 | 0.97 | 0.97 |
| | IV | 51.4 | 527 | 97 | 98 | 1.42 | 16.0 | 89 | 0.91 | 1.10 |
| | V | 58.5 | 532 | 110 | 110 | 1.65 | 14.9 | 111 | 1.01 | 0.99 |
| | VI | 57.5 | 530 | 108 | 112 | 1.77 | 15.8 | 112 | 1.00 | 0.97 |
| | Mean ±SEM | 56.0 ±1.2 | 526 ±7 | 107 ±3 | 107 ±3 | 1.60 ±0.06 | 15.7 ±0.3 | 104 ±5 | 0.97 ±0.02 | 1.01 ±0.03 |
| J. P.‖ | Mean ±SEM | 54.4 ±0.7 | 510 ±11 | 107 ±2 | 108 ±2 | 0.99 ±0.03 | 7.9 ±0.5 | 122 ±8 | 1.16 ±0.08 | 0.84 ±0.04 |
| J. D.‖ | Mean ±SEM | 46.4 ±1.5 | 420 ±11 | 111 ±3 | 110 ±3 | 0.66 ±0.03 | 7.4 ±0.2 | 94 ±4 | 0.87 ±0.03 | 1.17 ±0.04 |

* Plasma samples were obtained at the midpoints of the clearance periods.
‡ Urinary cyclic GMP-³H specific activity ($S_u$) divided by plasma cyclic GMP-³H specific activity ($S_p$).
§ The clearance periods correspond to the six successive 15-min periods depicted in Fig. 2.
‖ Experimental protocols were exactly as with subject L. T., but only the mean data are shown.

of the cyclic GMP in urine appears to be derived from plasma by glomerular filtration.

### Kinetic studies

*Cyclic AMP.* The distribution, plasma clearance, and rates of production, metabolism, and excretion of cyclic AMP were evaluated in the infusion studies (Table IV) and in studies utilizing the rapid intravenous injection technique (Table V).

The plasma disappearance curves of cyclic AMP-³H following a "pulse" intravenous injection of the tracer exhibited a multiexponential pattern, becoming monoexponential at approximately 40–50 min after injection (Fig. 3). Two components were resolvable from the curves, and the concentration of cyclic AMP-³H in plasma as a function of time was expressed as the sum of two exponentials, as shown in Table V. Both the initial distributive phase and the so-called elimination phase proceeded rapidly, with mean half-times of 6.0 and 28.8 min, respectively. In close agreement with this latter figure was the mean half-time of 32.2 min obtained in the constant infusion studies (Fig. 1 and Table IV).

The concentrations of endogenous cyclic AMP in plasma were essentially constant during the course of any one study (see insets in Fig. 3 and Table I), and the cyclic AMP-³H specific radioactivity, therefore, paralleled the plasma disappearance curves of cyclic AMP-³H. Thus, the rates of cyclic AMP input into and elimination from the sampled pool must have been approximately equal, defining a pool in dynamic steady state.

Extracellular fluid volume, as measured by inulin space, averaged 13.3 liters (17.2% of body weight),

and the "final" apparent volume of distribution of cyclic AMP-³H, as estimated by the infusion equilibrium method (Table IV), averaged 20.5 liters (26.5% of body weight). It appeared, therefore, that the cyclic AMP-³H was penetrating some intracellular pool of the nucleotide, an interpretation suggested also by the multiexponential curves of cyclic AMP-³H disappearance. In the injection studies (Table V), cyclic AMP-³H distributed initially in a volume averaging 10.0 liters 12.4% of body weight). A comparison of the rate constants for irreversible and total (irreversible and reversible) tracer loss from this initial volume revealed that approximately one-third of the tracer loss from this space was reversible (i.e., reentry was occurring).

The mean plasma clearance rate for cyclic AMP-³H was 644 ml/min in the injection studies (Table V) and 693 ml/min in the infusion studies (Table IV). The plasma clearance rate was subdivided into renal clearance and extrarenal or metabolic clearance. From a comparison of the relative values of these clearances in Tables IV and V, an average of 19.3% of the administered dose of cyclic AMP-³H would be predicted to appear in the urine, and the predictions agreed very well with the cumulative cyclic AMP-³H excretion which was measured (Fig. 4). Thus, renal excretion plays a relatively minor role in the elimination of cyclic AMP from the circulation, an average of approximately 80% of the loss of the nucleotide being extrarenal. As a consequence of the rapid plasma clearance, most of the cyclic AMP-³H which was excreted was collected within 2 hr after pulse injection or discontinuation of infusion (Fig. 4).

In the steady state, the rate of elimination of cyclic AMP from the circulation (i.e., the product of plasma

TABLE IV

*Kinetic Parameters Obtained from*

| Subject | Weight | Infusion rate (r) | t½ 0.693/β | Inulin space (Vd$_{in}$) | Final volume of distribution (Vd$_{inf\ eq}$) | Plasma cyclic NMP* (C$_{poNMP}$) | Plasma clearance rate (PCR) |
|---|---|---|---|---|---|---|---|
| | *kg* | *dpm/min* | *min* | *liters (% weight)* | *liters (% weight)* | *nM* | *ml/min* |
| Cyclic AMP-³H | | | | | | | |
| H. B. | 80.5 | 405,000 | 30.0 | 13.3 (16.5) | 19.50 (24.2) | 24.8 | 703 |
| J. C. | 72.7 | 367,130 | 34.5 | 13.2 (18.2) | 22.31 (30.7) | 15.4 | 726 |
| F. D. | 79.5 | 374,350 | 32.0 | 13.4 (16.9) | 19.53 (24.6) | 13.8 | 649 |
| Cyclic GMP-³H | | | | | | | |
| J. P. | 66.7 | 413,600 | 34.5 | 11.5 (17.2) | 23.64 (35.4) | 9.8 | 789 |
| F. D. | 80.9 | 436,320 | 43.0 | 11.5 (14.2) | 31.72 (39.2) | 7.4 | 1000 |
| L. T. | 58.0 | 432,390 | 27.1 | 10.6 (18.3) | 23.17 (40.0) | 15.7 | 807 |

* Cyclic NMP is a generic symbol for cyclic nucleotide.

clearance rate and plasma cyclic AMP concentration) must equal the rate of input of the nucleotide into the circulation, that is, the plasma production rate. The cyclic AMP production rates (by the plasma dilution method) ranged from 9.0 to 17.4 nmoles/min, and the average was 12.9 nmoles/min (Tables IV and V). In the infusion studies, the production rate could also be estimated by the urinary isotope dilution method, and there was good agreement between the plasma and urinary methods (Table IV).

Until approximately 1.5 hr after pulse injection and 3 hr after the initiation of an infusion, virtually all of the radioactivity occurring in both plasma and urine could be accounted for as unaltered cyclic AMP-³H (Fig. 4). The amount of the radioactive metabolites (as yet unidentified) of cyclic AMP excreted by 5–6 hr after tracer administration was small relative to the total quantity of cyclic AMP-³H excreted by this time and relative to the total dose of tritium administered. It is of interest that the cyclic nucleotides are the only nucleotides which have been detected in urine (2, 3).

*Cyclic GMP.* The kinetics of cyclic GMP-³H were evaluated exactly as described for cyclic AMP-³H, and the resultant kinetic parameters are shown in Tables IV and V.

Two exponential components were resolvable from the cyclic GMP-³H disappearance curves, with half-times very nearly equal to those seen in the cyclic AMP-³H experiments. The plasma levels of endogenous cyclic GMP (see insets in Fig. 5 and Table III) were

TABLE V

*Kinetic Parameters Obtained from*

| Subject | Weight | Dose | Plasma cyclic NMP-³H* $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ | t½ 0.693/α | t½ 0.693/β | Initial volume of distribution (V$_1$) |
|---|---|---|---|---|---|---|
| | *kg* | *μCi* | *C$_p$, A, B in dpm x 10⁶/liter α, β in min⁻¹ t in min* | *min* | *min* | *liter (% weight)* |
| Cyclic AMP-³H | | | | | | |
| H. B. | 80.5 | 13.58 | $1.824e^{-0.1145t} + 0.781e^{-0.0290t}$ | 6.05 | 23.90 | 11.47 (14.2) |
| T. F. | 91.4 | 12.12 | $2.350e^{-0.1288t} + 0.609e^{-0.0216t}$ | 6.61 | 30.53 | 9.01 (9.9) |
| J. C. | 72.7 | 10.45 | $2.046e^{-0.1288t} + 0.368e^{-0.0216t}$ | 5.38 | 32.08 | 9.43 (13.0) |
| Cyclic GMP-³H | | | | | | |
| P. R. | 75.5 | 7.84 | $1.250e^{-0.1552t} + 0.193e^{-0.0265t}$ | 4.47 | 26.15 | 11.95 (15.8) |
| L. T. | 58.2 | 11.34 | $3.144e^{-0.1378t} + 0.360e^{-0.0220t}$ | 5.03 | 31.50 | 7.12 (12.2) |
| J. P. | 66.7 | 11.88 | $2.771e^{-0.1375t} + 0.333e^{-0.0246t}$ | 5.04 | 28.17 | 8.42 (12.6) |

* Cyclic NMP is a generic symbol for cyclic nucleotide.

‡ Renal clearance values are those determined in the infusion experiments (Tables I and III) except for

*Constant Intravenous Infusion Experiments*

| Renal plasma clearance (Cr) | Metabolic plasma clearance (Cm) | Production rate (PRp) | Production rate (PRu) | Metabolic elimination (rm) | Renal excretory elimination (re) | Nephrogenous component (rk) | Total excretion rate (Re) |
|---|---|---|---|---|---|---|---|
| *ml/min* | *ml/min* | *nmoles/min* | *nmoles/min* | *nmoles/min* | *nmoles/min* | *nmoles/min* | *nmoles/min* |
| 132 | 571 | 17.42 | 16.30 | 14.14 | 3.28 | 1.05 | 4.33 |
| 135 | 591 | 11.13 | 10.73 | 9.05 | 2.08 | 2.15 | 4.23 |
| 109 | 540 | 8.95 | 8.75 | 7.45 | 1.50 | 1.17 | 2.67 |
| 108 | 681 | 7.75 | 7.88 | 6.69 | 1.06 | — | 1.04 |
| 110 | 890 | 7.40 | 6.27 | 6.59 | 0.81 | — | 0.68 |
| 107 | 700 | 12.68 | 11.70 | 11.00 | 1.68 | — | 1.58 |

quite stable during the individual studies, and the miscible pool of cyclic GMP was assumed to be in steady state.

Although the initial volume of distribution for cyclic GMP-$^3$H was similar to that estimated for cyclic AMP-$^3$H, the final apparent volume of distribution for cyclic GMP-$^3$H averaged 38.2% of body weight and, thus, was approximately 45% greater than the same estimate for cyclic AMP-$^3$H. The inulin space in these subjects averaged 16.6% of body weight. Approximately 29% of the loss of cyclic GMP-$^3$H from the initial volume of distribution reentered this space. As was the case in the cyclic AMP-$^3$H studies, the complex plasma disappearance curves, the large final apparent space for cyclic GMP-$^3$H, and the evidence for bidirectional tracer transfer are interpreted as indicating communication of the extracellular pool of cyclic GMP with an intracellular pool or pools of the nucleotide.

The plasma clearance rate was somewhat higher for cyclic GMP-$^3$H than for cyclic AMP-$^3$H, averaging 865 ml/min during the infusion studies (Table IV) and 845 ml/min during the injection studies (Table V). A direct comparison is possible for subject F. D. who received both cyclic AMP-$^3$H and cyclic GMP-$^3$H by infusion, with plasma clearance rates of 649 ml/min and 1000 ml/min, respectively.

By subdividing plasma clearance rate into its excretory and metabolic components, it is apparent that urinary excretion plays only a minor role in the elimination of cyclic GMP from the circulation. By pooling

*Single Intravenous Injection Experiments*

| Plasma cyclic NMP* (CpcNMP) | Plasma clearance rate (PCR) | Renal plasma clearance (Cr)‡ | Metabolic plasma clearance (Cm) | Reversible tracer flux (1 − f) | Production rate (PRp) | Production rate (PRu) | Total excretion rate (Re) |
|---|---|---|---|---|---|---|---|
| *nM* | *ml/min* | *ml/min* | *ml/min* | *%* | *nmoles/min* | *nmoles/min* | *nmoles/min* |
| 23.7 | 697 | 132 | 565 | 31.6 | 16.52 | — | 4.49 |
| 24.2 | 542 | 125 | 417 | 31.6 | 13.10 | — | 4.36 |
| 15.0 | 692 | 135 | 557 | 34.8 | 10.38 | — | 4.21 |
| 5.9 | 1124 | 125 | 999 | 31.8 | 6.60 | — | 0.65 |
| 14.5 | 636 | 107 | 529 | 29.0 | 9.23 | 8.46 | 1.56 |
| 9.2 | 776 | 108 | 668 | 26.5 | 7.10 | 7.08 | 1.07 |

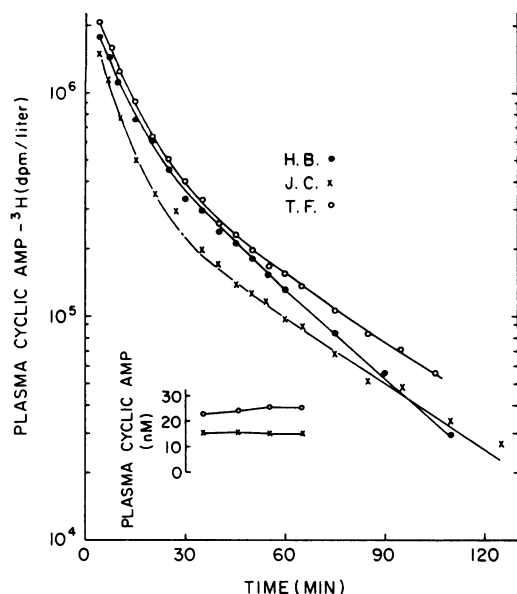T. F. and P. R., for whom a value of 125 ml/min is assumed.

FIGURE 3 Plasma cyclic AMP-$^3$H disappearance curves following single rapid intravenous injection of the tracer in man. Time is expressed in relation to the time of injection (t = 0). The experimental points are plotted on semilogarithmic coordinates, and the curves were obtained by least squares fits using a digital computer. The plasma levels of endogenous cyclic AMP are plotted in the insets (note Cartesian coordinates) for J. C. and T. F.; a single pooled plasma sample representing the same four points was obtained for H. B.

the data in Tables IV and V, an average of 13.3% of the administered tracer dose would be predicted to appear in the urine (vs. 19.3% for cyclic AMP-$^3$H). In the representative examples shown in Fig. 6, the measured cumulative cyclic GMP-$^3$H excretion agreed very well with the predicted excretion. As in the cyclic AMP-$^3$H experiments, the rapid plasma clearance of cyclic GMP-$^3$H resulted in a rapid decline in the rate of cyclic GMP-$^3$H excretion. Thus, for the pulse injection study depicted in Fig. 6, the urine collected in the first 30 min after tracer injection contained 83% of the total quantity of cyclic GMP-$^3$H excreted in 22.5 hr. Unidentified radioactive metabolites of cyclic GMP-$^3$H appeared in plasma and urine earlier and in greater abundance than in the cyclic AMP-$^3$H studies (Fig. 6).

As discussed previously, no nephrogenous contribution to urinary cyclic GMP could be demonstrated. Consequently, the production rates of cyclic GMP in the miscible pool could be estimated both in the injection studies and in the infusion studies by either the plasma dilution method or the urinary isotope dilution method. The general agreement of the two methods was good (see Tables IV and V). The values determined by the plasma method ranged from 6.6 to 12.7 nmoles/min with an average of 8.5 nmoles/min, or approximately 35% less than the average cyclic AMP production rate determined in similar fashion.
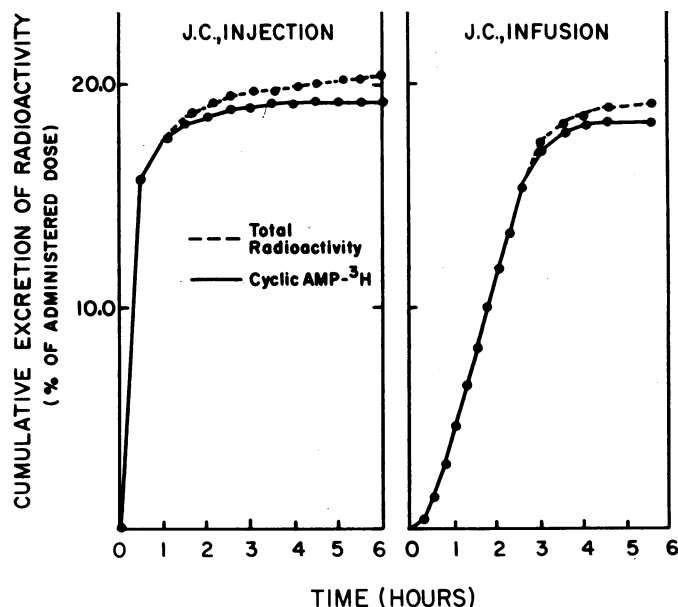


FIGURE 4 Cumulative urinary excretion of cyclic AMP-$^3$H (solid lines) and total radioactivity (broken lines) following cyclic AMP-$^3$H injection (left panel) and infusion (right panel), subject J. C. Time is expressed in relation to t = 0, the time of tracer injection or infusion initiation. Cumulative excretion is expressed as per cent of administered dose of cyclic AMP-$^3$H. The cumulative cyclic AMP-$^3$H excretion predicted from the relative magnitudes of plasma clearance rate and renal clearance was 19.5% for the injection study (Table V) and 18.6% for the infusion study (Table IV).

## DISCUSSION

*Clearances and sources of the urinary cyclic nucleotides.* The equivalent clearances of inulin and the tritium-labeled cyclic nucleotides could conceivably have resulted from plasma protein binding of the cyclic nucleotides-³H and counterbalancing renal tubular secretion of the compounds, or, alternatively, from exactly counterbalancing renal tubular reabsorption and secretion of the nucleotides. However, binding studies demonstrated that cyclic AMP-³H and cyclic GMP-³H were not detectably bound to plasma protein, and exactly equivalent and counterbalancing tubular reabsorption and secretion of a compound by the kidney would be an unprecedented mechanism of renal clearance. Moreover, as reported in an accompanying publication (8), the clearances of inulin and endogenous cyclic AMP were essentially equal when the circulating levels of cyclic AMP were elevated to 20–40 times control levels, conditions which might be expected to modify the renal clearance of a compound which was reabsorbed and/or secreted by the kidney. The clearances of inulin and endogenous cyclic GMP also have been found to be approximately equal in other studies not employing isotopic cyclic GMP (8). Thus, all of the data which we have accumulated are consistent with the interpretation that simple glomerular filtration is the mechanism of renal clearance of plasma cyclic AMP and cyclic GMP.
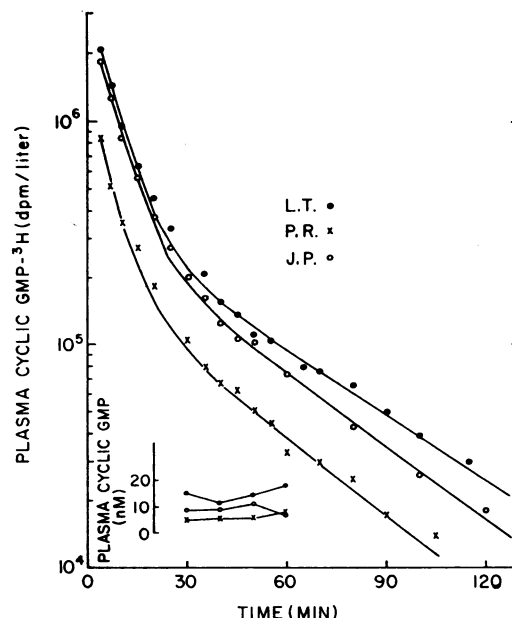


FIGURE 5 Plasma cyclic GMP-³H disappearance curves following single rapid intravenous injection of the tracer in man. Time is expressed in relation to the time of injection (t = 0). The experimental points are plotted on semilogarithmic coordinates, and the curves were obtained by least squares fits using a digital computer. The plasma levels of endogenous cyclic GMP are plotted in the insets (note Cartesian coordinates).
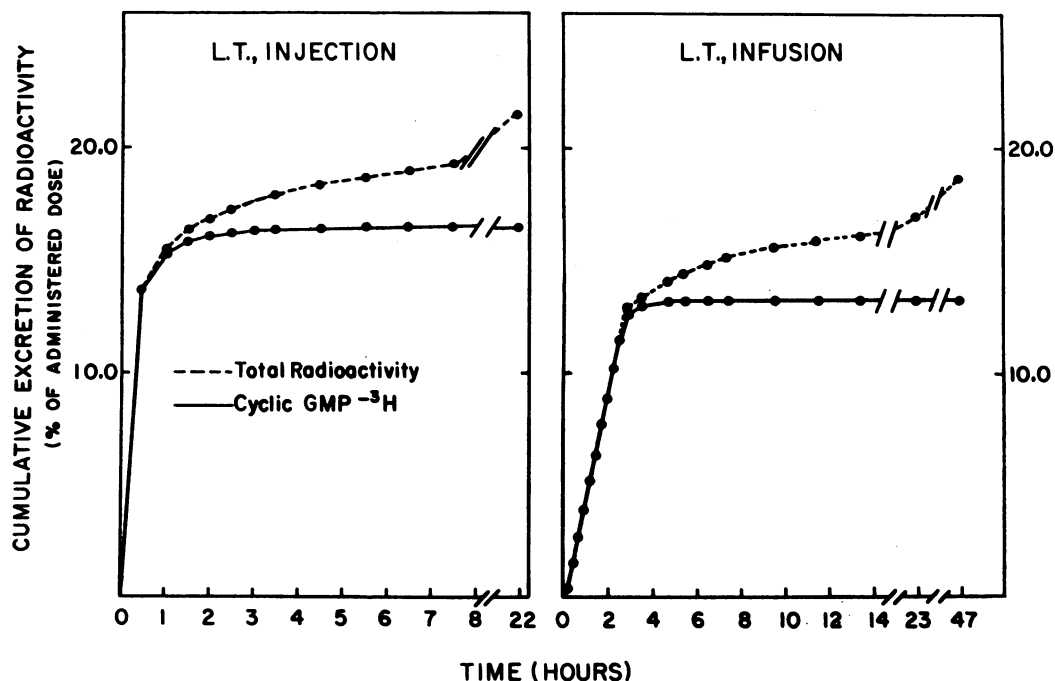


FIGURE 6 Cumulative urinary excretion of cyclic GMP-³H (solid lines) and total radioactivity (broken lines) following cyclic GMP-³H injection (left panel) and infusion (right panel), subject L. T. Time is expressed in relation to t = 0, the time of tracer injection or infusion initiation. Cumulative excretion is expressed as per cent of administered dose of cyclic GMP-³H. The cumulative cyclic GMP-³H excretion predicted from the relative magnitudes of plasma clearance rate and renal clearance was 16.8% for the injection study (Table V) and 13.3% for the infusion study (Table IV).

Virtually all the cyclic GMP in human urine appears to be filtered from plasma; no nephrogenous cyclic GMP was identified. Therefore, the levels of cyclic GMP in extracellular fluids presumably reflect formation of the compound by extrarenal tissues. Urinary cyclic AMP, on the other hand, is derived from both plasma and the kidney, and, as described in accompanying articles (8, 9), the contribution of either source may be hormonally altered. One implication of the findings of the present studies is that comparison of the variable cyclic AMP excretion rates in different subjects might be quite difficult since the absolute quantities of cyclic AMP derived from either plasma or the kidney vary considerably from one individual to another.

*Kinetics.* The techniques used in studying the kinetics of the cyclic nucleotides were, with slight modifications, those commonly employed in in vivo pharmacodynamic and endocrinologic experiments. The results demonstrated that the miscible pools of the compounds are extremely active, with rapid distribution, metabolism, and production of the nucleotides.

The calibrated infusion method of inulin space measurement and the infusion equilibrium method of determining cyclic nucleotide distribution were chosen because the techniques are formally equivalent, allowing for better comparison of results. Both cyclic AMP and cyclic GMP appeared to distribute in a space exceeding extracellular fluid volume. Our interpretation of the distribution data, the isotope disappearance curves, the apparent bidirectional tracer flux, and the ultimate disposition of the tracers is that there is some miscibility of extracellular and intracellular pools of the compounds.

The cyclic AMP plasma clearance rate was evaluated in two subjects (H. B. and J. C.) by both cyclic AMP-$^3$H injection and infusion, and the results for a given subject were almost identical with the two methods. Each subject maintained virtually the same plasma level and excretion rate of cyclic AMP on the separate occasions of study, which were about 1 month apart (see Tables IV and V). Similarly, two subjects, (J. P. and L. T.) were studied both by cyclic GMP-$^3$H injection and infusion. In subject J. P. practically identical plasma clearance rates were obtained on both occasions and by both methods, whereas in subject L. T. the plasma clearance rate as calculated from the infusion study was 27% higher than that from the injection study. The differences in cumulative cyclic GMP-$^3$H excretion measured after the two studies in L. T. correlated with the clearance figures in Tables IV and V and indicated that the variation in this subject was biological (see Fig. 6) rather than technological.

Approximately four-fifths of the administered cyclic AMP-$^3$H or cyclic GMP-$^3$H was rapidly eliminated from the circulation by extrarenal clearance. To what extent this is due to metabolism of the compounds, and to what extent it is due to other processes (e.g., biliary excretion) is unknown.

The plasma production rate measurements do not imply sampling of the total body tissue production of the cyclic nucleotides; rather, these determinations represent production only in that portion of the cellular cyclic nucleotide pool with which the compounds in plasma are miscible. Without modification, both plasma and urinary isotope dilution techniques are suitable for cyclic GMP, and the plasma technique is suitable for cyclic AMP. The urinary isotope dilution method for cyclic AMP may be used only in infusion studies in which the cumulative urinary specific activity may be appropriately corrected for nephrogenous cyclic AMP, making this usually simple technique rather laborious.

Urinary cyclic GMP is usually less than urinary cyclic AMP, and the relations pointed out in equation 6 (see Methods) offer some explanation for these differing excretion rates. That is, in comparisin to cyclic AMP, the production rate of cyclic GMP is generally lower, the relative rate of metabolic clearance is higher, and there is no appreciable nephrogenous contribution of cyclic GMP.

*Possible sites of production of the plasma cyclic nucleotides.* The adenyl cyclase, guanyl cyclase, and phosphodiesterase systems have been found to be widely distributed in mammalian tissues (1, 29–31). In spite of the demonstration of the extracellular occurrence of the cyclic nucleotides some years ago (1–3), few studies have been reported in which the phenomenon of cyclic nucleotide exit from specific mammalian tissues was considered.

In preliminary experiments, tracer quantities of cyclic AMP-$^3$H or cyclic GMP-$^3$H were incubated in vitro (37°C) with fresh, oxygenated, whole human blood. The decline of cyclic nucleotide-$^3$H radioactivity in the plasma was found to be roughly parallel to the decline in chemical levels of the nucleotides, the plasma specific radioactivity of the cyclic nucleotides remaining relatively unchanged. Therefore, the elements of blood do not appear to be a major source of the endogenous cyclic nucleotide levels found in normal human plasma. Cyclic AMP has been found to be present in the incubation medium containing rat epididymal fat pads (32) and fat cells (33). In ancillary studies we have found that in six patients undergoing pneumoencephalographic studies, the cerebrospinal fluid content of cyclic AMP ranged from 5 to 22 nM, while cyclic GMP levels ranged from undetectable to 7 nM. Thus, the central nervous system is a possible source of extracellular cyclic nucleotides. The two accompanying publications indicate that, with

appropriate hormonal stimulation, both the liver (8) and the kidney (9) may serve as sources of the cyclic AMP in human plasma. The elaboration of extracellular cyclic nucleotides by mammalian cells might, therefore, be a rather general phenomenon.

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