Metabolism and Excretion of Exogenous Adenosine 3':5'-Monophosphate and Guanosine 3':5'-Monophosphate

STUDIES IN THE ISOLATED PERFUSED RAT KIDNEY AND IN THE INTACT RAT*

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Isolated rat kidneys were perfused with a recirculating medium containing exogenous adenosine 3':5'-monophosphate (cyclic AMP) or guanosine 3':5'-monophosphate (cyclic GMP) at an initial concentration of 0.1 mM. Both cyclic nucleotides were rapidly removed from the perfusate. Urinary excretion accounted for about 20% and 40% of the respective cyclic AMP and cyclic GMP lost from the perfusate.

The metabolism of the cyclic nucleotides was studied by including 14C-labeled cyclic nucleotides in the perfusate. During 60 min, 30% of added cyclic [14C]AMP was metabolized to renal [14C]adenine nucleotides (ATP, ADP, and AMP) and 30% to perfusate [14C]uric acid. Similarly, 20% of cyclic [14C]GMP was metabolized to renal [14C]guanine nucleotides (GTP, GDP, and GMP) and 30% to perfusate [14C]uric acid. Urine contained principally unchanged [14C]-labeled cyclic nucleotide.

Addition of 0.1 mM cyclic AMP to the perfusate elevated the renal ATP and ADP contents 2-fold. Addition of 0.1 mM of either cyclic AMP or cyclic GMP to the perfusate also elevated the renal production of uric acid 2- to 3-fold.

The production and distribution of metabolites of exogenous cyclic nucleotides were also studied in the intact rat. Within 60 min after injection, 3.3 pmol of either 14C-labeled cyclic AMP or cyclic GMP was cleared from the plasma. Kidney cortex and liver were the principal tissues for 14C accumulation. Urinary excretion accounted for about 20 and 45% of the cyclic [14C]AMP and cyclic [14C]GMP lost from the plasma, respectively. The 14C found in the kidney and liver was present almost entirely as the respective purine mono-, di-, and tri-nucleotides. The other principal metabolite was [14C]allantoin, found in the urine and, to a lesser extent, the liver. The urine contained mostly unchanged [14C]-labeled cyclic nucleotide. Unlike the findings with the perfused kidney, [14C]uric acid was not a significant metabolite of the [14C]-labeled cyclic nucleotides in these in vivo experiments.

It has long been considered that the cell membrane is poorly permeable to exogenous cyclic nucleotides (2). Recent evidence from human in vivo experiments suggests that the cyclic nucleotides, adenosine 3':5'-monophosphate and guanosine 3':5'-monophosphate are very permeant in some tissues (3). Studies in the intact rat and dog have demonstrated that the cyclic AMP and, to a slightly lesser extent, the liver are the principal tissues involved in the removal of plasma cyclic AMP1 (4, 5). Studies with isolated perfused rat tissues have shown that the kidney (6) and the liver (7) are rapidly penetrated by exogenous cyclic AMP. In the perfused rat kidney it was concluded that the majority of the cyclic AMP removed from the perfusate was metabolized, since no significant intracellular accumulation of cyclic AMP was apparent and the urinary excretion of cyclic AMP accounted for only a small fraction of its total renal clearance (6). A similar conclusion could be made for the perfused rat liver (7). Cyclic GMP appears to share the ability of cyclic AMP to readily penetrate renal and hepatic tissues (5, 7) and is presumably also metabolized once it gains access to the cell.

In order to interpret the effects obtained with exogenous cyclic AMP and cyclic GMP in renal tissue, it is essential to understand the nature and distribution of the compounds produced from the metabolism of these cyclic nucleotides. Currently the metabolic fate of cyclic AMP and cyclic GMP in renal tissue is not known.

This communication describes and compares the handling and metabolic products of exogenous cyclic AMP and cyclic GMP in the isolated perfused rat kidney and the intact rat.

EXPERIMENTAL PROCEDURES

Materials—Unless otherwise indicated all nucleotides, nucleosides, purine bases, and enzymes were purchased from Boehringer Mann-
studies to prevent possible interference with nucleotide transport and described (9, 10). Unless indicated otherwise, kidneys were recirculated with uricase (8).

G-10, DEAE-Sephadex A-25, and blue dextran 2000 were from Sigma Chemical Co. Cyclic XMP were purchased from Sigma Chemical Co. Uricase was from Armour Pharmaceutical Co. Sephadex and its antibody were obtained from Schwarz/Mann. Cyclic

The radioactive content of tissues, urine, and plasma was determined by techniques previously described (4). Radioactivity was counted by liquid scintillation in Phase Combining System (Amersham/Searle) at an efficiency of 85% for $^{3}H$. Corrections were made for quenching by $^{3}H$.

Kidney Perfusion—Nonfasted male Charles River rats (350 to 450 g body weight) were used throughout these studies. Perfusion medium was prepared and kidneys were perfused by techniques previously described (9, 10). Unless indicated otherwise, kidneys were recirculated with 50 ml of perfusion medium consisting of Krebs-Henseleit bicarbonate buffer and 5 g of Fraction V bovine serum albumin per 100 ml. The antibiotics penicillin G and streptomycin, routinely employed in the perfusion medium in this laboratory, were omitted from these studies to prevent possible interference with nucleotide transport and metabolism. Other chemicals, when used, were present in the perfusion medium from the beginning of the perfusion and included insulin (400 $\mu$g/ml), cyclic AMP (0.1 mM) and cyclic GMP (0.1 mM). In some experiments additional cyclic AMP or cyclic GMP (2 or 5 mM, respectively) was infused directly into the perfusate oxygenation chamber of the perfusion apparatus at a rate sufficient to maintain the perfusate concentration at about 0.1 mM. Cyclic nucleotides labeled with $^{3}H$ were used to study metabolite formation. Previous experience with $^{3}H$-labeled cyclic AMP injected intravenously into rats revealed a significant production of $H_{2}O$, making this a poor choice of label for studying metabolite formation. Cyclic $[^{3}H]AMP$ and cyclic $[^{14}C]GMP$ were purified before use on columns (15 x 0.6 cm) of Dowex 50WX8 eluted with 0.1 N HCl. Kidneys were perfused with 0.1 mM (i.e. 5 pmol and 2 to 3 pCi) cyclic nucleotide. Urine samples were collected directly into preweighed tubes containing 1 ml of 1.7 M perchloric acid. Perfusate samples, taken at the beginning and end of all perfusions and at various times throughout the perfusion, were immediately acidified with an equal volume of 1.7 M perchloric acid. At the end of the experiment, the kidneys, while still being perfused, were frozen between aluminum blocks cooled in Dry Ice (11).

Intact Rat Studies—Rats were anesthetized with pentobarbital, the right carotid artery and left jugular vein were cannulated with PE 50 tubing (Clay Adams) and the bladder was catheterized with PE 90 tubing (Clay Adams). After an intravenous priming dose of 5 ml of 280 mm mannitol, 3.3 $\mu$mol and 2 to 4 $\mu$Ci of $[^{14}C]$-labeled cyclic nucleotide in 0.9 ml of 150 mM saline (0.9% NaCl solution) were rapidly injected into the vein and washed in with a further 1 ml of the saline. The amount of cyclic nucleotide was chosen to approximate 0.1 to 0.2 mM in the plasma assuming complete mixing. The mannitol solution was infused at 0.2 ml/min for the duration (60 min) of the experiment. Mannitol diuresis was maintained since normal urine flow was too low for an accurate estimate of urinary contents. Two consecutive 30-min urine collections were made into preweighed tubes containing 1 ml of 1.7 M perchloric acid. Likewise, approximately 1 ml of arterial blood was taken at 30 and 60 min after the isotope injection and added to similar preweighed tubes. Blood samples (0.2 ml) were also taken at intervals and centrifuged in heparin-treated tubes to obtain plasma. At the end of the experiment, 60 min after the isotope injection, one kidney and one liver lobe were rapidly excised and frozen in acetone/Dry Ice for the subsequent analysis of $[^{14}C]$-metabolites. The second kidney was excised and dissected into cortex and papillary section of the inner medulla before freezing in acetone/Dry Ice. A second liver lobe and other tissues were also removed and frozen in acetone/Dry Ice. The radioactive content of tissues, urine, and plasma was determined by liquid scintillation in Phase Combining System (Amersham/Searle) at an efficiency of 85% for $[^{14}C]$. Corrections were made for quenching by adding $[^{14}C]$-lucene to the scintillation vial and recounting.

Analyses—The analysis of cyclic AMP by a modified protein binding assay has been described (6). Cyclic GMP was measured by the method of Stein et al. (12) which was modified in that the antibody, succinyl cyclic GMP $[^{14}C]$iodotyrosine methyl ester complex, was added to Millipore filter (0.45 pm) and washed twice with 4 ml of 50 mM sodium acetate/15 mM sodium azide (pH 6.2) at 0$\circ$C in order to remove the excess unbound $[^{14}C]$-labeled derivative. Perchloric acid extracts of perfusate and urine were titrated to pH 4 to 6 with 10 M KOH and assayed for cyclic nucleotide without further purification.

Inulin was measured by the method of Heyrovsky (13) in perchloric acid extracts of perfusate and urine. During the course of these studies creatinine present in the perfusate at 13 mM was found to have significant effects on the handling of cyclic AMP by the kidney. Thus, inulin was routinely employed as a measure of the glomerular filtration rate in these studies.

For the study of $[^{14}C]$ metabolites in kidney and liver, the powdered frozen tissues were extracted into 2 volumes of 1.7 M perchloric acid at 0$\circ$C, the 4000 x g (20 min) pellet was re-extracted with 0.8 volume of 1.7 M perchloric acid, and the supernatants were pooled. The perchloric acid-insoluble pellet (containing RNA and DNA) contained less than 4% per kidney or liver of the administered radioactivity. The nature of the radioactivity in this pellet was not further studied. $[^{14}C]$-labeled cyclic nucleotides and their $[^{14}C]$ metabolites were identified in perchloric acid-soluble extracts by separation on columns of Sephadex G-10 and DEAE-Sephadex A 50. Purine bases and purine nucleosides were separated on columns (120 x 1.0 or 120 x 1.2 cm) of Sephadex G-10, eluted with 50 mM sodium phosphate/15 mM sodium azide (pH 7.0) as described by Sweetman and Nyland (14). Values for the void volume ($V_{o}$) and the void volume plus internal volume ($V_{v} + V_{i}$) are shown as $V_{i}$. Added...
Renal Metabolism of Exogenous Cyclic Nucleotides

TABLE I
Excretion and metabolism of exogenous cyclic nucleotides by isolated perfused rat kidney

| Cyclic nucleotide present in perfusate | Number of kidneys | Total cyclic nucleotide in perfusate | Infused \(^a\) cyclic nucleotide | Disposition of cyclic nucleotide from perfusate | \(\text{filtered}^{b} \) | Excreted \(^{c}\) | Secreted \(^d\) | Metabolized \(^d\) | Excreted \(^e\) |
|----------------------------------------|------------------|--------------------------------------|-----------------------------|------------------------------------------|---------|-----------|-----------|-----------|---------|
| Cyclic AMP 3                           |                  | 3640                                  | 6500\(^{f}\)                  | 10400                                    | 863     | 1580\(^{f}\) | 71\(^f\)  | 5880\(^f\) | 1.8\(^f\) |
| Cyclic GMP 2                           |                  | 4010                                  | 8800                        | 9940                                     | 480     | 3690      | 3210      | 6170      | 7.7     |

\(\text{nmol / 15 min / kidney}\)

\(^a\) Total quantity infused into the perfusate over 15 min.

\(^b\) Glomerular filtration rate estimated from the clearance of inulin.

\(^c\) Calculated as total loss from perfusate minus total appearing in urine. Intracellular accumulation of cyclic nucleotide is presumed not to influence this calculation since the perfusate concentration and hence, intracellular space were approximating a steady state.

\(^d\) Total cyclic nucleotide present in a perfusate volume of 50 ml.

\(^e\) Significantly different from values obtained with cyclic GMP, \(P < 0.01\).

RESULTS

Handling of Cyclic AMP and Cyclic GMP by Isolated Perfused Rat Kidney—Cyclic AMP or cyclic GMP, each present in the perfusate at an initial concentration of 0.1 \(\text{mM}\) and total content of 5 \(\text{nmol}\), were rapidly removed from the perfusate (Fig. 1, \(\text{upper panel}\)). A net transtubular secretion of both cyclic AMP and cyclic GMP was apparent from the greater amount of cyclic nucleotide excreted than filtered (Fig. 1, \(\text{lower panel}\)). Moreover, cyclic GMP was more extensively secreted than was cyclic AMP. In order to obtain more accurate and comparable data for the net secretion and metabolism of these cyclic nucleotides by the perfused rat kidney, cyclic nucleotide was infused into the perfusion medium to maintain the perfusate concentration at about 0.1 \(\text{mM}\) throughout the perfusion. The results of such experiments are shown in Fig. 1 and Table I. Cyclic GMP was metabolized at about the same rate as cyclic AMP (about 400 \(\text{nmol/min/kidney}\) but was excreted at more than 2-fold and secreted at more than 4-fold the corresponding rates for cyclic AMP (Table I). The higher rate of net transtubular secretion of cyclic GMP compared to cyclic AMP (about 200 versus 80 \(\text{nmol/min/kidney}\)) is probably caused by the more rapid disappearance of cyclic GMP from the perfusate.

Production and Distribution of \(^{14}\text{C}\)-Metabolites of Exogenous Cyclic \(^{14}\text{C}\text{AMP}\) in Isolated Perfused Rat Kidney—Fig. 2 shows the time course for the removal of cyclic \(^{14}\text{C}\text{AMP}\) from, and the appearance of \(^{14}\text{C}\)-metabolites in the perfusate and the simultaneous accumulation of cyclic \(^{14}\text{C}\text{AMP}\) and \(^{14}\text{C}\)-metabolites in the urine. \(^{14}\text{C}\text{AMP}\) was the principal metabolite found extrarenally, and it accumulated more rapidly in the perfusate than in the urine. Table II shows the results for the \(^{14}\text{C}\)-metabolite formation expressed as a percentage of the total administered radioactivity (counts per min). In experiments with the perfused rat kidney, the total renal content of \(^{14}\text{C}\) was estimated from the total weight of kidney, liver, and blood contents of \(^{14}\text{C}\) were estimated from the organ/body weight ratios for bile rate (19) and assuming a plasma volume of 4.5 ml/100 g of body weight or a whole blood volume of 7.5 ml/100 g of body weight.

Unless indicated otherwise, results are expressed as the mean ± S.E. The statistical significance of results was determined with Student's t test.

\(^{5}\) Secretion is the cyclic nucleotide excreted in excess of that amount filtered by the kidney. Thus a ratio greater than 1.0 for cyclic nucleotide excreted/cyclic nucleotide filtered indicates net secretion. In Fig. 1, the ratio was consistently lower than 1.0 when infinite cyclic nucleotide curves were compared to the respective noninfused cyclic nucleotide curves.
about 1 pmol of the perfusate cyclic AMP was lost in the urine contents about 2-fold over control values but to have no effect on perfusate was sufficient to raise the renal ATP and ADP, respectively. The latter increases are in close agreement with the amount of cyclic AMP incorporated into renal ATP and ADP after perfusion with 5 amol of cyclic [3H]AMP (Table II). This suggests that the adenine moiety of the cyclic nucleotide was utilized in the synthesis of extra ATP and ADP in addition to being incorporated into the pre-existing ATP, ADP and AMP pools by their normal turnover. The extent of the latter incorporation was demonstrated by a specific radioactivity of the renal adenine nucleotide pool (ATP, ADP plus AMP) which was about one-half of that of the original cyclic [3H]AMP added to the perfusate (data not shown).

Production and Distribution of [3H]-Metabolites of Exogenous Cyclic [3H]GMP in Isolated Perfused Rat Kidney—Fig. 3 shows the time course for cyclic [3H]GMP and its [3H]-metabolites disappearing from and appearing in the perfusate and urine during a kidney perfusion. Table IV shows the distribution of these [3H]-labeled compounds in kidney, final perfusate, and urine (10 to 60 min) after 60 min of perfusion. The total radioactivity was about equally distributed between kidney, final perfusate, and urine. In these experiments and analogous to the experiments with cyclic [3H]AMP, renal tissue radioactivity was found mostly as [3H]purine nucleotide (GTP, GDP, and GMP); perfusate radioactivity was principally as [3H]uric acid, whereas urinary (10 to 60 min) radioactivity was principally as unchanged cyclic [3H]GMP. In one perfused kidney, urine was collected throughout the perfusion (i.e. 0 to 60 min) and this resulted in a 1.6-fold more cyclic [3H]GMP appearing in the urine and 0.6-fold less [3H]urate in the final perfusate. However, the renal content and metabolite distribution of radioactivity were unaltered (data not shown).

Production of Uric Acid from Exogenous Cyclic AMP or Cyclic GMP in Isolated Perfused Rat Kidney—Because of the large metabolism of cyclic [3H]AMP or cyclic [3H]GMP to [3H]uric acid (Tables II and IV), it was of interest to determine whether these cyclic nucleotides replaced the endogenous substrates for nephrogenic uric acid, or whether they were additive to the endogenous synthesis. Table V demonstrates the latter postulate to be correct. The addition of 5 pmol of cyclic AMP or cyclic GMP to the perfused increased nephrogenic uric acid 2- to 3-fold. The increment in uric acid synthesis showed a very close agreement with the amount of [3H]-labeled cyclic nucleotide incorporated into [3H]uric acid (Table V). When urine was allowed to recirculate with the perfusate (i.e. was not collected), the increment in nephrogenic uric acid could account for up to 3 pmol of the 5 pmol of cyclic nucleotide originally added to the perfusate (Table V).

Production and Distribution of [3H]-Metabolites of Exogenous Cyclic [3H]AMP or Cyclic [3H]GMP in Intact Rat—Fig. 4 shows the multieponential disappearance curves for plasma radioactivity following the injection of cyclic [3H]AMP or cyclic [3H]GMP into rats. By inspection of these curves it is apparent that the plasma clearance of radioactivity is qualitatively similar for both cyclic nucleotides. In Table VI is shown the accumulation of radioactivity in various tissues relative to the plasma, taken 60 min after the [3H] isotope injection. The radioactivity from both [3H]-labeled cyclic nucleotides was
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**Production and distribution of \(^{14}\text{C}\)-metabolites of cyclic [\(^{8}\text{H}\text{C}\)]AMP in isolated perfused rat kidney**

Data are from two kidneys as described in legend to Fig. 2. After 60 min of perfusion, the kidneys were clamp-frozen and extracted into perchloric acid. Perchloric acid-soluble extracts of kidney, urine and final perfusate were fractionated and analyzed as described under "Experimental Procedures."

| Total \(^{14}\text{C}\)-compounds | Tissue distribution of \(^{14}\text{C}\)-compounds |
|-----------------------------|----------------------------------|
| per cent of total \(^{14}\text{C}\) added to perfusate at zero-time | kidney | perfusate | total urinary excretion (10 - 60 min) |
| Unchanged cyclic AMP | 17.0, 17.5 | 0, 0 | 0, 0 | 17.0, 17.5 |
| Uric acid | 37.3, 37.3 | 14.7, 14.7 | 28.7, 28.7 | 77, 77 |
| ATP | 20.4, 19.9 | 20.4, 19.9 | 0, 0 | 0, 0 |
| ADP | 5.7, 6.5 | 5.7, 6.5 | 0, 0 | 0, 0 |
| AMP | 2.6, 2.4 | 2.6, 2.4 | 0, 0 | 0, 0 |
| Inosine/allantoin \(^a\) | 1.5, 2.9 | 0, 0.5 | 1.0, 1.8 | 0.5, 0.6 |
| Xanthine | 1.4, 1.0 | 0, 0 | 0, 0 | 1.4, 1.0 |
| Hypoxanthine | 0.1, 0.6 | 0, 0 | 0, 0 | 0.1, 0.6 |
| Total \(^{14}\text{C}\)-compounds identified | 86.0, 88.1 | 30.1, 30.5 | 29.7, 29.1 | 26.2, 28.5 |
| Total \(^{14}\text{C}\) recovered | 94.2, 94.4 | 32.0, 32.9 | 34.3, 31.9 | 27.9, 29.6 |

\(^a\) Inosine and allantoin co-chromatographed on Sephadex G-10. The \(^{14}\text{C}\) metabolite located in this position was not further fractionated.

**Table III**

**Effect of exogenous cyclic AMP on renal concentration of adenine nucleotides**

Isolated rat kidneys were perfused for 60 min. Cyclic AMP, when present, was at an initial concentration of 0.1 mM in 50 ml of perfusate. Urine was either allowed to recirculate with the perfusate or was collected throughout the 60 min of perfusion. After 60 min, kidneys were clamp frozen whilst still being perfused and frozen kidney powder was extracted into perchloric acid. ATP, ADP, and AMP were analyzed in the perchloric acid-soluble extract. Data are presented as the mean ± S.E.

| Cyclic AMP present in perfusate | Urine recirculated with perfusate | Number of kidneys | ATP \(\mu\text{mol} / \text{g dry weight kidney}\) | ADP | AMP |
|-------------------------------|---------------------------------|------------------|-------------------|------|------|
|                              |                                 |                  |                   |      |      |
| -                             | ± \(^a\)                        | 4                | 4.14 ± 0.23       | 2.09 ± 0.18 | 0.49 ± 0.04 |
| +                             | +                               | 3                | 8.92 ± 0.55 \(^b\) | 3.48 ± 0.38 \(^c\) | 0.46 ± 0.10 |
| +                             | -                               | 3                | 6.30 ± 0.44 \(^c\) | 2.42 ± 0.13 | 0.42 ± 0.10 |

\(^a\) Two controls performed with and two controls performed without urine collection exhibited no difference in their adenine nucleotide contents and therefore these data were pooled.

\(^b\) Significantly different from respective control value (P<0.001).

\(^c\) Significantly different from respective control value (P<0.02).

most extensively concentrated in the kidney (principally the cortex) and, to a lesser extent, the liver. Similar results have been obtained previously with cyclic [\(^{3}\text{H}\)AMP (4). A small amount of the radioactivity from cyclic [\(^{8}\text{H}\text{C}\)]AMP was found to accumulate in the heart, small intestine, and lung. In comparison to the radioactivity from cyclic [\(^{14}\text{C}\)]AMP, radioactivity from cyclic [\(^{14}\text{C}\)]GMP accumulated less in the kidney but to an equal extent in the liver. No significant accumulation of radioactivity from cyclic [\(^{14}\text{C}\)]GMP was found in the other tissues examined.

Tables VII and VIII show the chemical nature of the radioactivity in whole blood, urine, liver, and kidney, 60 min after the injection of cyclic [\(^{14}\text{C}\)]AMP or cyclic [\(^{14}\text{C}\)]GMP. Two-thirds of the total radioactivity injected as cyclic [\(^{14}\text{C}\)]AMP could be recovered in the sum of urine (0 to 60 min), kidneys, and liver. The renal and hepatic radioactivity was
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The majority of "C-labeled cyclic nucleotides entering the renal cell is metabolized. An estimated rate of metabolism of 400 nmol/min/kidney for both cyclic nucleotides (Table I) was about half the reported cyclic AMP phosphodiesterase activity for rat kidney cortex (24). Since, for reasons previously described (above), the extracellular metabolism of cyclic nucleotides was assumed to be negligible, then it is probable that the "C-uric acid found in the perfusate and urine during perfusion of the rat kidney with cyclic ["C]AMP or cyclic ["C]GMP (Figs. 2 and 3, and Tables II and IV). The characteristics of cyclic nucleotide flux into the kidney have not been extensively studied. It is possible that renal cyclic AMP influx may occur at the peritubular boundary by an organic acid transport system since cyclic AMP transtubular transport is inhibited by substances such as probenecid (6), and cyclic AMP competes with the renal transport of paraaminobenzoate (22). In at least two species, rat (Table VI) and dog (23), the kidney cortex appears to be the principal tissue involved in the clearance (and metabolism) of exogenous cyclic AMP and cyclic GMP. Moreover, in both species, exogenous cyclic GMP was more extensively secreted into the urine than exogenous cyclic AMP (5), whereas the rat kidney exhibited identical rates of metabolism (Table I).

Renal Metabolism of Cyclic Nucleotides—The experiments reported here with the isolated perfused rat kidney confirm previous observations that exogenous cyclic AMP is rapidly cleared and metabolized by the kidney (6), and extend these observations to show that exogenous cyclic GMP is similarly treated by the kidney. Although it has been suggested that cyclic AMP is dephosphorylated prior to entry into the lymphocyte or thyroid cell (20, 21), there is no evidence that this is a necessary prerequisite for cyclic AMP (or cyclic GMP) entry into the renal cell. On the contrary the evidence suggests that intact cyclic nucleotide and not some circulating metabolite penetrates the cell membrane. Thus, in the isolated perfused rat kidney a transtubular secretory process has been demonstrated whereby intact cyclic AMP and cyclic GMP gain access to the urine (Fig. 1, Table I) and, other than ["C]uric acid, no "C-metabolites were found to accumulate in the perfusate during perfusion with ["C]cyclic AMP or ["C]cyclic GMP (Figs. 2 and 3, and Tables II and IV). The characteristics of cyclic nucleotide flux into the kidney have not been extensively studied. It is possible that renal cyclic AMP influx may occur at the peritubular boundary by an organic acid transport system since cyclic AMP transtubular transport is inhibited by substances such as probenecid (6), and cyclic AMP competes with the renal transport of paraaminobenzoate (22). In at least two species, rat (Table VI) and dog (23), the kidney cortex appears to be the principal tissue involved in the clearance (and metabolism) of exogenous cyclic AMP and cyclic GMP. Moreover, in both species, exogenous cyclic GMP was more extensively secreted into the urine than exogenous cyclic AMP (5), whereas the rat kidney exhibited identical rates of metabolism (Table I).

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**Renal Metabolism of Exogenous Cyclic Nucleotides**

**Table IV**

*Production and distribution of $^{14}\text{C}$-metabolites of cyclic [S-$^\text{14}$C]GMP in isolated perfused rat kidney*

Data refer to the experiment described in Fig. 3. After 60 min of perfusion the kidney was clamp-frozen and extracted into perchloric acid. Perchloric acid-soluble extracts of kidney, final perfusate, and urine were fractionated and analyzed as described under "Experimental Procedures."

| Total $^{14}$C-compounds | Kidney | Perfusate | Total urinary excretion (10 - 60 min) |
|--------------------------|--------|-----------|-------------------------------------|
| **Unchanged cyclic GMP** | 29.4   | 0.9       | 28.5                                |
| **Uric acid**            | 35.2   | 1.2       | 28.8                                |
| **GTP**                  | 13.4   | 13.4      | 0                                   |
| **GDP**                  | 3.3    | 3.3       | 0                                   |
| **GMP**                  | 3.6    | 2.3       | 0.8                                 |
| **Inosine/allantoin**    | 1.5    | 0.3       | 1.0                                 |
| **Xanthine**             | 0.9    | 0.1       | 0.8                                 |
| **Total $^{14}$C-compounds identified** | 87.3 | 21.5 | 30.6                              |
| **Total $^{14}$C recovered** | 94.5 | 22.4 | 35.8                              |

*Note:* Inosine and allantoin co-chromatographed on the Sephadex G-10. The $^{14}$C-metabolite located in this position was not further fractionated.

**Table V**

*Effect of exogenous cyclic nucleotides on the renal synthesis of uric acid*

Isolated rat kidneys were perfused for 60 min. Cyclic nucleotides, when present, were at an initial perfusate content of 5 µmol. Urine was either recirculated with perfusate throughout the perfusion or was collected for a part (10 to 60 min) of the perfusion. Uric acid production is expressed as the uric acid present in the final perfusate (at 60 min) plus, where applicable, the urinary excretion of uric acid. The latter accounted for 10 to 30% of total nephrogenic uric acid. The final (60 min) renal content of uric acid contributed only about 5% to the estimated uric production and was not included in these calculations. Results are expressed as the mean ± S.E. or as individual values when n = 2 or 1. Kidney weights before perfusion, based on the weights of the nonperfused contralateral kidney, ranged from 1.4 to 2.0 with a mean ± S.E. of 1.66 ± 0.05 g wet weight (n = 13).

| Cyclic nucleotide present in perfusate | Number of kidneys | Uric acid production | Metabolism of cyclic nucleotide to uric acid *a* |
|---------------------------------------|-------------------|----------------------|-----------------------------------------------|
|                                       |                   | mean increment over control |                          |                            |
|                                       |                   | total                  | rumol / 60 min / kidney |                      |
| **None (control)**                    | 4                 | ± b                   | 1570 ± 150                     | N.M. c                   |
| **Cyclic AMP**                        | 3                 | +                     | 4470 ± 300 2900              | 1870 , 1870              |
| **Cyclic GMP**                        | 2                 | -                     | 2800 , 3060 1810            | 1870 , 1870              |
| **Cyclic GMP**                        | 3                 | +                     | 3220 ± 500 1650             | N.M.                      |
| **Cyclic GMP**                        | 1                 | -                     | 3350 1780 1760             |                            |

*Note:* Calculated from the specific radioactivity of the $^{14}$C-cyclic nucleotide precursor and the radioactivity in the $^{14}$C-uric acid product. See Tables II and IV.

*Note:* Two controls without and two controls with urine collection showed no difference in total uric acid production and these data are pooled.

*Note:* N.M., not measured.
Cyclic [\(^{14}\)C]AMP has been demonstrated in rat liver (30), and excreted in the urine (29). Production of L-allantoin from kidney and that hepatic uricase converts most of this to uric acid but not allantoin was found in both urine and blood after the injection of [\(^{14}\)C]uric acid. It is possible that, in the intact rat, this may be the character of an unidentified biliary [\(^{14}\)C].

In the intact rat, by contrast to the isolated perfused rat kidney, [\(^{14}\)C]uric acid did not accumulate, whereas [\(^{14}\)C]allantoin was a precursor of [\(^{14}\)C]uric acid but not [\(^{14}\)C]allantoin (28). In the studies reported herein, it is known to be about one-tenth the renal ATP.

The extensive metabolism of cyclic AMP and cyclic GMP to metabolite accumulating after perfusion of the rat liver with cyclic [\(^{14}\)C]AMP (31). The metabolism of radioactively labeled cyclic AMP in other tissues such as rat liver slices (30), toad bladder (32, 33), and bovine thyroid cells (21) has produced a considerable significance with regard to the effects of exogenous cyclic AMP in the studies reported herein on the perfused rat kidney and bladder (32, 33), and bovine thyroid cells (21) has produced a considerable significance with regard to the effects of exogenous cyclic AMP (35). The mechanism for exogenous cyclic AMP-stimulated thymidine transport could, therefore, be via the synthesis of extra ATP. Likewise, other endocrine renal processes such as gluconeogenesis (36) may be stimulated by exogenous cyclic AMP due to an increase in the production of renal ATP.

Although the renal GTP pool was not measured in the studies reported herein, it is known to be about one-tenth the size of the renal ATP pool (37), and it seems probable that some renal effects of exogenous cyclic GMP might be mediated by alterations in the production of renal GTP.

### TABLE VI

| Tissue                               | Cyclic [\(^{14}\)C]AMP | Cyclic [\(^{14}\)C]GMP |
|--------------------------------------|------------------------|------------------------|
| Kidney (whole)                       | 218.0 ± 41.1           | 68.5, 119.0            |
| Kidney (cortex)                      | 277.0 ± 44.1           | 106.0, 191.0           |
| Kidney (inner medulla)               | 23.0                   | 4.2, 6.2               |
| Liver                                | 265.5 ± 3.3            | 19.9, 38.6             |
| Heart                                | 24.4 ± 1.3             | 0.5, 1.1               |
| Brain                                | 0.1 ± 0.1              | 0.2, 0.1               |
| Adipose                              | 0.4 ± 0.2              | 0.2, 0.0               |
| Lung                                 | 3.3 ± 1.2              | 0.6, 1.6               |
| Skeletal muscle                      | 0.3 ± 0.1              | 0.3, 0.2               |
| Spleen                               | 1.2 ± 0.1              | 0.6, 1.0               |
| Skin                                 | 0.4 ± 0.2              | 0.3, 1.2               |
| Testis                               | 0.6 ± 0.5              | 0.3, 0.5               |
| Small intestine                      | 2.1 ± 0.3              | 0.8, 1.3               |

*Mean ± S.E. from three animals except kidney (inner medulla) which is the result from a single animal.

*Individual values from two animals.

*Values for counts per min/ml in the final plasma are indicated in Fig. 4.
### TABLE VII

Production and distribution of \(^{14}\)C-metabolites of exogenous cyclic \([8-^{14}\)C]AMP in intact rat

Rats were injected intravenously with 3.3 amol of cyclic \([8-^{14}\)C]AMP (6.9 \(\times\) 10^8 cpm). For the next 60 min mannitol was infused intravenously at a rate of 56 amol/min and two consecutive 30-min urine samples were collected directly into 1.7 M perchloric acid. Arterial blood samples were taken 30 and 60 min after the isotope injection and immediately added to 1.7 M perchloric acid. One hour after the injection one kidney and one liver lobe per animal were rapidly excised and frozen in acetone/Dry Ice, pulverized, and extracted into 1.7 M perchloric acid-soluble extracts of urine, blood, kidney and liver were fractionated as described under “Experimental Procedures.” Data from two animals are presented.

|                        | Total \(^{14}\)C-compounds | Liver \(^a\) | Kidney \(^a\) | Blood \(^b\) | Urine (0-30 min) | Urine (30-60 min) |
|------------------------|---------------------------|-------------|--------------|-------------|-----------------|-----------------|
| Unchanged cyclic AMP   | 25.5, 16.2                | 0, 0        | 0, 0         | 0, 0        | 24.3, 15.0      | 1.2, 1.2        |
| Uric acid              | 1.7, 1.3                  | 0, 0        | 0.4, 0       | 0.1, 0      | 0.7, 0.6        | 0.5, 0.7        |
| ATP                    | 8.6, 17.6                 | 5.9, 7.2    | 2.5, 10.2    | 0.2, 0.2    | 0, 0            | 0, 0            |
| ADP                    | 11.6, 12.1                | 4.2, 4.4    | 7.3, 7.6     | 0.1, 0.1    | 0, 0            | 0, 0            |
| AMP                    | 13.5, 5.2                 | 2.1, 1.5    | 11.2, 2.9    | 0.2, 0.6    | 0, 0            | 0, 0            |
| IMP                    | 4.1, 1.2                  | 0.8, 0.1    | 3.3, 1.1     | 0, 0        | 0, 0            | 0, 0            |
| Allantoin \(^c\)       | 6.4, 5.8                  | 1.5, 0.7    | 0.8, 0.4     | 0.5, 0.9    | 1.4, 1.3        | 2.2, 2.5        |
| Xanthine               | 0.6, 0.7                  | 0, 0        | 0.4, 0       | 0, 0        | 0.2, 0.6        | 0, 0.1          |
| Hypoxanthine           | 0.5, 0                    | 0, 0        | 0.4, 0       | 0, 0        | 0.1, 0          | 0, 0            |
| Total \(^{14}\)C-compounds identified | 72.5, 60.1 | 14.5, 13.9 | 26.3, 22.2 | 1.1, 1.8    | 26.7, 17.7      | 3.9, 4.5        |
| Total \(^{14}\)C recovered | 74.8, 62.3 | 14.7, 14.6 | 27.3, 22.6 | 1.1, 1.8    | 27.3, 18.8      | 4.4, 4.5        |

\(^a\) Total tissue weights estimated from organ/body weight data for bled rats (19).

\(^b\) Data for total blood (estimated as 7.5 ml/100 g body weight) obtained 60 min after \([^{14}\)C]cyclic AMP injection. Blood obtained 30 min after injection contained about the same amount of radioactivity (about 2% per animal) with a similar composition of radioactive metabolites, plus a trace (0.5% per animal) of \([^{14}\)C]cyclic AMP.

\(^c\) \([^{14}\)C]allantoin was identified on Sephadex G-10 as described in the "Experimental Procedures".

### TABLE VIII

Production and distribution of \(^{14}\)C-metabolites of exogenous cyclic \([8-^{14}\)C]GMP in intact rat

Rats were injected intravenously with 3.3 amol of cyclic \([8-^{14}\)C]GMP (3.7 \(\times\) 10^8 cpm). The experimental procedure is described in Table VII. The data are from two animals.

|                        | Total \(^{14}\)C-compounds | Liver \(^a\) | Kidney \(^a\) | Blood \(^b\) | Urine (0-30 min) | Urine (30-60 min) |
|------------------------|---------------------------|-------------|--------------|-------------|-----------------|-----------------|
| Unchanged cyclic GMP   | 44.3, 45.8                | 0, 0        | 0, 0         | 0, 0        | 43.2, 44.7      | 1.0, 1.2        |
| Uric acid              | 1.3, 0.1                  | 0, 0        | 0, 0         | 0, 0        | 0.9, 0          | 0.4, 0.1        |
| GTP                    | 13.6, 11.7                | 9.1, 6.8    | 4.0, 4.8     | 0.5, 0.1    | 0, 0            | 0, 0            |
| GDP                    | 10.8, 7.0                 | 5.8, 4.0    | 4.5, 2.9     | 0.5, 0.1    | 0, 0            | 0, 0            |
| CMP                    | 3.8, 3.7                  | 1.8, 1.8    | 1.4, 1.0     | 0, 0        | 0, 0            | 0, 0.3          |
| Allantoin \(^c\)       | 7.3, 7.0                  | 0.7, 0.4    | 0.2, 0.2     | 0.4, 0.5    | 2.7, 2.8        | 3.3, 3.0        |
| Total \(^{14}\)C-compounds identified | 80.2, 53.3 | 17.4, 13.0 | 10.1, 8.9 | 1.4, 0.8    | 46.0, 47.1      | 5.6, 5.9        |
| Total \(^{14}\)C recovered | 81.7, 78.1 | 17.4, 14.0 | 10.1, 9.3 | 1.5, 0.8    | 46.5, 47.3      | 6.2, 6.7        |

\(^a\) Total tissue weights estimated from the organ/body weight ratio for bled rats (19).

\(^b\) Data for total blood (estimated as 7.5 ml/100 g body weight) obtained 60 min after \([^{14}\)C]cyclic GMP injection. Blood obtained 30 min after injection contained about the same amount of radioactivity (about 2% per animal) with a similar composition of radioactive metabolites. No \([^{14}\)C]cyclic GMP was detected in the blood at either time.

\(^c\) \([^{14}\)C]allantoin was identified on Sephadex G-10 as described in the “Experimental Procedures.”
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