Urinary excretion of cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate in malignancy

CARLO GENNARI, GUIDO FRANCINI*, MARCO GALLI, AND FAUSTO LORE

From the Institutes of Medical Pathology and Thoracic Surgery, University of Siena, Italy*

SUMMARY The urinary excretion of cyclic adenosine 3',5'-monophosphate (cAMP), corrected for urinary creatinine, was determined in 177 patients with primary or metastatic tumours and in 149 normal subjects. In 26 patients with malignancy and in 10 control subjects the excretion of cyclic guanosine 3',5'-monophosphate (cGMP) was also evaluated. The urinary cAMP/Cr ratio in human neoplasms of epithelial origin was often significantly lower than normal, irrespective of the extension of malignancy. Surgical resection of the tumour, radiotherapy, or theophylline treatment increased urinary excretion of the nucleotide. In patients with malignancy, intravenous infusion of glucagon failed to produce the degree of elevation of plasma cAMP seen in normal subjects. Urines from patients with malignant neoplasms had low values of cAMP/Cr ratio with increased values of cGMP/Cr ratio. These findings could be the result of systemic alteration in synthesis or breakdown of the nucleotides.

Most of the urinary cyclic adenosine 3',5'-monophosphate (cAMP) is derived from the plasma by glomerular filtration (Broadus *et al.*, 1970). About one-third of the total amount is produced by the kidney (Kaminsky *et al.*, 1970) as a result of parathyroid hormone (PTH) action on the proximal nephron (Chase and Aurbach, 1967, 1968). The evaluation of urinary cyclic AMP corrected for urinary creatinine provides an index of parathyroid activity, which is useful for clinical discrimination between patients with PTH and non-PTH-mediated hypercalcaemia (Dohan *et al.*, 1972; Murad and Pak, 1972; Neelon *et al.*, 1973). An increased urinary cAMP has been found in hypercalcaemic patients who had cancer of the lung or other tumours that secrete a PTH-like peptide (ectopic PTH) (Drezner *et al.*, 1976; Shaw *et al.*, 1977). It has been stated that when patients with malignant lesions and hypercalcaemia have normal urinary cAMP output, the pathogenesis is not PTH-related. In these cases hypercalcaemia is probably due to prostaglandins or other osteolytic factors (Mundy *et al.*, 1974; Seyberth *et al.*, 1975).

Contrary to what might have been expected,

recent studies on hypercalcaemia associated with lung cancer have shown that the urinary cAMP excretion is abnormally low in most patients (Dohan *et al.*, 1972; Francini and Galli, 1977). Therefore we designed a study to compare urinary cAMP excretion in normal subjects and in patients with malignancy.

Methods

A total of 177 patients with primary or metastatic tumours were studied. There were 85 women and 92 men (age range 22-95 (mean 59) years); 122 patients were in hospital and 55 were outpatients. Informed consent was obtained for all studies. The diagnosis was established by histopathological techniques after biopsy or surgical resection of the tumour. Altogether 153 cases of neoplasms were of epithelial origin, including carcinoma of various organs: 61 lung (classified as epidermoid carcinoma in 40 patients, large-cell anaplastic carcinoma in 6, adenocarcinoma in 7, and small-cell in 8), 38 breast (1 male), 10 uterus, 3 ovary, 3 lip, 2 tongue, 2 pharynx, 16 stomach, 3 colon, 6 rectum, 2 pancreas (non-islet), 1 liver, 2 kidney, 1 prostate, 1 testis, and 2 thyroid. Nineteen cases were of mesenchymal

Received for publication 12 January 1978

origin: 5 lymphoma, 4 Hodgkins, 3 reticulosarcoma, 3 osteosarcoma, 2 chondrosarcoma, 1 myosarcoma, and 1 liposarcoma. There were also five cases of astrocytoma.

One hundred and forty-nine patients (84 men and 65 women, aged 22-86 years; mean body surface area 1.69 m^2 , range 1.31 to 2.29 m^2) had not previously received radiotherapy or chemotherapy. Seven patients with carcinoma of the lung were receiving theophylline treatment and 21 (11 breast, 3 uterus, 1 lip, 1 tongue, 5 astrocytoma) radiotherapy at the time they were studied. In six cases of carcinoma of the lung the study was performed before and after a theophylline treatment (0.9 mg/kg/h for three days), in 10 cases (5 lung, 3 breast, 1 pharynx, 1 lymphoma) before and after radiotherapy (15-20 days, 200 rads daily, total dose 3000 to 4000 rads). In 15 cases of carcinoma of the lung the study was repeated one week after surgical resection of the tumour.

In all cases the study included the evaluation of hepatic and renal function by conventional laboratory tests. A reduced hepatic function was ascertained in six patients (1 liver, 2 stomach, 1 colon, 2 pancreas). All patients but three (2 kidney, 1 prostate) had normal renal function, as assessed by serum creatinine concentrations of less than 1.4 mg/dl . In 83 untreated patients (54 lung, 27 breast, 2 kidney) the study included serum calcium evaluation and a complete radiological skeletal survey to determine the presence or absence of bone metastases.

The control group included 149 normal subjects; 55 of them were healthy volunteers (aged 21-35 years), mostly university students, who were on a random diet and unrestricted activity; 94 were hospital inpatients (aged 31-93 years) without symptoms or history of peptic ulcer disease, hypercalcaemia, bone disease, renal stones, chronic renal failure, essential hypertension, or diabetes. The conditions of the 149 normal subjects with reference to age (21 to 93 years), sex (72 men and 77 women), and body surface area (mean 1.70 m^2 , range 1.28 to 2.30 m^2) were similar between groups for comparison with the untreated patients with malignancy.

Twenty-four-hour urines beginning at 0800 hours were collected under refrigeration. At the end of the urine collection periods aliquots of urine were frozen at -20°C until analysed for their content of cAMP and creatinine. A $50\text{-}\mu\text{l}$ aliquot from each sample was assayed in duplicate by the 'cyclic AMP assay kit' of the Radiochemical Centre, Amersham, Bucks, UK. The assay is based on the competition between unlabelled cAMP and a fixed quantity of the tritium-labelled compound for binding to a protein which has a high specificity and affinity for cAMP (Tovey

et al., 1974).

Blood samples for the analysis of serum calcium and creatinine were drawn at 0800 hours after an overnight fast. Urinary creatinine (Cr) was measured by the 'creatinine kine-test' of the Istituto Sclavo, Siena, Italy (normal range $14\text{--}26 \text{ mg/kg/24 h}$). Serum calcium was measured by atomic absorption spectrophotometry (normal range $8.8\text{--}10.5 \text{ mg/dl}$). Urinary excretion of cAMP was expressed as a function of urinary creatinine (micromoles per gram creatinine). The cAMP/Cr ratio is the most frequently used parameter in cyclic nucleotide research; it allows partial correction for changes in glomerular filtration rate and inaccuracies in urine collection; furthermore, urinary creatinine can be considered as an indirect measure of lean body mass (Nistrup Madsen *et al.*, 1976; Shaw *et al.*, 1977).

In 26 untreated patients with malignancy (10 breast, 7 lung, 9 miscellaneous), who showed low values of urinary cAMP, and in 10 normal subjects, the 24-hour urinary excretion of cyclic guanosine 3',5'-monophosphate (cGMP) was measured as well. Urinary cGMP was analysed by the 'cyclic GMP RIA kit' of the Radiochemical Centre, Amersham. The assay is based on the competition between unlabelled cGMP and a fixed quantity of the tritium-labelled compound for binding to an antiserum which has a high specificity and affinity for cGMP. Urinary excretion of cGMP was expressed as cGMP/creatinine ratio ($\mu\text{mol/g}$).

We have also evaluated the response of the adenyl-cyclase system to glucagon by measuring plasma cAMP levels after infusion of glucagon (Glucagon crystalline, Eli Lilly) in six normal subjects and in 3 cases of carcinoma (2 lung and 1 pancreas). The patients were fasting for eight hours before the test. Glucagon, 1 mg , was diluted in 50 ml of isotonic saline and administered intravenously over a 10-minute period. Plasma samples were obtained before and 30, 60, and 90 minutes after glucagon infusion. The concentration of cAMP was determined as described above and expressed as pmol/ml of plasma.

Results

In normal subjects a highly significant negative correlation was found between age and urinary cAMP/Cr ratio (regression line $y = 4.25 - 0.02x$, $r = -0.45$, $P < 0.01$) according to our previous studies (Gennari *et al.*, 1976). There was also a highly significant positive correlation between body surface area and cAMP/Cr ratio (regression line $y = -1.10 + 2.59x$, $r = 0.45$, $P < 0.01$). No correlation was present in the 149 patients with untreated neoplasms (age versus cAMP/Cr: $r = 0.10$; body

Fig. 1 Urinary cAMP/Cr ratio in 149 normal subjects and 177 cases of malignancy (mean $\pm$ standard error of the mean).

surface area versus cAMP/Cr: $r = 0.11$). The mean total urinary cAMP excretion/gram creatinine was significantly higher in control subjects (3.36 ± 0.10 , mean $\pm$ standard error) than in untreated patients with malignancy (2.36 ± 0.10 , $P < 0.001$) (Fig. 1).

The 149 patients with neoplasms were divided into six groups: group I to V included epithelial tumours (I lung, II breast, III uterus, IV gastrointestinal tract, V miscellaneous), group VI included mesenchymal tumours. In all except group VI the mean value of cAMP/Cr ratio was significantly lower than in normal subjects, as shown in the Table.

The cAMP/Cr ratio was significantly higher than normal in the seven patients with malignancy of the lung who were receiving theophylline treatment at the time they were tested (4.35 ± 0.81 , $P < 0.05$) and in the 21 patients on radiotherapy (5.70 ± 0.74 , $P < 0.001$) (Fig. 1).

Table Urinary cAMP/Cr ratio ($\mu\text{mol/g}$) in 149 untreated patients with malignancy

Group	No.	cAMP/Cr (M $\pm$ SE)	P	No. normal (3.36 $\pm$ 0.20)	No. higher > 3.56	No. lower < 3.16
I	54	2.36 $\pm$ 0.16	< 0.001	2 (3.7)	7 (12.9)	45 (83.4)
II	27	1.65 $\pm$ 0.13	< 0.001	1 (3.7)	—	26 (96.3)
III	8	1.87 $\pm$ 0.28	< 0.001	1 (12.5)	—	7 (87.5)
IV	30	2.80 $\pm$ 0.22	< 0.05	4 (13.3)	6 (20.0)	20 (66.7)
V	11	2.11 $\pm$ 0.20	< 0.001	—	—	11 (100.0)
VI	19	3.32 $\pm$ 0.39	ns	3 (15.7)	5 (26.3)	11 (58.0)
Total	149			11 (7.4)	18 (12.1)	120 (80.5)

Percentages are given in parentheses

Both urinary cAMP/Cr and serum calcium were evaluated in 83 untreated cancer patients; 14 of them (16.8%) were hypercalcaemic and six (7.2%) hypocalcaemic. In hypercalcaemia a cAMP/Cr ratio greater than 4.6 (normal mean value + SD) indicates a PTH-mediated hypercalcaemia whereas a cAMP/Cr ratio lower than 2.1 (normal mean value - SD) could indicate a non-PTH-mediated hypercalcaemia. Of the 14 patients with hypercalcaemia, four (2 epidermoid carcinoma, 2 adenocarcinoma of the lung) were in the PTH-mediated area (one patient had bone metastases) and six (2 epidermoid carcinoma, 1 adenocarcinoma of the lung; 2 breast, 1 kidney) fell in the non-PTH-mediated area (all had bone metastases). Of the six hypocalcaemic patients, five (2 small-cell carcinoma of the lung, 3 breast) had low values of urinary cAMP/Cr ratio; none had bone metastases.

A three-day theophylline treatment caused significant increases in cAMP/Cr ratio in all but one patient tested (before— 1.99 ± 0.23 ; after— 3.37 ± 0.39 ; $t = 3.78$, $P < 0.02$) (Fig. 2).

A striking and highly significant increase in urinary cAMP/Cr ratio was observed in all the patients who underwent radiotherapy (before— 1.45 ± 0.15 ; after— 4.34 ± 0.56 ; $t = 6.42$, $P < 0.001$) (Fig. 2).

Surgical resection of lung carcinoma caused a significant increase in cAMP/Cr ratio in 12 out of 15 cases (before— 2.33 ± 0.30 ; after— 3.92 ± 0.44 ; $t = 3.96$, $P < 0.01$) (Fig. 3). After surgical removal of the tumour all patients had a normal plasma calcium level, including the four hypercalcaemic subjects (Fig. 3).

In six normal subjects a brisk increase in plasma cAMP concentration was seen after intravenous administration of glucagon, the normal maximal response being 191 ± 19 pmol/ml. In patients with neoplasms, the maximal plasma concentration was lower than in normal subjects (140, 72, and 50 pmol/ml) (Fig. 4).

The 24-hour urinary excretion of cAMP and cGMP was evaluated in 26 untreated patients with malignancy and in 10 normal subjects (Fig. 5). The cAMP/Cr ratio was subnormal in all our patients (1.20 ± 0.08 in patients with malignancy, $3.47 \pm$

Fig. 2 Urinary cAMP/Cr ratio before and after theophylline treatment (3 days) and radiotherapy (15-20 days). Open squares: mean $\pm$ standard error of the mean.

Fig. 3 Effect of surgical removal of tumour on urinary cAMP/Cr ratio and serum calcium levels in 15 cases of bronchogenic carcinoma of lung (hatched area: normal range).

Fig. 4 Plasma cAMP response to glucagon (GCG: 1 mg intravenously) in six normal subjects and in three patients with malignancy.

Fig. 5 Urinary cAMP/Cr in 10 normal subjects and in 26 patients with malignancy (mean $\pm$ 2 standard deviations: $M \pm 2SD$).

0.11 in normal subjects, $P < 0.001$), whereas the cGMP/Cr ratio was significantly raised (0.79 ± 0.13 in patients with malignancy, 0.37 ± 0.03 in normal subjects, $P < 0.05$).

Discussion

The results of the present study demonstrate that: urinary excretion of cAMP in human neoplasms of epithelial origin is often significantly lower than normal, irrespective of the extension of malignancy; in patients with mesenchymal tumours the cAMP/Cr ratio is normal; the few cases of lung carcinoma with elevated urinary cAMP/Cr ratio also show hypercalcaemia, suggesting an ectopic secretion of PTH;

surgical resection of the tumour frequently results in a rapid and striking increase in urinary cAMP/Cr ratio; in patients with malignancy radiotherapy or short-term treatment with increased doses of theophylline causes a significant rise in cAMP/Cr ratio; in patients with malignancy the intravenous infusion of glucagon raises the plasma level of cAMP only very slightly; in patients with neoplasms with low values of urinary cAMP/Cr ratio the values of urinary cGMP/Cr ratio are higher than normal.

In mammalian tissues cAMP acts as an intracellular mediator for many hormonal activities; some of the intracellular cAMP escapes from its cell of origin into the plasma, so that alterations in the extracellular level reflect the cellular content because of the rapid dynamic equilibrium that exists between the two spaces (Broadus *et al.*, 1971). Many tissue sources, not yet all known, maintain the basal plasma level, thus affecting the excretion of urinary cAMP (Wehmann *et al.*, 1974). With the exception of reduced renal production of the nucleotide due to hypoparathyroidism or renal failure, a decreased urinary excretion of cAMP is due to a reduced supply to plasma from the tissues.

The cellular level of cAMP is the result of the activity of two enzymes—adenylcyclase, which controls its production, and phosphodiesterase, which controls its breakdown. Therefore, in patients with neoplasms two possible mechanisms could account for the reduced urinary excretion of the nucleotide: firstly, a reduced rate of synthesis by adenylycyclase, and, secondly, an increased rate of degradation by phosphodiesterase.

Low cAMP levels have been found in some experimentally induced tumours (Thomas *et al.*, 1973; Hickie *et al.*, 1974). In man, the data available on cAMP levels in malignancy are limited. Recent studies have shown that the cAMP content is significantly lower in adenocarcinoma of the human colon than in the normal adjacent mucosa (DeRuberis *et al.*, 1976). Phosphodiesterase activity was found to be three to five times greater in a normal lung than in a carcinomatous one (Curtis-Prior *et al.*, 1976); this finding is compatible with low concentrations of cAMP in neoplastic tissue (Sheppard, 1972).

However, in view of the widespread presence of the adenylycyclase system, a reduction in the limited area of the neoplasm seems an unlikely explanation of the reduced urinary excretion of cAMP found in malignancy. In fact, if the tumour is small and localised—which is the case in many of the patients

studied—it is difficult to explain a decrease in adenylycyclase reactivity without supposing that the tumour is capable of producing an inhibiting substance.

The hypothesis that the low cAMP urinary excretion found in malignancy is due to the presence of inhibitors produced by the neoplastic tissue is also supported by the fact that a week after surgical removal of the tumour the levels of urinary cAMP/Cr ratio are higher; this increase cannot be ascribed to the operation because it has been shown that high plasma cAMP levels during surgical trauma fall to normal within a few hours (Gill *et al.*, 1975).

Similarly, a significant increase in urinary cAMP/Cr ratio has been observed after radiotherapy; however, we cannot exclude the possibility that this increase may be due to cellular lysis; in fact, research carried out on rabbits has demonstrated a cAMP hyperexcretion after total body gamma irradiation (Thiery *et al.*, 1976).

In normal subjects it has been demonstrated that glucagon increases both plasma and urinary cAMP levels (Broadus *et al.*, 1970; Taylor *et al.*, 1970); the effect on plasma cAMP appears to be mainly the result of increased hepatic release, and the increased urinary excretion appears to be derived from plasma by glomerular filtration (Broadus *et al.*, 1970). The hypothesis of a decreased activation of adenylyl cyclase in neoplasms of epithelial origin is supported by the partial loss of response to glucagon observed in the present study in patients with tumours not involving the hepatic tissue.

Theophylline is the most potent known inhibitor of cAMP phosphodiesterase (Butcher and Sutherland, 1962); it follows that theophylline treatment increases cAMP levels, as it does in some patients with malignancy in our study. This finding suggests that the sensitivity of phosphodiesterase to theophylline is intact in patients with malignancy. Thus it does not seem likely that increased cAMP degradation by phosphodiesterase plays an important part, although this possibility cannot definitely be excluded.

Finally, in our study, patients who showed low values of urinary cAMP/Cr ratio had a higher cGMP/Cr ratio than did normal subjects. Although mechanisms responsible for the increase in cGMP urinary excretion are at present unknown, it is certain that all the urinary cGMP in normal subjects is derived from plasma by glomerular filtration (Broadus *et al.*, 1970). Increased levels of cGMP have been observed in tissues or in the urine of animals with transplantable liver and kidney tumours (Thomas *et al.*, 1973; Murad *et al.*, 1975; Criss and Murad, 1976), and it has been suggested that a reduced tissue cAMP/cGMP ratio might favour

unregulated cellular proliferation (Goldberg *et al.*, 1975).

In conclusion, patients with tumours of epithelial origin excrete significantly lower amounts of cAMP and higher amounts of cGMP than do normal subjects; it is not clear why the urinary excretion of cAMP is normal in patients with mesenchymal tumours. The changes observed in patients with malignancy could result from systemic alteration in the synthesis or degradation of the nucleotides; for cAMP we prefer the hypothesis of a reduced activation of adenylate cyclase.

Obviously further information on cyclic nucleotides metabolism in malignancy is needed before one can begin to draw pathophysiological conclusions.

We gratefully acknowledge the technical help of Mrs A. Avanzati Vattimo and of Mr A. Torricelli. These investigations were supported partly by a grant from the National Research Council CNR 76.01349.04.

References

- Broadus, A. E., Hardman, J. G., Kaminsky, N. I., Ball, J. H., Sutherland, E. W., and Liddle, G. W. (1971). Extracellular cyclic nucleotides. *Annals of the New York Academy of Sciences*, **185**, 50-66.
- Broadus, A. E., Kaminsky, N. I., Hardman, J. G., Sutherland, E. W., and Liddle, G. W. (1970). Kinetic parameters and renal clearance of plasma adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in man, *Journal of Clinical Investigation*, **49**, 2222-2236.
- Butcher, R. W., and Sutherland, E. W. (1962). Adenosine 3',5'-phosphate in biological materials. I. Purification and properties of cyclic 3',5' nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3',5'-phosphate in human urine. *Journal of Biological Chemistry*, **237**, 1244-1250.
- Chase, L. R., and Aurbach, G. D. (1967). Parathyroid function and the renal excretion of 3',5'-adenylic acid. *Proceedings of the National Academy of Sciences of the United States of America*, **58**, 518-525.
- Chase, L. R., and Aurbach, G. D. (1968). Renal adenylyl cyclase: anatomically separate sites for parathyroid hormone and vasopressin. *Science*, **159**, 545-546.
- Criss, W. E., and Murad, F. (1976). Urinary excretion of cyclic guanosine 3',5'-monophosphate and cyclic adenosine 3',5'-monophosphate in rats bearing transplantable liver and kidney tumors. *Cancer Research*, **36**, 1714-1716.
- Curtis-Prior, P. B., Gibbons, J. R. P., and Yin-Ha Chan (1976). Cyclic nucleotide phosphodiesterase activity of human normal and carcinomatous lung tissue. *Lancet*, **2**, 1224-1225.
- DeRubertis, F. R., Chayoth, R., and Field, J. B. (1976). The content and metabolism of cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate in adenocarcinoma of the human colon. *Journal of Clinical Investigation*, **57**, 641-649.
- Dohan, P. H., Yamashita, K., Larsen, P. R., Davis, B., Defetos, L., and Field, J. B. (1972). Evaluation of urinary cyclic 3',5'-adenosine monophosphate excretion in the differential diagnosis of hypercalcemia. *Journal of Clinical Endocrinology and Metabolism*, **35**, 775-784.
- Drezner, M. K., Neelon, F. A., Curtis, H. B., and Lebovitz, H. E. (1976). Renal cyclic adenosine monophosphate: an accurate index of parathyroid function. *Metabolism*, **25**, 1103-1112.
- Francini, G., and Galli, M. (1977). Hypercalcemia and urinary excretion of cyclic AMP in bronchogenic carcinoma. *World Journal of Surgery*, **1**, 527-530.
- Gennari, C., Galli, M., and Montagnani, M. (1976). Urinary cyclic adenosine monophosphate in young adults and elderly subjects. *Journal of Clinical Pathology*, **29**, 69-72.
- Gill, G. V., Prudhoe, K., Cook, D. B., and Latner, A. L. (1975). Effect of surgical trauma on plasma concentrations of cyclic AMP and cortisol. *British Journal of Surgery*, **62**, 441-443.
- Goldberg, M. L., Burke, G. C., and Morris, H. P. (1975). Cyclic AMP and cyclic GMP content and binding in malignancy. *Biochemical and Biophysical Research Communications*, **62**, 320-327.
- Hickie, R. A., Walker, C. M., and Croll, G. A. (1974). Decreased basal cyclic adenosine 3',5'-monophosphate levels in Morris hepatoma 5123 t.c. (h). *Biochemical and Biophysical Research Communications*, **59**, 167-173.
- Kaminsky, N. I., Broadus, A. E., Hardman, J. G., Jones, D. J., Jr., Ball, J. H., Sutherland, E. W., and Liddle, G. W. (1970). Effects of parathyroid hormone on plasma and urinary adenosine 3',5'-monophosphate in man. *Journal of Clinical Investigation*, **49**, 2387-2395.
- Mundy, G. R., Luben, R. A., Raisz, L. G., Oppenheim, J. J., and Buell, D. N. (1974). Bone-resorbing activity in supernatants from lymphoid cell lines. *New England Journal of Medicine*, **290**, 867-871.
- Murad, F., Kimura, H., Hopkins, H. A., Looney, W. B., and Kovacs, C. J. (1975). Increased urinary excretion of cyclic guanosine monophosphate in rats bearing Morris hepatoma 3924A. *Science*, **190**, 58-60.
- Murad, F., and Pak, C. Y. C. (1972). Urinary excretion of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate. *New England Journal of Medicine*, **286**, 1382-1387.
- Neelon, F. A., Drenzer, M., Birch, B. M., and Lebovitz, H. E. (1973). Urinary cyclic adenosine monophosphate as an aid in the diagnosis of hyperparathyroidism. *Lancet*, **1**, 631-634.
- Nistrup Madsen, S., Badawi, I., Shønau Jørgensen, F., Skovsted, L., and Transbøl, I. (1976). Urinary cyclic AMP. *Acta Medica Scandinavica*, **200**, 195-199.
- Seyberth, H. W., Segre, G. V., Morgan, J. L., Sweetman, B. J., Potts, J. T., Jr., and Oates, J. A. (1975). Prostaglandins as mediators of hypercalcemia associated with certain types of cancer. *New England Journal of Medicine*, **293**, 1278-1283.
- Shaw, J. W., Oldham, S. B., Rosoff, L., Bethune, J. E., and Fichman, M. P. (1977). Urinary cyclic AMP

- analyzed as a function of the serum calcium and parathyroid hormone in the differential diagnosis of hypercalcemia. *Journal of Clinical Investigation*, **59**, 14-21.
- Sheppard, J. R. (1972). Difference in the cyclic adenosine 3',5'-monophosphate levels in normal and transformed cells. *Nature New Biology*, **236**, 14-16.
- Taylor, A. L., Davis, B. B., Pawlson, L. G., Josimovich, J. B., and Mintz, D. H. (1970). Factors influencing the urinary excretion of 3',5'-adenosine monophosphate in humans. *Journal of Clinical Endocrinology and Metabolism*, **30**, 316-324.
- Thiery, G., Golse, B., Valle, C., Ducouso, R., and Pasquier, C. (1976). Origine de l'hyperexcrétion urinaire d'AMPc chez l'animal irradié. *Annales de Biologie Clinique*, **34**, 255-258.
- Thomas, E. W., Murad, F., Looney, W. B., and Morris, H. P. (1973). Adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate: concentrations in Morris hepatomas of different growth rates. *Biochimica et Biophysica Acta*, **297**, 564-567.
- Tovey, K. C., Oldham, K. G., and Whelan, J. A. M. (1974). A simple direct assay for cyclic AMP in plasma and other biological samples using an improved competitive protein technique. *Clinica Chimica Acta*, **56**, 221-234.
- Wehmann, R. E., Blonde, L., and Steiner, A. L. (1974). Sources of cyclic nucleotides in plasma. *Journal of Clinical Investigation*, **53**, 173-179.

Requests for reprints to: Professor Carlo Gennari, Institute of Medical Pathology, University of Siena, 53100 Siena, Italy.