

Cyclic Nucleotide Concentrations in Relation to Renal Growth and Hypertrophy

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In rats no consistent change in the concentration of cyclic GMP or cyclic AMP concentration was found in the renal cortex between 2 hours and seven days after unilateral nephrectomy. In regenerating liver tissue, between 2 hours and seven days after removal of one-third of the liver, there were no consistent changes in cyclic GMP concentrations, but cyclic AMP concentrations were higher than in controls. During postnatal growth, no consistent changes occurred in the cyclic GMP concentration of the spleen, the testes, the kidney cortex, the renal papilla, the liver or the ventricle between two and sixty days after birth. Cyclic AMP concentration on the other hand, in all these tissues with the exception of the spleen, was depressed between the twenty-first and fortieth day after birth, i.e., at a period of rapid growth. In the spleen, the concentration of cyclic AMP increased continuously from the second to the fifth day after birth. During renal parenchymal hyperplasia induced by a large intravenous dose of folic acid two days before sacrifice, the concentration of cyclic GMP in renal cortical tissue increased consistently. A model is proposed to explain the different patterns of changes in the cyclic nucleotide concentrations found.

For the past several years, studies have been reported wherein cyclic nucleotides were implicated in the regulation of cell division. In the earliest reports, the predominant findings in cultured cells and tumors were that increased concentrations of cyclic GMP were associated with an increase in mitosis [1], while high levels of cyclic AMP inhibited mitosis [2,3]. In addition, increase in cyclic GMP was accompanied or followed by a decrease in cyclic AMP. As a result, the Yin Yang hypothesis of regulation of cell division was proposed which stated that cyclic GMP stimulated mitosis, whereas cyclic AMP was an inhibitor [4,5].

Other studies, however, indicated that regulation of cell growth by nucleotides is not as simple as presented in the Yin Yang hypothesis. Thus in liver regeneration, it is possible to find an increase in cyclic AMP [6]. In salivary glands, stimulation to divide by β -adrenergic stimulation was shown to be associated with an increase in both cyclic AMP and cyclic GMP [7]. We decided, therefore, to determine if and how the cyclic nucleotides may be involved in renal growth and hypertrophy.

In the original series of studies, male Wistar rats weighing 180-200 g were anaesthetized with ether and the left kidneys were either removed (nephrectomized animals) or manipulated (sham controls). The kidneys which were removed during nephrectomy were weighed immediately and used as unoperated controls. At various times after nephrectomy, the animals were reanaesthetized and the remaining kidney of the nephrectomized animal as well as the right kidney of the sham controls was removed and weighed. Two days after unilateral nephrectomy the remaining kidney showed a 23 percent increase in kidney weight. Immediately after weighing of each

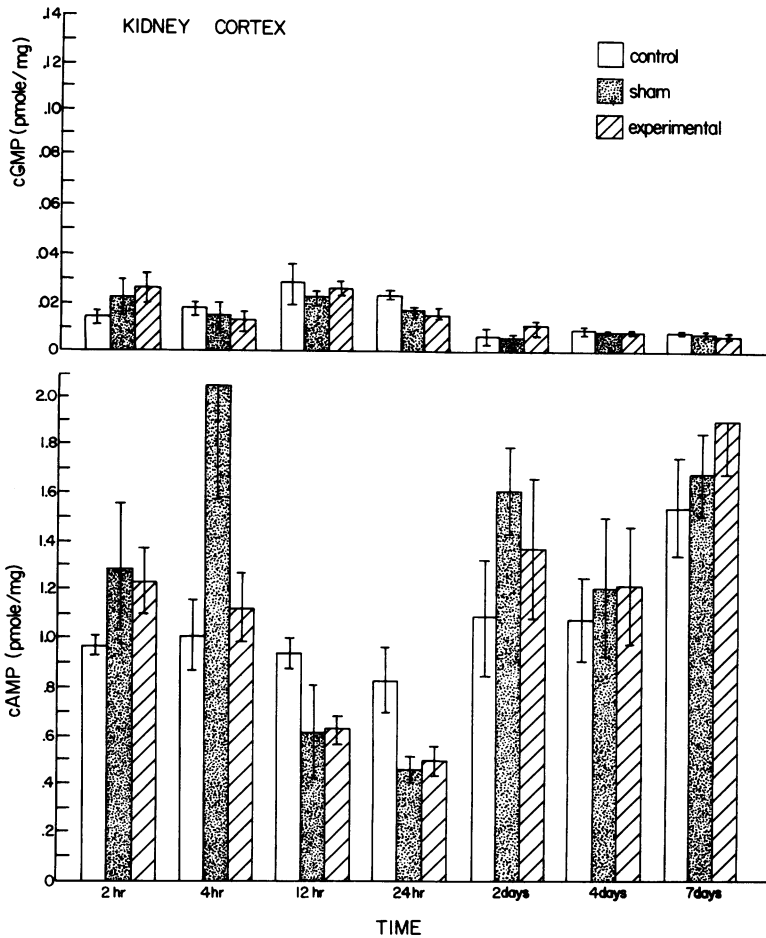


FIG. 1. Cyclic AMP and cyclic GMP levels in kidney cortex at various times after unilateral nephrectomy. Each bar represents the mean \pm SEM of 6 rats. See text for definition of experimental groups.

kidney, pieces of cortex were removed, weighed, frozen in liquid nitrogen and homogenized in ice-cold trichloroacetic acid. Extracts were then analyzed for nucleotide content using radioimmunoassay. Results of these studies are shown in Fig. 1 with the upper panel indicating changes in cyclic GMP, while the lower panel shows the data for cyclic AMP. Although some small variations of nucleotide contents are found at different times, no significant consistent differences between nephrectomized animals and controls for either nucleotide were found. As a result, it was concluded that changes in cyclic nucleotide content were not involved in the response which accompanies renoprival hypertrophy.

At about the time that these studies were being completed, the work of Schlondorff and Weber [8] appeared which indicated that cyclic GMP and cyclic AMP changed during compensatory renal hypertrophy in accordance with the Yin Yang hypothesis. At present the differences in our results are not readily explainable. As a biological control of our studies, it was decided to determine if nucleotide changes in regenerating liver could be found in this laboratory. One lobe of the liver was removed by cautery in hepatectomized animals. The amount removed approximated one-third of

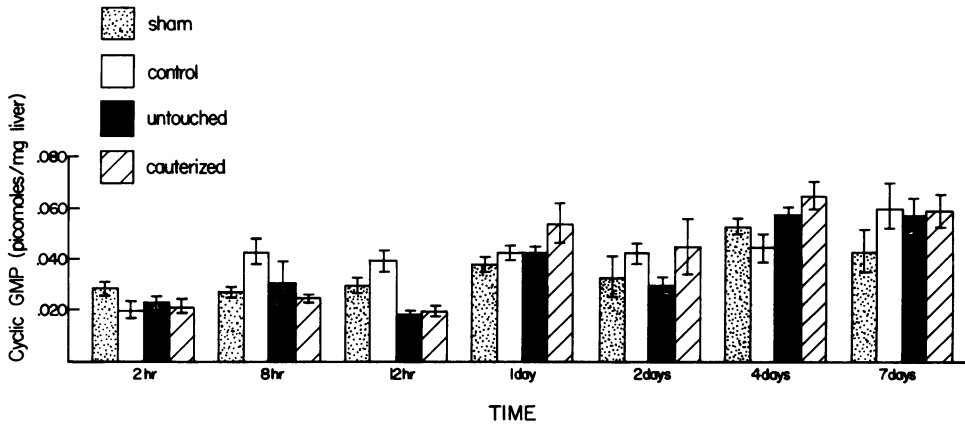


FIG. 2. Cyclic GMP levels in liver at various times after partial hepatectomy. Each bar represents the mean \pm SEM of 6 rats. See text for definition of experimental groups.

the liver. A piece of the removed lobe (as far removed as possible from the cautery site) served as unoperated controls. Pieces of liver from sham-operated animals and hepatectomized animals were sampled at various times after surgery. In the hepatectomized animals, one piece was taken from the regenerating lobe and another from an untouched lobe. The pieces of liver were weighed, frozen and analyzed for nucleotide as described above.

Changes in cyclic GMP are shown in Fig. 2. At twelve hours after partial hepatectomy, a significant reduction ($p < .05$) in cyclic GMP was found in both untouched and cauterized lobes. At no other time were consistent differences found between control tissues and those from hepatectomized animals. There is some tendency for cyclic GMP to increase with longer experimental times for all tissues. We are unable to explain this observation.

In contrast, cyclic AMP concentrations are significantly higher in both the surgically manipulated and untouched sections of the liver one day post-operatively (Fig. 3). Thereafter, cyclic AMP of the untouched lobe returned to control levels while the content of the cauterized lobe remained high. The increase of cyclic AMP preceded an increase in thymidine incorporation (an indicator of mitosis) of regenerating liver by one day (Fig. 4). Removal of the liver caused an 807 percent increase in thymidine incorporation in the remaining portion of the cauterized lobe 48 hours after surgery. The remaining part of the liver not directly affected by the surgical procedure showed a 385 percent increase in thymidine incorporation. The change in the mitotic rate was always greater in the surgically manipulated lobe than in the untouched lobe at all time intervals analyzed. It was also observed that the differences in cyclic AMP between cauterized and untouched lobes paralleled comparable differences in thymidine incorporation. It would therefore appear that an increase in cyclic AMP was associated with the hyperplasia of liver regeneration.

A possible role of the nucleotides in normal tissue growth was also investigated. Various tissues were removed, weighed, and analyzed at weekly intervals between two and sixty days postnatally. No pattern of change in tissue cyclic GMP levels was found (Fig. 5). In all tissues except spleen, cyclic AMP was significantly lower between 23 and 37 days of age than at other times (Fig. 6). In spleen, cyclic AMP continued to increase with age. There was, however, a tendency to plateau during the 23- to 37-day period. In this laboratory, it has been found that there is a period of

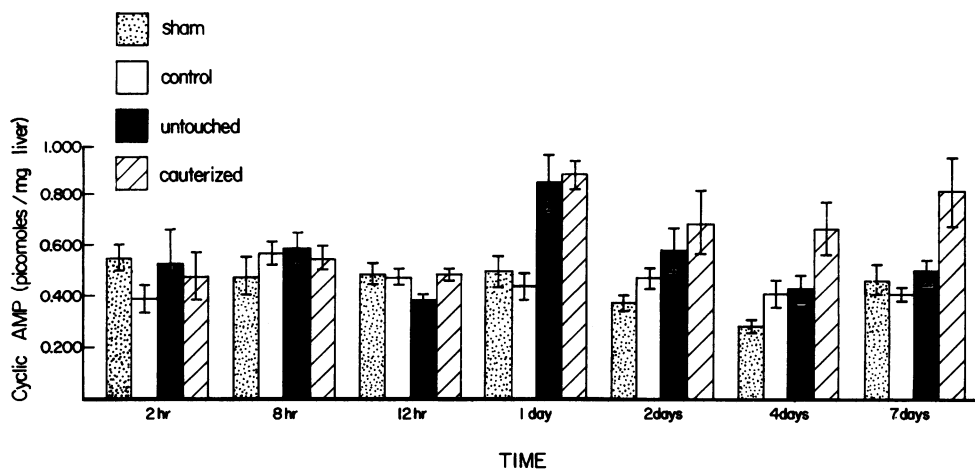


FIG. 3. Cyclic AMP levels in liver at various times after partial hepatectomy. Each bar represents the mean \pm SEM of 6 rats. See text for definition of experimental groups.

rapid growth between weaning (21 days) and 40 days of age. The observed decrease in cyclic AMP coincides then with this period of increased growth and most likely of cell division. These data are consistent with at least part of the Yin Yang hypothesis, i.e., that a decrease in cyclic AMP is associated with increased cell division.

A major difference in the studies on liver regeneration and postnatal growth as compared to renoprival hypertrophy is that, in the latter, kidney growth occurs primarily through an increase in cell size, while in the former studies the response is primarily the result of an increase in cell numbers. It was therefore decided to

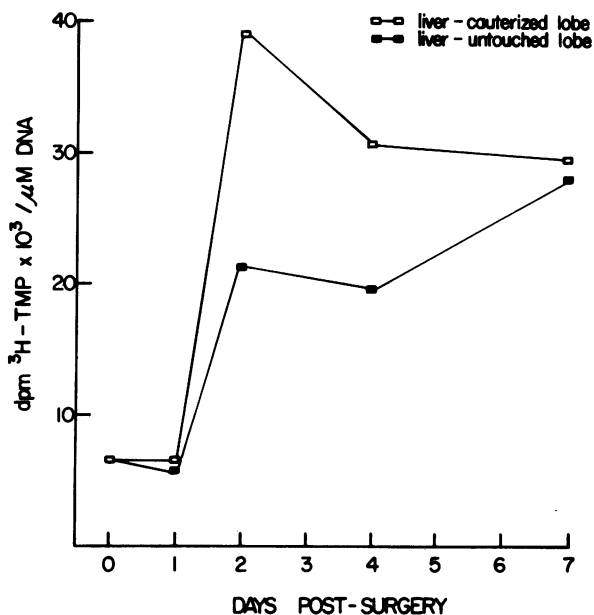


FIG. 4. Changes in thymidine incorporation in liver at various times after partial hepatectomy. Each point represents the mean of 4-6 rats. See text for definition of experimental groups.

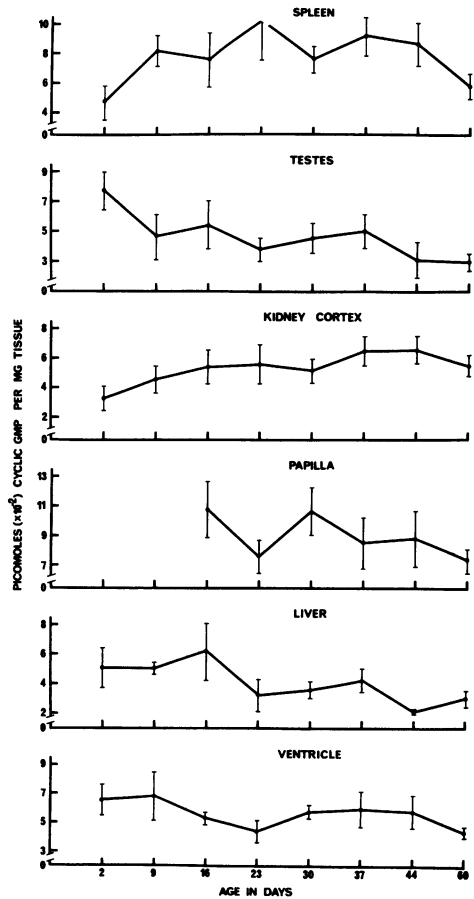


FIG. 5. Tissue levels of cyclic GMP at various days after birth. Each point represents the mean \pm SEM of 4–8 rats.

determine if changes in nucleotide contents could be detected under conditions when renal parenchymal hyperplasia was induced. To this end, rats were administered folic acid (250 mg/kg via the jugular vein) [9]. Two days later, the kidneys were removed and pieces of cortex analyzed for both cyclic AMP and cyclic GMP. In the first series of studies, a significant increase in cyclic AMP and cyclic GMP was found in the folic-treated animals as compared to medium-injected controls (Table 1). Since the absolute levels of cyclic AMP were high in this study as compared to other data obtained in this laboratory, this study was repeated. In the second series, no change was found in cyclic AMP but again a significant increase in cyclic GMP was found.

Consideration of the total literature as well as the results which are reported here indicates that the involvement of cyclic nucleotides in cell division and regeneration is probably not nearly as simple as originally proposed [4]. In fact, it would appear that several types of changes in nucleotide levels may be associated with regulation of cell division, depending on the organ and the specific stimulus under investigation. The problem resolves itself into one of reconciling the different changes in cyclic AMP and cyclic GMP which have been observed. In Fig. 7, a speculative scheme is presented which indicates the possible kinds of interactions which could be considered. It is assumed that a trigger to stimulate cell division can result in an increase in either cyclic AMP, cyclic GMP, or both. It is further hypothesized that some factor

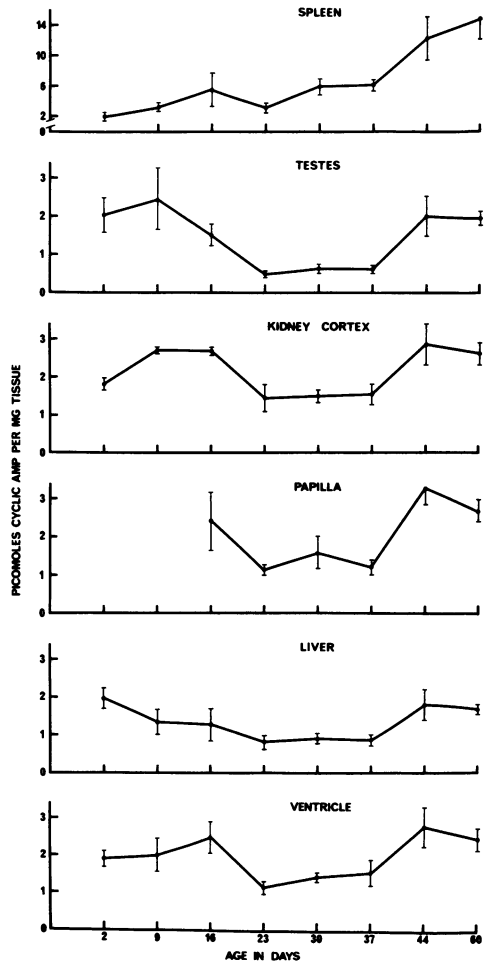
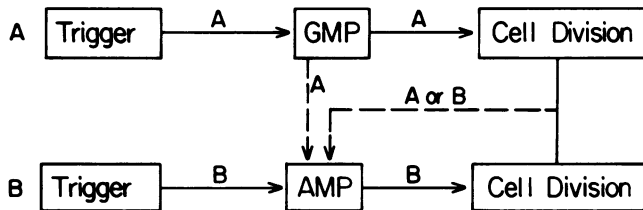


FIG. 6. Tissue levels of cyclic AMP at various days after birth. Each point represents the mean \pm SEM of 4-8 rats.

Speculative scheme of interaction of AMP and GMP in regulating cell division.



A and B represent two alternate pathways depending on stimulus and cell system.
 Solid lines indicate stimulatory action
 Broken lines indicate inhibitory action

FIG. 7. A speculative scheme indicating the possible mechanisms whereby initiation of cell division can result in various kinds of changes in nucleotide content.

TABLE 1
Effect of Folic Acid on Renal cAMP and cGMP Content

	<i>n</i>	Kidney Wt (g)	cAMP (pmole/mg)	cGMP (pmole/mg)
First series control	12	.756 ± .018	4.518 ± .361*	.077 ± .0073*
First series folate	10	1.129 ± .035	6.328 ± .475*	.153 ± .010*
Second series control	8	.971 ± .011	2.643 ± .292	.065 ± .010*
Second series folate	8	1.397 ± .071	2.906 ± .346	.138 ± .036*

n indicates number of kidneys.

*indicates folate treated different from control $p < .05$.

resulting from cell division can feed back and suppress cyclic AMP contents. Another alternative pathway is that cyclic GMP itself may result in a reduction of cyclic AMP concentration possibly by increasing the tissue concentration of phosphodiesterase. Using this scheme, an increase in cell division could be associated with an increase in cyclic GMP and suppression of cyclic AMP (Yin Yang hypothesis) [4,5], an increase in both nucleotides [7], or an increase or decrease in cyclic AMP or cyclic GMP alone [1; this study].

In summary, changes in kidney cyclic nucleotide levels were not found in the remaining kidney following unilateral nephrectomy, a process which results primarily in cellular hypertrophy. When an increase in cellular hyperplasia is observed rather than cellular hypertrophy, changes in nucleotides were detected in both kidney and liver as well as in rapid stages of growth. The changes in nucleotide contents under the various experimental conditions do not follow a consistent pattern but depend on stimulus and tissue under study. A possible model is proposed to explain the differences in the patterns found.

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