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Polyamine metabolism in compensatory renal growth

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Compensatory renal growth, following renal mass extirpation, is accompanied by multiple biochemical alterations including increased nucleic acid, protein, and polyamine synthesis. The aliphatic polyamines-putrescine, spermidine, and spermine are found in most living organisms and appear to participate in many forms of augmented growth including embryonic, regenerative, hormone-induced, and neoplastic [1–3]. While the precise biochemical function of these compounds has not been defined, increased levels of polyamines and their biosynthetic enzymes occur in association with enhanced nucleic acid and protein synthesis in rapidly growing tissues [3–6]. Polyamines apparently contribute to nucleic acid accumulation by promoting biosynthesis and retarding degradation [6–8]. The decarboxylation of ornithine to putrescine is the rate-limiting step in polyamine synthesis. The catalyst, ornithine decarboxylase, is very inducible and has a short half-life in both normal and regenerating liver (10 to 11 min) [5]. Changes in enzyme activity precede or occur simultaneously with increases in RNA, DNA, and protein concentrations [3].

In this study, alterations in renal polyamine synthesis were investigated in conjunction with other evidences of stimulated kidney growth following unilateral nephrectomy. These data are discussed in the context of previous observations regarding polyamine biosynthesis during enhanced renal growth.

Methods

Male Sprague-Dawley rats were maintained at a constant temperature with a light-dark phase of 14 and 10 hr. Free access to rat chow and water was permitted. Rats, 200 to 230 g in weight, were subjected to left unilateral nephrectomy (uni) or sham operation (sham) via a flank incision under ether anesthesia. Immediately after operation, rats received 10 ml of normal saline by gavage to minimize dehydration. The time of surgery varied, but all rats were sacrificed at approximately 9 A.M. The kidneys were rapidly removed and placed in cold saline.

Ornithine decarboxylase activity. Kidney samples were homogenized with a motor driven teflon pestle in a medium containing 0.25 M sucrose, 10 mM Tris buffer (pH 7.5), 0.5 mM EDTA, and 2 mM dithiothreitol. Enzyme activity was determined in the supernatant fraction following centrifugation of the homogenate (20%, w/v) at ×115,000*g* for 60 min. Aliquots of supernate were added to incubation flasks containing 0.2 ml of 0.05 M Tris buffer (pH 7.8), 0.2 μmole of pyridoxal phosphate in 0.3 ml distilled water, and 0.25 μmole DL ornithine and 0.33 μCi 1-ornithine-1-^{14}C (New England Nuclear Corporation, Boston, Massachusetts) in 0.4 ml distilled water. Tris buffer (10 mM, pH 7.5) was added to achieve a final incubation volume of 1.5 ml. Released carbon dioxide was trapped in 0.2 ml soluene, a strongly alkaline solution, contained in a plastic cup suspended above the incubate. After 30 min at 37°C, the reaction was terminated by the addition of 1.0 ml 1 M citric acid. The incubation was continued for another 60 min to insure complete collection of released carbon dioxide in soluene. For β counting, the cups were then placed in liquid scintillation vials containing dioxane as the solvent, PPO was the primary scintillator and dimethyl POPOP as the secondary scintillator.

Polyamine determinations. After separation on a cation exchange phosphocellulose column [10], putrescine, spermidine, and spermine concentrations were determined colorimetrically by the 2,4 dinitrofluorobenzene method described by Dubin [9].

Nucleic acid determinations. DNA was measured by the diphenylamine method and RNA by the orcinol procedure using the method described by Schmidt and Thannhauser [11] for separation of nucleic acids.

In-vitro experiments. To further investigate the early changes in polyamine synthesis induced by growth stimuli, we employed an in vitro system which has provided evidence for a circulating renal growth factor following unilateral nephrectomy [12–17]. In these experiments, we studied the effects of uni and sham plasma (obtained 18 hr postoperatively after uni or sham) upon ornithine decarboxylase activity and [^{3}H] thymidine incorporation into DNA of normal kidney tissue (Fig. 1). Heparinized blood underwent centrifugation in the cold, and the plasma was used promptly.

Renal cortical fragments were obtained from control rats by forcing cortices through a nylon sieve (2 × 1.5 mm) obtained from the framework of a twin coil dialyzer (Travenol Laboratories, Morton Grove, Illinois). Previous investigations have demonstrated that the fragments: *(1)* contain an average of 11 glomeruli, *(2)* appear morphologically normal by electron microscopy after 90 min of incubation, and *(3)* manifest linear incorporation of [^{3}H]-thymidine into DNA over 3 hr [13, 15].

Fragments were incubated at 25°C in a modified Krebs-Ringer solution containing Na^+ 126 mM, K^+ 5 mM, Cl^- 131 mM, phosphate buffer (pH 7.4) 10 mM, uni or sham plasma (10%, v/v) and gassed with 100% O_2. Approximately 2 μCi of methyl-[^{3}H] thymidine (New England Nuclear Corporation) was added at the beginning of incubation to observe the effect of uni or sham plasma on renal DNA synthesis.

After 15-, 30- or 45-min incubation, the ornithine decarboxylase activity of the renal cortical fragments was measured by

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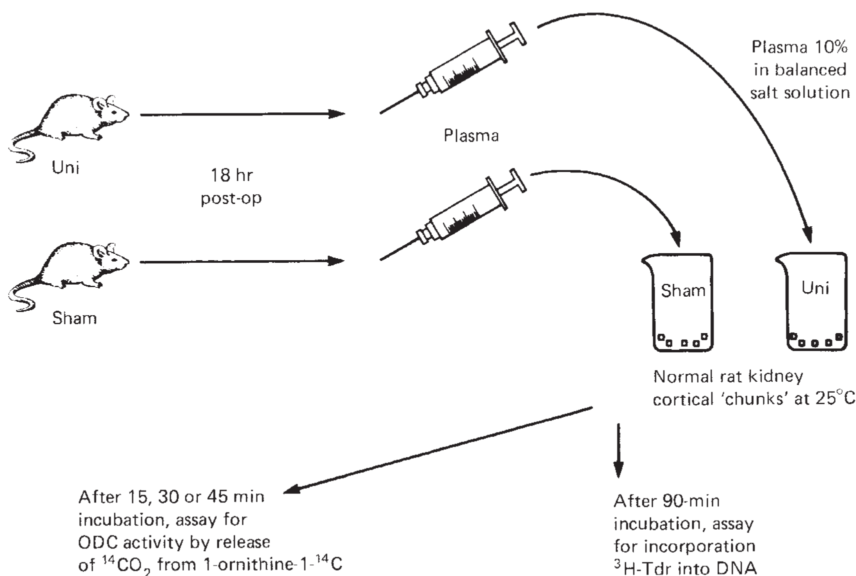

Fig. 1. *In vitro assay.* (See **Methods**).

release of $^{14}CO_2$ from 1-ornithine-1-^{14}C as described above. The incorporation of [^{3}H] thymidine into DNA of renal cortical fragments was determined at the end of a 90-min incubation. DNA was extracted by the method of Fleck and Munro [18] as modified by Halliburton and Thomson [19]. DNA was quantitated by the indole-HCl reaction of Ceriotti [20], modified by using 0.06% indole and 2.5 N HCl as described by Keck [21]. Standard curves were prepared with calf thymus DNA. β counting was performed using dioxane as the solvent, 2,5-diphenyloxazole as the primary scintillator, and 1,4-bis [2-4-(methyl-5-phenyloxazolyl)] benzene as the secondary scintillator. Cab-O-Sil (Packard Instrument Co., Inc., Downers Grove, Illinois) was added to the scintillation mixture resulting in a gel which prevented the DNA from settling out. Results were expressed as the specific activity of DNA in the renal cortical fragments.

Statistics were done by Student's t test using group or paired analysis. Statistical significance was set at $P < 0.05$.

Results

Changes in kidney weight, RNA, and DNA during compensatory renal growth. Three days following surgery, kidney wet weight, and the ratio of kidney weight to body weight were significantly greater for uni than sham-operated animals (Table 1). These differences, indicative of compensatory renal growth following unilateral nephrectomy, persisted over the remainder of the 15 days of observation.

Total renal RNA content increased 10% within 48 hr postunilateral nephrectomy and essentially kept pace with increasing renal mass during compensatory renal growth (Table 1). In contrast, a similar increment in renal DNA content was not apparent until 9 days after uni resulting in decreased renal DNA concentrations following unilateral nephrectomy (Table 1). As a consequence of these alterations, concordant with previous observations [22], the ratio of renal RNA/DNA was significantly greater in uni compared to sham-operated animals — a biochemical manifestation of compensatory renal hypertrophy subsequent to loss of functional renal tissue [19].

Changes in polyamine metabolism during compensatory renal growth. Following renal mass extirpation, ornithine decarboxylase (ODC) activity rose in the remaining kidney as early as 45 min after surgery (Fig. 2). Renal ODC activity peaked 6 to 12 hr following unilateral nephrectomy and remained significantly elevated compared to sham-operated animals for 9 days postoperatively.

Forty-eight hours after surgery, the concentrations of renal putrescine and spermidine (but not spermine) rose in uni compared to sham-operated animals (Fig. 3). The spermidine level gradually declined, while a second increase in renal putrescine was apparent 6 days following unilateral nephrectomy, possibly related to the modest and variable increase in renal ODC activity detected 4 to 6 days postoperatively. Of note, enhanced renal putrescine concentrations were seen 3 days after uni and sham operations, suggesting that factors other than stimuli specific for compensatory renal growth influenced renal polyamine metabolism in the in vivo experiments.

In vitro experiments. In vitro experiments were performed using a system which previously provided evidence for a circulating renal growth factor following unilateral nephrectomy [12–17]. We studied the effect of plasma obtained 18 hr after uni or sham operations upon ODC activity and [^{3}H] thymidine incorporation into DNA of normal kidney tissue (see **Methods**). Uni plasma enhanced ODC activity of renal cortical fragments incubated for 15, 30, and 45 min in a balanced salt solution (Fig. 4). The combined results from 25 paired experiments over 45 min of incubation revealed that uni plasma compared to sham plasma caused greater renal ODC activity in 24 of the experiments, 42.5% ± 9.0 (SEM) T = 4.76, $P < 0.001$.

Another index of growth, the incorporation of [^{3}H] thymidine into renal DNA, was also determined in 19 of these in vitro experiments. In 16 of 19 paired experiments, uni plasma com-

Table 1. Changes in kidney weight, renal nucleic acid and polyamine concentrations after uni and sham[a]

Type of operation	Days after surgery	KW/BW $\times 10^{-4}$	RNA *mg/g*	DNA *mg/g*	RNA/ DNA	Putrescine *μEq/g*	Spermidine *μEq/g*	Spermine *μEq/g*
Uni	1	42	7.311	3.582[b]	2.038	0.26	1.096	2.43
		±2.7	±0.296	±0.09	±0.059	±0.019	±0.149	±0.156
	2	47	7.643	2.515	3.018	0.382	1.30[b]	2.21
		±1.3	±0.315	±0.131	±0.145	±0.111	±0.143	±0.15
	3	50[b]	6.096	2.052[b]	2.962[b]	0.494	0.803	1.61
		±1.4	±0.456	±0.109	±0.126	±0.029	±0.043	±0.115
	5	45[b]	—	—	—	0.301	0.631	1.50
		±1.6				±0.14	±0.181	±0.16
	6	48[b]	6.09	2.057[b]	2.95	0.583[b]	0.663	1.50
		±0.8	±0.536	±0.109	±0.195	±0.125	±0.083	±0.099
	9	48[b]	6.15	1.89	3.245	0.164	0.415	1.94
		±1.8	±0.733	±0.168	±0.196	±0.051	±0.079	±0.077
	12	50[b]	5.468	1.775	3.09	0.204	0.573	1.702
		±1.4	±0.508	±0.20	±0.053	±0.027	±0.143	±0.106
	15	47[b]	5.865	2.32	2.563	0.11	0.169	1.89
		±1.6	±0.281	±0.202	±0.171	±0.01	±0.023	±0.068
Sham	1	41	7.713	3.928	1.96	0.203	1.058	2.455
		±2.3	±0.337	±0.127	±0.076	±0.04	±0.201	±0.182
	2	43	7.008	2.568	2.76	0.152	0.409	2.175
		±1.3	±0.115	±0.127	±0.165	±0.05	±0.062	±0.119
	3	40	6.633	2.642	2.514	0.406	0.695	1.73
		±1.3	±0.548	±0.184	±0.044	±0.086	±0.136	±0.145
	5	37	—	—	—	0.148	0.602	1.29
		±2.0				±0.009	±0.141	±0.082
	6	38	7.372	2.715	2.76	0.196	0.565	1.54
		±1.0	±0.508	±0.098	±0.277	±0.022	±0.059	±0.123
	9	40	5.399	2.02	2.83	0.130	0.444	2.104
		±0.9	±0.276	±0.301	±0.363	±0.022	±0.073	±0.052
	12	40	5.25	1.895	2.86	0.201	0.491	1.831
		±2.0	±0.427	±0.233	±0.189	±0.05	±0.152	±0.213
	15	38	5.53	2.328	2.398	0.144	0.232	2.012
		±1.6	±0.416	±0.191	±0.162	±0.011	±0.061	±0.097

Abbreviations: KW, kidney weight; BW, body weight; Uni, left unilateral nephrectomy; Sham, sham operation.
[a] Values are the means ±SEM.
[b] $P < 0.05$.

pared to sham plasma enhanced this indicator of renal DNA synthesis, 16.2% ± 4.6 (SEM) $P < 0.01$ (Fig. 4).

Discussion

The aliphatic polyamines, putrescine, spermidine, and spermine, are natural constituents of most living organisms and appear to play an important role in growth phenomena. The concentrations of polyamines and their biosynthetic enzymes increase in association with a broad range of growth processes including embryonic, regenerative, neoplastic, and hormone-induced [1, 2]. Micro-organisms have been described in which there is an absolute growth requirement for polyamines [3].

While the exact biochemical function of polyamines is unknown, several lines of evidence suggest that they may be involved in the biosynthesis and accumulation of nucleic acids and proteins [4–8]. During rapid growth, alterations in polyamine synthesis usually precede or coincide with increases in RNA, DNA, and protein concentrations [3]. Although polyamines appear to be preferentially bound to chromatin, nucleoli, and cytoplasmic ribosomes, experiments attempting to discern the intracellular distribution of polyamines are technically difficult and should be interpreted with caution [8]. Nevertheless, polyamines, because of their polycationic nature and conformational flexibility, bind tightly to nucleic acids in vitro [3, 8]. Thus the metabolism and biosynthesis of RNA and DNA may be influenced by the effect of polyamines on their secondary and tertiary structure. Polyamines can stabilize nucleic acids against denaturation and shearing; presumably this effect is due to neutralization of phosphate groups reducing their repulsive action [3]. Furthermore, the effect of polyamines on a broad range of reactions involving RNA and DNA may reflect the impact of polyamines on nucleic acid conformations rather than on the requisite enzymes, although this is unproven. Thus a potential explanation is provided for the diverse in vitro effects of polyamines on DNA and RNA polymerases, methylases, hydrolases, and nucleotidyl transferases as well as on reactions involving tRNA, ribosomal RNA, and mRNA leading to protein synthesis [8].

The prompt enhancement of ODC activity following unilateral nephrectomy [23], partial hepatectomy [24], or hormone administration [25] provides additional circumstantial evidence that polyamines are involved in numerous growth processes. ODC, which catalyzes the decarboxylation of L-ornithine to putrescine, is the rate-controlling enzyme in the biosynthesis of polyamines. It is rapidly inducible and has the shortest half-life (10 to 11 min) ever reported for a mammalian enzyme [5].

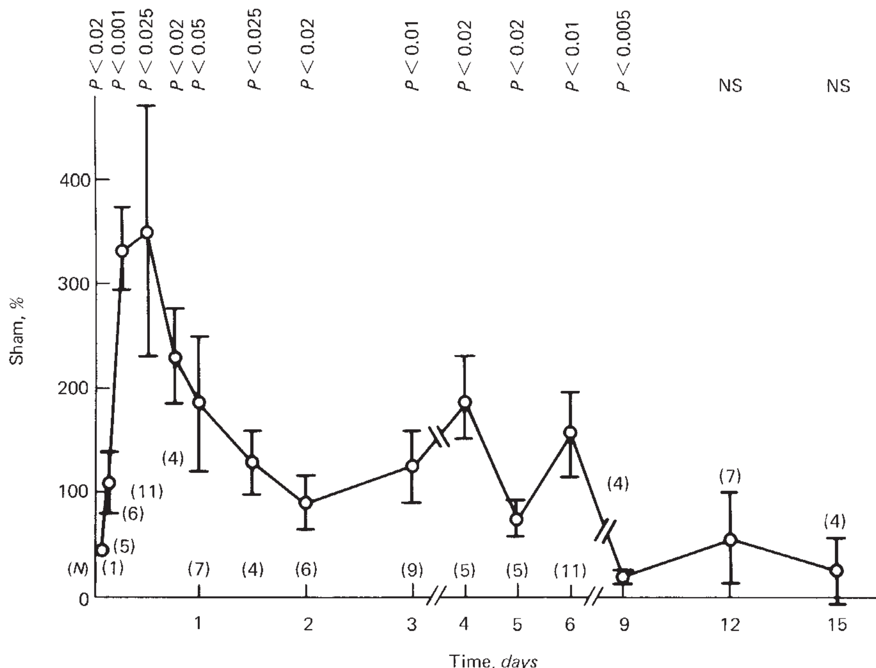

Fig. 2. *Enhanced ornithine decarboxylase (ODC) activity (○) after unilateral nephrectomy versus sham operation.* ODC activity during compensatory renal growth was measured in the ×115,000*g* supernatant fraction of renal tissue by the release of $^{14}CO_2$ from l-ornithine-1-^{14}C during 30-min incubation. Results are expressed as a percentage increase relative to concurrent sham.

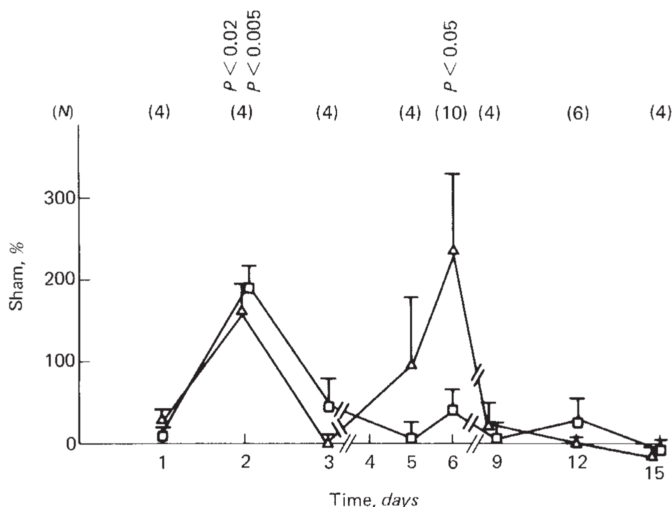

Fig. 3. *Changes in polyamine concentrations after unilateral nephrectomy versus sham operation.* Putrescine (△), spermidine (□), and spermine concentrations were determined colorimetrically with 2,4 dinitrofluorobenzene after separation from renal homogenate on phosphocellulose column. Results are expressed as a percentage increase relative to concurrent sham. Insignificant alterations in spermine levels were detected.

Hence polyamine biosynthesis may be an exquisitely controlled process associated with the onset and perpetuation of various growth phenomena.

During compensatory renal growth following renal mass extirpation, we found ODC activity rose in the remaining kidney as early as 45 min after surgery. Peak ODC activity occurred 6 to 12 hr following unilateral nephrectomy as described previously [23]. A second increase in ODC activity was noted beginning approximately 3 to 4 days postoperatively, somewhat later than the second peak observed by Brandt, Pierce, and Fausto (36 hr after uni) [23]. During prolonged observations of renal ODC activity after surgery, we found significantly elevated levels for 9 days after uni. Concurrently, increased RNA content was detectable in the remaining kidney 2 days after surgery, and a similar increment in renal DNA content was evident 9 days postoperatively.

Alterations in polyamine concentrations followed changes in ODC activity. Within 2 days after surgery, renal putrescine and spermidine rose in uni compared to sham-operated animals commensurate with previous observations [23, 26]. While a subsequent decline in spermidine levels has been consistently observed [23, 26], a secondary rise in putrescine concentration 6 days after uni was detected by Desiderio, Sessa, and Perin [26] and ourselves, but not by Brandt, Pierce, and Fausto [23]. These differences probably reflect the modest and variable nature of enhanced renal ODC activity 4 to 6 days after uni.

Alterations in renal polyamine metabolism have been studied following various growth stimuli including chemically induced kidney growth. Four hours after a single intravenous injection of folic acid, increased ODC activity was noted in rat kidneys, followed by elevated putrescine levels [6]. Enhanced RNA polymerase activity was detected 6 to 12 hr after folic acid treatment suggesting a correlation between renal polyamine and RNA synthesis [6].

Increased ODC activity and putrescine accumulation has been described following the nephrotoxic injury caused by a single injection of methyl-mercury into winter flounder. A sevenfold elevation of renal ODC activity occurred within 2 days; thus polyamines may be involved in the recovery phase following a toxic dose of methyl-mercury [27].

Hormonal regulation of renal polyamine biosynthesis has been the subject of a number of investigations. Rat kidney ODC

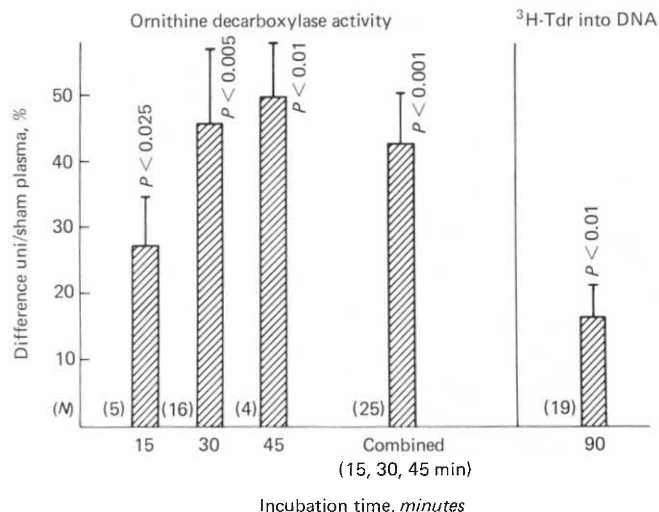

Fig. 4. *In vitro renotropin assays to determine the effect of uni versus sham plasma upon ODC activity and [^{3}H]thymidine incorporation into DNA of normal kidney tissue incubating in balanced salt solution.* Results are expressed as a percentage difference associated with uni versus sham plasma.

activity appears to vary in parallel with the diurnal rhythm in plasma corticosteroid concentration. The data suggest that the pituitary influences renal ODC activity, particularly through the rhythmical secretion of growth hormone and ACTH [28]. For this reason, in our experiments, enzyme activities and polyamine levels were determined on kidneys obtained at a uniform time of the day.

The stimulatory effect of hydrocortisone and growth hormone on renal ODC activity has been further investigated employing single intraperitoneal injections of hormone into intact rats. Enhanced enzyme activity was apparent approximately 4 hr after installation of either hormone [23]. The stimulatory effect of hydrocortisone as well as unilateral nephrectomy was negated by a single injection of actinomycin D [23]. These results are comparable to those involving hepatic ODC in which actinomycin D or cycloheximide prevented the increase in enzyme activity induced by growth hormone or partial hepatectomy [5]. Taken together, these observations suggest that increased RNA and protein synthesis contribute to the induction of ODC activity under a variety of circumstances including regenerative and hormone-induced growth.

A broad range of hormones influence renal ODC activity. In hypophysectomized rats, enhanced enzyme activity was detected within 5 hr of injections of parathyroid hormone, calcitonin, vasopressin, L-triiodothyronine, pentagastrin, serotonin, d-aldosterone [25], and prolactin [29]. The diversity of hormones capable of stimulating ODC activity suggests that several mechanisms for enzyme induction are probably important and that cAMP, although a stimulator of ODC activity [30], is not the common mediator of enzyme induction [25].

In another experimental model of hormonally induced renal growth, testosterone injections into castrated mice promoted renal hypertrophy associated with enhanced renal ODC activity and polyamine levels [31]. Of interest, 1,3-diaminopropane (a structural analogue of putrescine and inhibitor of ODC) effectively suppressed testosterone-induced renal hypertrophy. Coincidentally 1,3 diaminopropane appeared to diminish renal RNA and polyamine biosynthesis in testosterone-treated mice [32]. Evidence is provided suggesting that this effect of 1,3 diaminopropane is not simply the consequence of nephrotoxic injury [33]. However, it has yet to be established that the impact of 1,3 diaminopropane on testosterone-induced renal growth is actually mediated by inhibition of polyamine synthesis.

Since enhanced polyamine synthesis is associated with neoplastic growth, the impact of various chemical carcinogens upon ODC activity and polyamine levels of target tissues is of interest. Estradiol, which is carcinogenic to hamster kidneys, increased renal ODC activity in castrated male hamsters [34]. Binding of estradiol to a cytoplasmic receptor protein appears to be a prerequisite for enzyme induction [34, 35]. Further investigations will be necessary to define the physiologic role of polyamines in normal and neoplastic growth.

Similarly, the precise interactions of polyamine biosynthesis and compensatory renal growth have yet to be elucidated. Because of the broad range of factors that influence renal ODC activity, the results of in vivo experiments are difficult to interpret. In our studies, some augmentation of putrescine levels occurred in sham-operated animals. Surgical stress induces release of corticosteroids and B-endorphin, both of which increase renal ODC activity [23, 36]. The importance of surgical stress is further underscored by investigations demonstrating that increased enzyme activity observed 4 hr after uni can be prevented by maintaining nembutal anesthesia for 4 hr postoperatively or by treating with metapyrone which inhibits 11 B-hydroxylation of steroids [37]. Parenthetically, it is unlikely that adrenal hormones mediate compensatory renal growth [38].

Furthermore, changes in food intake postoperatively may influence levels of polyamines and their biosynthetic enzymes. As an example of the effect of dietary alterations on polyamine metabolism, enhanced renal and hepatic ODC activity were demonstrable in rats fed protein after 3 days on a protein-free diet [39].

Considering that multiple factors can promote renal polyamine biosynthesis, it is probable that investigations of causal interactions would benefit from the application of in vitro systems. Thus experiments were performed utilizing an in vitro method which has provided evidence for a circulating renal growth factor, called renotropin, following unilateral nephrectomy [12–17]. We assayed the effect of plasma obtained 18 hr after uni or sham operations on polyamine and DNA synthesis of normal renal tissue incubating in a balanced salt solution. Uni plasma compared to sham plasma resulted in enhanced ODC activity within 15 min and greater incorporation of [^{3}H] thymidine into DNA within 90 min. The rapid induction or preservation of renal ODC activity following growth stimuli is evident. Also the close association of polyamine and nucleic acid biosynthesis is further illustrated.

This in vitro system, or modifications employing isolated glomeruli or tubules, could be utilized to further investigate the impact of the purported circulating renal growth regulator, as well as other growth stimuli, on renal polyamine and nucleic acid synthesis.

Parenthetically, renotropin, whose existence is favored by experimental data obtained at several centers, probably contributes to compensatory responses induced by loss of functional renal parenchyma due to surgery or disease (recently re-

viewed [40]). Our in vitro observations further support the presence of a circulating renal growth factor following renal mass extirpation and corroborate the results of other in vitro investigations employing mitotic index [41], isotopic incorporation into nucleic acids [12–16, 42] and phospholipids [43], autoradiography [17], protein changes, and dry weight [44] as indices of growth. To date, the source and the exact chemical nature of a circulating regulator remain uncertain. Despite the well documented impact of multiple hormones upon renal growth and polyamine biosynthesis, it is unlikely that renotropin emanates from the pituitary [45, 46], adrenals [38], thyroid [47], or testes [48].

These issues represent but one facet of investigations into factors which initiate and control various growth processes. The many unresolved questions pertaining to compensatory, neoplastic, and embryonic growth clearly provide an important challenge for further study.

Summary. The aliphatic polyamines, putrescine, spermidine, and spermine, appear to play an important role in many forms of rapid growth including embryonic, regenerative, hormone-induced, and neoplastic. While the exact biochemical function of polyamines is unclear, current evidence suggests they are probably involved in the biosynthesis and accumulation of nucleic acids and proteins. Increased levels of polyamines and their biosynthetic enzymes are associated with augmented kidney growth stimulated by renal mass extirpation, as well as by various hormones, toxins, and carcinogens. These observations are reviewed and additional data is provided pertaining to alterations in polyamine metabolism during compensatory renal growth following unilateral nephrectomy (uni). To further explore the effect of growth stimuli on renal polyamine synthesis, an in vitro system was employed which previously provided evidence for a circulating renal growth factor after unilateral nephrectomy. These in vitro observations *(1)* underscore the rapid inducibility of ornithine decarboxylase, the rate limiting enzyme for polyamine biosynthesis; *(2)* illustrate the association of polyamine and nucleic acid synthesis during enhanced kidney growth; and *(3)* support the existence of a circulating renal growth regulator which apparently contributes to compensatory responses following loss of functional renal parenchyma.

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