Further Studies on a Renotropic System in Rats

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Based upon many investigations, the existence of short-lived, specific, circulating substances which incite and/or regulate compensatory renal growth has been proposed. In our studies, we find that sera and plasma from unilaterally nephrectomized rats compared to sera and plasma from sham-operated rats stimulate the incorporation of \(^{3}\)H-thymidine monophosphate, \(^{3}\)H-thymidine and \(^{14}\)C-uridine into the DNA of incubating rat kidney fragments. While extracts from growing rat kidneys are not excitatory, they produce a relative enhancement to incorporation of isotope into DNA when combined with sera from uninephrectomized rats—more than the sera do alone. The above is found also for the incorporation of \(^{14}\)C-uridine into RNA of incubating rat kidney fragments. Sera from uninephrectomized rats fail to stimulate DNA synthesis in liver slices from rats but do so in the presence of extracts from growing kidneys. Renotropic factors in sera and extracts do not appear to work by diluting the isotopes, by enhancing transport, or by effecting overall metabolism of the renal cells. The above described serum and liver factors may play a role in compensatory renal growth.

Compensatory renal growth following unilateral nephrectomy (uninephrectomy) occurs both by hypertrophy and hyperplasia [1]. The mechanisms that initiate and regulate compensatory renal growth are uncertain despite the existence of many varied theories—neurogenic, circulatory-mechanical [2,3], work-load [4], and humoral [5,6,7]. Based upon numerous experiments of varied design, many investigators favor that compensatory renal growth is controlled, to some extent, by circulating renotropic factors. Support for the existence of renotropins derives from finding: (1) that increases in DNA synthesis and/or growth are seen in the untouched kidneys of cross-circulated rats following nephrectomies in their partners [8,9], (2) that compensatory renal growth occurs in transplanted kidneys given to anephric recipients [10], (3) that injections of sera or plasma from unilaterally nephrectomized animals into normal animals augment renal DNA synthesis [11,12,6,13], and (4) that a precedent has been set because the regeneration of another organ, i.e., liver, may be controlled by circulating factors [14,15].

Braun-Menendez [16] popularized the concept that there are circulating renotropins. When he created hypertension in animals, kidneys grew under many circumstances. To explain this, he hypothesized the presence of humoral factors, renotropins, which could incite renal growth and perhaps indirectly cause hypertension. However, no evidence was given to prove that renotropins exist. In 1954, Ogawa and Nowinski [5] showed that sera from unilaterally nephrectomized rats stimulate mitoses in renal medullary explants. Encouraged by this, we began our attempts to develop an in vitro assay for renotropin [7]. Our original assay consisted of kidney slice halves incubated in a balanced salt medium containing \(^{3}\)H-thymidine mono-
phosphate and \( ^{14}\)C-uridine. We used the incorporation of these isotopes into DNA and RNA as an estimate of synthesis. One slice half was bathed in plasma from sham-operated rats, the other, in plasma from uninephrectomized rats.

Figure 1 depicts data from some of these first studies. Compared to the addition of plasma from sham-uninephrectomized rats ("sham"), plasma from uninephrectomized rats ("uni") (24 hours post-operation) added to the medium in concentrations between 8 percent to 50 percent v/v augmented the incorporation of isotope into DNA of slice halves. In contrast, "uni" plasma do not stimulate the incorporation of isotope into liver slice DNA or RNA, spleen slice DNA or lung slice DNA. A finding that intrigued us during these studies was that azotemic plasma from rats 24 hours after bilateral nephrectomy (BI), unlike plasma from "uni" rats, did not stimulate the incorporation of \(^3\)H-thymidine monophosphate into renal slice DNA.

After the initial work, we continued our studies with two general goals in mind: (1) to improve the reproducibility and sensitivity of the assay, and (2) to determine why sera from bilaterally nephrectomized rats do not stimulate isotopic incorporation into DNA and RNA [7]. To accomplish the former, the assay was modified (Fig. 2). We replaced kidney slice pairs with renal fragments. The fragments were made by forcing rat renal cortices, diced into small pieces, through a nylon sieve [17]. Each fragment contained an average of 11 glomeruli, showed linear incorporation of \(^3\)H-thymidine into DNA over 3 hours, and by electron microscopy, proved morphologically normal after 90 minutes of incubation. Details of the assay have been reported [18,19]. By autoradiography, \(^3\)H-thymidine incorporation was found in the nuclear elements of cells from the glomeruli, proximal and distal tubules and the loop of Henle. Most important for our purposes, the replication of \(^3\)H-thymidine incorporation into kidney tissue among flasks in a given set was better than the duplication between slice pairs [18].

**FIG. 1.** The effects of different concentrations of plasma on the incorporation of \(^1\)H-thymidine monophosphate into the DNA of rat kidney slices. Results are expressed as a percent of change by slices incubated in plasma from uninephrectomized rats as compared to plasma from sham-operated rats. Numbers in parentheses equal number of experiments. SEM is shown.
FIG. 2. Schematic diagram of the in vitro assay for the renotropic and tissue factors.

Using fragments, we studied the temporal appearance of the circulating renotropic factors [19]. Within 10 hours after surgery, a relative stimulation to incorporation by sera from uninephrectomized rats was noted. This stimulation lasted approximately 30 hours after unilateral nephrectomy and then declined. As in previous studies, sera from rats with both kidneys removed (B1) (10 and 20 hours earlier) produced no stimulation to DNA synthesis in renal fragments.

Figure 3 summarizes our findings to date on the stimulation of isotope incorporation into renal DNA. The first three bars depict the effects of “uni” plasma and sera relative to “sham” plasma and sera on the incorporation of various precursor isotopes into DNA (S). The first bar delineates the original studies and shows that “uni” plasma enhances the incorporation of $^3$H-thymidine monophosphate into renal slice DNA. The data depicted by the next bar show that “uni” sera can relatively enhance the incorporation of $^3$H-thymidine into the DNA of incubating renal fragments, and the last bar in the series depicts the results when $^{14}$C-uridine is the precursor. With this precursor, the relative stimulation to incorporation into renal DNA by “uni” sera is even greater than with $^3$H-thymidine monophosphate or $^3$H-thymidine. Therefore, using three different precursors for DNA, “uni” plasma and sera compared to “sham” plasma and sera enhance isotope incorporation into DNA.

If the removal of one kidney can produce a stimulatory factor in sera then why does not the removal of two kidneys produce as much or even more? The lack of stimulation to renal DNA synthesis by azotemic sera from bilaterally nephrectomized rats suggests two possibilities:

a. substances in azotemic sera inhibit renotropic activity by some means, and/or,
FIG. 3. Histogram depicting the effects of sera (S), renal extracts (E) and a combination of sera and extracts (C) from unilaterally nephrectomized rats compared to sham-operated rats on the incorporation of various isotopes into the DNA of incubating renal tissue.

- $^3$H-thymidine monophosphate into the DNA of kidney slices
- $^3$H-thymidine into the DNA of renal fragments
- $^{14}$C-uridine into the DNA of renal fragments
- * indicates statistical significance

b. renal tissue produces the circulating renotropic factors.

To detect an azotemic inhibitory factor, we investigated sera obtained from bilaterally nephrectomized rats before and after dialysis [19]. Whereas azotemic sera from rats bilaterally nephrectomized 20 hours earlier (BUN = 120 mg/100 ml) did not stimulate $^3$H-thymidine incorporation into renal fragment DNA, stimulation was present when these same sera were dialyzed for 24 hours. From this, we conclude that augmented concentrations of renotropin are present in sera from rats made azotemic by having both kidneys removed 20 hours earlier and that this renotropic factor can remain even after 24 hours of dialysis. Since both kidneys were absent for 20 hours before obtaining sera, kidneys could not produce the circulating factor responsible for enhanced DNA synthesis.

If renal tissue does not produce renotropin, can it activate it? This question may relate to another question, i.e., why is renotropin so highly specific for renal tissue? Saetren [20] reported in 1956 that intraperitoneal extracts of macerated kidneys inhibited rather than enhanced renal compensatory hyperplasia. While Williams [21] described similar findings, he noted that this depression was associated with a greatly reduced food intake. A general feeling has emerged that there are no kidney specific effects produced by injections of renal tissue [22]. When we added extracts from growing or control kidneys to renal fragments incubating in $^3$H-thymidine or $^{14}$C-uridine, no enhancement of DNA synthesis was demonstrated (E) (Fig. 3). However, the incorporation of precursors into DNA is enhanced when extracts from growing kidneys and “uni” sera (C) are added to the medium compared to the results when “sham” sera and extracts are combined in the medium. The stimulation by the “uni” combination exceeds the stimulation by “uni” sera whether $^3$H-thymidine or $^{14}$C-uridine is the marker. This suggests that within 20 hours after uninephrectomy two
factors contribute to enhanced DNA synthesis—one in the remaining kidneys, the other in the circulation.

Figure 4 deals with the incorporation of $^{14}$C-uridine into renal RNA. The first bar in Fig. 4 depicts the results from the 1970 studies [7]. "Uni" plasma compared to "sham" plasma stimulated $^{14}$C-uridine incorporation into renal slice RNA. The next bar depicts newer data showing that sera from rats 20 hours after uninephrectomy stimulated incorporation of $^{14}$C-uridine into renal fragment RNA. The effects of "uni" plasma on slices and "uni" sera on fragments are of a similar magnitude. Again, renal extracts alone do little to affect isotope incorporation into RNA, while the combination of "uni" sera and "uni" renal extracts proves even more stimulatory than "uni" sera alone.

Compensatory renal growth appears to be specific. It is generally accepted that removal of kidney tissue results in extensive compensatory growth in kidneys alone; and, in turn, partial hepatectomy results solely in hepatic regeneration. In vitro this seems to be the case also. "Uni" sera does not enhance the incorporation of $^{3}$H-thymidine into the DNA of incubating hepatic slices. Likewise, we found that sera from 2/3 partially hepatectomized rats enhance DNA synthesis in incubating liver slices, whereas sera from partially hepatectomized rats decrease $^{3}$H-thymidine incorporation into the DNA of renal fragments (Table 1).

How can we explain the specificity of stimulation by sera from uninephrectomized and partially hepatectomized rats? Perhaps, specific excitatory factors for the circulating renotropic and hepatotropic factors could be located in the renal and hepatic tissues. Such a possibility would explain the specificity of circulating tropic factors for their respective tissues. Renotropins are activated by kidney tissue and

![Graph](image.png)

**FIG. 4.** Histogram depicting the effects of sera (s), renal extracts (e) and a combination of sera and extracts (c) from unilaterally nephrectomized rats compared to sham-operated rats on the incorporation of $^{14}$C-uridine into the RNA of incubating renal tissue.

- $^{14}$C-uridine into RNA of kidney slices
- $^{14}$C-uridine into RNA of kidney fragments
- * indicates statistical significance
TABLE 1

Comparison Between Sera and Sera Plus Liver Extracts from 2/3 Partially Hepatectomized Rats (PH) and Sera and Sera Plus Liver Extracts from Sham-Operated Rat (SHAM) on Rat Liver Slice DNA Synthesis

| Excitor               | Δ Spec Act (PH-SHAM) | %   |
|-----------------------|----------------------|-----|
| Sera                  | +12.4 ± 4.3 (SEM)    | +9.9% (*p < .01)* |
|                       | *n = 20*             |     |
| Sera + Extracts       | +23.4 ± 6.8 (SEM)    | +19.0% (*p < .01)* |
|                       | *n = 20*             |     |

Δ Spec Act = difference in specific activity.
% change from the control situation is shown also.
n = number of experiments.

hepatotropins, by hepatic tissue. This raises an intriguing possibility. If both components in the renotropic system (sera and renal extracts) were present in the medium, could we stimulate hepatic DNA synthesis? We performed the experiments depicted in Figs. 5 and 6 to answer this question. Compared to liver slices incubating in medium alone, the stimulation by UNI sera is not significantly greater than that seen with “sham” sera (Fig. 5). The black bar at the end of the grouping graphically depicts the small difference. In addition, renal extracts from the remaining kidney of “uni” and from “sham” kidneys depress ³H-thymidine incorporation by similar amounts; so again, no marked differences are noted. However, the combination of “uni” sera and renal extracts significantly stimulates DNA synthesis (*p < .05*), whereas “sham” sera and extracts do not significantly alter ³H-thymidine incorporation into DNA. In another series of 8 experiments (Fig. 6) where the various

![FIG. 5. The effects of sera, kidney extracts, and combined sera and extracts from unilaterally nephrectomized and sham-operated rats on the incorporation of ³H-thymidine into liver slice DNA](image-url)

- [ ] sham-operated
- [X] unilaterally nephrectomized
- [ ] difference

Data are from ten experiments. Only the differences in the studies where sera and extracts are combined are statistically significant. SEM shown.
combinations of sera and extracts from sham-operated and uninephrectomized rats on the incorporation of $^3$H-thymidine into liver slice DNA were followed, only a combination of "uni" sera and "uni" extracts were statistically stimulatory ($p < .05$). In contrast, addition of only one of the "uni" components has been shown previously to enhance $^3$H-thymidine incorporation into renal DNA [19].

Before accepting that our renotropic system is specifically enhancing DNA and RNA syntheses, we must test other possibilities. Many in vivo studies concerned with the incorporation of $^3$H-thymidine into DNA have been criticized on the grounds that dilution of isotope in the circulation or in the kidney milieu could affect results in such a way to suggest increased DNA production where there is none. In vitro studies concerned with isotope incorporation have some advantages over in vivo studies in this regard. In the latter, it is obvious that the presentation of the radionuclide to the kidney by the circulation or changing dilution by the precursor pools in the kidney could affect incorporation. This could not be the case in our in vitro studies where the initial pools in the same renal tissue have to be similar and where there can be no doubt that the same amount of isotope is presented to the tissues under study. Could sera and renal extracts from "uni" cause less isotope dilution and thus only seemingly appear to enhance precursor incorporation into DNA and RNA? Two findings make this seem unlikely. First, differences in isotope dilution by "uni" and "sham" sera should result in enhanced incorporation in tissues other than renal—liver, spleen, and lung. This does not occur. Second, the incorporation of $^3$H-thymidine is not different in the presence of extracts from the kidneys of sham-operated and uninephrectomized rats. Finally, dilution of isotopes also would not explain why "uni" extracts combined with "uni" sera cause an even greater incorporation of $^3$H-thymidine into DNA than "uni" sera does alone.

Could "uni" sera enhance transport of both $^3$H-thymidine and $^{14}$C-uridine into
renal tissue and increase incorporation? This is not the case. The acid soluble portion of each isotope found in renal tissue after 30, 60, and 90 minutes of incubation was not different whether "uni" or "sham" sera and "uni" or "sham" combinations (sera plus extract) were added to the medium.

Perhaps "uni" sera and extracts enhance the overall metabolism of renal tissue and are not specific for the synthesis of DNA and RNA. Figure 7 depicts the relative effects of "uni" sera, "uni" extracts, and combined "uni" sera and extracts compared to "sham" components on two transport functions and two metabolic functions of incubating kidney slices. For comparison, the first bar in each group depicts the findings when slices are removed from renal tissue undergoing compensatory renal growth in vivo. It has been found on the day after unilateral nephrectomy that the growing renal tissue transports more PAH (organic anion) but not TEA (organic cation) [23]. At the same time, we could find no increase in oxygen consumption (QO₂). Finally, renal ammonia production four days after "uni" is enhanced [24]. Suffice it to say, neither "uni" sera, extracts nor combinations of "uni" sera and extracts affect any of these functions in our assay system. Thus, it seems unlikely that the renotropic system enhances the synthesis of DNA and RNA by indirect means.

Prior to accepting the above conclusions, one should explain the rapidity of the

**FIG. 7.** The effects of sera (s), renal extracts (e) and combined sera and extracts (c) from unilaterally nephrectomized rats compared to sham-operated rats on PAH and TEA transport, QO₂ and ammonia production under the conditions used in the in vitro assay for renotropins. For comparison, these same studies are carried out on renal tissue removed from the remaining kidney after uninephrectomy. For PAH, TEA, and QO₂, tissue was removed one day after uninephrectomy. For ammonia production, tissue was obtained four days after uninephrectomy.
stimulation to synthesis of DNA and RNA. It has generally been accepted that augmented DNA synthesis in the course of compensatory renal growth may take many hours to occur [25], although renal DNA synthesis has been reported to occur as early as six hours after unilateral nephrectomy [26]. Comparing our in vitro studies with renal compensatory growth in vivo is difficult. Our material (sera and extracts) is obtained 20 hours after uninephrectomy. Only then is it placed in contact with tissue. Delays in synthesis of DNA and RNA in vivo could be secondary to the time involved in the production of the excitor rather than the time necessary for DNA synthesis to be enhanced by a stimulator. Levi and Zeppa [27] used such reasoning to explain the ability of their factors to enhance hepatic DNA synthesis over a two-hour period. There is less problem explaining augmented RNA synthesis during a 90-minute incubation. RNA synthesis is known to be accelerated soon after nephrectomy [25]. In addition, Lyons, Evans, McLaren and Solomen [28], using tissue cultures from hamsters, showed that the addition of “uni” sera enhances cytoplasmic RNA synthesis in four hours and nuclear RNA synthesis in two hours.

From all this, we hypothesize that following unilateral nephrectomy, a non-renal substance builds up in the blood and another in the renal tissue that can affect the synthesis of DNA and RNA. Both appear to be relatively specific for the synthesis of DNA and RNA. “Uni” sera alone stimulates only renal tissue, not hepatic slices. When both components (circulating and tissue) are added to liver slices, they can stimulate DNA synthesis although not as greatly as they do renal DNA synthesis. Since “uni” sera is specific for renal tissue, it may be that enough of the excitatory factor is present in normal renal tissue to activate the circulating factor and enhance the production of RNA and DNA. A specific location in the kidney of the renotropic tissue activator may explain why “uni” sera affect only renal tissue.

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REFERENCES

1. Johnson HA, Vera Roman JM: Compensatory renal enlargement. Hypertrophy vs. hyperplasia. Am J Path 49:1-13, 1966
2. Dicker SE, Shirley DG: Mechanism of compensatory renal hypertrophy. J Physiol 219:507-523, 1971
3. Krohn AG, Peng BB, Antel HL, Stein S, Waterhouse K: Compensatory renal hypertrophy—the role of immediate vascular changes in its production. J Urol 103:564-568, 1970
4. Allen RB: Experiments on the physiology and course of compensatory renal hypertrophy. Proc Staff Meet Mayo Clin 9:333-336, 1934
5. Ogawa K, Niwinson WW: Mitosis stimulating factor in serum of unilaterally nephrectomized rats. Proc Soc Exp Biol Med 99:350-353, 1958
6. Silk MR, Homsey GE, Merz T: Compensatory renal hyperplasia. J Urol 98:36-39, 1967
7. Preuss HG, Terry EF, Keller AI: Renotropic factor(s) in plasma from uninephrectomized rats. Nephron 7:459-470, 1970
8. Kurnick NB, Lindsay PA: Compensatory renal hypertrophy in parabiotic mice. Lab Invest 19:45-48, 1968
9. Van Vroonhoven TJ, Soler-Montesinos L, Malt RA: Humoral regulation of renal mass. Surgery 72:300-305, 1972
10. Ogden DA: Donor and recipient function 2 to 4 years after renal homotransplantation. Ann Int Med 67:998-1006, 1967
11. Lowenstein LM, Stern A: Serum factor in renal compensatory hyperplasia. Science 142:1479-1480, 1963
12. Bury HPR, Crane WAJ, Dutta LP: Cell proliferation in compensatory renal growth. Br J Urol 37:201-210, 1965
13. Connolly JG, Demelker J, Promislow C: Compensatory renal hyperplasia. Canad J Surg 12:236-240, 1969
14. Grisham JW, Kaufman DG, Alexander RW: 3H-thymidine labeling of rat liver cells cultured in plasma from sham or partially hepatectomized rats. Fed Proc 26:624, 1967
15. Hays DM, Tedo I, Matsushima Y: Stimulation of in vivo growth of rat liver cells with calf serum drawn following partial hepatectomy. J Surg Res 9:133–137, 1969
16. Braun-Menendez E: Evidence for renotropin as a casual factor in renal hypertension. Circulation 17:696–701, 1958
17. Goldin H, Zmudka M, Tio F, Vasquez A, Preuss HG: Paraaminohippurate and tetraethylammonium transport in fragments of rat renal cortex. Proc Soc Exp Biol and Med 144:692–696, 1974
18. Preuss HG, Goldin H: Humoral regulation of compensatory renal growth. Med Clin North America 59:771–780, 1975
19. Preuss HG, Goldin H: A renotropic system in rats. J Clin Invest 57:94–101, 1976
20. Saetren H: A principle of autoregulation of growth. Production of organ specific mitosis-inhibitors in kidney and liver. Exp Cell Res 11:229–1232, 1956
21. Williams GEG: Studies on the control of compensatory hyperplasia of the kidney in the rat. Lab Invest 11:1295–1302, 1962
22. Goss R J: Mitotic responses of compensating rat kidney to injections of tissue homogenates. Cancer Res 23:1031–1035, 1963
23. Goldberg VJ, Weiss FR, Keller Al, Preuss HG: Function in hypertrophying kidneys: organic acid and base transport. Am J Physiol 218:1065–1069, 1970
24. Preuss HG, Goldin H: Ammoniagenesis in growing nephrons of uninephrectomized rats. Lab Invest 31:454–457, 1974
25. Malt RA: Biochemistry in regeneration of liver and kidney, Bacher NLR, Malt RA, eds. Boston, Massachusetts, Little Brown and Company, 1971, pp 213–225
26. Toback FG, Lowenstein LM: Uridine metabolism during normal and compensatory renal growth. Growth 38:17–34, 1974
27. Levi JU, Zeppa R: Source of the humoral factor that initiates hepatic regeneration. Ann Surg 174:364–370, 1971
28. Lyons HJ, Evan AP, McLaren LC, Solomen S: In vitro evidence for a renotrophic factor in renal compensatory hypertrophy. Nephron 13:198–211, 1974