A Renotropic System in Rats

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ABSTRACT While plasma or sera obtained from rats 20 h after removal of one kidney (uninephrectomy) stimulated [^{3}H]thymidine incorporation into the DNA of kidney tissue incubating in vitro, azotemic plasma or sera obtained from rats 20 h after removal of both kidneys had no apparent effect. Dialysis of this azotemic sera resulted in its ability to stimulate isotope incorporation into renal DNA to the same degree as sera from uninephrectomized rats. This stimulatory factor (renotropin) was found to rise significantly within the first 26 h after uninephrectomy. Renotropin worked only on renal tissue, and we found that a factor could be extracted in large amounts from the remaining kidney 20 h after uninephrectomy that would stimulate renal DNA synthesis in the presence of sera. Based on these findings and others, we postulate that after uninephrectomy there is an elevation in circulating renotropin as well as a tissue factor in the remaining kidney. Both factors together probably produce an excitor which enhances [^{3}H]thymidine into DNA. The latter is tightly bound to renal tissue, and its production and/or activity is modified by circulating inhibitors that are especially prominent in azotemia.

INTRODUCTION

Results from parabiotic and transplant studies suggest that compensatory renal growth is initiated and/or controlled to some extent by circulating factors, renotropins (1, 2). In 1970, we found that plasma obtained from rats 24 h after one kidney was removed (uninephrectomy) compared to plasma removed from rats having a sham procedure stimulated [^{3}H]thymidine monophosphate incorporation into the DNA and [^{14}C]-uridine incorporation into the RNA of incubating rat kidney cortical slices (3). Some specificity of this factor was indicated by the inability of similar plasma to stimulate isotope incorporation into the DNA of rat liver, renal medullary, spleen, and lung slices (4). While the source of this circulating renotropic factor was not identified, an interesting observation was the absence of stimulation by plasma from rats having both (bilateral nephrectomy) rather than one kidney removed (3). These initial studies did not reveal why removing two kidneys instead of one resulted in a loss of our renotropic effect, but two hypotheses seemed attractive: (*a*) azotemic factors inhibit the effects of the renotropic factor, and (*b*) renal tissue is necessary to produce the circulating renotropic factor. A major purpose of this investigation was to evaluate each hypothesis. The results indicate that both an extrarenal sera and renal factor are necessary to produce our previously described effect, i.e. enhancement by plasma from uninephrectomized rats of [^{3}H]thymidine incorporation into renal DNA. In addition, there are circulating factors that inhibit this reaction; and they are very prominant in azotemic sera.

METHODS

Male Sprague-Dawley rats (150–250 g) were maintained in a constant temperature room with a light-dark phase of 14 and 10 h and were allowed free access to rat chow and water. Later, they were killed by a blow on the head, and the kidneys were removed rapidly and placed in cold saline. We obtained tissue fragments by forcing cortices diced into small pieces with scissors, through a nylon sieve (2 × 1.5 mm) (5). This nylon sieve was obtained from the framework of a twin coil dialyzer (Travenol Laboratories, Morton Grove, Ill.). The bottom of the sieve was scraped with a spatula to recover as much tissue as possible, and the material was placed in cold oxygenated medium. We allowed the tissue to settle for 2–3 min and then decanted the supernate. The fragments were washed twice by mixing with cold oxygenated medium.

The basic medium used in washing and incubating fragments was a modified Krebs-Ringer solution composed of the following anions and cations: Na^+ 126 mM, K^+ 5 mM, Mg^{++} 1.2 mM, Ca^{++} 1.0 mM, $SO_4^=$ 1.2 mM, Cl^- 133 mM, phosphate buffer (pH 7.4) 10 mM, and gassed with 100% O_2. To each flask containing 3 ml of medium and 30–60 mg wet wt of tissue fragments, approximately 2 μCi of methyl-

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[^{3}H]thymidine (specific activity 15.2 Ci/mmol) was added at the beginning of incubation. In this system, less than 2% of the total counts disappeared from the medium over the course of incubation.

At the end of a 90-min incubation at 25°C, cortical fragments were trapped with suction on circular filter paper (Whatman 40 2.4 cm radius). The DNA in the fragments was extracted from the filter paper by the method of Fleck and Munro (6) as modified by Halliburton and Thomson (7). DNA was estimated by the indole-HCl reaction of Ceriotti (8), modified by using 0.06% indole and 2.5 N/HCl according to Keck (9). Standard curves were prepared with calf thymus DNA.

We performed β-counting on a Packard Tri-Carb 2420 liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) with samples corrected back to 100% absolute efficiency using a standard quench curve which related automatic external standard counts to absolute efficiency. Dioxane was used as a solvent, 2,5-diphenyloxazole was the primary scintillator, and 1,4-bis[2-4-(methyl-5-phenyloxazolyl)]benzene, the secondary scintillator. Since DNA extracted in 0.3 N KOH tended to settle out, Cab-O-Sil (Packard Instrument Co., Inc.) was added to the scintillation mixture to form a gel.

In the usual experiments, plasma or serum was obtained from rats 20 h after uninephrectomy (UNI) [1] or bilateral nephrectomy (BI) and after an appropriate sham operation (SHAM). In some studies, plasma was obtained at different times after the operation. Nephrectomy was carried out by a flank incision, and SHAM consisted of exposure and replacement of the kidney. To obtain plasma or sera from the lower aorta, rats were lightly anaesthetized with ether, and blood was drawn into chilled glass syringes. When studying plasma, clotting was prevented by the addition of 3.8% EDTA (final volume blood: EDTA = 100: 1). Blood was immediately centrifuged in the cold in plastic tubes. The plasma was then ultrafiltered through Collodion bags (model 4675-B12, Arthur H. Thomas Co., Philadelphia) and the ultrafiltrate was collected into cold plastic tubes. In addition, some sera were dialyzed for 24 h against the incubation medium used in these studies (cellulose dialyzer tubing, model 3787-D40. Arthur H. Thomas Co.). This procedure was carried out in a room maintained at 2°C.

Two different types of investigations were performed. First, we studied plasma ultrafiltrates or sera from rats having SHAM procedures, removal of one kidney (UNI) or removal of both kidneys (BI) after various intervals postoperation. In the usual experiment, we ascertained the effects of plasma ultrafiltrates or sera from the nephrectomized rats (12% vol/vol) on [^{3}H]thymidine incorporation of isotope into the DNA of fragments incubating in medium alone. Also we studied the incorporation of isotope into the DNA of fragments incubating in plasma ultrafiltrates from SHAM rats (12% vol/vol). These results are depicted in Fig. 1. The ability of various types of sera, pre- and postdialysis, to affect [^{3}H]thymidine incorporation into renal DNA is depicted in Fig. 2.

To assess [^{3}H]thymidine transport into renal fragments, two studies were performed in a manner used for p-aminohippurate (PAH) and tetraethylammonium (TEA) transport (5). At fixed intervals of incubation, fragments and

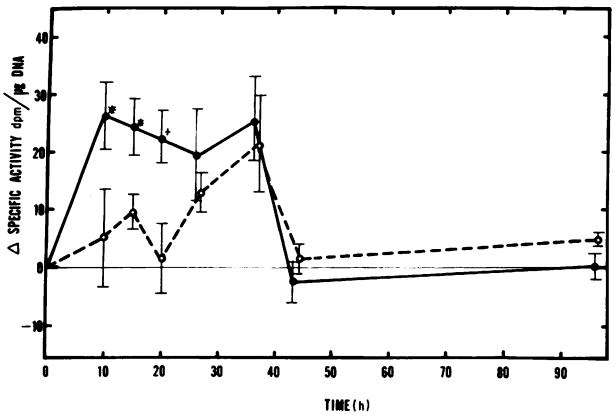

FIGURE 1 Incorporation of [^{3}H]thymidine into the DNA in renal fragments incubating in plasma obtained at different time intervals from UNI rats (●) and SHAM rats (○) compared to control. Points represent the means of observations on three to six plasma ultrafiltrates; the SEM is also depicted. * $P < 0.05$, + $P < 0.01$.

medium were separated, and the [^{3}H]thymidine was released from the fragments by homogenization in 10% TCA. A tissue to medium rate ratio (T/M) was calculated by comparing the dpm in 1 g of fragments to the dpm in 1 ml of medium.

In a second series of studies, extracts from rat kidney were investigated. Whole kidneys were removed from SHAM rats or those with a single remaining kidney 20 h after UNI and homogenized in the same cold medium used for incubation (1 g kidney/5 ml medium). Homogenization was carried out using a motor-driven Teflon pestle. After centrifugation at 500 g, the supernates were collected and kept cold until study. Supernates from the remaining growing kidneys from UNI and kidneys from SHAM were added (12% vol/vol) along with [^{3}H]thymidine to incubating fragments. Controls (addition of 12% vol/vol medium only) were run simultaneously. In some studies, pooled sera (12% vol/vol) from control, SHAM, UNI, and BI rats were included in the medium. Again, after 90 min incubation, comparison was made among the specific activities of DNA in each flask. These results are depicted in Tables I–V.

Results from any experimental group are the average of a minimum of four flasks. Statistics are by paired analysis using Student's t test. Statistical significance was set at $P < 0.05$. Some of the results are expressed as the ratio between the specific activity of the DNA in fragments incubating under the various conditions. When the specific activity of the fragments incubating under the test situation (T) exceeded the specific activity of the fragments incubating under control conditions (C) the ratio was obtained by (T/C) − 1.00 and the number became positive. If the C was greater, the ratio was expressed as a negative number using the formula 1.00 − (C/T).

RESULTS

In this fragment preparation, [^{3}H]thymidine incorporation into DNA was linear over the 90 min of incubation whether the fragments were incubating in medium alone, in the presence of sera, (UNI or SHAM) or in the presence of kidney extract (UNI or SHAM).

[1] *Abbreviations used in this paper:* BI, bilateral nephrectomy; PAH, p-aminohippurate transport; SHAM, sham operation; TEA, tetraethylammonium transport; UNI, uninephrectomy.

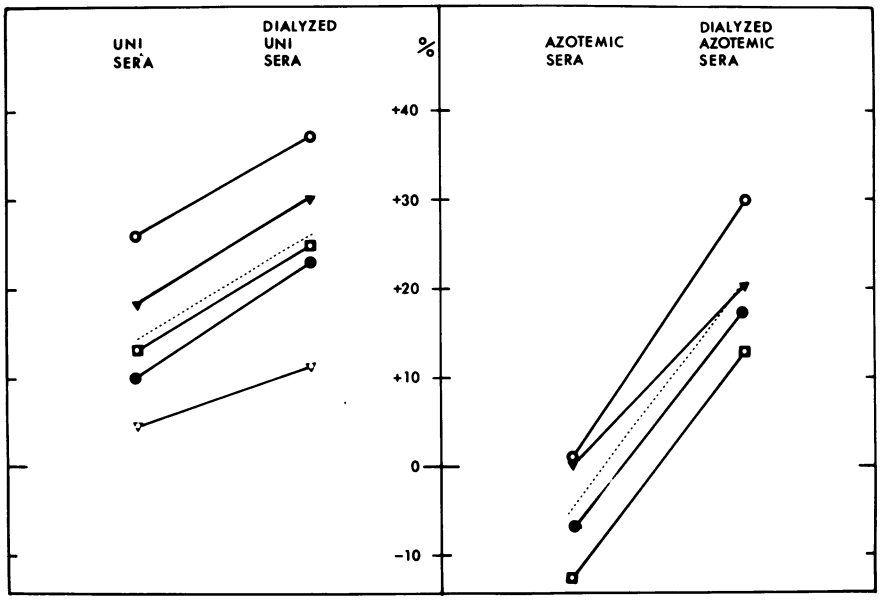

FIGURE 2 The ability of pre- and postdialysis sera from UNI rats (20 h) and from BI rats (20 h) compared to SHAM sera collected and treated in the same manner to stimulate [^{3}H]thymidine incorporation into renal fragment DNA. Each line connects the same sera pairs tested pre- and postdialysis. The dotted lines indicate the average for each group.

Autoradiography showed that the label was in the nuclei of proximal tubular cells (47.3%) and distal tubular cells (12.2%) (4). The remaining label was found in cells of the loop of Henle (23.6%) and cells of the glomeruli (16.9%).

The addition of plasma ultrafiltrates from rats between 10 and 30 h after UNI significantly augmented the incorporation of isotopic thymidine into DNA of rat renal fragments, while addition of plasma from SHAM rats had a less stimulatory effect. Fig. 1 depicts the time course for the circulating stimulator to DNA synthesis. The averages and the SEM for studies performed at each time postsurgery are shown. As early as 12 h after UNI, plasma from these rats significantly stimulated [^{3}H]thymidine incorporation into the DNA of renal fragments when compared to those fragments incubating in no plasma. This stimulation continued in plasma assayed at 16, 20, 26, and 36 h post-UNI. There was a markedly different pattern when this same parameter was investigated in fragments incubating in plasma from SHAM rats. A slight but lesser enhancement to [^{3}H]thymidine incorporation was seen over the first 26 h. While individually, no stimulation at any point was statistically significant, the over-all average increase over control between 12 and 26 h post-SHAM procedure was + 5.1%, $P < 0.05$. The over-all stimulation, by UNI plasma ultrafiltrates compared directly to SHAM plasma ultrafiltrates between 12 and 26 h postoperation, was + 13%, $P < 0.001$. At 36 h postsurgery, fragments incubating in SHAM plasma seemed to stimulate some incorporation of [^{3}H]thymidine into DNA (+ 21.4 dpm/μg DNA ±8.2 (SEM), + 14.7%, $P < 0.1$). This value did not prove to be statistically significant from control due to the large range of results. Why this SHAM plasma appeared to show more stimulation is not clear beyond the surmise that such changes might reflect a diurnal variation. At 44 and 96 h postsurgery, no significant stimulatory response by either plasma was seen.

In the series of studies depicted in Fig. 2, the effects of dialysis on sera were followed. When five sera, rather than plasma ultrafiltrates, from rats 20 h after UNI were compared to the effects of sera obtained from rats 20 h after SHAM, a stimulation of + 12.2 dpm/μg DNA±2.5 (SEM), (↑14.2%) ($P < 0.02$) was seen. After these same sera were dialyzed for 24 h against medium, the "UNI sera" compared to "SHAM sera" showed as much and perhaps more ability to enhance [^{3}H]thymidine incorporation into the DNA of renal fragments, + 14.2 dpm/μg DNA±1.6 (SEM), (↑25.2%) ($P < 0.01$). The difference between the groups was not statistically significant; however, the percent increase was greater after dialysis as controls in these studies were lower. In each pair of sera, the relative stimulation by UNI sera compared to SHAM sera as percent of control was greater after 24 h of dialysis (Fig. 2). When four azotemic sera from rats receiving a BI 20 h earlier were compared to sera obtained from SHAM rats, no enhancement to [^{3}H]thymidine incorporation was seen, − 2.8 dpm/μg DNA

TABLE I

Effects of Kidney Extracts from UNI and SHAM Rats (20 h postoperation) on [³H]Thymidine Incorporation into DNA of Renal Fragments

Exp.	Average specific activity: Control (no extract)	SHAM extract	UNI extract
		dpm/μg DNA	
1	78.0 (73.0–84.5)	64.0 (47.9–83.0)	63.8 (51.1–73.8)
2	83.4 (74.3–94.6)	67.0 (66.6–67.7)	65.2 (59.9–71.0)
3	142.7 (134.1–155.4)	111.9 (103.1–119.1)	116.1 (98.8–125.1)
4	125.9 (123.6–128.3)	101.0 (97.1–104.4)	92.2 (91.4–100.7)

Control = Fragments incubating in the presence of no renal extracts. No sera is present in any of the flasks. Values are an average of results from four flasks. Range of values are shown in parentheses. The average difference in specific activity between fragments incubating in extracts from SHAM rats compared to control is −21.5 dpm/μg DNA±3.9(SEM), $P < 0.02$; and between fragments incubating in extracts from UNI rats compared to control is −23.2 dpm/μg DNA±4.4(SEM), $P < 0.02$.

±1.6 (SEM), (↓5.0%) (P — NS). In contrast, this sera from BI rats after dialysis compared to SHAM sera after dialysis enhanced incorporation significantly, + 12.4 dpm/μg DNA±1.8 (SEM), (↑20.0%) ($P < 0.02$). Azotemic sera allowed to stand 24 h at 2°C without dialysis compared to SHAM sera subjected to the same procedure do not produce enhanced [³H]thymidine incorporation into renal DNA.

To determine if stimulation by UNI plasma was secondary to augmented [³H]thymidine transport into renal cells, we performed studies modeled after those used for PAH and TEA transport (5). Within 30 min, the maximal tissue to medium ratio (T/M) in these studies was approached and remained close to 1.0. No difference existed between transport in the presence of SHAM or UNI plasma.

Addition of supernate from kidneys homogenized in medium tended to depress [³H]thymidine incorporation into DNA of rat renal fragments whether the tissue was obtained from the remaining kidney 20 h after UNI or a kidney 20 h after SHAM (Table I). The depression was similar with either group of homogenates so that there was no significant difference in the depression between the two groups. However, when the renal fragments were bathed in common pooled sera (12% vol/vol) (Table II), the extract from the remaining kidney of UNI rats stimulated isotopic incorporation ($P < 0.01$) while the extract from the kidneys of SHAM rats still depressed this index when both were compared to fragments incubating in sera alone. Within a given experiment, the ability of renal extracts from UNI rats with sera compared to extracts from SHAM rats with sera to stimulate [³H]thymidine incorporation into renal DNA was highly significant ($P < 0.001$).

In the next studies, we expanded our investigations to compare the effects of renal extracts from SHAM (20 h) and UNI rats (20 h) in the presence of plasma from SHAM, UNI, and BI rats on [³H]thymidine incorporation into DNA of renal fragments obtained from control rats. Table III shows data from seven experiments. Within each experiment, the flasks containing sera and renal extracts from SHAM rats are used as base lines to compare various combinations in this humoral-tissue system. In these studies, the combination of UNI sera in the presence of SHAM extract, increased incorporation to the same extent as the stimulation seen in UNI plasma or sera alone at 20 h +15% ($P < 0.05$) (Figs. 1 and 2). The combination of SHAM sera and UNI extract compared to SHAM sera and SHAM extract stimulated renal DNA synthesis significantly, + 31.4%, ($P < 0.01$), as it did when compared to the effects of SHAM sera alone (Table II). The pairing of UNI plasma and UNI extract resulted in the most stimulation, + 52%, ($P < 0.01$).

Our next series of six studies (Table IV) was performed to discern the effects of azotemic sera from BI rats 24 h postoperation. Two findings were not unexpected, i.e. that azotemic sera in the presence of SHAM extract did not cause marked stimulation, + 6.0%, ($P < 0.05$), while SHAM sera and UNI extract stimulated as before, + 38.3%, ($P < 0.05$). A new finding was that UNI extract in the presence of azotemic sera (blood urea nitrogen, [BUN] 120 mg/100 ml) can stimulate [³H]thymidine incorporation into DNA,

TABLE II

Effects of Kidney Extracts from UNI and SHAM Rats (20 h postoperation) in the Presence of Serum on [³H]Thymidine Incorporation into DNA of Renal Fragments

Exp.	Average specific activity: Control (no extract)	SHAM extract	UNI extract
		dpm/μg DNA	
1	41.1 (38.9–43.9)	31.2 (28.6–32.8)	42.1 (38.7–44.4)
2	66.1 (53.6–81.3)	57.2 (51.8–65.6)	78.8 (65.3–88.7)
3	75.0 (65.2–90.2)	68.7 (62.1–72.1)	85.4 (82.2–96.1)
4	120.6 (113.7–133.7)	106.0 (91.6–114.5)	119.8 (109.8–131.8)
5	51.6 (47.2–56.1)	44.5 (38.0–48.9)	67.2 (61.5–77.1)
6	83.2 (76.5–88.5)	91.9 (83.3–97.8)	111.9 (99.5–119.1)
7	76.0 (72.0–79.7)	71.3 (69.9–73.1)	79.9 (71.8–93.3)
8	77.6 (75.0–80.9)	56.7 (43.8–67.0)	102.0 (85.5–123.9)
9	65.0 (56.4–74.5)	47.6 (44.7–50.7)	77.6 (75.4–79.5)

Control = Fragments incubating in the presence of sera but no renal homogenates. Control sera (12% vol/vol) is present in all flasks. Values are the average of results from four flasks. Range of values are shown in parentheses. The average difference in specific activity between fragments incubating in extracts from SHAM rats and normal sera compared to control is −9.0 dpm/μg DNA±2.9(SEM), $P < 0.02$; and between fragments incubating in extracts from UNI rats and control sera compared to control is 12.1 dpm/μg DNA±3.3(SEM), $P < 0.01$.

TABLE III

Effects of Combinations of Extracts and Sera from UNI and SHAM Rats on [^3H]Thymidine Incorporation into DNA of Rat Renal Fragments

Extract sera	Average specific activity			
	SHAM SHAM	SHAM UNI	UNI UNI	UNI SHAM
	dpm/μg DNA			
1	168.0 (161.8–180.0)	214.0 (193.0–226.0)	293.0 (258.0–321.0)	228.0 (188.0–238.0)
2	82.0 (77.4–86.5)	95.0 (90.1–99.8)	124.0 (105.0–140.0)	91.9 (80.6–105.0)
3	97.9 (97.8–98.0)	95.5 (72.3–123.0)	138.0 (124.0–149.0)	122.0 (112.0–132.0)
4	123.0 (121.0–126.0)	137.0 (128.0–145.0)	171.0 (168.0–174.0)	153.0 (150.0–158.0)
5	89.8 (88.1–91.6)	103.0 (95.9–117.0)	103.0 (100.0–105.0)	106.0 (98.5–115.0)
6	74.7 (72.2–77.5)	99.5 (94.4–106.0)	148.0 (129.0–156.0)	124.0 (111.0–132.0)
7	60.4 (52.3–66.1)	61.4 (59.0–66.7)	92.4 (88.1–95.4)	84.6 (80.1–91.1)

Average of results and range from four flasks are shown to the left for all combinations of sera and extracts from UNI (20 h) and SHAM (20 h) rats. Compared to fragments incubating in sera and kidney extracts from SHAM rats, the average difference in specific activity of fragments incubating in sham extract and UNI sera is 15.7 dpm/μg DNA±6.1(SEM), $P < 0.05$; incubating in UNI sera and UNI extract is 53.4 dpm/μg DNA ±13.7(SEM), $P < 0.01$, and incubating in UNI extract and sham sera is 30.5 dpm/μg DNA±6.8(SEM), $P < 0.01$.

+ 16.5%, ($> 0.05 < 0.1$). Of course, this stimulation was less than that produced by UNI extracts in the presence of SHAM or UNI plasma (Table III).

The four studies shown in Table V were performed to check how dialysis of azotemic sera affected these reactions. The dialyzed SHAM sera in the presence of UNI extract still caused a significant stimulation, + 24%, ($P < 0.05$) (compare Tables II, III, IV); and dialyzed azotemic sera in the presence of SHAM extract significantly stimulated incorporation, + 28.1%, ($P < 0.05$). The combination of dialyzed azotemic sera and UNI extract caused the greatest enhancement of incorporation, + 53.8%, ($P < 0.01$).

Finally, two studies were performed to see if the active renotropic stimulator was released from renal tissue. Kidney slices obtained from four rats 20 h after a SHAM (eight flasks) and four rats 20 h after UNI (eight flasks) were incubated in control sera and with pooled fragments from two control rats. While the slices from the remaining kidney 20 h after UNI compared to slices from a SHAM kidney, as expected, incorporated significantly more [^{3}H]thymidine into their

TABLE IV

Effects of Combinations of Extracts from UNI and SHAM Rats and Sera from BI and SHAM Rats on [^3H]Thymidine Incorporation into DNA of Rat Renal Fragments

Extract sera	Average specific activity			
	SHAM SHAM	SHAM BI	UNI BI	UNI SHAM
	dpm/μg DNA			
1	63.2 (56.6–71.0)	64.6 (59.5–69.8)	66.7 (58.2–71.8)	79.0 (70.6–91.9)
2	53.6 (48.6–58.5)	56.6 (52.7–59.4)	60.4 (53.8–67.8)	64.8 (60.7–68.0)
3	61.8 (57.7–63.4)	62.5 (58.6–65.9)	84.3 (79.7–92.3)	86.2 (81.6–96.0)
4	48.1 (41.4–60.0)	53.4 (50.8–57.3)	52.6 (47.3–59.4)	55.9 (50.0–63.5)
5	42.9 (40.8–45.1)	50.2 (47.3–52.9)	45.3 (38.1–50.5)	70.1 (61.8–85.3)
6	99.2 (79.4–109)	99.5 (91.9–110)	129 (114–153)	165 (153–170)

Average of results and range from four flasks are shown to the left for all combinations of extracts from UNI (20 h) and SHAM (20 h) rats and sera from BI (20 h) and SHAM (20 h) rats. Compared to fragments incubating in sera and kidney extracts from SHAM rats, the average difference in specific activity of fragments incubating in SHAM extract and BI sera is 3.0 dpm/μg DNA ±1.1(SEM), $P < 0.05$; incubating in UNI extract and BI sera is 11.6 dpm/μg DNA±4.7(SEM) $P > 0.05 < 0.1$; and incubating in UNI extract and SHAM sera is 25.4 dpm/μg DNA±8.6(SEM), $P < 0.05$.

TABLE V

Effects of Combinations of Extracts from UNI and SHAM Rats and Dialyzed Sera from BI and SHAM Rats on [³H]Thymidine Incorporation into DNA of Rat Renal Fragments

Extract dialyzed sera	Average specific activity			
	SHAM SHAM	SHAM BI	UNI BI	UNI SHAM
	dpm/μg DNA			
1	117 (107–124)	126 (117–131)	136 (131–142)	135 (131–139)
2	112 (108–116)	139 (133–151)	168 (165–173)	150 (138–165)
3	85.2 (83.4–86.7)	122 (112–133)	144 (136–148)	104 (88.8–121)
4	58.4 (57.1–58.7)	80.5 (76.9–82.8)	106 (94.6–111)	72.9 (65.4–80.9)

Average results and range from four flasks are shown to the left for all combinations of extracts from UNI (20 h) and SHAM rats (20 h) and dialyzed sera from BI (20 h) and SHAM rats (20 h). Compared to fragments incubating in dialyzed sera and extracts from SHAM rats, the average difference in specific activity in fragments incubating in SHAM extract and dialyzed BI sera is 23.7 dpm/μg DNA±5.8(SEM), $P < 0.05$; incubating in UNI extract and dialyzed BI sera is 45.3 dpm/μg DNA±9.0(SEM), $P < 0.01$; and incubating in UNI extract and dialyzed SHAM sera is 22.3 dpm/μg DNA±5.3(SEM), $P < 0.05$.

DNA (421.3 dpm/μg DNA±10.2 [SEM] compared to 204.7 dpm/μg DNA±12.7 [SEM] [$P < 0.01$]), the renal fragments from control rats bathing with the UNI slices incorporated no more [³H]thymidine than those bathing with the kidney slice from the SHAM rat (323.5 dpm/μg DNA±26.4 [SEM] compared to 323.0 dpm/μg DNA±25.1 [SEM]).

DISCUSSION

Existence of circulating renotropins, factors affecting some aspect of renal cellular growth, is supported by the ability of sera from UNI animals to enhance isotopic labeling of nucleic acids in renal tissue cultures and intact kidneys (10–16). In 1970, we utilized an in vitro assay to detect a circulating renotropin that stimulates DNA synthesis as measured by isotopic labeling of DNA in rat kidney slices (3). We directed our initial efforts in the present study toward determining the temporal appearance of circulating renotropin in an improved assay system. In the new assay, we chose fragments over slice pairs because, in our hands, there is a closer replication of results within a given experiment (compare Table II with experiments in reference 3). [³H]Thymidine is substituted for [³H]thymidine monophosphate because of its greater transport into cells. We use ultrafiltrates of plasma or whole sera because addition of plasma to fragments produces clotting, indicating that excess amounts of calcium and/or magnesium are present in the medium to overcome the effects of EDTA. In agreement with our previous findings (3), we discovered that a circulating renotropin is present after UNI. The new information gained was that renotropin is present as early as 10 h postoperation but is gone by 36 h.

Did the presence of EDTA in plasma ultrafiltrates affect our results? This is unlikely. Toback and Lowenstein (17) used an in vitro system to measure the rate of DNA synthesis and reported that EDTA can inhibit [³H]thymidine incorporation into renal slice DNA but only in medium devoid of calcium. Our present studies using minimal amounts of EDTA as well as the earlier ones (3) were always performed in medium containing calcium. Then, too, our effect can be shown using sera instead of plasma (Fig. 2). In the original studies, (3), we felt that the renotropic factor was more apparent in EDTA plasma than sera. For this reason, our initial assays were performed with the plasma ultrafiltrates. When we found that sera showed as much renotropic activity as plasma ultrafiltrates (cf. Figs. 1 and 2), we continued our assays with sera.

After improvement of the in vitro assay, our prime objective was to comprehend why azotemic sera from BI rats shows no renotropic activity (3). Two possibilities seemed likely: (*a*) that renal tissue is necessary to produce the substance causing the effect and/or (*b*) circulating inhibitors in azotemia prevent stimulation despite the presence of renotropins. We pursued the former possibility first.

In 1956, Saetren (18) reported that intraperitoneal extracts of macerated kidneys inhibited rather than enhanced renal compensatory hyperplasia. While Williams described similar findings (19), 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 (20). When we added extracts from growing kidneys to renal fragments incubating in [³H]thymidine, depression rather than excitation to DNA syn-

thesis was demonstrated (Table I). However, extracts from growing kidneys in the presence of serum showed enhanced renal DNA synthesis when compared to fragments incubating in serum alone (Table II). This suggests a two-part system, a factor that increases its concentration in the remaining kidneys after UNI and another in the circulation.

A comparison between the findings depicted in Tables I and II is important. [^{3}H]thymidine incorporation into DNA has been used by many as a sensitive indicator of DNA metabolism (13, 17, 21–23). However, interpretation of results using isotopes is dependent upon the specific activity of the immediate precursor pools of nucleic acids. The use of incubating renal fragments offers certain advantages for, in the assay, little doubt can exist concerning the similarity of the intracellular precursor pools for DNA within a given experiment. Still, a nonspecific extracellular dilution of tracer by SHAM extracts and/or sera as compared to test extract and/or sera could increase isotope incorporation into DNA in the test fragments despite no actual change in DNA synthesis. The results using liver slices in the assay (3, 4) help to dispel the possibility that changes in the precursor pools for DNA in the sera have brought about the positive results. An increase in isotopic incorporation into DNA by such a nonspecific mechanism as dilution of tracer should appear with slices from any organ. Since this is not the case, it seems likely that a humoral factor stimulating [^{3}H]-thymidine into kidney DNA truly exists. Likewise, the equal depression to [^{3}H]thymidine incorporation into renal fragment DNA by extracts from SHAM and the remaining kidney after UNI (Table I) should dispel the possibility that the differences in results seen in Tables II (as well as Tables III–V) when both sera and extract are added are secondary to nonspecific dilution of tracer by materials in the extracts.

How do the factors during the renal growth period after UNI (sera and tissue) compare in their ability to stimulate renal DNA synthesis with those present during a nongrowth state? From the data depicted in Table III, we surmise that both sera and renal factors increase after UNI and that an elevation in either factor alone in the presence of normal concentrations of the other can increase stimulation to [^{3}H]thymidine incorporation into renal DNA. Finally, since the greatest stimulation to DNA synthesis in renal fragments is seen in the present of combined UNI sera and UNI extract, both factors are additive to the excitatory phenomenon.

Our question, whether kidneys produced the originally described circulating renotropic factor (3), was answered in the studies on azotemic sera. Whereas azotemic sera from rats bilaterally nephrectomized 20 h 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 h. From this, we must conclude that augmented concentrations of renotropin are present in sera from rats made azotemic by having both kidneys removed 20 h earlier and that this renotropic factor can remain active even after 24 h of dialysis. Since both kidneys were absent for 20 h before obtaining this sera, it seems unlikely that kidneys produce the circulating factor responsible for enhanced DNA synthesis. Also because UNI sera stimulates [^{3}H]thymidine incorporation into DNA to a greater extent based on percent of control after dialysis (Fig. 2), it is possible that inhibitors to renotropic activity, while more apparent in azotemic sera, are present in nonazotemic UNI sera as well. We cannot state how the stimulator augments DNA synthesis, but we do know that it does not work by enhancing [^{3}H]thymidine transport into renal cells.

In our last two experiments, we found that slices from UNI rats (20 h postoperation) in the presence of sera show enhanced incorporation of [^{3}H]thymidine into their own DNA but do not pass the stimulation to control renal fragments incubating in the same medium. Thus, while kidney extracts from UNI in the presence of sera can enhance DNA synthesis in control renal fragments, slices from UNI in the presence of sera cannot. These findings suggest to us that the excitor produced in the presence of the renal and sera factors from UNI does not appear in the medium in active form but is converted to an active form within renal tissue and remains there.

Perhaps the original renotropin in plasma and sera from UNI (3) (Figs. 1 and 2) is the same as the sera factor necessary in the system depicted in Tables II–V. It occurred to us that our original renotropic factor in plasma or sera from UNI (3) enhanced DNA synthesis only in kidney tissue. Specific localization of tissue activator and its excitatory product could explain why only renal cortical tissue not liver, spleen, or lung tissue is affected by UNI plasma or sera (3, 4). This is consistent with the possibility that renotropin is inactive until it is in the presence of renal tissue. In support of this, either the production by sera and renal extracts of the excitor from renotropin and/or its activity is lessened by dialyzable inhibitors present in azotemic sera (Table V). This is similar to the findings depicted in Fig. 2 where azotemic sera alone is assayed.

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