GENETIC INFLUENCE ON THE DEVELOPMENT OF RENOPRIVAL HYPERTENSION IN PARABIOTIC RATS

Evidence That a Humoral Hypertensinogenic Factor Is Produced in Kidney Tissue of Hypertension-Prone Rats*

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(Received for publication 25 August 1969)

Experimental hypertension in the rat is modified by genotype; a given procedure may cause severe hypertensive disease in one rat, induce a moderate elevation of blood pressure in another and leave a third unaffected. By selective breeding for such traits, two colonies of Sprague-Dawley rats have been established; one is called the S strain and its members have proved extremely sensitive to all tested procedures for experimental hypertension; members of the other strain, called R, have high resistance (1–3).

From evidence presented in recent reports (4, 5), we concluded that a hypertensinogenic agent was produced in S animals on a high salt diet or after certain renal operations. It was not naturally present in R rats, but if transmitted in parabiosis, R rats would respond to its influence. We found in single S rats, that adrenal function was necessary for development of hypertension, although adrenal tissue has not been proved to produce the strain-specific hypertensinogenic agent (6).

In this report we present evidence, from nephrectomy of one member of a pair of rats united in parabiosis, which indicates that kidney tissue is necessary for production of the S factor.

Materials and Methods

All rats were from our R and S colonies. Details on care, breeding, blood pressure measurements, and the operations of parabiosis and nephrectomy have been published (1–5).

Two types of diets were used. One was a standard Purina rat chow with a low sodium content (0.4% NaCl, dry weight) made to order. The other, prepared by us, was a mixture of corn oil, sucrose, purified casein (Vitamin-free Casein, Nutritional Biochemicals Corp., Cleveland, Ohio), vitamins, and minerals, but no added sodium. By analysis, this diet contained 0.008–0.013% NaCl. The two diets will be referred to as "low sodium" and "sodium-free", respectively; their compositions are compared in Table I.

* This work was supported by the United States Atomic Energy Commission.

Drinking fluid was distilled water; tap water (0.003% NaCl); or 0.45% NaCl solution with 2.5% sucrose. The latter was used to increase the sodium intake, rather than adding NaCl to the solid food and such animals were considered to be on a "high sodium" intake.

Statistical analysis was by analysis of variance.[1] The influences of strain and operation on blood pressures of both partners were tested in 2 × 2 factorial designs.

Among the factors to be considered before analysis was possible, the high mortality caused most concern. In some unpublished studies, we found no evidence, however, that it introduced a systematic bias. The animals which died after nephrectomy of one of the members in parabiosis had uremia; this occurred mainly in pairs with a slow rate of exchange of extracellular fluid across the parabiosis junction. R-R pairs (i.e., parabionts, both members of which be-

TABLE I

Composition of Test Diets

	Low Sodium	Sodium-free
Carbohydrate	40–50% (from starches and molasses)	72% (from sucrose)
Fat	Approx. 5%	5% (corn oil)
Crude fiber	" 6%	None
Ash	" 9%	5% (mineral mixture)
NaCl	0.4%	0.01%
Protein	" 23%	18% (casein)
Arginine	1.09%	0.45%
Aspartic acid	1.85%	1.00%
Glycine	1.14%	0.25%
Alanine	1.09%	0.43%

Amino acid contents were equivalent, except for the ones that are included in the table.

We thank Dr. R. Aronson for performing the amino acid analyses of the diets.

longed to the R strain) generally had the highest exchange rate (as determined by ^{22}Na injected i.v. into one partner) and the highest survival ratio. However, within each class, variance of the exchange rate, and hence mortality, appeared to be caused by random influences. A more serious problem arose from efforts to improve the survival ratio by performing the nephrectomy in two stages at 8 wk intervals. Uninephrectomized S rats have a tendency to develop hypertension (5) and the bias introduced by this 8 wk period of uninephrectomy and parabiosis had to be evaluated and considered.

Since hypertension is known to be a complication of the renoprival state after high salt intake, we also had to compare the results on different sodium intake levels. In the sodium-free diet, the sources of protein and carbohydrate differed from that in the regular rat chow but, quantitatively, we attempted to make the diets equivalent. The only intended difference was in sodium content. The literature pertaining to those problems was reviewed recently (7).

In all series, the operations were identical. A total of 269 pairs of 4-wk old rats was united in parabiosis at which time one partner was also uninephrectomized. The low salt diet and tap water on which they had been maintained from weaning were continued for 8 more wk. Preliminary studies had shown that about 8 wk were necessary to establish sufficient exchange between the parabionts to prevent a prohibitively high mortality after the second nephrectomy. At the end of the 8 wk period blood pressures were recorded and analyzed to find the effect of

[1] We thank Keith Thompson for help with this analysis.

uninephrectomy on parabionts on a low sodium diet. The diet was then adjusted according to the experimental design (below) and the second kidney removed from the previously uninephrectomized partner of the 242 surviving pairs. The original protocol called for at least 6 wk of observation thereafter. However, rats on sodium-free diets showed weight loss, dull furs, and increased mortality at 5 wk. For these reasons, the statistical comparisons were restricted to the 4-wk values of sodium-free and low sodium groups. Because nearly all of the high sodium rats died within less than 3 wk, results from these groups are presented without statistical evaluation.

The following notations will be used: The letters R and S symbolize rats from one of our two colonies; R-S, S-S, R-R represent parabiotic pairs. When one member of a parabiotic pair is to be identified separately it will be presented first with the identity of its partner shown in parenthesis, e.g., S(R). A subscript ($_{op}$) will denote a uni- or bilaterally nephrectomized rat. Thus, R_{op}-S is a parabiotic pair in which the R partner is nephrectomized; in such a pair R_{op}(S) identifies the operated R partner, while S(R_{op}) identifies the intact S partner.

Study 1

Effect of Uninephrectomy of One Partner in Parabiosis: Low Sodium Diet.—This study is an analysis of blood pressures on the 242 surviving pairs obtained after 8 wk of uninephrectomy/parabiosis; i.e., before the second nephrectomy.

Study 2

Effect of Total Nephrectomy on One Partner in Parabiosis: Sodium-Free Diet.—Two days prior to the second nephrectomy, 104 pairs were changed to the sodium-free diet and distilled water. During the next 4 wk on this diet, blood pressures were measured twice a week. Time was recorded from the day of the second nephrectomy.

Study 3

Effect of Total Nephrectomy of One Partner in Parabiosis: Low Sodium Diet.—The low-sodium diet was continued among the remaining 138 pairs for 2–4 wk after the second nephrectomy. Blood pressures were measured twice weekly.

Study 4

Effect of Total Nephrectomy of One Partner in Parabiosis: High Sodium Intake.—29 pairs among the 138 mentioned above were taken from Study 3 after 2–4 weeks on low-sodium diet. They were given 0.43% saline with 2.5% sucrose, instead of tap water, and thereafter observed on this high-sodium intake. Blood pressures were continued twice weekly.

OBSERVATIONS

Study 1

Effect on Blood Pressure of Uninephrectomy of One Partner in Parabiosis: Low Salt Diet (Table II).—Table II sums up the average results. Uninephrectomy did not result in hypertension in rats from either strain united to R rats, whereas the same operation induced significant increase in the blood pressures of similar rats united with S partners. Parabiosis appeared to dampen the hypertensive response of S rats after uninephrectomy; union with intact R rats was more effective in this respect. In a previous study we found that single uninephrectomized S rats on low salt diet had an average blood pressure of 149 mm Hg

(5), whereas in the present experiment $S_{op}(S)$ rats averaged 135 mm Hg and $S_{op}(R)$ rats 124 mm Hg. The intact parabionts remained normotensive.

Study 2

Effect on Blood Pressure of Complete Nephrectomy of One Partner in Parabiosis: Sodium-Free Diet (Table III, Fig 1).—104 pairs were used for this study of which 46 survived 4 wk and are included in Table III; data on all surviving pairs each wk are included in Fig. 1. As mentioned above, after 5 wk on this sodium-deficient diet, the animals appeared sick; thus no data after the 4th wk have been included in the analysis. The bias introduced by earlier uni-

TABLE II

Blood Pressures of Parabionts on Low Sodium Diet, 8 Wk after Uninephrectomy of One Partner

Class*	No. in Class	Mean Systolic Blood Pressure (*mm Hg*)	(±SE)
$R_{op}(R)$	56	118	(1.52)
$S_{op}(R)$	69	124	(1.59)
$R_{op}(S)$	66	136	(2.43)
$S_{op}(S)$	51	135	(1.93)
$R(R_{op})$	56	115	(1.51)
$R(S_{op})$	69	125	(2.00)
$S(R_{op})$	66	123	(1.52)
$S(S_{op})$	51	126	(1.47)

Factorial analysis of variance showed that among uninephrectomized parabionts: $S_{op}(S) \sim R_{op}(S) > S_{op}(R) \sim R_{op}(R)$ ($P < 0.01$), i.e., genetic factors from the intact parabionts provided the only significant source of variance.

* See text for explanation of symbols.

nephrectomy was apparent at the beginning of the experiment. (See Study 1). At 0 wk uninephrectomized rats with S partners had significantly higher blood pressures than the other groups (Fig. 1). Nephrectomized partners subsequently developed frank hypertension only if they were united with intact S partners, but not if united with R partners ($P < 0.01$).

Blood pressures of the *intact* parabionts of both strains on a sodium-free diet were unaffected by bilateral nephrectomy of the partner, even when the latter developed significant renoprival hypertension.

Study 3

Effect on Blood Pressure of Nephrectomy of One Partner in Parabiosis: Low Sodium Diet (Table IV, Fig. 2).—Table IV lists the individual blood pressures of the 45 pairs alive at 4 wk on this regimen. Of the original 138 pairs, 19 had been transferred to Study 4 (below) at the end of the 2nd wk. The rest were dead (74 pairs). Of the 45 pairs in Table IV, 10 pairs were transferred to Study

TABLE III

Blood Pressures of Parabionts on Sodium-free Diet for 4 Wk after Complete Nephrectomy of One Partner

R_{op}-R*

Pair	0 Wk‡		4 Wk§		Sex
	R_{op}	R	R_{op}	R	
	(*mm Hg*)		(*mm Hg*)		
1	122	112	152	122	F
2	110	122	102	120	F
3	108	114	132	93	F
4	120	112	190	120	M
5	128	130	170	122	M
6	110	122	120	128	M
7	120	120	162	118	M
8	110	114	124	120	M
9	130	122	122	120	M
10	120	114	128	127	M
11	96	120	102	112	M
12	122	134	140	124	M
13	104	102	94	111	M
n	13		13		
Mean	115	118	134	118	
SE (mean)	2.7	2.3	7.9	2.5	

S_{op}-R

Pair	0 Wk		4 Wk		Sex
	S_{op}	R	S_{op}	R	
	(*mm Hg*)		(*mm Hg*)		
14	139	132	92	122	F
15	148	120	161	122	F
16	132	124	120	104	F
17	104	164	142	154	F
18	128	126	138	132	F
19	104	102	138	122	F
20	118	112	144	124	M
21	124	122	132	118	M
22	138	132	124	132	M
23	104	138	104	132	M
24	115	122	102	122	M
n	11		11		
Mean	123	127	127	126	
SE (mean)	4.5	4.9	6.3	3.7	

R_{op}-S

Pair	0 Wk		4 Wk		Sex
	R_{op}	S	R_{op}	S	
	(*mm Hg*)		(*mm Hg*)		
25	152	114	162	108	F
26	156	94	152	118	F
27	126	134	172	120	F
28	132	124	152	120	F
29	150	130	154	122	F
30	142	131	142	120	M
31	132	118	154	122	M
32	140	126	142	144	M
33	142	115	150	102	M
34	138	126	174	132	M
35	124	110	184	150	M
n	11		11		
Mean	140	120	158	123	
SE (mean)	3.1	3.5	4.1	4.2	

S_{op}-S

Pair	0 Wk		4 Wk		Sex
	S_{op}	S	S_{op}	S	
	(*mm Hg*)		(*mm Hg*)		
36	151	118	144	112	F
37	133	122	132	110	F
38	128	120	151	142	F
39	134	130	150	104	F
40	159	120	172	134	F
41	140	135	172	130	F
42	141	132	132	152	M
43	120	142	160	136	M
44	142	132	152	140	M
45	122	130	194	142	M
46	140	122	198	138	M
n	11		11		
Mean	137	128	160	131	
SE (mean)	3.5	2.3	6.7	4.7	

Factorial Analysis of variance showed: 0 Wk, operated partner: $S_{op}(S) \sim R_{op}(S) > S_{op}(R) \sim R_{op}(R)$ ($P < 0.01$); 4 Wk, operated partner: $S_{op}(S) \sim R_{op}(S) > S_{op}(R) \sim R_{op}(R)$ ($P < 0.01$); 0 Wk, intact partner: $S(S_{op}) \sim R(S_{op}) > S(R_{op}) \sim R(R_{op})$ ($P < 0.05$); 4 Wk, intact partner: $S(S_{op}) \sim R(S_{op}) > S(R_{op}) \sim R(R_{op})$ ($P < 0.05$).

A comparison by *t* tests for the increase within each class during the 4-wk period showed: $S_{op}(S)_{4\,wk} \sim R_{op}(S)_{4\,wk} > S_{op}(S)_{0\,wk} \sim R_{op}(S)_{0\,wk}$ ($P < 0.001$); $R_{op}(R)_{4\,wk} > R_{op}(R)_{0\,wk}$ ($P < 0.05$). The other classes did not change ($P > 0.05$).

* See text for explanation of symbols.

‡ Systolic blood pressures obtained prior to second nephrectomy and change of diet, i.e., after 8 wk of unilateral nephrectomy of one partner.

§ Blood pressures obtained in the same pairs 4 wk after the second nephrectomy of one partner and change to sodium-free diet.

4 after the 4th wk. Fig. 2 a–d summarize the blood pressure data of all animals on low sodium during the 4 wk observation period.

In contrast to the parabionts on the sodium-free diet, all 4 classes of *nephrectomized* parabionts suffered a significant increase in blood pressure during 4 wk on the low sodium diet. Intraclass correlations showed that genetic factors were not a significant source of variance ($P > 0.05$). The *intact* S parabionts tended

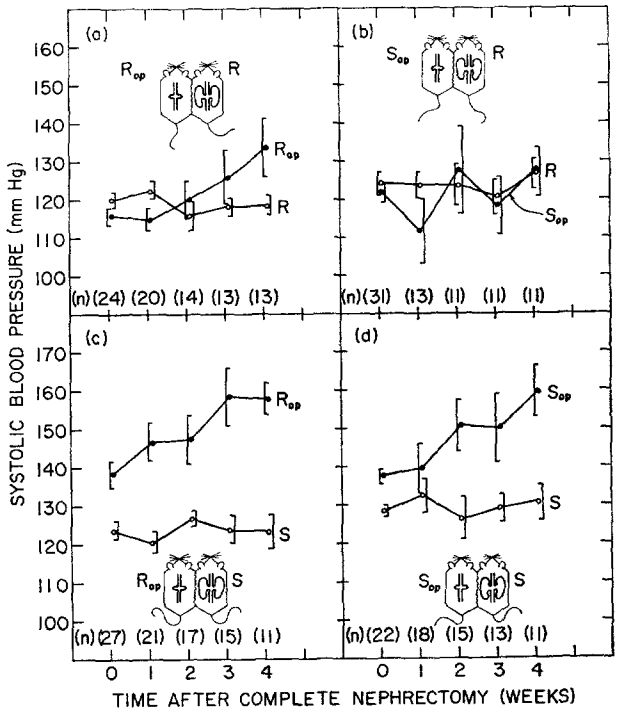

FIG. 1. Development of blood pressures of parabionts on sodium-free diet after bilateral nephrectomy of one partner. For explanation see text and legend to Table III.

to develop hypertension (Fig. 2 c, d), whereas the intact R parabionts did not. The development of hypertension in the intact S animals could be due to transfer of a humoral agent from the nephrectomized partners, or be a result of the S animals' own metabolism. Since intact R parabionts failed to develop hypertension when exposed to similar influences, the observations argue against the presence of a transmitted humoral agent from the nephrectomized side. The essence of these observations is that even when nephrectomized S rats did develop hypertension, there was no general tendency for this to be transmitted to intact R partners.

TABLE IV

Blood Pressures of Parabionts on Low Sodium Diet 4 Wk after Complete Nephrectomy of One Partner

R_{op}-R

Pair	0 Wk		4 Wk		Sex
	R_{op}	R	R_{op}	R	
	(*mm Hg*)		(*mm Hg*)		
47	152	120	146	130	F
48	116	110	140	126	F
49	118	104	136	124	F
50	120	96	116	102	F
51	92	104	110	116	F
52	110	126	136	122	F
53	110	112	128	102	M
54	114	132	156	122	M
55	122	140	142	120	M
56	124	116	148	120	M
57	116	120	140	120	M
58	124	116	126	110	M
59	126	136	144	136	M
60	126	104	142	126	M
n	14		14		
Mean	119	117	136	120	
SE (mean)	3.5	3.5	3.4	2.6	

S_{op}-R

Pair	0 Wk		4 Wk		Sex
	S_{op}	R	S_{op}	R	
	(*mm Hg*)		(*mm Hg*)		
61	108	110	120	102	F
62	125	124	102	124	F
63	140	122	164	98	F
64	118	148	140	128	F
65	140	100	160	120	M
66	120	110	168	140	M
67	118	108	158	136	M
68	148	120	140	126	M
69	112	120	120	122	M
70	126	138	162	148	M
71	116	118	152	132	M
n	11		11		
Mean	125	120	144	125	
SE (mean)	5.3	4.1	6.6	4.5	

R_{op}-S

Pair	0 Wk		4 Wk		Sex
	R_{op}	S	R_{op}	S	
	(*mm Hg*)		(*mm Hg*)		
72	115	135	116	108	F
73	128	130	162	108	F
74	110	108	150	152	F
75	120	140	130	182	F
76	116	104	160	106	F
77	172	122	154	122	M
78	120	110	160	152	M
79	124	132	170	164	M
80	120	142	142	170	M
81	154	142	184	138	M
82	111	98	166	120	M
83	112	114	136	118	M
n	12		12		
Mean	125	123	153	137	
SE (mean)	5.4	4.6	5.4	7.7	

S_{op}-S

Pair	0 Wk		4 Wk		Sex
	S_{op}	S	S_{op}	S	
	(*mm Hg*)		(*mm Hg*)		
84	100	110	140	114	F
85	120	110	150	126	M
86	138	130	182	146	M
87	145	126	120	156	M
88	120	118	128	140	M
89	122	124	144	122	M
90	134	142	148	160	M
91	124	122	174	140	M
n	8		8		
Mean	125	123	148	138	
SE (mean)	4.9	3.7	7.4	5.8	

Factorial analysis of variance showed: 0 Wk, operated partner: $S_{op}(S) \sim R_{op}(S) \sim S_{op}(R) \sim R_{op}(R)$; 4 Wk, operated partner: $S_{op}(S) \sim R_{op}(S) \sim S_{op}(R) \sim R_{op}(R)$; 0 Wk, intact partner: $S(S_{op}) \sim S(R_{op}) \sim R(S_{op}) \sim R(R_{op})$; 4 Wk, intact partner: $S(S_{op}) \sim S(R_{op}) > R(S_{op}) \sim R(R_{op})$ ($P < 0.05$).

A comparison by *t* test within each class showed that average blood pressure of all nephrectomized parabionts increased during the 4 wk ($P < 0.05$). Among the intact partners, only S parabionts showed significant increase ($P < 0.05$).

The $R_{op}(S)$ and $S_{op}(S)$ rats which survived 4 wk on low salt intake, started at 0 Wk with average blood pressure 125 mm Hg. The populations they came from had average blood pressures 134 and 132 mm Hg, respectively. (See Fig. 2c, d) This could suggest a higher survival rate for rats with lower pressures; however, analysis of variance indicated that the difference might be due to chance alone ($P > 0.05$).

Study 4

Effect on Blood Pressure of Nephrectomy of One Partner in Parabiosis: High Sodium Intake (Table V).—Increasing the salt intake by drinking saline, (0.45% NaCl solution with 2.5% glucose) instead of tap water, produced hypertension in nearly all nephrectomized partners of both strains and killed most pairs within 3 wk.

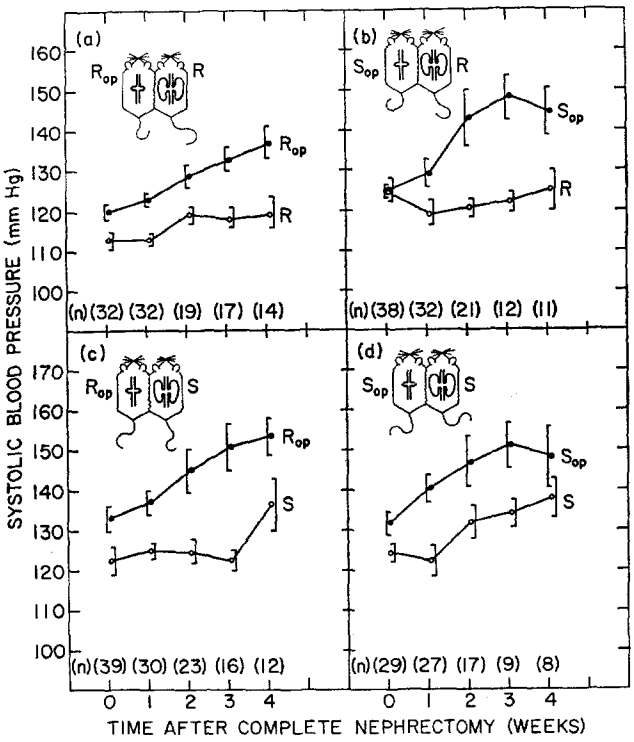

FIG. 2. Development of blood pressures of parabionts on low sodium diet after bilateral nephrectomy of one partner. For explanations, see text and legend to Table IV.

Table V lists the results during 3 wk of study. The data are presented without statistical evaluation, because of unequal groups and the short and variable duration of observation caused by the high mortality. The pressures represent the last reliable recording in the period 2 days to 3 wk on high salt. In conformity with the two other studies above, hypertension in renoprival S rats had little or no tendency to induce hypertension in intact R partners.

DISCUSSION

The central question in this study was whether S kidneys could be shown to be the source of a hypertensinogenic agent that, in earlier studies, had been

TABLE V

Blood Pressures of Parabionts on High Sodium Intake after Bilateral Nephrectomy of One Partner

R_{op}-R

Pair	Starting values: Day after nephrectomy	Starting values: Blood pressure R_{op}	Starting values: Blood pressure R	Final values: Days on high salt	Final values: Blood pressure R_{op}	Final values: Blood pressure R	Sex
		(*mm Hg*)			(*mm Hg*)		
92	14	140	126	21	158	130	F
93	34	146	128	19	150	158	F
94	28	144	126	17	154	126	F
95	34	116	102	2	118	110	F
96	34	110	116	2	No BP obtnd		F
97	14	116	114	21	156	120	M
98	14	144	136	21	130	138	M
99	14	108	100	21	146	120	M
100	14	134	106	14	141	110	M
101	14	108	120	14	164	121	M
102	34	126	110	7	118	120	M
103	14	122	110	2	No BP obtnd		M
104	28	142	126	2	No BP obtnd		M
	n	13		n	10		
	Mean	127	117	Mean	144	125	

S_{op}-R

Pair	Starting values: Day after nephrectomy	Starting values: Blood pressure S_{op}	Starting values: Blood pressure R	Final values: Days on high salt	Final values: Blood pressure S_{op}	Final values: Blood pressure R	Sex
		(*mm Hg*)			(*mm Hg*)		
105	14	160	122	21	152	140	F
106	32	120	108	21	144	112	F
107	30	140	120	2	No BP obtnd		F
108	14	164	116	21	186	144	M
109	14	130	124	21	150	120	M
110	14	70	158	14	166	162	M
111	14	90	110	14	160	129	M
112	14	120	122	14	200	134	M
113	14	93	124	2	No BP obtnd		M
114	14	140	112	2	No BP obtnd		M
	n	10		n	7		
	Mean	123	122	Mean	165	134	

R_{op}-S

Pair	Starting values: Days After Nephrectomy	Starting values: Blood pressure R_{op}	Starting values: Blood pressure S	Final values: Days on high salt	Final values: Blood pressure R_{op}	Final values: Blood pressure S	Sex
		(*mm Hg*)			(*mm Hg*)		
115	14	118	154	21	182	188	F
116	32	154	112	2	No BP obtnd		F
117	14	110	116	11	150	162	M
	n	3		n	2		
	Mean	127	127	Mean	166	175	

S_{op}-S

Pair	Starting values: Day after nephrectomy	Starting values: Blood pressure S_{op}	Starting values: Blood pressure S	Final values: Days on high salt	Final values: Blood pressure S_{op}	Final values: Blood pressure S	Sex
		(*mm Hg*)			(*mm Hg*)		
118	32	142	130	15	156	148	F
119	14	154	166	21	168	210	M
120	14	130	122	6	146	128	M
	n	3		n	3		
	Mean	142	139	Mean	157	162	

To allow for recovery, the high sodium addition was delayed for 2–4 wk after bilateral nephrectomy. This observation period is given in second column.

Most of the S_{op}-S and R_{op}-S pairs assigned to this study died before they could be switched to high sodium diet. Because of this and of the variable observation periods, a statistical evaluation is not presented.

Even R_{op}(R) parabionts developed hypertension on this regimen.

demonstrated to be transmittable across a parabiosis junction (4, 5). We found little or no evidence for such an agent after complete nephrectomy of the S rat and conclude that the agent is produced by the S kidney or needs the presence of kidney tissue for its production. A nephrectomized rat developing renoprival hypertension did not induce high blood pressure in its intact partner. Rather, when sodium intake was negligible, the development of renoprival hypertension in these parabionts depended on the union with an intact S partner. When salt intake increased, hypertension developed in nephrectomized rats, even when the intact partner belonged to the R strain. In the S_{op}-R pairs on the sodium-free diet (Table III), only one intact R(S_{op}) parabiont had hypertension (154 mm Hg) at the end of the 4th wk (pair 17) and in this instance the pressure had been elevated to even higher levels (164 mm Hg) *before* complete nephrectomy. Neither was there much hypertension among the S_{op}(R) rats, only 3 of which had such elevations at the end of the 4th wk (161, 142, 144 mg Hg; pairs 15, 17, 20). On low sodium diet (Table IV), in members of the corresponding group (S_{op}-R), similar results obtained in the intact R partners: only 2 of 11 animals were hypertensive (140, 148 mm Hg; pairs 66 and 70), whereas 8 of 11 of the nephrectomized rats were hypertensive (140–168 mm Hg). Finally, on high sodium intake, all 7 nephrectomized S rats in the S_{op}(R) group became hypertensive (144–200 mm Hg), whereas 4 out of 7 intact R partners remained normotensive. This list is in striking contrast with earlier observations on salt-fed intact parabionts, where all of the R partners rapidly developed significant hypertension prior to their S mates (4).

The influence of the intact parabionts on the development of renoprival hypertension in their operated partners, as mentioned above, was demonstrated among groups on the sodium-free diet (Table III): 20 of the 22 nephrectomized animals united with the intact S partners developed hypertension, whereas only 8 of 24 became hypertensive when the intact partner was from the R strain ($P < 0.01$). When dietary sodium was added to the regimen of similarly treated animals, the clear-cut difference just described became blurred; on the high sodium diet (Table V), for instance, only 4 of 22 nephrectomized rats remained normotensive and the influence of the intact partner was no longer apparent.

The study also raises some of the general problems of renoprival hypertension. This syndrome has been studied in man and in dog during treatment with artificial dialysis. In the rat, it has mainly been studied in parabiosis preparations. Jeffers, Lindauer, Twaddle, and Wolferth (8) were the first to report hypertension in renoprival parabiotic rats. They removed 3 kidneys from each pair of rats, examined the animals 2–90 days thereafter, with a median survival of 10 days. 13 of the bilaterally nephrectomized rats among 19 pairs showed a terminal elevation of blood pressure above 140 mm Hg. The food was standard

Purina dog chow, cabbage, celery, and carrots. Grollman and Rule (9) found in two pairs of parabiotic rats that removal of the kidneys in one animal sufficed to induce hypertension. Braun-Menéndez and Von Euler, however, found this to be the case in only 3 out of 23 pairs (10). Ledingham (11) confirmed the observations of Grollman and Rule in 12 out of 13 nephrectomized partners in parabiosis. The period of observation ranged from 9 to 55 days after nephrectomy. The diet contained 1 % NaCl, by weight. In this and subsequent studies of parabiosis, Ledingham (12) and Floyer (13) concurred in the conclusion that the main specific role of the kidney in blood pressure regulation was to inhibit an extrarenal pressor factor.

Toth and Bartfai (14) described the transmission of renal hypertension in parabiosis; they claimed, also, that the antihypertensive action of kidneys was based upon an endocrine effect, and could be transferred to a partner in parabiosis. None of their experiments lasted longer than 6 days.

The possible exchange of humoral factors between partners sets the parabiosis preparation apart from dialysis studies. Our first definite evidence that experimental hypertension was associated with the presence of a transmittable humoral agent derived from the studies of parabionts on high salt diet (4). In R-S parabionts, there was a temporary reversal of response compatible with the transfer of a pressor agent from S to R, or of an anti-hypertensive agent from R to S, or both. When renal hypertension was induced in one member of R-S parabionts, they were equally sensitive to the Goldblatt operation (unilateral nephrectomy and contralateral renal artery constriction). However, only the S parabionts—not the R—so operated, managed to induce hypertension in an intact partner (5). This would indicate that only the S rats produced a transmittable pressor agent. We have interpreted the data in the present studies as indicating that a pressor agent from the intact S rat can induce hypertension in nephrectomized partners on sodium-free diets and that such a factor is absent after bilateral nephrectomy of S rats.

Blood pressure is the final result of a delicate balance of homeostatic forces. Many of the so-called pressor agents are normal constituents of the organism, and they are balanced by anti-pressor factors. Although our interpretation appears sufficient, it may turn out to be too simplistic in the final analysis; however, as a working hypothesis we assume:

1. S rats produce a hypertensinogenic agent not present in R rats.
2. The agent is produced by kidney tissue.
3. The agent is transmittable in parabiosis.
4. The agent needs functioning adrenals to produce hypertension.
5. The agent is not a *sine qua non* for hypertension. Goldblatt hypertension in R rats (5), and renoprival hypertension in $R_{op}(R)$ parabionts on high salt developed, presumably, without the presence of this agent.

SUMMARY

Rats from two strains with opposite constitutional predisposition to hypertension were joined in parabiosis and one partner was nephrectomized. The influence of genetic factors and of diet on the blood pressures of the two classes of parabionts, operated and intact, indicated that renoprival hypertension occurred with equal frequency in rats from both strains; that the development of renoprival hypertension depended on the influence from an intact S partner, or on a high salt intake, or on both.

A nephrectomized S rat developing renoprival hypertension did not induce high blood pressure in its intact R partner. In this respect renoprival hypertension differs from salt and renal hypertension.

The findings are interpreted to mean that the hypertensinogenic agent specific for S rats is produced by S kidneys.

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