Table 1. Overall frequency of *t*-alleles in male mice from the vicinity of Calgary, Alberta.

No. of mice	Genotypes	Frequency	Gene frequency
134	++	0.761	$+ = 0.081$
36	$+t^{w5}$	0.205	$t^{w5} = 0.102$
6	$+t^{wx}$	0.034	$t^{wx} = 0.017$

to 84, with a mean of 10.4 individuals for buildings where two or more mice were found. On the basis of the behavioral observations of Eible-Eibesfeldt (*6*) on *Mus*, and the segregation of social groups observed in a Norway rat colony by Calhoun (*7*), each building might be expected to contain one or more family groups reproductively isolated from other such groups through defense of communal territories. The pattern of gene distribution I have observed appears to support such a hypothesis. Adjacent groups differ in genetic composition and these differences have persisted for 2 years despite gross disturbances incurred when males were removed for testing in 1962. During these sampling operations, 65 percent of male mice known to be present through previous mark-and-release censuses were removed. Of 13 buildings inhabited in both years, none of six buildings where heterozygotes were not found in 1962 had acquired *t*-alleles in 1963. Of the seven buildings where heterozygotes did occur in 1962, all but two produced animals heterozygous for the same allele in 1963.

Table 2. Distribution of genotypes among male mice from seven sets of farm buildings.

Farms*	Genotypes 1962†			Genotypes 1963‡		
	++	$+t^{w5}$	$+t^{wx}$	++	$+t^{w5}$	$+t^{wx}$
			Ellis			
A	0	1	0	2	0	0
B	0	4	0	2	1	0
G				0	1	0
I	1	0	0	3	0	0
J				0	1	0
K	3	0	0	3	0	0
L				1	3	0
N	0	1	0	1	2	0
O	1	1	0	3	4	0
			Stryker			
A				1	0	0
B	0	0	0			
C				1	0	0
D	1	0	0	3	0	0
E				5	0	0
F	1	1	0			
G	2	0	0			
H				2	3	0
I	1	0	0			
J	5	1	1	2	0	0
			Dunn			
A				3	0	0
B	1	0	0	3	0	0
C	0	1	0	2	1	0
D	1	1	0	10	1	0
			Peterson			
A				0	1	0
B	3	0	0	1	0	0
D				4	1	0
			Soderburg			
A				0	1	0
			Cary			
B	0	1	0			
C	1	0	0	11	0	0
E				5	0	0
F				7	0	0
			Perry			
B				1	0	2
C				0	0	2
D				2	0	0
F				4	0	1
H				31	4	0
			Totals			
36	21	12	1	113	24	5

* Letters indicate individual buildings. † In 1962 some buildings were sampled a second time. Tests included in this table refer only to the first sampling in each specific building. ‡ In 1962 only adult males were tested. In 1963 juvenile and immature males were also retained for testing.

The pattern of distribution on the Perry farm is particularly interesting. There the very large population in building *H* produced mice heterozygous for t^{w5}, but almost certainly did not contain mice heterozygous for t^{wx}, which was carried by mice in buildings *B*, *C* and *F* only a few meters distant.

In 1962 one t^{w5} heterozygote was taken on the Cary farm, in building *B*. In 1963 this deme had become extinct, but a total of 23 males were tested from three other granaries and an abandoned hen house, all within 30 meters, without discovery of a heterozygote. It thus appears that no deme on this farm now carries the allele.

The patterns of local distribution observed suggest that immigration of mice into established populations is very rare. Such a possibility has been suggested by the work of Godfrey (*8*) with voles. The demes discussed herein are of very small size, and the random pattern of distribution of *t*-alleles may be interpreted as supporting the contention of Lewontin and Dunn (*4*) that stochastic processes may play a significant role in the evolutionary dynamics of these alleles.

PAUL K. ANDERSON

Department of Biology,
University of Alberta, Calgary

References and Notes

1. L. C. Dunn, A. B. Beasley, H. Tinker, *J. Mammal.* **41**, 220 (1960).
2. L. C. Dunn, *Proc. Natl. Acad. Sci. U.S.* **43**, 158 (1957).
3. D. Bruck, *ibid.*, p. 152.
4. R. C. Lewontin and L. C. Dunn, *Genetics* **45**, 705 (1960).
5. L. C. Dunn, *ibid.* **42**, 299 (1957).
6. I. Eibl-Eibesfeldt, *Z. Tierpsychol.* **7**, 558 (1950).
7. J. B. Calhoun, *U.S. Public Health Serv. Publ. 1008* (1963).
8. J. Godfrey, *Proc. Roy. Phys. Soc. Edinburgh* **27**, 47 (1958).
9. I thank L. C. Dunn of Columbia University for supplying test stock and for conducting cross-testing of alleles in 1962. My work was supported by a grant from the National Research Council of Canada.

18 May 1964

Hypertensive Vascular Disease Produced by Homologous Renin

Abstract. *Administration of rat renin to uninephrectomized rats reproduced most, if not all, the changes (hypertension, vascular disease, hypertrophy of the zona glomerulosa of the adrenals) found after partial constriction of the renal artery. This is taken as evidence that the renal pressor system plays a major role in the pathogenesis of renal hypertension.*

On the assumption that renal hypertension results from increased release of renin, various, but unsuccessful, attempts have been made to reproduce the course of renal hypertensive disease by administering renin to normal animals. Subcutaneous injections or intravenous infusions of renin caused a slight rise in arterial pressure but no vascular lesions (*1*, *2*). These failures have been used as supporting evidence by those who believe that the renal pressor system has no part in the pathogenesis of hypertension.

Recently (*3*), we found that unlike hog renin, crude extracts of rat kidneys which had been subjected to reduced perfusion pressure reproduced the early manifestations of renal hypertension when injected subcutaneously into uninephrectomized rats. We then postulated that renal hypertension was caused by the release of renin and of a potentiating substance. The possibility remained, however, that the ineffectiveness of hog renin was due to its heterologous nature. We therefore decided to study the effects of rat renin alone or in combination with crude kidney extracts. By injecting renin into uninephrectomized animals we hoped to reproduce the changes associated with hypertension in rats with unilateral and partial clipping of the renal artery: if the injections were to replace the endocrine functions of the clipped kidney, the remaining kidney of the uninephrectomized rats should show the same changes as those seen in the one contralateral to the clipped kidney.

Female Sprague-Dawley rats weighing between 130 to 155 g were uninephrectomized and divided into four groups of six animals each. The animals in each group received injections as follows: group 1, crude kidney extracts; group 2, rat renin; group 3, rat renin plus crude kidney extracts; the rats in group 4 received saline injections. Crude extracts were prepared by grinding fresh normal rat kidneys with distilled water (2 ml per kidney) in a Potter homogenizer and centrifugation at 3600*g* for 20 minutes. The supernatant was injected at a total rate of 1.2 ml per day. Rat renin was prepared from 3 kg of normal kidneys (*4*) according to the procedure of Haas *et al.* (*5*); this included ammonium sulfate fractionation. The final product tested against hog renin (*6*) had a specific activity of 6 Goldblatt units per milligram of solids (*7*). The total daily dose was 60 units dissolved in 1.2 ml of saline. Animals of group 3 received a total daily volume of 2.4 ml consisting of 1.2 ml of the rat renin solution and of 1.2 ml of kidney extracts mixed together. The different preparations were administered daily in three equally divided subcutaneous injections every 8 hours. Treatment was started about 4 hours after uninephrectomy.

The animals were placed in metabolism cages, fed Purina Fox Chow and given tap water to drink. Blood pressure was measured by tail sphygmography just prior to the 5 o'clock injection. Urine flow and body weight were also recorded daily. Because of a limited supply of renin, animals were killed on the 10th day, starting 6 hours after the last injection. Blood was collected during ether anesthesia, ethylenediaminetetraacetic acid (EDTA) (3 to 5 $\times$ $10^{-3}M$) being used as anticoagulant and angiotensinase inhibitor (*8*). After centrifugation in the cold, the cell-free plasma was kept in the freezer and then assayed for pressor activity following incubation at 37°C for 2 hours. The assay was carried out in pentolinium-treated rats. Tissues were weighed, examined for gross lesions, and prepared for histologic studies. A part of each kidney was kept frozen until pressor activity of saline extracts could be assayed in rats nephrectomized 24 hours previously.

Treatment did not interfere with normal growth except in rats which received renin plus crude kidney extracts (Table 1). These animals also showed subcutaneous induration at the sites of injection. Urine flow was slightly increased in animals receiving injections of crude extracts (group 1). This increase was more pronounced in groups 2 and 3, diuresis occurring on the 2nd day of treatment and persisting at the same level until the end. Blood pressure values in the control group 4 showed the normal variations expected from the method (Fig. 1). The rats in group 1 remained normotensive; those in groups 2 and 3 showed normal values during the first 4 days, then a progressive rise to hypertensive levels. On the 10th day, blood pressure averages with standard deviations for each of the four groups were 127 ± 4.2, 194 ± 19.1, 180 ± 17.6, and 117 ± 9.9 mm-Hg, respectively. The degree of hypertension was similar in groups 2 and 3, indicating that addition of kidney extracts (group 3) did not modify the effects of renin in spite of subcutaneous inflammatory reactions. Heart weights, either absolute or expressed as percentages of body weight, paralleled changes in pressure; they were normal in group 1 and significantly increased in the two groups which showed hypertension ($< .01$). In groups 2 and 3 the kidneys were slightly enlarged and the thymus slightly atrophic; this atrophy reflects adrenal stimulation probably resulting from the severity of hypertensive disease.

For the four groups the average weights of the adrenal glands were 61.3 ± 7.7, 67.7 ± 9.25, 68 ± 6.2, and 59.2 ± 6.5 mg, respectively. The increase in groups 2 and 3 over the control value of 59.2 is significant ($< .05$). The width of the zona glomerulosa in groups 2 and 3 was about twice that in groups 1 and 4. Histologically, the zona glomerulosa in groups 1 and 4 consisted of closely packed cells with little cytoplasm and few lipids; in groups 2 and 3, cells were larger with a clear cytoplasm, arranged in well-defined clusters and loaded with lipid droplets. On gross examination, the kidneys of rats in group 1 appeared normal; those of groups 2 and 3 were somewhat enlarged, edematous, and exhibited irregular and mottled surfaces. Some had small petechiae. On histological examination, the renal lesions consisted of marked deposits of hyalin material in the glomerular tufts, tubular dilatations and casts, and arteriolar necrosis. These changes are similar to those seen in kidneys contralateral to clamped kidneys. In three rats of group 2, and in four rats of group 3, the hearts had an irregular surface with prominent white patches of necrosis mostly on the right ventricular wall; the same animals showed small nodules characteristic of arteritis, on the branches of the hepatic artery. These lesions were verified by histologic examination.

Table 1. Effects of rat renin in uninephrectomized rats.

Group and treatment	Body weight (g)		Heart (mg)*	Kidney (mg)	Thymus (mg)	Urine flow (ml/day)
	Initial	Final				
1. Kidney extract	140±8.5	160± 8.5	331±12.5	893±46.8	398±14.9	14.2± 5.2
2. Rat renin	135±7.0	151± 7.4	414±27.9	992±74.2	228±49.2	37.5±11.1
3. Rat renin and kidney extract	148±7.8	153±12	422±35.4	1042±84	213±80.9	26.3± 7.5
4. Control	145±7.4	162± 6.6	332±15.4	902±89.3	363±44.1	6.8± .87

* Estimated as percentage of body weight.

Assay of nonincubated and incubated plasma showed that the pressor activity in the rats which received rat renin (groups 2 and 3) was about 5 times as great as that found in the control group (7.28 ± 4.6 nanograms as compared with $1.29 \pm .5$ ng of angiotensin equivalents per milliliter). The renin content of the kidneys was slightly but not significantly decreased in rats receiving kidney extracts (group 1), but markedly depressed in groups 2 and 3 treated with renin. The following values were obtained for each of the four groups: 5.3 ± 1.5, 1.9 ± 1.3, 1.5 ± 1.3, and 7.8 ± 4.7 Goldblatt units per gram of tissue.

Our results show that injections of rat renin into uninephrectomized rats reproduced not only hypertension and hypertensive vascular disease as caused by various renal manipulations or hormonal treatments, but also the specific changes seen in hypertensive rats with the left renal artery partially clipped and the right renal artery left untouched. In the latter there is a marked decrease in renin content of the right kidney (*9*) and an increase in aldosterone secre-

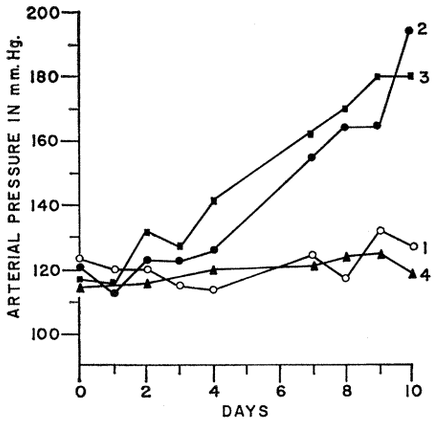

Fig. 1. Curves of the blood pressure of rats injected with extracts of rat kidneys (curve 1), rat renin (curve 2), rat renin and extracts of rat kidneys (curve 3), and saline (curve 4).

tion (*10*). Similarly, we found a decrease in the renin content of the remaining kidneys of uninephrectomized rats treated with renin and an increase in the width of the zona glomerulosa of the adrenals, which is the generally accepted site of aldosterone synthesis.

Evidently, homologous renin reproduced the effects of the endocrine secretions of clipped kidneys, thus eliminating the need to postulate the existence of another renal factor. Although the renin preparation was crude, it is likely that renin and not impurities were responsible for the effects described since treatment with angiotensin can also elicit sustained elevation of blood pressure accompanied by inhibition of renin formation and stimulation of aldosterone release (*11*). The amounts of renin administered may appear enormous when compared with the intravenous dose necessary to produce a similar acute pressor effect; however, presumably only an infinitesimal part reaches the blood stream, since no increase in blood pressure was detected before the 4th day.

The acute pressor activity which follows intravenous administration might best explain the development of hypertension after prolonged treatment. All the evidence indicates a more complex mechanism. Indeed, one point common to this type of experiment with renin or angiotensin (*11*, *12*) is the delayed appearance of hypertension. This latent period suggests that a critical amount of pressor material should accumulate before it raises arterial pressure by direct effect on the vascular system. This is unlikely because of the short half-life of angiotensin of less than 30 minutes (*13*) and the abrupt fall in pressure on cessation of angiotensin infusion (*12*). As an alternative, the existence of secondary mechanisms which may be nervous or endocrine has to be considered. One possible nervous mechanism is based on the ability of angiotensin to intensify normal neurogenic vasomotor activity (*14*). It has also been proposed (*12*) that angiotensin may cause specific cerebral vasoconstriction, which would in turn alter the activity of medullary vasoconstrictor centers.

The endocrine effects of angiotensin are better documented. Administration of renin, or angiotensin, in subpressor doses stimulates specifically aldosterone secretion; clinical and experimental renal hypertension are associated with aldosteronemia; aldosterone causes hypertension; administration of renin to aldosterone-treated rats precipitates a syndrome reminiscent of malignant hypertension (*15*). There is, however, no evidence that during the prehypertensive phase the renin-angiotensin-aldosterone system is activated to the point of being a determining factor in the evolution of hypertension. Hypertension probably cannot be explained according to a single scheme; each of these mechanisms may participate from the beginning, acting either together or in succession, and in the course of hypertension one of them may become predominant.

GEORGES M. C. MASSON
CHUJIRO KASHII
MASATO MATSUNAGA
IRVINE H. PAGE

Research Division,
Cleveland Clinic Foundation,
Cleveland, Ohio

References and Notes

1. G. M. C. Masson, A. C. Corcoran, I. H. Page, *Am. J. Physiol.* **162**, 379 (1950).
2. R. B. Blacket, A. Depoorter, G. M. Pickering, A. L. Sellers, G. M. Wilson, *Clin. Sci.* **9**, 223 (1950).
3. G. M. C. Masson, C. Kashii, J. C. Panisset, S. Yagi, I. H. Page, *Circulation Res.* **14**, 150 (1964).
4. Our thanks to S. Hoobler, University of Michigan, and W. Wagner, Ciba Pharmaceutical Products Inc., for the supply of normal kidneys.
5. E. Haas, H. Lamfrom, H. Goldblatt, *Arch. Biochem. Biophys.* **48**, 256 (1954).
6. This preparation graciously supplied to us by Harry Goldblatt had a specific activity of 15 units per milligram.
7. E. Haas, H. Goldblatt, *Am. J. Physiol.* **197**, 1103 (1959).
8. P. A. Khairallah, F. M. Bumpus, I. H. Page, R. R. Smeby, *Science* **140**, 672 (1963).
9. D. Regoli, H. Brunner, G. Peters, F. Gross, *Proc. Soc. Exptl. Biol. Med.* **109**, 142 (1962).
10. B. Singer, C. Losito, S. Salmon, *Acta Endocrinol.* **44**, 505 (1963).
11. A. J. Marx, H. W. Deane, T. F. Mowles, H. Scheppard, *Endocrinology* **73**, 329 (1963).
12. C. J. Dickinson, J. R. Lawrence, *Lancet* **1963-I**, 1354 (1963).
13. P. A. Khairallah, I. H. Page, F. M. Bumpus, R. R. Smeby, *Science* **138**, 523 (1963).
14. J. W. McCubbin and I. H. Page, *ibid.* **139**, 210 (1963).
15. Reviewed in "International Symposium on Angiotensin, Sodium and Hypertension," *Can. Med. Assoc. J.* **90**, 153 (1963).
16. Supported in part by grant H-6835 from the National Heart Institute.

20 May 1964

Attention, Vigilance, and Cortical Evoked-Potentials in Humans

Abstract. *Computer-averaged potentials evoked from the cortex were recorded to nonsignal stimuli and to randomly interspersed signal stimuli requiring detection and response during prolonged visual vigilance. As detection efficiency diminished over time, the amplitude of evoked responses to nonsignal stimuli decreased and latency increased. Fluctuations in vigilance (attentiveness) during the course of the task also were accompanied by corresponding changes in evoked-potentials to nonsignal stimuli. More specific lapses of attention, revealed by detection failures, resulted in average evoked-responses of lower amplitude to missed as compared with detected signals.*

Recent research has been focused on possible neural mechanisms mediating fluctuations in attentiveness. Several reports have dealt with changes in evoked-responses to sensory stimuli during attentive and inattentive states (*1*). Most of this work has been conducted with animals, although there have been a few reports of experiments carried out with human subjects (*2*). Thus far, the results have been largely inconclusive, due, we believe, to methods of varying and measuring attentiveness.

One area of psychological investigation which has been intensively concerned with short- and long-term fluctuations of attention is that dealing with performance on "vigilance tasks" (*3*). Most workers in this field agree that failures in signal detection and the long-term decrement and fluctuations in performance that characterize such tasks can be attributed to changes in the observer's state of attentiveness. Accordingly, the experiment described herein was designed for investigating changes in the potentials evoked from the human cortex averaged during a vigilance task requiring visual discrimi-