EFFECTS OF CHRONIC EXCESS SALT INGESTION

Inheritance of Hypertension in the Rat*

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In man, hereditary factors are recognized as essential in the etiology of hypertension. The genetic expression is, however, difficult to assess because it is not known how many genes are involved, how they interact with one another and with environmental factors, or by what mechanisms they exert their influence.

In rats, the genetic influence on blood pressure regulation is definitely established. If similar factors operate in humans, rat colonies may provide prototypes of heredity and blood pressure regulation for the study of human health and disease. Work at this laboratory demonstrated in 1962 that the development of salt-induced hypertension strongly depended upon the genetic background of the rats used (1, 2). By selective breeding, two colonies were derived from a single Sprague-Dawley strain. In one colony the rats rapidly developed hypertension on the same salt (NaCl) intake to which members of the other colony were resistant. Later we confirmed these differences with other techniques generally used to induce experimental hypertension. These techniques included: DOCA plus NaCl, unilateral renal artery compression without NaCl, cortisone, adrenal regeneration, and uninephrectomy without NaCl (3–5). If these experimental models have relevance to man, then several forms of human hypertension would also require for their emergence an appropriate genetic substratum which depends upon environmental determinants for its expression (4).

In the present work, which extended over a six year period, we have explored the mode of inheritance of hypertension in these two strains of rats. Approximately 2000 animals were studied in an effort to determine the number of genes involved. The data indicated that regulation of blood pressure is multigenic; the probable number of genes was calculated to be from two to four. The simplest model compatible with our data consists of two nonlinked, autosomal, diallelic loci with a sex-modified expression of the genes at one locus.

Materials and Methods

Rats were housed three to a cage in air-conditioned rooms, lighted for 10 hr each day. The food pellets were made to order and contained a total of 8% NaCl (w/w). Pellets and

tap water were always available. Systolic blood pressures (BP)[1] were measured by our modification of the tail microphonic method of Friedman and Freed (6).

All rats were weaned at 21–23 days of age and started on the 8% NaCl diet; they were observed continuously thereafter for 52 wk unless they died from natural causes before that time. BP and weight were recorded once a week from wk 4–12 after weaning, every other week from wk 14–24, and every 4 wk thereafter. If there was a sustained weight loss of more than 10 g the values obtained after the weight-loss started were not used in the analyses, thus avoiding the inclusion of secondary effects. In many cases this precaution may be unnecessary, but it has been consistently applied in all our recent work (7):

The generations were designated as indicated in Fig. 1: R and S stand for parent generations, F_1 and F_2 for the first and second filial generations, BR and BS for the backcrosses between F_1 and the R or S parent population, respectively.

Parent Populations.—R and S rats from our own colonies were used. The development of these strains, and their reaction to dietary salt has been reported in several papers (1–4, 7–9). On a low sodium diet both strains thrive and develop normally; S rats will slowly reach a BP of about 140 mm Hg after a year, whereas R rats will average about 120 mm Hg. A diet containing 8% NaCl is remarkably well tolerated by R rats, but it devastates S rats; after 3–4 wk their BP starts to climb and between 6 and 12 wk after start of this diet most die with a BP in excess of 200 mm Hg. In the R colonies, on the other hand, females have been uniformly resistant; even after 1 yr there is no significant difference in their weight and only a moderate increase in BP when compared with control groups on low salt diets. R males are less predictable as they grow older and we are still collecting data on their long-term reaction to 8% NaCl. After 1 yr on this diet, they have shown individual variations in weight, morbidity, and blood pressure; many animals have presented systolic BP in the range 140–160 mm Hg.

We discuss our R and S colonies as if they were genetically homogeneous, but variations occur in both colonies. We therefore test our breeders by the performance of their first litters: weanling S rats are given 8% NaCl in their food, are expected to develop blood pressures above 180 mm Hg after 8 wk, and to die within 3 months. R rats are given 8% NaCl in their food and triiodothyronine (Cytomel[R]) 0.05 mg/liter in their drinking water; on this regimen their blood pressures should remain below 140 mm Hg after 8 wk. Only if the first litter passes the test will the parents participate in the breeder pool. The original breeders for both strains, furthermore, came from the same line of Sprague-Dawley rats (1, 2). The early generations were obtained by brother–sister matings, but when polydactyly, syndactyly, runting, and infertility began to occur we developed breeding pools of 40 males and females from each strain by testing each pair on the basis of their progeny.

Crossbred Populations.—From 13 R and 13 S litters by tested breeders, one male and one female rat were selected and mated as shown in Fig. 1. The matings were planned to insure equal representation of the same genetic lines in both reciprocal mating types. Nonproductive matings forced us, however, to compromise both in the F_1 and other hybrid populations.

We followed each individual rat for a year or until it died. The rats that participated in the study could therefore not be mated and we had to duplicate the F_1 generation to get breeders for the F_2 and backcrosses. The F_1 rats used for further breeding were kept on a low sodium diet and the same was true for the R and S parents. The information about parental response to high salt applies, therefore, to the population from which the breeders were drawn, and is not based on direct observations of the actual parents. To analyze the results we used the methods developed for population genetics (10). For each of the permutations shown in Fig. 1 we observed approximately 100 rats.

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[1] Abbreviations used in this paper: BP, systolic blood pressure; SHR, spontaneously hypertensive rats; V_g, genetic variance; V_r, random variance; V_t, total variance.

OBSERVATIONS

F_1 *Generation.*—From 26 matings we obtained 19 litters of F_1 rats for observation. A group of 7 litters had no reciprocal matching of same lineage, while 12 litters could be arranged in 6 pairs of double first cousins. The average blood pressure of the whole group was intermediate to the R and S values. When the sexes were analyzed separately, the male average was close to the midparental average while the female mean was closer to the R parents. Sex, therefore, appeared in F_1 rats to have a profound influence on the phenotypic expression of the genetic and environmental determinants. This was also reflected in the
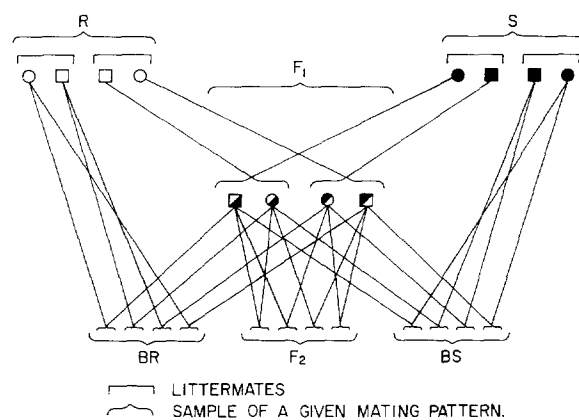

FIG. 1. Diagram of matings for genetic study. From 13 R and 13 S litters, a male and a female were selected and crossmatched to produce the F_1 generation. For the F_2 and backcrosses (BR and BS) a sufficient number of littermates were selected to have each line represented in all the permutations of mating patterns.

fact that within every litter but one the males had the higher blood pressures. Table I presents the cumulative average values after 24 wk of observation.

On the other hand there was no evidence for a sex-linked inheritance. Factorial analysis of the matching litters supported the conclusion that reciprocal matings produced equivalent offspring. A hierarchical analysis of variance involving the entire F_1 generation in the progression (sex, mating type, litter, individuals) further showed that in comparing rats of the same sex, littermates were likely to be more uniform than rats chosen at random from different litters. This is interpreted as evidence that there was a genetic variance in the parent populations.

For Tables I and II, the last reliable blood pressures of dead animals (cumulative values) were included in the results. To show the influence of mortality on the results, we entered in Table III "actual" and "cumulative" averages for each sex at selected intervals. The actual values are those obtained on the

TABLE I

Average Blood Pressure Values, Standard Error, and Number of Rats of Each Sex in F₁ Litters

Mean blood pressure *, SEM, and No.‡ of rats

Mating type (dam × sire)	Sex		Nonmatching litters					Matching litters						Totals Sex	Totals Cross	Totals All
(S × R)	Female	Mean				125	135	156	136	129[d]	123	136[d]	149	135	(Mean) 147	(Mean) 148
		SEM				4.0	5.1	11.2	3.9	5.1	4.7	6.4	8.1	2.4	(SEM) 2.6	(SEM) 1.6
		No.				8	8	5	5	10	6	7	7	56	(No.) 93	(No.) 212
	Male	Mean				147	182	180	158	143[d]	172	148[d]	185	164		
		SEM				5.8	10.1	10.8	9.6	7.0	5.9	9.8	9.3	4.0		
		No.				3	3	5	4	7	5	4	6	37		
(R × S)	Female	Mean	124	135	142[d]	126	165[d]	128	133	126	128	124	126	132	149	
		SEM	5.0	14.2	4.5	2.3	11.1	4.0	3.5	5.9	5.0	2.0	4.1	2.2	2.0	
		No.	3	4	9	5	3	7	3	6	8	2	4	54	119	
	Male	Mean	150	160	141[d]	160	178[d]	158	172	159	173	170	156	162		
		SEM	4.4	5.3	4.0	6.0	4.4	4.5	2.2	2.5	11.6	3.2	3.5	1.9		
		No.	8	6	3	5	8	6	2	8	5	8	6	65		

Except in one litter, males had higher average pressure than female littermates. The difference was significant ($P < 0.05$) in all litters except for the four marked d. Each column represents one set of reciprocal matings of littermates. No entry indicates that only one of the reciprocal matings succeeded. Values are calculated from readings taken 24 wk after weaning, or from the last reliable pressure previous to that date.

* Mean, mean blood pressure in millimeters of Hg.

‡ No., number of rats.

survivors at a given time. The table also gives the number and fraction of survivors. In the F_1 generation males had both a higher blood pressure and a higher rate of mortality than females.

F_2 *Generation.*—Similar observations for the F_2 generation (also given in Table III) revealed that male F_2 rats had average blood pressures equivalent to

TABLE II

Analysis of Variance of F_1 Generation

SOURCE	DF	MS Main	MS Parti-tioned	F	P
Among sex	1	44773		189.24	0.0000
Among mating type, within sex	2	166		0.70	0.4962
Female	1		203	0.86	0.3551
Male	1		129	0.55	0.4604
Among litter, within mating type and sex	34	808		3.41	0.0000
Female, S × R	7		789	3.33	0.0024
Female, R × S	10		527	2.23	0.0183
Male, S × R	7		1488	6.29	0.0000
Male, R × S	10		625	2.64	0.0051
Among individuals, within litter, mating type, and sex	174	237		1.00	
Total		211			

The sex difference was highly significant (F = 189.24, P < 0.01). There was no difference between the reciprocal mating types (F < 1; P > 0.2) indicating no sex-linked inheritance. The differences between litters were significant for both sexes and both mating types (F = 2.23–6.29; P < 0.02) indicating a genetic heterogeneity in one or both parental strains. DF, degrees of freedom; MS, variance; F, Snedecor's F ratio; P, probability. The column headed "partitioned" refers to subdivisions within each main source of variance.

male F_1 rats, but a different mortality curve; the F_2 had a higher mortality at the beginning of the year, and a higher survival ratio at the end. The F_2 females had higher average blood pressures than F_1 females. The sex influence on blood pressure phenotype was thus less obvious in the F_2 than in the F_1 generation. Although the average for males was significantly higher than for females (P < 0.01), the absolute difference between sexes was less, and there were litters in which females had higher pressures than males.

Backcrosses.—In the backcrosses the average pressures were intermediate to

TABLE III

Average Blood Pressure Values, Standard Error, and No. of Rats of Each Sex in the Crossbred Populations after Different Periods on High Salt Diet

Cross	Sex	4 wk				12 wk				24 wk				36 wk				48 wk			
		Ave.*	Cum.†	No.§	f‖	Ave.	Cum.	No.	t	Ave.	Cum.	No.	t	Ave.	Cum.	No.	t	Ave.	Cum.	No.	t
		mm	mm			mm	mm			mm	mm			mm	mm			mm	mm		
F$_1$	F	141 (1.35)	141 (1.35)	110	1.00	133 (1.50)	133 (1.50)	110	1.00	134 (1.69)	134 (1.63)	104	0.95	141 (2.21)	141 (2.09)	97	0.88	138 (2.30)	142 (2.03)	69	0.63
	M	147 (1.45)	147 (1.45)	102	1.00	152 (1.86)	155 (2.03)	96	0.94	159 (1.70)	163 (1.89)	90	0.88	179 (3.91)	175 (1.97)	31	0.30	171 (4.73)	173 (1.75)	8	0.08
F$_2$	F	137 (1.44)	137 (1.44)	202	1.00	135 (1.87)	137 (1.96)	184	0.91	143 (2.20)	148 (2.29)	172	0.85	147 (2.51)	154 (2.48)	164	0.81	153 (2.09)	161 (2.21)	136	0.67
	M	142 (1.34)	142 (1.34)	216	1.00	148 (1.89)	154 (1.93)	178	0.82	160 (1.66)	166 (1.68)	156	0.72	166 (1.55)	171 (1.48)	113	0.52	162 (1.76)	170 (1.47)	75	0.35
BR	F	113 (1.01)	113 (1.01)	209	1.00	119 (1.11)	119 (1.17)	205	0.98	122 (1.06)	123 (1.12)	202	0.97	128 (1.27)	128 (1.32)	199	0.95	126 (1.07)	127 (1.17)	188	0.90
	M	121 (0.96)	121 (0.96)	209	1.00	130 (1.25)	129 (1.25)	203	0.97	139 (1.28)	139 (1.30)	198	0.94	145 (1.45)	146 (1.45)	185	0.89	145 (1.40)	148 (1.38)	143	0.68
BS	F	155 (1.55)	155 (1.55)	185	1.00	168 (3.11)	192 (2.62)	89	0.48	170 (3.16)	196 (2.43)	70	0.38	177 (3.04)	201 (2.15)	58	0.31	175 (3.70)	202 (2.09)	38	0.20
	M	159 (1.63)	159 (1.63)	196	1.00	176 (4.15)	197 (2.16)	53	0.27	176 (2.97)	200 (1.95)	37	0.19	183 (2.88)	202 (1.84)	21	0.11	171 (6.14)	201 (1.88)	7	0.04

All reciprocal mating types in each cross are combined; sexes are presented separately. Males have a significantly higher blood pressure than females in F$_1$, F$_2$ and BR crosses, but not in BS. In all crosses, males have a higher mortality than females.

* Ave., average blood pressure ($\pm$ standard error) for surviving rats.

† Cum., cumulative values (last reliable pressures of dead rats included in results).

§ No., Number of surviving rats.

‖ f, fraction of survivors relative to the number present at 4 wk.

mm, millimeters of Hg.

F_1 and the respective parent strains. BR rats ($R \times F_1$) evinced a demonstrable sex influence on the phenotype while no sex difference in blood pressure could
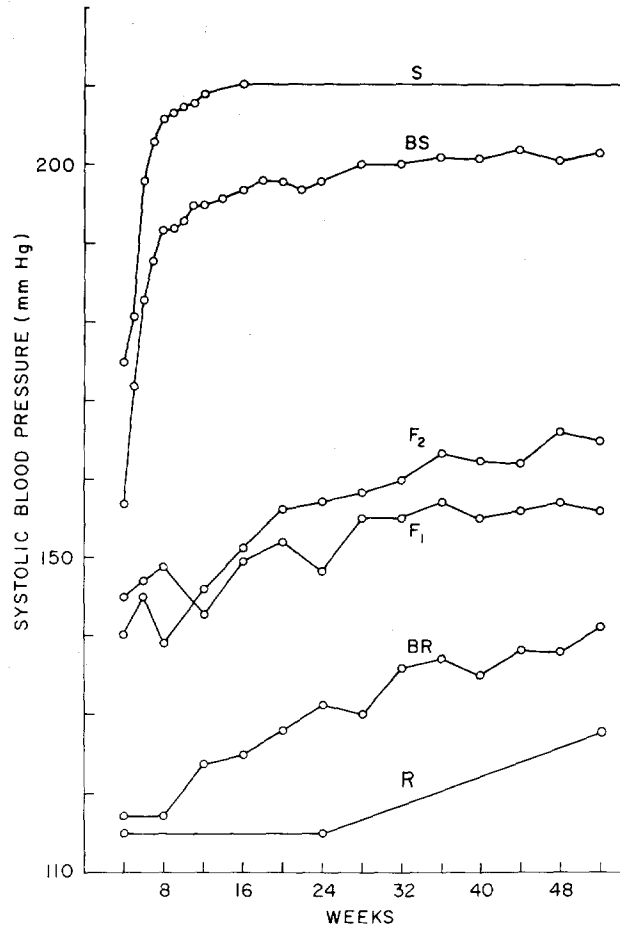

FIG. 2. Average blood pressures of different rat populations on 8% NaCl. The curves give the average cumulative systolic blood pressure for each cross during a year of observation. Although R, BR, F_1, and F_2 showed significant differences in BP based on sex (males higher than females), the values have been pooled for the purpose of this graph which illustrates the definite genetic influence on the reaction to salt intake.

be shown for the BS ($S \times F_1$) rats. In all crosses represented in Table III the males had higher mortality rates than females.

The information tabulated in Table III is presented graphically at closer time intervals in Figs. 2 and 3, which also includes data from representative R and S populations.

The data show conclusively that mendelian inheritance provides etiological factors for hypertensive disease developing as a result of high salt intake. To
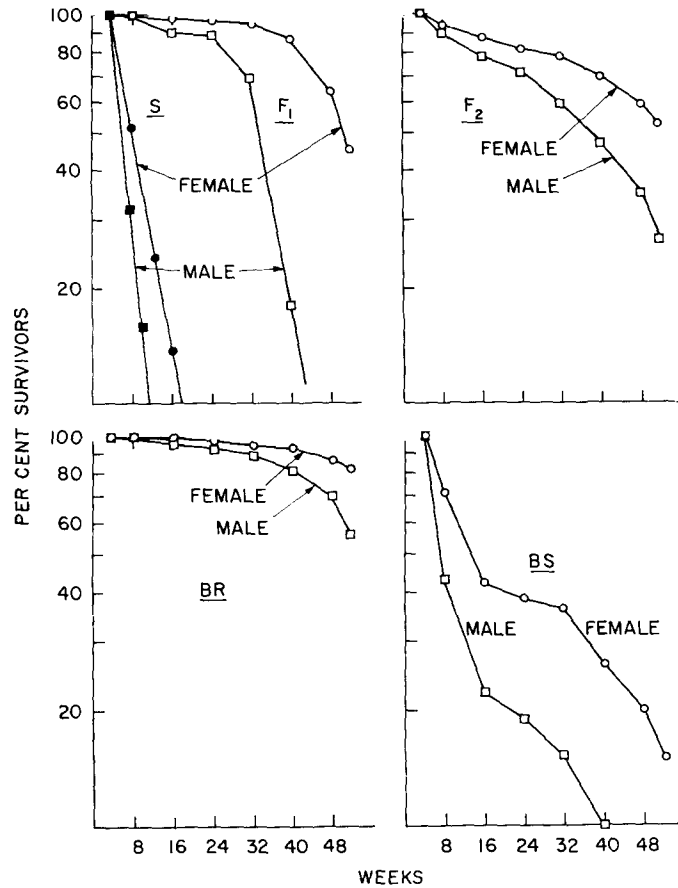

FIG. 3. Survivors in different rat populations on 8% NaCl. The graphs demonstrate how mortality on 8% NaCl parallels blood pressure development (see Fig. 2) and has a significant element of genetic determination. R rats are not shown. Mortality among R females during the first year cannot be correlated to salt or blood pressure; data on R males indicate practically no mortality in the first 36 wk and better than 80% survival after 48 wk. In all these populations exposed to high salt diet we have thus observed a higher mortality rate among males than among females. Note that coordinate is logarithmic.

investigate the mode of inheritance the cumulative values at 24 wk (the last reliable pressures of dead rats are included in the averages) are presented with sample statistics in Table IV. The choice of 24 wk was a compromise. The time was long enough to separate "pure" S rats from hybrids and provide some spread in the blood pressures of the latter, but short enough to avoid the high

mortality figures caused by chronic hypertensive disease. However, the sex influence on BP of R rats was not fully demonstrated; the observed sex difference was not statistically significant, but increased in the following months and was probably real. The statistics show that S rats had the highest averages and

TABLE IV

Observations after 24 Wk on 8% NaCl

Cross	Sex	No. of rats observed	Mean blood pressure	Variance			SD due to genetic variance $(=\sqrt{V_g})$	Heritability $V_g/V_t \times 100$
				Total V_t (measured)	Random V_r (estimated)	Genetic V_g $= V_t - V_r$		
			mm Hg				*mm Hg*	%
R	Female	50	114	94	94	0	0	
	Male	28	123	151	150	0	0	
S	Female	70	212	660	660	0	0	
	Male	48	210	1146	650	396	20	
F_1	Female	108	134	294	220	74	8.6	
	Male	102	163	366	390	<0		
F_2	Female	202	148	1038	300	758	27.5	74
	Male	216	166	613	400	213	14.6	35
BR	Female	209	123	262	150	112	10.6	43
	Male	209	139	353	240	113	10.6	32
BS	Female	185	196	1090	570	520	22.8	48
	Male	196	200	743	390	153	12.4	20

Last reliable pressures of dead rats are included in the results. Compare SD to estimates from model in Table VI.

V_t, total variance. Value calculated from data. V_r, variance caused by random factors. For the R populations this variance is determined by independent tests. For the other populations it has been estimated by assuming the total S female variance to be random, and interpolate for average blood pressures. V_g, genetic variance. Calculated by subtracting V_r from V_t. The considerable genetic variance in S male and F_1 female is probably an indication of the uncertainty of the V_g estimates. $V_g/V_t \times 100$, heritability, or degree of genetic determination. Part of V_g in females is due to dominance. Strictly, the expression should include additive genetic variance, only. The male data may therefore be more relevant.

the largest variances. Repeated weekly readings on R rats have shown that most of the variance found in R populations is due to uncontrollable factors such as physiologic fluctuations, metabolic state, and anesthesia. The rapid blood pressure increases in the terminal phase of the S rats prevented a similar test for that strain and must have worked to increase the observed variance in the present study. Since blood pressures were read at weekly intervals the last reliable pressure could have been recorded in some animals days before the peak

value was reached. Hence, in the S parent population, the observed average values are presumably lower, and the variance larger, than we might have found by a continuous, or even daily, record of the pressures.

Genetic variances in the F_2, BR, and BS populations are only partly responsible for the observed phenotypic variance (V_t) entered in Table IV, as these populations must be subject to the same random fluctuations as the more homogeneous groups. If we assume that the data on R and S females represent the random variances at average readings of 114 and 212 mm Hg respectively, and further assume a linear relationship between random variance and average BP, we may estimate the expected random variance of the other population samples by interpolation. These estimates are given in the column V_r in Table IV. The environment was kept as uniform as possible: there was no change in rooms, personnel, food supply, or working routine during the years of observation. The difference between total variance (V_t) and random variance (V_r) is therefore the best available estimate of genetic variance (V_g). Table IV lists the estimated genetic variances and the corresponding standard deviations (SD).

INTERPRETATION

The high variance of the S males, and the high average BP of the BS females do not fit a simple, noncontroversial genetic model. Except for these data the findings are consistent with the hypothesis that blood pressure regulation and the constitutional tendency to hypertensive disease depend on several autosomal genes, that in males the effects of these genes are additive while in females the allele for lower blood pressure is dominant at one or more of the loci. Fig. 4 displays mean blood pressures from Table IV. The male averages fall close to a straight line, while the female averages show dominance. To fit a straightforward genetic pattern the BS females should have averaged 175 mm Hg.

We can arrive at estimates of the degree of genetic determination (heritability) and the number of genes involved by using the equations derived by Wright (10). From the male data we calculated two to four genes and a heritability of 20-35% (Table IV). Due to dominance these equations are not as applicable to the female data. The strains were fairly well established by the third generations (1, 2), indicating that the number of genes involved must be small; this is consistent with the present estimates.

The observations that blood pressures increase with age at different rates in different rats, that mortality acts as a selection against high pressures, that the parent populations probably are not uniform, that only the males seem to express an additive effect of the genes, and that environmental factors have different effects on the different genotypes all warn against placing too much emphasis on any given number. We have tested models with two, three, and

four nonlinked autosomal diallelic loci. There is no unique solution; in general the more complex models can be manipulated to correspond better with the observations. We feel, however, that any complex model should be justified by independent evidence. Random fluctuations so cloud the results that any purely genetic model which describes these particular data with reasonable success is
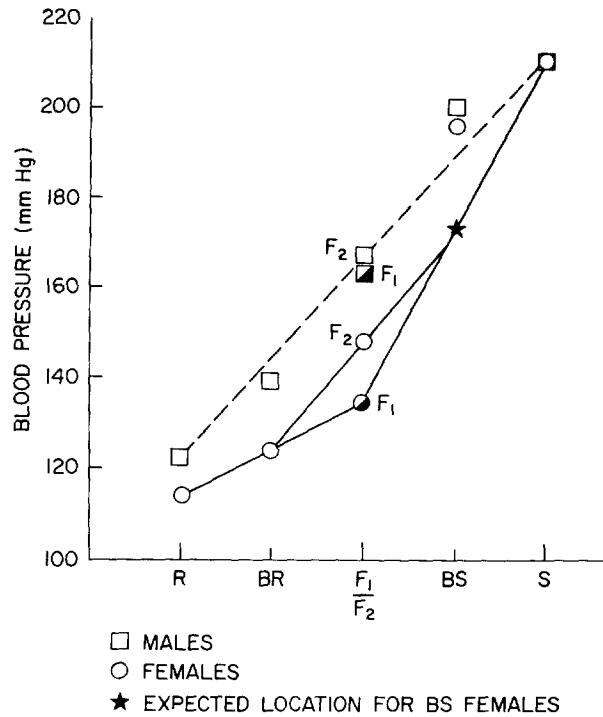

FIG. 4. Mean blood pressure of each sex in R, S, and cross bred populations at 24 wk. The values are consistent with an additive genetic effect in the males, and dominance in the females. However, the BS females broke the pattern; the asterisk indicates the location which would have fitted with the other data.

likely to be as correct as one which reproduces them in great detail based on speculative assumptions.

We also tested the consequences of logarithmic transforms of the observations. They improved the form of the distribution curves and made the variances of the different populations more equal. They did not, however, remove the difficulties with the S male, and BS female data. As in the case of complicated models, we feel that transforms should be justified by physiological reasoning, not introduced solely to improve sets of data.

If we tentatively ascribe the deviations as due to sampling variance in the

selection of breeders, the simplest model that gives a fair prediction of the findings consists of two nonlinked diallelic loci. The allelic effects are additive in the male but the R allele dominates at one locus in the female. Ascribing the gene values 0 to the R alleles and 1 to the S alleles, the locus where R is dominant can only contribute 2 (for genotype 11), or 0 (for genotype 01 and 00) to the total genotypic values of the females.

If we make the simplifying assumption that R and S strains are uniform (00)/(00) and (11)/(11) respectively, we may calculate probabilities of combina-

TABLE V

Calculation of Genotype Distribution in F_2 Generation

				Locus A				
				Male			Female	
	Genotype			11	01	00	11	01 00
		Value		2	1	0	2	0
			f	0.25	0.50	0.25	0.25	0.75
Locus B	11	2	0.25	0.0625	0.125	0.0625	0.0625	0.1875
(Both sexes)	01	1	0.50	0.125	0.250	0.125	0.125	0.375
	00	0	0.25	0.0625	0.125	0.0625	0.0625	0.1875

	Genotype value				
	0	1	2	3	4
Female, f	0.1875	0.375	0.250	0.125	0.0625
Male, f	0.0625	0.250	0.375	0.250	0.0625

Assumptions: 2 autosomal, nonlinked, diallelic loci. R and S parental strains homozygous at both loci. Values additive over loci. At one locus, in the female only, the 0 allele dominates. Otherwise the allelic effects are additive.

Genotype distributions for the other crossbred populations were calculated in the same manner.

f, frequency.

tions as demonstrated for the F_2 generation in Table V. The predicted distributions for all crosses are given in Table VI. Plotting average genotypic values from this table against the observed average blood pressures in Table IV gives a good fit for all but the female BS rats (Fig. 5). The rank correlation between the predicted genotypic and the observed phenotypic variances is also significant. ($p < 0.05$).

DISCUSSION

Colonies with genetically determined elevations of BP have been studied by others. In 1958, Smirk and Hall first reported on one such colony isolated by

selective breeding (11). Their spontaneous hypertension was not as severe as ours and environmental influences have not been deemed significant. They found higher BP in males than in females, as did we in crossbred colonies and R rats. They found a higher pressor response to many vasoactive drugs in their

TABLE VI

Genotype Distribution in Crossbred Populations Predicted by Model, with Average Blood Pressure and Standard Deviations Expected and Observed for Each Cross

Cross	Sex	Distribution of genotypic value in arbitrary units					Predicted genotype			Mean blood pressure		SD due to genotype	
		0	1	2	3	4	Mean	MS	SD	ex-pected	ob-served	ex-pected	ob-served
		%	%	%	%	%	units	units	units	mm Hg	mm Hg	$\frac{mm}{Hg}$	$\frac{mm}{Hg}$
R	Female	100					0	0	0	120	114	0	0
	Male	100					0	0	0	120	123	0	0
S	Female					100	4	0	0	210	212	0	0
	Male					100	4	0	0	210	212	0	20
F_1	Female		100				1	0	0	143	134	0	9
	Male			100			2	0	0	165	163	0	0
F_2	Female	19	38	25	12	6	1.5	1.2	1.2	154	148	27	28
	Male	6	25	38	25	6	2	1	1	165	166	23	15
BR	Female	50	50				0.5	0.25	0.5	131	123	11	11
	Male	25	50	25			1	0.5	0.7	143	139	15	11
RS	Female		25	25	25	25	2.5	1.5	1.2	176	196	27	23
	Male			25	50	25	3	0.5	0.7	187	200	15	12

Frequency distribution of genotypic values, average genotypic value, and genotypic variance, by cross and sex. The observed values are taken from Table IV. The expected values are based on a model discussed in the text. Each genotypic unit is given the value 22.5 mm Hg for the calculation of expected means and standard deviation of blood pressure; the means were calculated from the equation: BP (mm Hg) = 120 + 22.5 x (genotype, mean).

hypertensive strain (12); we have reported similar findings (13). Their pathological findings (14) resemble those on our S rats except that the advanced periarteritis nodosa found in their rats is infrequent in ours. However, their rats develop this vascular lesion particularly after a year or two of observation whereas our S-strain rats on salt diets never reached old age. It is still possible that the alleles they have isolated are similar to ours.

Okamoto and Aoki (15) have developed the spontaneously hypertensive (SHR) strain of Wistar rats, a review of which was published recently (16).

Their rats differ from ours in at least one respect; their's develop hypertension on low salt intake (17). In a study of the inheritance in SHR rats, Louis, Tabei, Sjoerdsma, and Spector (18) found several autosomal loci and concluded that the SHR were not completely homogeneous as regards their hypertensive genetic complement. They also found higher average values in males than in females.

Our findings are thus in general agreement with those on similar rat populations and raise the possibility that all these strains have become selected for the same mutations. If not, the completed model must be more complex than the one under discussion here.

Alexander, Hinshaw, and Drury (19, 20) studied a strain of spontaneously hypertensive rabbits and concluded that the trait was inherited. They found higher values in males than in females and the range of BP was similar to that of rat and man. Schlager and Weibust (21-23) have analyzed the genetic control of BP in mice. Among several strains, they found that variations within the normal range were genetically determined. By crossing the strains, they concluded that several genes were involved. The effects were additive over loci in the males, whereas the female mouse represses the elevation of BP (23). They calculated the heritability as 20% in males and 77% in females (21, 22). These conclusions agree with ours. They furthermore reported in their early studies a possibly greater influence of the genetic contribution of the sire (22), an observation compatible only with some of our preliminary results (24). We later found, however, that spurious evidence for sex-linked inheritance appeared because a high-risk line of the R rats were disproportionately represented in one of the reciprocal matings. (In subsequent mouse studies, no evidence for sex-linked inheritance has been observed. [Schlager, G., personal communication]).

We have also tested our model by trying to predict segregation into parental phenotypes and by estimating frequency distributions of blood pressures in each cross. We further tested the consequences of extrapolating the model to man. There is a peril in manipulation of numbers on the basis of assumptions. Nonetheless, the results suggest that model and data are compatible. The additional assumptions appear reasonable, were consistently applied, and may constitute clues to as yet unexplored relations.

Segregation of Hybrid Colonies into Parental Phenotypes.—We define the parental phenotypes as follows: R animals live 48 wk on 8% NaCl in the diet and their blood pressures remain below 140 mm Hg; S animals die within 16 wk on the same diet. These definitions are too strict as nearly half of the R males fail to live up to this ideal, but they permit a comparison of crosses by counting, rather than by biometric methods. In the F_1 population, 30% of the females and none of the males were phenotype R. We therefore assigned all of genotype 0 and 30% of genotype 1 to phenotype R; we further assumed that geno-

types 3 and 4 gave rise to phenotype S and that genotype 2 was in between. On this basis we predicted the number of phenotypes in each cross and compared this with the actual counts. Table VII gives the results. The model with these assumptions predicts a higher segregation into R phenotypes in the male F$_2$ and BR rats than we observed. To remove this discrepancy, the model and assumptions must be modified to allow for a sex difference in the R rats.

Frequency Distributions of Blood Pressures in Hybrid Colonies.—We may use the model to predict frequency distributions in each cross if we assign a blood

TABLE VII

Predicted and Observed Segregations into Parental Phenotypes

Cross	Sex	R		S	
		Predicted	Observed	Predicted	Observed
		%	%	%	%
R	Female	100	100		
	Male	100	50		
S	Female			100	85
	Male			100	100
F$_1$	Female	30	30	0	2
	Male	0	0	0	9
F$_2$	Female	32	20	18	15
	Male	14	2	31	24
BR	Female	66	61	0	2
	Male	41	18	0	3
BS	Female	8	0	50	58
	Male	0	0	75	78

pressure range to each genotype. Fig. 5 indicates that each unit increase in genotype adds 20–25 mm Hg to the average pressure. This and the increase in variance from R to S phenotypes formed the basis for assuming the following correlations:

Genotype	Systolic BP (mean $\pm$ SD)	
	Female	Male
0	115 $\pm$ 10	120 $\pm$ 10
1	140 $\pm$ 14	140 $\pm$ 14
2	165 $\pm$ 18	165 $\pm$ 18
3	190 $\pm$ 22	190 $\pm$ 22
4	215 $\pm$ 26	215 $\pm$ 26

Then we applied these distribution curves to the genotype combinations presented in Table VI, and compared them with the observed distributions. The results and the goodness of fit are presented in Table VIII and in Figs. 6 and 7.

Frequency Distributions of Blood Pressures in Human Populations.—In applying the model to data from human populations we assumed, in analogy to the rat, that genotypes 3 and 4 give rise to malignant hypertension, that 1 and 2 provide candidates for essential benign hypertension, and that genotype 0 is normotensive. (Further, to make estimates of gene frequencies possible, we stipulated that malignant hypertension occurs in less than 1% of adults, twice as
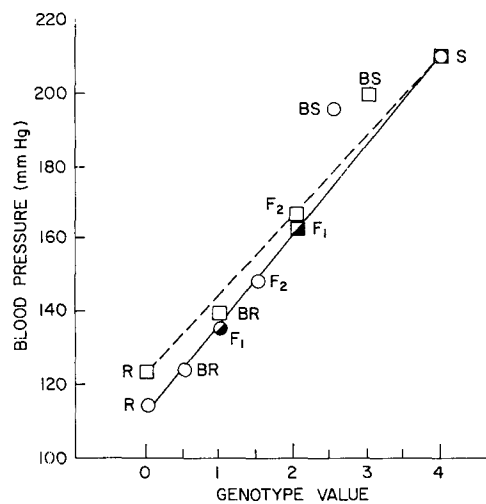
Fig. 5. Mean blood pressure of each cross and sex vs. mean genotype value. □, male; ○ female.

frequently in males as in females, while 70% or more of the population is normotensive.) The gene frequency $P = 0.13$ for the hypertensive allele at both loci led to a satisfactory development. In a randomly mating population, these gene frequencies give the genotype distributions calculated in Table IX. The same correlations between blood pressure ranges and genotypes that we used for rats (see above) lead to the frequency distributions of blood pressures which are presented in Table X and Fig. 8. They compare favorably with observations on adults in the age group 35–44 yr (25). The critical issue is not so much the "goodness of fit" in this example. Other age groups in the same study (25) showed different distributions, and other studies have presented other distributions for this particular age group. But all studies of young adult human populations show a pattern similar to panel d in Fig. 8, and this is the type of distribution pattern the model generates. (Fig. 8, panel c). The example there-

TABLE VIII

Expected and Observed Distribution of Blood Pressures in Different Rat Populations

(mm Hg)	F_1 Female Exp.* No.	F_1 Female Obs. No.	F_1 Male Exp. No.	F_1 Male Obs. No.	F_2 Female Exp. No.	F_2 Female Obs. No.	F_2 Male Exp. No.	F_2 Male Obs. No.	BR Female Exp. No.	BR Female Obs. No.	BR Male Exp. No.	BR Male Obs. No.	BS Female Exp. No.	BS Female Obs. No.	BS Male Exp. No.	BS Male Obs. No.
<99	1.7	3			3.0	4	0.5		7.9	2	1.0	1				
100–109	7.1	15			10.8	16	2.9	2	28.0	34	9.2	6	0.8	1		
110–119	18.4	27	0.6	1	19.7	17	8.7	2	46.7	58	25.2	17	3.2	6	0.3	
120–129	29.2	34	2.1	1	22.5	26	15.4	6	41.5	56	35.9	48	8.8	3	1.3	1
130–139	28.5	17	6.0	8	25.4	23	21.3	17	33.5	32	37.5	42	15.4	7	3.8	1
140–149	17.1	1	12.6	14	26.7	27	25.6	26	27.6	8	34.5	36	18.9	9	8.6	7
150–159	6.3	7	19.5	22	22.9	22	26.9	40	16.2	8	26.2	28	18.9	9	15.2	3
160–169	1.4	3	22.3	22	18.1	20	26.4	32	5.9	4	17.4	13	18.3	14	21.5	10
170–179	0.2	3	18.9	13	14.6	8	24.2	28	1.3	2	11.0	11	18.0	22	25.7	21
180–189			11.9	10	11.5	12	20.1	23	0.2		6.3	6	17.2	26	26.9	22
190–199			5.5	8	8.6	9	15.4	20			2.8	0	15.8	15	25.4	29
200–209			1.9	1	6.3	9	11.0	10			1.0	1	13.9	18	21.7	26
210–219			0.5	0	4.5	2	7.5	5			2.0		11.5	23	16.8	20
220–229			0.1	2	3.1	4	4.7	3					8.8	14	11.8	21
230–239					2.0	1	2.7	1					6.2	5	7.7	17
240–249					1.2	2	1.5	1					3.9		4.6	14
250–259					1.0		1.2						2.2		2.5	2
260–269													1.1		1.2	2
270–279													0.7		0.8	
X^2		36.9		3.9		9.1		25.5		31.9		11.0		75.6		50.3
DF		5		6		11		11		7		8		12		11
P		<0.001		>0.50		>0.50		<0.01		<0.001		>0.20		<0.001		<0.001
Total no. obs.		110		102		202		216		209		209		184		196

The calculations of predicted distributions are based on the assumptions outlined in the text. The observed (Obs.) data are the cumulative observations after 24 wk. (Last reliable values of dead rats are included). The distributions are graphically presented in Figs. 6 and 7.
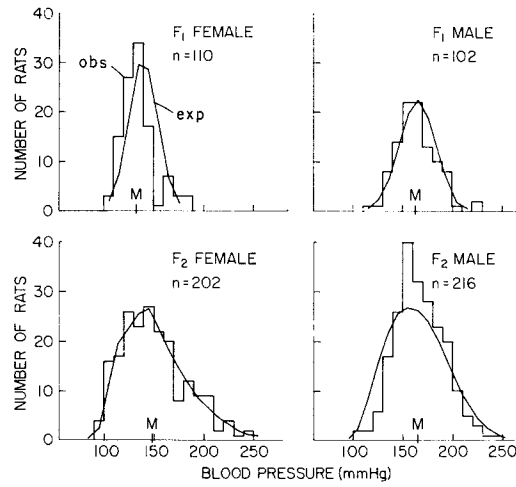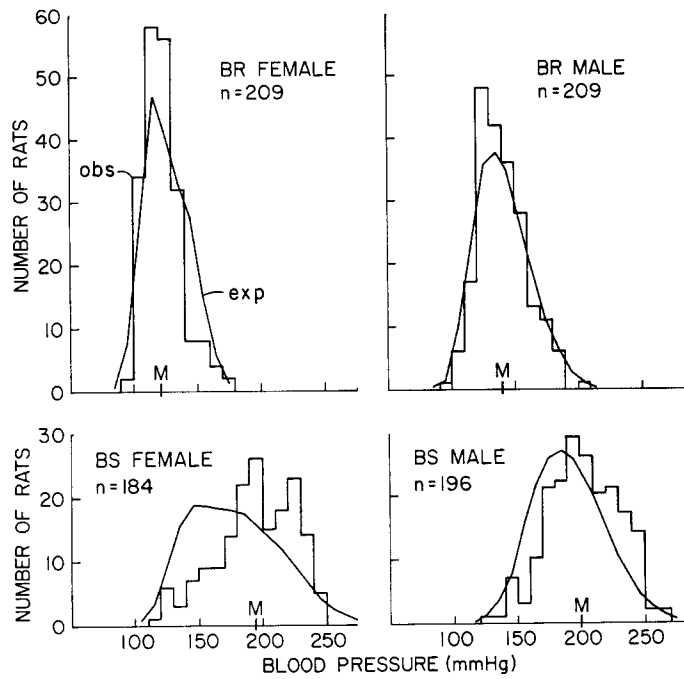
* No., number of rats.

FIG. 6. Comparisons of expected and observed blood pressure distributions for each cross and sex. Histograms present observations, frequency polygons present expectations. M stands for mean.

FIG. 7. Comparisons of expected and observed blood pressure distributions for each cross and sex. Histograms present observations, frequency polygons present expectations. M stands for mean.

fore strengthens our hope that it will be possible to design a genetic model based on rat experiments and apply that model to a set of human data.

The administration of a number of drugs and hormones as well as the development of endocrine and renal diseases affect the blood pressure of rat and man in a similar manner. Whether the analogy extends to epidemiological and genetic studies still has to be proven, but circumstantial evidence holds forth promise in this respect. Doyle and Fraser studied the correlation between vascular reactivity and hypertensive disease and showed that a hereditary pattern is com-

TABLE IX

Genotype Distributions for a Randomly Mating Population Where the Gene Frequency is 0.13 at Each Locus for the Hypertension-Producing Allele

				Locus A					
				Male			Female		
Genotype				11	01	00	11	01	00
		Value		2	1	0	2	0	
			f	0.0169	0.2262	0.7569	0.0169	0.9831	
Locus B	11	2	0.0169	0.0003	0.0038	0.0128	0.0003	0.0166	
(Both sexes)	01	1	0.2262	0.0038	0.0512	0.1712	0.0038	0.2224	
	00	0	0.7569	0.0128	0.1712	0.5729	0.0128	0.7441	

	Genotype value				
	0	1	2	3	4
Female, f	0.7442	0.2224	0.0294	0.0038	0.0003
Male, f	0.5729	0.3424	0.0768	0.0076	0.0003

Explanation of assumptions in text. The calculations are based on the proposed model and an assumed frequency of the allele for high pressure of 0.13 at each locus.

f, frequency.

mon to both these aspects of circulatory function (26). The analogy to the findings that hypertension-prone rats have increased vascular reactivity is obvious (12, 13). The epidemiological studies of Dahl and Love (27) and of Isaacson, Modlin, and Jackson (28) on the correlation between average salt intake and prevalence of hypertension in different populations, parallel closely the findings of Meneely et al. on unselected rat populations (29). Our S and R colonies indicate that salt sensitivity is dependent on the presence of certain genes in the rat. It is reasonable to expect that the same may be found to be true for man and would be compatible with the conclusions of Thomas and Cohen that genetic factors are modified by environmental agents (30).

Many studies on selected human populations have been published with a number of contradictory observations and often conflicting interpretations of

TABLE X

Expected and Observed Blood Pressure Distributions in Human Populations, Based on the Model and Observations of the U.S. Health Survey

Group	Measure	<90	90-99	100-09	110-19	120-29	130-39	140-49	150-59	160-69	170-79	180-89	190-200	>200	Total No. observed
Expectation	Female, %	0.5	5.0	19.1	29.9	20.9	10.2	6.5	4.1	2.0	0.9	0.5	0.2	0.1	
Expectation	Male, %		1.5	8.9	22.2	25.0	16.8	10.9	6.9	3.7	2.0	1.1	0.6	0.5	
Female 25-34 yr	Exp., No.	60	563	2163	3378	2363	1150	736	457	230	100	54	26	24	11291
Female 25-34 yr	Obs., No.	102	928	2440	4174	2255	935	222	116	73	34	14			
Female 35-44 yr	Exp., No.	66	614	2361	3687	2580	1255	804	499	240	109	55	29	25	12325
Female 35-44 yr	Obs., No.	16	391	2134	3571	2794	1761	747	456	245	87	102	21	20	
Female 35-44 yr	Obs., %	0.13	3.17	17.31	28.97	22.67	14.29	6.06	3.54	1.99	0.71	0.83	0.17	0.16	
Male 25-34 yr	Exp., No.		158	911	2286	2567	1728	1124	708	381	203	112	57	44	10281
Male 25-34 yr	Obs., No.		203	1129	2390	3187	2025	927	311	88	8	13			
Male 35-44 yr	Exp., No.		175	1007	2528	2839	1912	1243	783	421	224	124	63	50	11373
Male 35-44 yr	Obs., No.		95	1012	2755	2894	2153	1171	703	267	240	21	12	50	
Male 35-44 yr	Obs., %		0.83	8.89	24.22	25.44	18.93	10.30	6.18	2.35	2.11	0.18	0.11	0.44	

Predicted values are calculated from the frequency distributions in Table IX, transformed into blood pressure values in the same manner as explained for Table VIII. Observed data are from U.S. Health Survey. 1960-1962. **23:** 7, 22, 25, 29, 30.

the same data. A polarization of opinions is personified by Platt and Pickering. Their views have the virtue of representing extremes and providing a convenient framework for discussions.
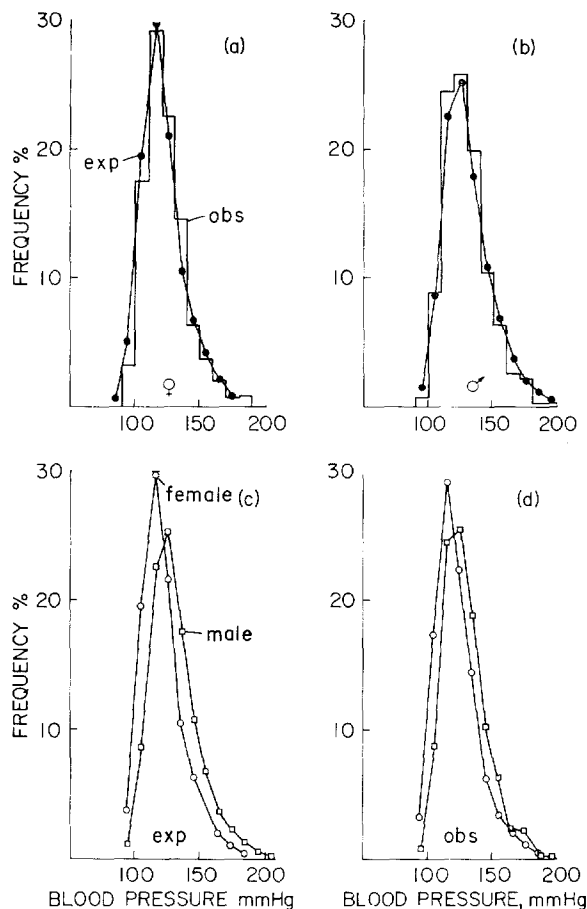

FIG. 8. Comparisons of expected and observed frequency distributions in human populations. Data for the graphs are taken from Table X. (a) Histogram of the male age group 35-44 yr superimposed on the predicted frequency polygon for males. (b) Histogram of the female age group 35-44 yr superimposed on the predicted frequency polygon for females. (c) Comparison of male and female distributions generated by model. □, male; ○, female. (d) Comparison of male and female curves observed in age group 35-44 yr.

Platt views essential hypertension as a clinical entity, qualitatively different from the normotensive state, and characterized by an inherited tendency to develop high blood pressure in middle life. He does not rule out multigenic inheritance but considers a single gene with partial dominance as a sufficient hy-

pothesis. Environmental variance does not occupy a major place in his arguments. Pickering claims that a number of genes are involved, that blood pressure resembles stature in that it has a continuous and unimodal frequency distribution, that no natural dividing line can be drawn between normotension and hypertension, and consequently that the concept "essential hypertension" has no useful function in the clinic. Both schools base their analyses on distribution curves in the general population, comparison of relatives of hypertensives and of controls, and studies of blood pressures in twins.

At the root of these controversial interpretations is the fact that a number of nongenetic influences confound the results. While most studies permit the conclusion that heredity is important for the development of blood pressure diseases, the data do not yield to an impartial analysis of the quantitative aspects of this inheritance. One has to adopt a point of view and look for inconsistencies; if none occur, the data are compatible with the postulated model. Whenever discrepancies disturb, imponderable features may be invoked to explain them. Pickering and Platt do not agree on the ground rules. Pickering's school uses sex- and age-adjusted scores developed by Roberts (31) rather than the raw data as a basis for analysis. Platt's thesis denies the validity of such scores; some persons will show an increase in blood pressure with age, others will not. In Platt's words: "If we are going to study essential hypertension it is necessary to study people at the age of 50 to 60 or at the widest, 45 to 65." (32)

We followed our rats for one year. The natural life span of the rat is 2–4 yr and the reproductive period is about 15 months (33). By analogy it would correspond to epidemiological studies in man restricted to the age group below 30–50 yr, the age when Platt wants studies to start. Since we have not observed the rats into senium, we have no way of knowing whether the scores developed by Pickering's school have any relevance to our rat populations; conversely, we can test our model only against data obtained on young adults.

For this reason we have been unable to compare specific predictions of our model with the data presented by Miall and Oldham from two populations in Wales (34). They calculated regression coefficients for the scores of first degree relatives and found average values of 0.3. Our model would predict regression coefficients for the raw data from 0.3 to 0.5, but we cannot match their values or make allowance for the effects of scoring except in general terms.

Our observations suggest that modifying factors, genetic and environmental, are multiple but that the main tendency to hypertensive disease may be explained by as little as two determining genetic components. The difference between malignant hypertension and the more common varieties of essential hypertension may be one of degree; it takes a combination of strong genetic and environmental agents to produce the malignant forms. In one respect the data favor Platt's view; there is an inherited difference among rats in the rise in blood pressure we observe with age. Miall and Lovell found that the increase in man observed over a 4 yr interval depended on the level of the initial pressure rather

than on the age of the individual (35). The same pattern is evident in our rats. Individuals with high pressures have a tendency to rise further, while those in the low blood pressure ranges tend to remain low. Age adjusted scores may therefore be of value in comparing populations of mixed genetic composition, but be quite misleading when applied to individuals or to members of a single family.

SUMMARY

Two strains of rat have been developed by selective breeding: one strain (R rats) is resistant to salt hypertension, the other strain (S rats) is highly susceptible. The inheritance of these traits has been explored in the first (F_1) and second (F_2) generation of crossbred rats and in backcrosses between parent and first filial ($F_1 \times R$, $F_1 \times S$) generations. Male F_1 rats had an average blood pressure close to the mid-parental (R and S) values, and the average of F_2 males was equivalent to that of F_1. Male offspring of F_1 with R, or F_1 with S also showed averages close to the respective mid-parental values. Female offspring showed deviations from this linear relationship, indicating a significant dominance in the female for the genes of normal blood pressure. A model of two autosomal, nonlinked diallelic loci, with a dominance deviation at one locus in the female, gave predictions with a reasonable agreement to the observed values. The same model also appeared compatible with human data if we assume a gene frequency of 0.13 for the hypertensinogenic allele on both loci. Random fluctuations in blood pressure, and incomplete homogeneity of parental strains permit several alternative models. The major conclusions are: that more than one locus is needed to explain the findings though as few as two loci may possibly suffice; the allelic effect seems additive in males, but there is a sex-determined influence on the expression in females; there is no consistent evidence for sex-linked inheritance. Furthermore, this model developed from the study of rats may provide a framework for analysis of human data.

REFERENCES

1. Dahl, L. K., M. Heine, and L. Tassinari. 1962. Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. *Nature (London)*. **194**:480.
2. Dahl, L. K., M. Heine, and L. Tassinari. 1962. Effects of chronic excess salt ingestion. Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J. Exp. Med.* **115**:1173.

3. Dahl, L. K., M. Heine, and L. Tassinari. 1963. Effects of chronic excess salt ingestion: role of genetic factors in both DOCA-salt and renal hypertension. *J. Exp. Med.* **118**:605.

4. Dahl, L. K., M. Heine, and L. Tassinari. 1965. Effects of chronic excess salt ingestion. Further demonstration that genetic factors influence the development of hypertension. Evidence from experimental hypertension due to cortisone and to adrenal regeneration. *J. Exp. Med.* **122**:533.

5. Iwai, J., K. D. Knudsen, L. K. Dahl, M. Heine, and G. Leitl. 1969. Genetic influence on the development of renal hypertension in parabiotic rats. Evidence for a humoral factor. *J. Exp. Med.* **129**:507.

6. Dahl, L. K. 1960. Effects of chronic excess salt feeding: elevation of plasma cholesterol in rats and dogs. *J. Exp. Med.* **112**:635.

7. Dahl, L. K., K. D. Knudsen, M. Heine, and G. Leitl. 1968. Effects of chronic excess salt ingestion. Modification of experimental hypertension in the rat by variations in the diet. *Circ. Res.* **22**:11.

8. Knudsen, K. D., J. Iwai, M. Heine, G. Leitl, and L. K. Dahl. 1969. 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. *J. Exp. Med.* **130**:1353.

9. Iwai, J., K. D. Knudsen, and L. K. Dahl. 1970. Genetic influence on the renin-angiotensin system. Evidence for a renin inhibitor in hypertension-prone rats. *J. Exp. Med.* **131**:543.

10. Wright, S. 1968. *In* Evolution and the Genetics of Populations. Vol. I. University of Chicago Press, Chicago, Ill. 383.

11. Smirk, F. H., and W. H. Hall. 1958. Inherited hypertension in rats. *Nature (London).* **182**:727.

12. Smirk, F. H., and I. Eryetishir. 1959. Pressor Responses in Rats Bred to Develop Spontaneous Hypertension. *Proc. Univ. Otago Med. Sch.* **37**:28.

13. Dahl, L. K., M. Heine, and L. Tassinari. 1964. Effects of chronic excess salt ingestion: vascular reactivity in two strains of rats with opposite genetic susceptibility to experimental hypertension. *Circulation.* **29–30** (Suppl. 2):11.

14. Smirk, F. H. 1967. Genetic Hypertension in Rats. *In* The Epidemiology of Hypertension. J. Stamler, R. Stamler, and T. N. Pullman, editors. Grune and Stratton, New York. 39.

15. Okamoto, K., and K. Aoki. 1963. Development of a strain of spontaneously hypertensive rats. *Jap. Circ. J.* **27**:282.

16. Okamoto, K. Spontaneous hypertension in rats. 1969. *Int. Rev. Exp. Pathol.* **7**:227.

17. Louis, W. J., R. Tabei, S. Spector, and A. Sjoerdsma. 1969. Studies on the spontaneously hypertensive rat. *Circ. Res.* **23**(Suppl. 1):93.

18. Louis, W. J., R. Tabei, A. Sjoerdsma, and S. Spector. 1969. Inheritance of high blood pressure in the spontaneously hypertensive rat. *Lancet.* **1**:1035.

19. Alexander, N., L. B. Hinshaw, and D. R. Drury. 1954. Development of a strain of spontaneously hypertensive rabbits. *Proc. Soc. Exp. Biol. Med.* **86**:855.

20. Alexander, N., L. B. Hinshaw, and D. R. Drury. 1956. Further observations on development of a colony of spontaneously hypertensive rabbits. *Proc. Soc. Exp. Biol. Med.* **92**:249.

21. Schlager, G. 1965. Heritability of blood pressure in mice. *J. Hered.* **56**:278.

22. Schlager, G., and R. S. Weibust. 1967. Genetic control of blood pressure in mice. *Genetics*. **55**:497.

23. Schlager, G. 1968. Genetic and physiological studies of blood pressure in mice. *Can. J. Genet. Cytol.* **10**:853.

24. Dahl, L. K. 1967. Effects of chronic excess salt ingestion-experimental hypertension in the rat: correlation with human hypertension. *In* The Epidemiology of Hypertension. J. Stamler, R. Stamler, and T. N. Pullman, editors. Grune and Stratton, New York. 218.

25. National Center for Health Statistics. 1964. Blood Pressure of Adults by Age and Sex. U. S. Department of Health, Education, and Welfare. Series II, No. 4.

26. Doyle, A. E., and J. R. E. Fraser. 1961. Essential hypertension and inheritance of vascular reactivity. *Lancet.* **II**:509.

27. Dahl, L. K., and R. A. Love. 1954. Evidence for relationship between sodium (chloride) intake and human essential hypertension. *Arch. Intern. Med.* **94**:525.

28. Isaacson, L. C., M. Modlin, and W. P. U. Jackson. 1963. Sodium intake and hypertension. *Lancet.* **I**:946.

29. Meneely, G. R., R. G., Tucker, W. J., Darby, and S. H. Auerbach. 1955. Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure. *J. Exp. Med.* **98**:71.

30. Thomas, C. B., and B. H. Cohen. 1955. The familial occurrence of hypertension and coronary artery disease, with observations concerning obesity and diabetes. *Ann. Intern. Med.* **42**:90.

31. Hamilton, M., G. W. Pickering, J. A. F. Roberts, and G. S. C. Sowry. 1954. The aetiology of essential hypertension. II. Scores for arterial blood pressures adjusted for differences in age and sex. *Clin. Sci. (London).* **13**:37.

32. Platt R. 1961. Essential hypertension. Incidence, course, and Heredity. *Ann. Intern. Med.* **55**:1.

33. Farris, E. J., and J. Q. Griffith, Jr., editors. 1963. The Rat in Laboratory Investigation. Hafner Publishing Co. Inc., New York.

34. Miall, W. E., and P. D. Oldham. 1963. The hereditary factor in arterial blood pressure. *Brit. Med. J.* **1**:75.

35. Miall, W. E., and H. G. Lovell. 1967. Relation between change of blood pressure and age. *Brit. Med. J.* **2**:660.