# EFFECTS OF CHRONIC EXCESS SALT INGESTION*

## Evidence That Genetic Factors Play an Important Role in Susceptibility to Experimental Hypertension

By LEWIS K. DAHL, M.D., MARTHA HEINE, and LORRAINE TASSINARI

*(From the Medical Research Center, Brookhaven National Laboratory, Upton, New York)*

(Received for publication, March 5, 1962)

There is considerable evidence which can be interpreted to indicate a familial trend in human essential hypertension (1–5). A familial disease could be due exclusively to common environmental factors, exclusively to common genetic patterns, or to an interaction of the two.

For some years we have been exploring the effects of chronic excess salt ingestion in both man and animals and have brought forward evidence to support the thesis that dietary salt plays an etiologic role in human essential hypertension (6–12). However, it was observed repeatedly that some individuals, and some rats, remained normotensive despite the fact that they were chronically consuming large amounts of salt.

In our nutritional experiments with rats (13–15) the control of environmental factors was rigid. In spite of this, in a given group, some salt-fed animals never developed hypertension whereas a few became hypertensive after 1 month on the diet and rapidly developed fulminating hypertension. It was thought that such wide variations in response to excess salt consumption represented either the statistical limits of a homogeneous populatoin or the extreme consequences of genetic heterogeneity. If the population were homogeneous, it would not lend itself to fractionation by genetic techniques. By contrast, if sensitivity to salt were genetically controlled, it should be possible to separate two strains that differ demonstrably in the incidence and gravity of hypertension developing from excess salt consumption.

The present paper is a report of experiments that resulted in the separation of two strains of rats differing markedly from one another in their susceptibility to the development of experimental hypertension from excess salt ingestion.

### *Experiment 1*

We were aware that thyroid hormone enhanced the development of experimental hypertension produced by salt and desoxycorticosterone acetate (16–20).

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

Furthermore, Selye (16, 21) and his associates, as well as Masson, Corcoran, and Page (20), had observed in uninephrectomized salt-fed rats that the administration of thyroxin alone could lead rapidly but not constantly to hypertension.

We thought it likely that administration of thyroid hormone would enhance the development of hypertension in non-operated salt-fed animals, as well. If so, observations on the development of hypertension could be completed in perhaps 3 or 4 months as opposed to the much slower rate at which experimental hypertension ordinarily develops in salt-fed rats (13–15, 22). The initial experiments, therefore, were designed to evaluate the effect of thyroid hormone on the development of hypertension in intact, salt-fed animals.

21 unselected weanling female Sprague-Dawley rats were studied. The care, diet, and technique of blood pressure measurement were the same as reported in earlier papers (13–15), and only items pertinent to the present study will be given here. 7 animals were fed the low sodium control diet; 10 were fed chow containing 8 per cent NaCl, and 4 received chow with 11.6 per cent sea salt equivalent to about 7.3 per cent NaCl (14). At approximately 3 weeks of age, usually 1 day after being placed on their respective diets at weaning, all animals received intraperitoneally 50 $\mu$g of sodium L-triiodothyronine (*cytomel*®, S.K.F.)[1]. This was dissolved in 0.2 ml of saline made slightly alkaline with dilute NaOH. It was administered daily for 4 doses initially, and thereafter 5 $\mu$g in the same volume were given 4 times per week for 3 months (Nos. 797, 798, and 799 for 4 months); at the end of this time the triiodothyronine ($T_3$) was stopped but the diet continued unchanged for 8 more months (7 months for Nos. 797, 798, and 799) for a total of 11 months of observation. Blood pressures were measured every 2 to 3 weeks during administration of the $T_3$, 48 hours after the last dose of $T_3$, and at monthly intervals thereafter until sacrifice of the animals at approximately 1 year of age.

*Results.*—The important data are summarized in Table I.

None of the control animals developed continuing hypertension although several had occasional elevations (not shown) of between 140 and 148 mm Hg during the period of $T_3$ administration.

By contrast, most of the salt-fed animals developed hypertension rapidly, and this was not significantly affected when the $T_3$ was stopped: systolic pressures 48 hours as well as 1 month after cessation of $T_3$ were very similar. The rapidity of onset of sustained hypertension was significantly faster than we have observed with salt-feeding alone (13, 14) but in other respects the animals resembled our standard colonies of salt-fed animals (13–15).

From this experiment it appeared that triiodothyronine enhanced the development of hypertension in intact, salt-fed rats. As in earlier observations (12, 20, 21), we found this enhancement to be inconstant. Arbitrarily, we assigned this inconstancy of response to variations in genetic sensitivity to the regimen. The fact that $T_3$ alone had no effect on the development of hypertension was interpreted to implicate NaCl as the primary offending agent.

[1] We are indebted to Dr. H. Greenberg, Research Division, Smith, Kline and French, Philadelphia, for all the cytomel® used in this study.

Finally, it was postulated that the development of strains of rats both sensitive and insensitive to the $T_3$-salt regimen might indicate similar response to salt alone.

TABLE I

*Experiment 1*

| No. rats | Rat No. | Diet | Regimen | Blood pressure | | |
|---|---|---|---|---|---|---|
| | | | | End $T_3$ | One month after last $T_3$ injection | Average B.P. after 10 + 11 months on diet |
| | | | | *mm Hg* | *mm Hg* | *mm Hg* |
| 7 | 7-99* | Control | $T_3$ 50 μg/day i.p. for 4 days, then 5 μg 4×/wk. for 3 months. | 126 | 128 | 133‡ |
| | 8-19 | | | 138 | 130 | 133 |
| | 8-20 | | | 126 | 130 | 126 |
| | 8-21 | | | 112 | 124 | 126 |
| | 8-22 | | | 116 | 112 | 129 |
| | 8-23 | | | 131 | 126 | 125 |
| | 8-25 | | | 124 | 124 | 126 |
| 10 | 7-97* | 8 per cent NaCl | Same as above | 182 | 183 | 222 |
| | 7-98* | | | 170 | 182 | 197 |
| | 8-10 | | | 164 | 148 | 175 |
| | 8-11 | | | 130 | 136 | 147 |
| | 8-12 | | | 154 | 152 | 181 |
| | 8-13 | | | 196 | 202 | 177 |
| | 8-15 | | | 163 | 154 | 175 |
| | 8-16 | | | 128 | 130 | 143 |
| | 8-17 | | | 146 | 156 | 158 |
| | 8-18 | | | 156 | 160 | 181 |
| 4 | 8-05 | 11.6 per cent sea salt | Same as above | 186 | 192 | 223 |
| | 8-07 | | | 128 | 150 | 138 |
| | 8-08 | | | 182 | 158 | 154 |
| | 8-09 | | | 164 | 162 | 175 |

Blood pressure of female rats on diets with high or low salt content after injection of L-triiodothyronine ($T_3$) intraperitoneally.

* Received 5 μg $T_3$ 4×/wk. for 4 months rather than 3 months.

‡ Average of pressures at 9 and 10 months; respiratory infection at 11 months.

*Experiment 2*

An unselected group of 24 female and 23 male 3-week-old Sprague-Dawley weanlings constituted the initial stock from which two strains were developed. The regimen for this group and the generations derived from it differed somewhat from that in Experiment 1. Initially 50 μg of $T_3$ was injected daily for 4 days, as before, after which the animals received only ten 5 μg doses in the next 21 days, for a total of 250 μg in approximately 4 weeks. Blood pressures were measured 48 hours later; immediately after this measurement animals saved for breeding were placed on the low sodium, control diet and the remainder were sacrificed.

The regimen was changed from that used in Experiment 1 in the belief that animals which became hypertensive on the abbreviated design were genetically the most sensitive to salt. However, the shorter regimen might allow some less sensitive, but potentially hypertensive, animals to be accepted as resistant to the hypertensogenic effects of NaCl.

In order to simplify feeding procedure, all animals received only one salt-containing food, namely the "11.6 per cent sea salt chow" (14). On this identical regimen, no difference in the hypertensive potential has been found for the 8 per cent NaCl or the 11.6 per cent sea salt chows: this is in contrast to long-term feeding experiments in which the sea-salt was found to produce significantly more severe hypertension in rats (14).

In order to be sure that our particular group of Sprague-Dawley rats was not unique, other unselected groups of Sprague-Dawley rats were tested for their hypertensive response to the above regimen during the 2 years of this study. In these instances, each such experiment was confined to the unselected animals without subsequent selective breeding. From these experiments we were able to ascertain the range of response to this regimen that might be expected in a mixed population of Sprague-Dawley rats. 4 such groups were studied, distributed by sex and number as follows:[2]

| | ♀ | ♂ |
|---|---|---|
| Unselected strain $U_1$ A | 23 | 17 |
| " " $U_1$ B | 29 | 30 |
| " " $U_1$ C[2] | 25 | — |
| " " $U_1$ D[2] | 21 | — |
| Total | 98 | 47 |

*Selective inbreeding.*—From the blood pressure response 48 hours after the last $T_3$ injection of members in the original unselected group (called $U_1$), males and females with the highest blood pressures were selected for inbreeding within this group; those with the lowest pressures were similarly bred. Mating ordinarily was begun during the week following blood pressure measurement although occasionally the interval was longer. During the period of mating and subsequent gestation, all animals were maintained on the low sodium, control diet: we have observed that female rats on a high salt diet produce smaller litters which appears to be due to resorption of fetuses. Offspring ordinarily were weaned at 21 days although in the case of large litters if the young animals were small, this was extended up to a maximum of 28 days. At weaning, they were put on the sea-salt chow and $T_3$ administration was begun

[2] We are indebted to Dr. Eckart Schackow for allowing us to use the blood pressure data on Groups $U_1$ C and D.

as described above. Animals derived from parents with high blood pressure were called *sensitive* (S) and each generation subsequent to the unselected group ($U_1$) was labeled $S_1$, $S_2$, or $S_3$, respectively. Animals from parents that did not respond with high blood pressure were called *resistant* (R) and succeeding generations were labeled $R_1$, $R_2$, or $R_3$, respectively. It was usually possible to select for inbreeding brothers and sisters that had responded in similar fashion. In selection of the breeders for the successive *resistant* strains, animals showing the least blood pressure response to the regimen were used and the remaining rats were discarded. Animals responding in the reverse manner were selected as breeders for successive *sensitive* strains. Following the initial designation of $U_1$ animals as sensitive or resistant no crossbreeding between the 2 strains was permitted.

Analysis of the data from $S_2$ and $R_2$ indicated that two strains were being successfully separated one of which was more, while the other was less, resistant to developing hypertension on this regimen than was the original $U_1$ group. Therefore, from litters in $S_3$ and $R_3$ animals were selected randomly and placed in 2 subgroups at weaning after sex was determined. 1 subgroup was treated in the standard fashion described above with $T_3$ and sea salt chow. The 2nd subgroup did not receive $T_3$ but was maintained only on the control or sea salt chows as described in Experiment 3 (below).

*Results.—*

*Response of original $U_1$ strain compared with other unselected Sprague-Dawley rats:* In Table II are summarized the blood pressure responses to the $T_3$–sea salt regimen of 192 unselected weanling rats, groups of which were subjected to the same regimen, at intervals throughout this study. The response of the $U_1$ strain which was used as the original breeder stock, did not differ significantly ($p > 0.05$) from the other groups. A contrary finding would only have reinforced the suspicion that differences in genetic susceptibility to salt exist. It was concluded that the response of the $U_1$ animals was characteristic of the Sprague-Dawley strain from which they originated.

*Comparison of blood pressure responses to triiodothyronine–sea salt regimen among successive generations:*

*1.* Mean systolic blood pressure (Tables III and IV). The diminution in mean systolic blood pressure with succeeding generations *of resistant* (R) animals is summarized in Table III; the corresponding increase among *sensitive* (S) animals is shown in Table IV. The high mean value observed among the $S_1$ animals is probably fortuitous: an epidemic of pneumonia swept the $R_1$-$S_1$ animals during the first 2 weeks of the regimen and among the 113 animals at risk, 31 died. Interestingly, 25 of the deaths occurred in the $S_1$ strain, leaving only 15 survivors of each sex. In both sexes, the mean systolic blood pressure of the $S_3$ animals was higher while that of the $R_3$ animals was lower, than that of the $U_1$ animals ($p < 0.01$).

TABLE II

*Experiment 2*

| Strain | Total No. rats in group | Sex | Number (No.) of rats and fraction (*f*) of group with systolic B.P. (mm Hg) as indicated | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | <140 | | 140 to 179 | | 180+ | |
| | | | No. | *f* | No. | *f* | No. | *f* |
| $U_1$ | 24 | Females | 7 | 0.29 | 13 | 0.54 | 4 | 0.17 |
| $U_1$ A | 23 | " | 3 | 0.13 | 16 | 0.70 | 4 | 0.17 |
| $U_1$ B | 29 | " | 9 | 0.31 | 17 | 0.59 | 3 | 0.10 |
| $U_1$ C | 25 | " | 4 | 0.16 | 14 | 0.56 | 7 | 0.28 |
| $U_1$ D | 21 | " | 2 | 0.10 | 14 | 0.67 | 5 | 0.24 |
| All females.. | 122 | " | 25 | 0.21 | 74 | 0.61 | 23 | 0.19 |
| $U_1$ | 23 | Males | 3 | 0.13 | 15 | 0.65 | 5 | 0.22 |
| $U_1$ A | 17 | " | 3 | 0.18 | 8 | 0.47 | 6 | 0.35 |
| $U_1$ B | 30 | " | 4 | 0.13 | 21 | 0.70 | 5 | 0.17 |
| All males.... | 70 | " | 10 | 0.14 | 44 | 0.63 | 16 | 0.23 |
| All..... | 192 | Both | 35 | 0.18 | 118 | 0.62 | 39 | 0.20 |

Effect of $T_3$–sea salt regimen on unselected Sprague-Dawley rats of both sexes. $U_1$ animals were used as original breeding stock and $U_1$ A through $U_1$ D as checks on expected distribution of blood pressure response among similarly treated, unselected animals.

Chi-square tests reveal no significant differences in the dispersion of blood pressure responses among all groups of females, among the groups of males, or between all females and all males.

TABLE III

*Experiment 2*

| Strain | Total No. | Sex | Average B.P. (±S.D.) |
|---|---|---|---|
| | | | *mm Hg* |
| $U_1$ | 24 | Females | 157.6 (±24.2) |
| $R_1$ | 28 | " | 153.2 (±18.8) |
| $R_2$ | 35 | " | 137.6 (±14.6) |
| $R_3$ | 32 | " | 136.1 (±14.5) |
| $U_1$ | 23 | Males | 161.6 (±18.5) |
| $R_1$ | 24 | " | 152.5 (±16.5) |
| $R_2$ | 23 | " | 136.0 (±15.4) |
| $R_3$ | 29 | " | 139.5 (±15.4) |

Development of increasing resistance to $T_3$–sea salt regimen: decline in average blood pressure response by selective inbreeding. $U_1$ = Unselected; $R_1$, $R_2$, $R_3$ = 1st, 2nd, and 3rd generation, respectively.

By Student's $t$ test, the mean B.P. of $♀R_3 < ♀U_1$, $t$ 4.10, $p < 0.001$; $♂R_3 < ♂U_1$, $t$ 4.72, $p < 0.001$.

*2.* Detailed systolic blood pressures of $U_1$ *versus* $S_3$ and $R_3$ animals. The individual pressures recorded for all survivors among the $U_1$ and $S_3$-$R_3$ strains in Experiment 2 are summarized in detail in Table V and VI. A graphic demonstration of the difference between the pressures among the $U_1$ and the $S_3$ and $R_3$ animals is provided by Fig. 1. The difference in distribution of blood pressure responses among $U_1$ and $S_3$-$R_3$ animals is summarized in Table VII, where the members have been distributed according to their systolic blood pressures as

TABLE IV

*Experiment 2*

| Strain | Total No. | Sex | Average B.P. (±S.D.) |
|---|---|---|---|
| | | | *mm Hg* |
| $U_1$ | 24 | Females | 157.6 (±24.2) |
| $S_1$ | 15* | " | 188.2 (±17.4) |
| $S_2$ | 24 | " | 166.3 (±24.1) |
| $S_3$ | 24 | " | 182.3 (±18.3) |
| $U_1$ | 23 | Males | 161.6 (±18.5) |
| $S_1$ | 15* | " | 186.3 (±18.8) |
| $S_2$ | 27 | " | 178.5 (±15.7) |
| $S_3$ | 25 | " | 179.3 (±20.2) |

Development of increasing sensitivity to $T_3$–sea salt regimen: increase in average blood pressure by selective inbreeding. $U_1$ = Unselected; $S_1$, $S_2$, $S_3$ = 1st, 2nd, and 3rd generation, respectively. By Student's $t$ test, the mean B.P. of $\varphi S_3 > \varphi U_1$, with $t$ 3.99, $p < 0.001$; $\sigma S_3 > \sigma U_1$ with $t$ 3.15, $p < 0.005$.

* A severe pneumonia epidemic occurred among the $S_1$–$R_1$ animals with death primarily among the $S_1$ strain: there were 25 deaths among 55 $S_1$ animals in contrast to only 6 deaths among 58 animals in the $R_1$ strain. The increase in average B.P. among $S_1$ animals, therefore, may be only due to chance survival of the more salt-sensitive animals among the small number of animals remaining.

follows: < 140 mm Hg (normal), 140 to 179 mm Hg (mild to moderate hypertension), 180+ mm Hg (severe hypertension).

In Tables V and VI the mean and median systolic pressures are shown to be significantly ($p < 0.01$) higher in the $S_3$ strain, and lower in the $R_3$ strain, than in the original $U_1$ strain. The difference in frequency of "severe" and "normal" pressures is striking when the $S_3$ and $R_3$ animals are compared. From Table VII it will be seen that among the 61 resistant ($R_3$) animals of both sexes, none with severe (180+) hypertension was observed. Yet, the failure to respond is even more striking than is indicated by Table VII, for among females there was none, and among males only 1 animal (No. 3-52), with a pressure in excess of even 160 mm. By contrast, among the 49 sensitive ($S_3$) animals, 26 had pressures of 180 mm or more (*i.e.*) severe hypertension);

TABLE V

*Experiment 2. Females*

| Strain..... | $U_1$ | | $S_3$ | | $R_3$ | |
|---|---|---|---|---|---|---|
| | Rat No. | Systolic B.P. | Rat No. | Systolic B P. | Rat No. | Systolic B.P. |
| | | *mm Hg* | | *mm Hg* | | *mm Hg* |
| | 1 | 134 | 3-14 | 194 | 3-01 | 150 |
| | 2 | 210 | 3-15 | 187 | 3-02 | 116 |
| | 3 | 152 | 3-16 | 186 | 3-04 | 142 |
| | 4 | 170 | 3-17 | 174 | 3-05 | 125 |
| | 5 | 154 | 3-18 | 168 | 3-06 | 147 |
| | 6 | 134 | 3-19 | 166 | 3-07 | 136 |
| | 7 | 174 | 3-20 | 156 | 3-08 | 130 |
| | 8 | 172 | 3-25 | 210 | 3-12 | 108 |
| | 9 | 160 | 3-26 | 194 | 3-21 | 128 |
| | 10 | 126 | 3-27 | 204 | 3-28 | 140 |
| | 11 | 186 | 4-06 | 174 | 3-29 | 135 |
| | 12 | 156 | 4-07 | 174 | 3-30 | 124 |
| | 13 | 121 | 4-25 | 186 | 3-31 | 118 |
| | 14 | 136 | 4-26 | 228 | 3-32 | 158 |
| | 15 | 110 | 4-27 | 200 | 3-33 | 124 |
| | 16 | 144 | 4-28 | 162 | 3-34 | 112 |
| | 17 | 171 | 4-29 | 171 | 3-35 | 144 |
| | 18 | 130 | 4-44 | 183 | 3-36 | 158 |
| | 19 | 162 | 4-45 | 200 | 3-43 | 144 |
| | 20 | 181 | 4-46 | 166 | 3-44 | 147 |
| | 21 | 177 | 4-47 | 170 | 4-05 | 110 |
| | 22 | 176 | 4-48 | 172 | 4-08 | 134 |
| | 23 | 184 | 4-49 | 154 | 4-09 | 154 |
| | 24 | 163 | 4-50 | 196 | 4-12 | 139 |
| | | | | | 4-20 | 153 |
| | | | | | 4-21 | 152 |
| | | | | | 4-22 | 150 |
| | | | | | 4-23 | 131 |
| | | | | | 4-24 | 116 |
| | | | | | 4-41 | 148 |
| | | | | | 4-42 | 141 |
| | | | | | 4-43 | 142 |
| *n*..... | 24 | | 24 | | 32 | |
| Mean..... | 157.6 | | 182.3 | | 136.1 | |
| S.D..... | ±24.2 | | ±18.3 | | ±14.5 | |
| Median..... | 161 | | 178.5 | | 139.5 | |

Effect of triiodothyronine–sea salt regimen on systolic blood pressures of female rats: comparison of unselected ($U_1$) group with 3rd generation of animals inbred for sensitivity ($S_3$) and resistance ($R_3$) to hypertension from above regimen.

By Student's $t$ test, mean B.P. for $S_3 > U_1$ ($t$, 3.99, $p < 0.001$) and $R_3 < U_1$ ($t$, 4.10, $p < 0.001$).

TABLE VI

*Experiment 2. Males*

| Strain..... | $U_1$ | | $S_3$ | | $R_3$ | |
|---|---|---|---|---|---|---|
| | Rat No. | Systolic B.P. | Rat No. | Systolic B.P. | Rat No. | Systolic B.P. |
| | | *mm Hg* | | *mm Hg* | | *mm Hg* |
| | 51 | 172 | 3-65 | 161 | 3-51 | 143 |
| | 52 | 156 | 3-66 | 128 | 3-52 | 164 |
| | 53 | 124 | 3-68 | 184 | 3-55 | 122 |
| | 54 | 190 | 3-69 | 194 | 3-56 | 155 |
| | 55 | 154 | 3-70 | 162 | 3-62 | 123 |
| | 56 | 174 | 3-71 | 182 | 3-63 | 146 |
| | 57 | 174 | 3-72 | 198 | 3-67 | 128 |
| | 58 | 166 | 3-73 | 190 | 3-74 | 128 |
| | 59 | 164 | 4-55 | 182 | 3-82 | 160 |
| | 60 | 146 | 4-56 | 174 | 3-83 | 118 |
| | 61 | 173 | 4-57 | 207 | 3-89 | 130 |
| | 62 | 154 | 4-58 | 190 | 3-90 | 146 |
| | 63 | 164 | 4-59 | 192 | 3-91 | 114 |
| | 64 | 182 | 4-60 | 194 | 3-99 | 156 |
| | 65 | 183 | 4-75 | 182 | 4-00 | 132 |
| | 66 | 126 | 4-76 | 220 | 4-01 | 152 |
| | 67 | 184 | 4-77 | 170 | 4-11 | 122 |
| | 68 | 144 | 4-78 | 176 | 4-51 | 128 |
| | 69 | 143 | 4-79 | 166 | 4-52 | 148 |
| | 70 | 180 | 4-80 | 168 | 4-53 | 156 |
| | 71 | 166 | 4-81 | 162 | 4-54 | 160 |
| | 72 | 162 | 4-82 | 144 | 4-61 | 148 |
| | 73 | 136 | 4-83 | 164 | 4-62 | 160 |
| | | | 4-95 | 204 | 4-65 | 134 |
| | | | 4-96 | 188 | 4-66 | 154 |
| | | | | | 4-73 | 144 |
| | | | | | 4-74 | 132 |
| | | | | | 4-93 | 118 |
| | | | | | 4-94 | 124 |
| *n*..... | 23 | | 25 | | 29 | |
| Mean..... | 161.6 | | 179.3 | | 139.5 | |
| S.D..... | ±18.5 | | ±20.2 | | ±15.4 | |
| Median..... | 164 | | 182 | | 143 | |

Systolic blood pressure in male rats. Otherwise as in Table V. By Student's $t$ test, mean B.P. for $S_3 > U_1$ ($t$, 3.15, $p < 0.005$) and $R_3 < U_1$ ($t$, 4.72, $p < 0.001$).

only 1 animal with a pressure below 140 mm was observed and only 4 of the $S_3$ animals had pressures below 160 mm.

Thus, among animals inbred for their failure to develop hypertension on the $T_3$–sea salt regimen the fraction responding with severe hypertension disap-

peared and the number responding with normal pressure more than doubled compared with the original strain. By contrast, among animals inbred for their tendency to develop hypertension on this same regimen, the fraction

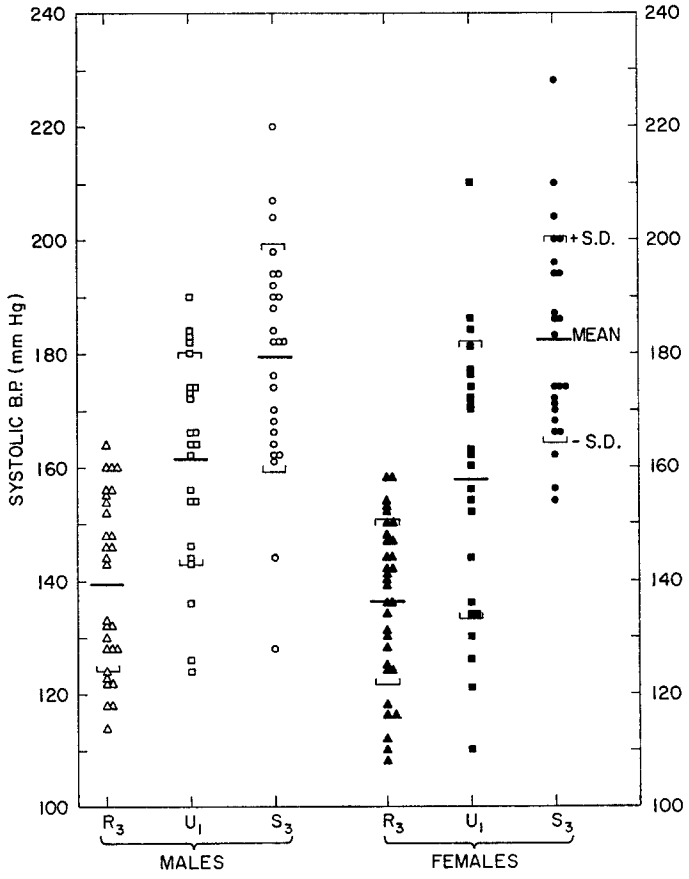


FIG. 1. Comparison of systolic blood pressures, among unselected ($U_1$) rats with 3rd generation of animals inbred for resistance ($R_3$) and sensitivity ($S_3$) to hypertension from triiodothyronine–sea salt regimen. Difference between mean blood pressures was significant: $S_3 > U_1 > R_3$ ($p = < 0.005$).

responding with severe hypertension increased $2\frac{1}{2}$ times while those responding with normal pressure virtually disappeared (1 out of 49 animals). If comparison is made not with the original ($U_1$) strain but between $S_3$ and $R_3$, the differences in responses are even more striking. As indicated in Table VII, these differences are highly significant when tested by chi-square.

On the basis of the foregoing evidence, it was concluded that a difference in

susceptibility to the triiodothyronine–sea salt regimen had been developed after 3 generations of selective inbreeding although it was clear that neither the $S_3$ nor the $R_3$ strains were as yet pure.

It remained to be demonstrated that susceptibility as indicated by the response to the $T_3$–sea salt regimen was an index of susceptibility to sea salt alone. The data in Experiment 3 show this to be true.

TABLE VII

*Experiment 2*

| Sex | B.P. | $U_1$ | | $S_3$ | | $R_3$ | |
|---|---|---|---|---|---|---|---|
| | | No. | *f* | No. | *f* | No. | *f* |
| | *mm Hg* | | | | | | |
| Males | <140 | 3 | 0.13 | 1 | 0.04 | 14 | 0.48 |
| | 140 to 179 | 15 | 0.65 | 10 | 0.40 | 15 | 0.52 |
| | 180+ | 5 | 0.22 | 14 | 0.56 | 0 | 0 |
| Total..... | | 23 | | 25 | | 29 | |
| Females | <140 | 7 | 0.28 | 0 | 0 | 16 | 0.50 |
| | 140 to 179 | 13 | 0.52 | 12 | 0.50 | 16 | 0.50 |
| | 180+ | 5 | 0.20 | 12 | 0.50 | 0 | 0 |
| Total..... | | 25 | | 24 | | 32 | |
| Both | <140 | 10 | 0.21 | 1 | 0.02 | 30 | 0.49 |
| | 140 to 179 | 28 | 0.58 | 22 | 0.45 | 31 | 0.51 |
| | 180+ | 10 | 0.21 | 26 | 0.53 | 0 | 0 |
| Total..... | | 48 | | 49 | | 61 | |

Distribution of blood pressure responses after $T_3$–sea salt regimen: comparison of unselected ($U_1$) animals with 3rd generation inbred for sensitivity ($S_3$) and resistance ($R_3$) to regimen.

By the chi-square test, the differences in distribution were highly significant: *Males*, $U_1$ versus $S_3$, $\chi^2 = 17.02$, $p < 0.005$; $U_1$ versus $R_3$, $\chi^2 = 34.97$, $p < 0.005$; *Females*, $U_1$ versus $S_3$, $\chi^2 = 17.56$, $p < 0.005$; $U_1$ versus $R_3$, $\chi^2 = 11.95$, $p < 0.005$.

## *Experiment 3*

131 weanling $S_3$ and $R_3$ animals were used in Experiment 3; these were siblings of animals used in Experiment 2. As before, animals were fed either the control low salt chow or the 11.6 per cent sea salt chow but for this experiment no triiodothyronine was administered. Because of the various times at which litters were delivered, as well as because the $S_3$ strains tended to have smaller litters, a longer time was required to get sufficient numbers of $S_3$ rats, and the studies on some $S_3$ animals lagged about 3 months behind the $R_3$ strain. Thus, in Experiment 3, blood pressures of the $S_3$ strain after 3 months have been compared with pressures of the $R_3$ strain after 6 months on the diet. By this procedure an increase in sensitivity to salt would

tend to be minimized in the $S_8$ strain and therefore, differences in response of the two strains will be under-rather than overestimated.

*Results.*—The response to salt-feeding alone is summarized in Tables VIII and IX and Fig. 2. The difference in blood pressure response between salt-fed

TABLE VIII

*Experiment 3. Females*

| Strain | | $S_2$ | | $R_3$ | |
|---|---|---|---|---|---|
| | Diet | Rat No. | Systolic B.P. | Rat No. | Systolic B.P. |
| | | | *mm Hg* | | *mm Hg* |
| | 11.6 per cent sea salt chow | 5-07 | 132 | 3-03 | 110 |
| | | 5-08 | 136 | 3-09 | 108 |
| | | 5-09 | 152 | 3-10 | 126 |
| | | 5-10 | 146 | 3-11 | 92 |
| | | 5-11 | 189 | 3-13 | 103 |
| | | 5-12 | 132 | 3-22 | 108 |
| | | 5-13 | 184 | 3-23 | 86 |
| | | 5-15 | 152 | 3-24 | 107 |
| | | 5-16 | 194 | 3-38 | 92 |
| | | 5-18 | 182 | 3-45 | 122 |
| | | 5-19 | 144 | 3-46 | 108 |
| | | 5-20 | 158 | 3-47 | 102 |
| | | 5-22 | 198 | 3-48 | 109 |
| | | 5-23 | 141 | 3-49 | 88 |
| | | 5-24 | 140 | 3-50 | 100 |
| | | 5-25 | 170 | 3-81 | 114 |
| | | 5-26 | 130 | 4-16 | 118 |
| | | 5-27 | 134 | 4-17 | 84 |
| | | 5-28 | 127 | 4-18 | 122 |
| | | 5-30 | 172 | | |
| | | 5-31 | 134 | | |
| | | 5-32 | 123 | | |
| | | 5-33 | 240 | | |
| | | 5-34 | 132 | | |
| | | 5-35 | 170 | | |
| | | 5-36 | 142 | | |
| | | 5-37 | 138 | | |
| | | 5-41 | 150 | | |
| | | 5-42 | 154 | | |
| | | 5-14 | 216* | | |
| | | 5-43 | 148* | | |
| *n* | | 31 | | 19 | |
| Mean | | 156.8 | | 105.2 | |
| S.D. | | ±28.4 | | ±12.5 | |
| Median | | 146 | | 108 | |

TABLE VIII—*Continued*

| Strain..... | | $S_3$ | | $R_3$ | |
|---|---|---|---|---|---|
| | Diet | Rat No. | Systolic B.P. | Rat No. | Systolic B.P. |
| | | | *mm Hg* | | *mm Hg* |
| | Control | 5-01 | 100 | 4-03 | 100 |
| | | 5-02 | 118 | 4-04 | 104 |
| | | 5-03 | 102 | 4-10 | 130 |
| | | 5-04 | 120 | 4-13 | 110 |
| | | 5-05 | 114 | 4-14 | 102 |
| | | 5-06 | 110 | 4-15 | 114 |
| | | 5-17 | 107 | 4-19 | 104 |
| | | 5-21 | 122 | 4-38 | 92 |
| | | 5-38 | 118 | 4-40 | 102 |
| | | 5-39 | 114 | | |
| | | 5-40 | 109 | | |
| *n*..... | | 11 | | 9 | |
| Mean..... | | 112.2 | | 106.4 | |
| S.D..... | | ±7.3 | | ±10.7 | |
| Median..... | | 114 | | 104 | |

Effect of high salt intake *without* triiodothyronine on blood pressure response of female Sprague-Dawley rats that were selectively inbred for 3 generations to develop strains sensitive ($S_3$) and resistant ($R_3$) to the hypertensogenic effect of dietary salt. Blood pressures of $S_3$ strain were after only 3 months on diet whereas those of $R_3$ strain were after 6 months on same regimen.

By Student's $t$ test, the mean B.P. of the following were significantly different: $S_3$ salt > $S_3$ controls, $t$ 5.13, $p < 0.001$; $S_3$ salt > $R_3$ salt, $t$ 7.45, $p < 0.001$.

* = B.P. after 2 months on diet; animal died before B.P. measurement at 3 months.

$S_3$ and salt-fed $R_3$ animals was striking: after 6 months of salt feeding none of the 39 $R_3$ animals had hypertension whereas after only 3 months on the same program 49 of the 60 $S_3$ animals were hypertensive and many of them severely so. The hypertensive response of the $S_3$ salt-fed males was particularly marked among which the mean and median systolic pressure was 180 mm Hg. In the animals that died, large hearts with thick-walled hypertrophied left ventricles were observed uniformly.

By contrast, when members of this same $S_3$ strain were maintained on the control diet, hypertension did not develop. Thus, however strong the genetic tendency to hypertension may be in this strain, the addition of a high salt diet seems necessary to unmask the trait. Among the $R_3$ animals, the presence or absence of excess dietary salt did not seem to affect the blood pressure response.

Finally, comparison of the response of males with that of females on the

same regimen indicated that in each instance, the mean blood pressure of the males was significantly higher ($p < 0.01$) than that of the females.

From this experiment, it was concluded that marked differences in sensitivity to salt had been demonstrated in two strains of rats, and that this variability was genetically transmitted. The data suggested that males were more sensitive than females.

TABLE IX

*Experiment 3. Males*

| Strain | | $S_2$ | | $R_2$ | |
|---|---|---|---|---|---|
| | Diet | Rat No. | Systolic B.P. | Rat No. | Systolic B.P. |
| | | | *mm Hg* | | *mm Hg* |
| | 11.6 per cent sea salt | 5-54 | 172 | 3-53 | 108 |
| | | 5-55 | 194 | 3-54 | 114 |
| | | 5-56 | 131 | 3-57 | 104 |
| | | 5-57 | 201 | 3-58 | 118 |
| | | 5-58 | 200 | 3-59 | 110 |
| | | 5-60 | 174 | 3-61 | 92 |
| | | 5-66 | 180 | 3-64 | 132 |
| | | 5-67 | 204 | 3-76 | 134 |
| | | 5-68 | 214 | 3-77 | 134 |
| | | 5-69 | 176 | 3-78 | 137 |
| | | 5-74 | 192 | 3-79 | 112 |
| | | 5-75 | 208 | 3-80 | 130 |
| | | 5-78 | 154 | 3-84 | 104 |
| | | 5-79 | 160 | 3-85 | 139 |
| | | 5-81 | 164 | 3-86 | 130 |
| | | 5-82 | 172 | 3-87 | 125 |
| | | 5-84 | 197 | 3-88 | 138 |
| | | 5-85 | 182 | 3-93 | 104 |
| | | 5-86 | 180 | 3-94 | 126 |
| | | 5-90 | 160 | 4-02 | 102 |
| | | 5-91 | 182 | | |
| | | 5-61 | 156* | | |
| | | 5-62 | 204‡ | | |
| | | 5-63 | 204‡ | | |
| | | 5-71 | 172* | | |
| | | 5-72 | 162* | | |
| | | 5-73 | 172‡ | | |
| | | 5-76 | 176‡ | | |
| | | 5-83 | 182* | | |
| *n* | | 29 | | 20 | |
| Mean | | 180.2 | | 119.7 | |
| S.D. | | ±19.4 | | ±14.5 | |
| Median | | 180 | | 121.5 | |

TABLE IX—*Continued*

| Strain | | $S_3$ | | $R_3$ | |
|---|---|---|---|---|---|
| | Diet | Rat No. | Systolic B.P. | Rat No. | Systolic B.P. |
| | | | *mm Hg* | | *mm Hg* |
| | Control | 4-98 | 128 | 4-63 | 132 |
| | | 4-99 | 138 | 4-64 | 128 |
| | | 5-00 | 136 | 4-67 | 118 |
| | | 5-51 | 121 | 4-68 | 108 |
| | | 5-52 | 120 | 4-69 | 124 |
| | | 5-53 | 118 | 4-70 | 114 |
| | | 5-70 | 116 | 4-71 | 122 |
| | | 5-77 | 122 | 4-72 | 109 |
| | | 5-80 | 128 | 4-91 | 130 |
| | | 5-87 | 126 | 4-92 | 116 |
| | | 5-88 | 126 | | |
| | | 5-89 | 130 | | |
| *n* | | 12 | | 10 | |
| Mean | | 125.8 | | 120.1 | |
| S.D. | | ±6.8 | | ±8.5 | |
| Median | | 126 | | 119 | |

Blood pressure of male rats; otherwise legend as for Table VIII.

By Student's $t$ test, the mean B.P. of the following were significantly different: $S_3$ salt > $S_3$ controls, $t$ 9.38, $p < 0.001$; $S_3$ salt > $R_3$ salt, $t$ 11.80, $p < 0.001$.

By the same test, mean B.P. of the two sexes differed as follows: ♂ $S_3$ salt > ♀ $S_3$ salt, $t$ 3.71, $p < 0.001$; ♂ $R_3$ salt > ♀ $R_3$ salt, $t$ 3.23, $p < 0.005$; ♂ $S_3$ controls > ♀ $S_3$ controls, $t$ 4.55, $p < 0.001$; ♂ $R_3$ controls > ♀ $R_3$ controls, $t$ 3.10, $p < 0.01$.

* and ‡ = B.P. after only 1 or 2 months, respectively, on diet; animal died before B.P. measurement at 3 months.

## DISCUSSION

The present studies indicate that it is possible to evolve two strains of rats differing markedly from one another in their predilection to develop hypertension from a high salt diet. These variations in sensitivity to salt appear to be genetically determined. For the present, we have been concerned primarily with demonstrating that the frequency, and possibly the severity, of experimental hypertension induced by excess salt consumption is due to genetically transmitted sensitivity to salt. It will be necessary to further purify these two strains in order to characterize the genetic components accurately.

It was of interest to observe that in the strain most prone to develop hypertension from salt, the disease did not develop when excess dietary salt was omitted. Thus, while this strain was genetically predisposed to hypertension, the additive environmental factor of dietary salt appeared to be necessary in order for hypertension to develop. The effect of salt on this sensitive strain was

in sharp contrast to that on the other, resistant strain in which salt consumption failed to influence the blood pressure significantly.

Different experimentalists studying the effect of salt consumption on the

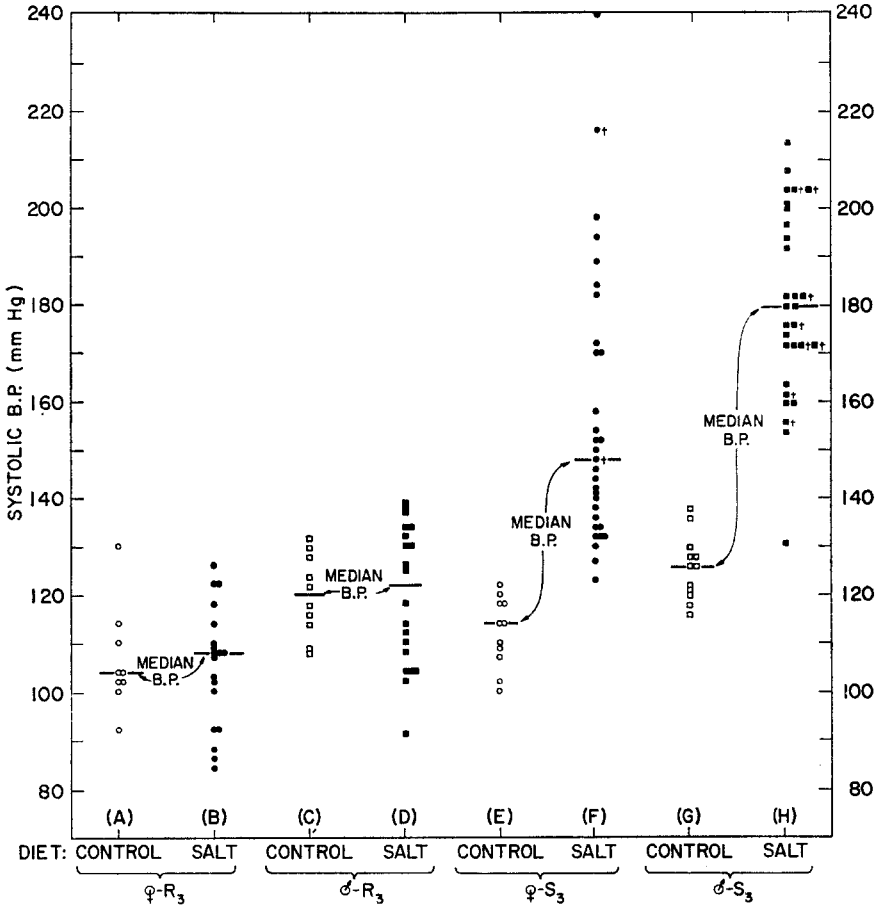


FIG. 2. Evidence of genetic variation in susceptibility to high salt diet. Effect of dietary salt, only, on blood pressure of rats inbred for resistance ($R_3$) and sensitivity ($S_3$) to triiodothyronine–salt regimen. Blood pressures of $R_3$ animals were recorded after 6 months whereas those on $S_3$ animals were observed after only 3 months on the same diets. † = Animal died before 3rd month; B. P. shown was recorded after only 1 or 2 months on regimen.

development of hypertension might arrive at diametrically opposite conclusions if chance selection of animals had led one to study a genetically sensitive, and the other a resistant, population.

It seems reasonable to expect that similar genetic factors operate in man. If salt plays the important etiological role that we believe it does in human hyper-

tension, and given the usual genetically heterogeneous population characteristic of man, it would be illogical to expect all individuals on similar salt intakes to have similar blood pressures. On the contrary, one would expect to find individuals on high average salt intakes who did not develop hypertension as well as individuals on lower average salt intakes who did develop hypertension. However, both the human and experimental data suggest that individuals on a lifelong low salt diet rarely develop essential hypertension. Because of the outbreeding ordinarily enforced by most societies, it is unlikely that more or less uniform strains of humans can be found with the sensitivity to salt evinced by our rats although studies of isolated populations might be very helpful. In the absence of such genetic homogeneity it is sufficient to re-emphasize that the available data relating salt intake to human hypertension indicate group rather than individual probability of developing hypertension.

## CONCLUSION

Using the genetic technique of selective inbreeding, it has been possible to quickly develop two statistically separable populations from one unselected strain of Sprague-Dawley rats. One of these is very sensitive, the other very resistant, to the development of experimental hypertension from a high salt diet. It was suggested that similar genetic factors operate in man.

The senior author wishes to thank Drs. Donald L. Shaver and Robert Steele of the Biology Department at Brookhaven, for reviewing the manuscript and making pertinent suggestions for its final form.

## REFERENCES

1. Bechgaard, P., Arterial Hypertension. A follow-up study of one thousand hypertonics, Copenhagen, NYT Nordisk Forlag, Arnold Busck, 1946, 102.
2. Platt, R., Heredity in hypertension, *Quart. J. Med.*, 1947, **16,** 111.
3. Søybe, P., Heredity in Essential Hypertension and Nephrosclerosis. A genetic-clinical study of 200 Propositi suffering from nephrosclerosis, Copenhagen, NYT Nordisk Forlag, Arnold Busck, 1948, 225.
4. Hamilton, M., Pickering, G. W., Robert, J. A. F., and Sowry, G. S. C., Aetiology of essential hypertension: arterial pressure in a general population, *Clin. Sci.*, 1954, **13,** 11.
5. McKusick, V. A., Genetic factors in cardiovascular diseases. I., *Mod. Concepts Cardiovascular Dis.*, 1959, **28,** 535.
6. Dahl, L. K., and Love, R. A., Relation of sodium choride intake to essential hypertension in humans, *Fed. Proc.*, 1954, **13,** 426.
7. Dahl, L. K., and Love, R. A., Evidence for relationship between sodium (chloride) intake and human essential hypertension, *Arch. Int. Med.*, 1954, **94,** 525.
8. Dahl, L. K., and Love, R. A., Etiological role of sodium chloride intake in essential hypertension in humans, *J. Am. Med. Assn.*, 1957, **164,** 397.
9. Dahl, L. K., Salt intake and salt need, *New England J. Med.*, 1958, **258,** 1152, 1205.

10. Dahl, L. K., Salt intake, adrenocortical function and hypertension, *Nature*, 1958, **181,** 989.
11. Dahl, L. K., Possible role of salt intake in the development of essential hypertension, *in* Essential Hypertension. An International Symposium. (K. D. Bock and P. T. Cottier, editors), Berlin, Springer-Verlag, 1960, 53.
12. Dahl, L. K., Smilay, M., Silver, L., and Spraragen, S. C., Prolonged biological half-life of Sodium-22 in patients with essential hypertension, *Nature*, 1961, **192,** 267.
13. Dahl, L. K., Effects of chronic excess salt feeding. Elevation of plasma cholesterol in rats and dogs, *J. Exp. Med.*, 1960, **112,** 635.
14. Dahl, L. K. and Heine, M., Effects of chronic excess salt feeding. Enhanced hypertensogenic effect of sea salt over sodium chloride, *J. Exp. Med.*, 1961, **113,** 1067.
15. Dahl, L. K., Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats, *J. Exp. Med.*, 1961, **114,** 231.
16. Selye, H., Stone, H., Nielsen, K., and Leblond, C. P., Studies concerning the effects of various hormones upon renal structures, *Canad. Med. Assn. J.*, 1945, **52,** 571.
17. Green, D. M., Saunders, F. J., Wahlgren, N., and Craig, R. L., Self-sustaining, post-DCA hypertensive cardiovascular disease, *Am. J. Physiol.*, 1952, **170,** 94.
18. Selye, H., and Bois, P., Thyroxin as sensitizing agent in production of renal and cardiovascular lesions with corticoids, *Proc. Soc. Exp. Biol. and Med.*, 1956, **92,** 164.
19. Salgado, E., and Green, D. M., Mechanisms of desoxycorticosterone action. XII: Influence of the thyroid, *Am. J. Physiol.*, 1957, **188,** 519.
20. Masson, G. M. C., Corcoran, A. C., and Page, I. H., Effects of renin and thyroxin in rats treated with corticosteroids and in rats with regenerating adrenals, *Endocrinology*, 1957, **61,** 409.
21. Selye, H., Production d'une hypertension et d'une néphrosclérose maligne par la thyroxine chez le rat, Rev. Canad. Biol., 1950, **9,** 475.
22. Meneely, G. R., Tucker, R. G., Darby, W. J., and Auerbach, S. H., Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure, *J. Exp. Med.*, 1953, **98,** 71.