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EFFECTS OF CHRONIC EXCESS SALT INGESTION
Experimental Hypertension in the Rat
Correlation with Human Hypertension

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By

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EFFECTS OF CHRONIC EXCESS SALT INGESTION
Experimental Hypertension in the Rat
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Although the role of salt consumption in human hypertension has been a controversial subject for some years, the writer has accumulated evidence which suggests that there is a correlation between the level of salt intake and the prevalence of elevated blood pressure in a community: among societies in which the average salt intake was found to be high, there was a significantly greater prevalence of hypertension in the adult population than among societies in which average salt consumption was low. For this reason, as well as others based on both clinical and experimental data, it has been proposed that chronic excess salt consumption plays an etiologic role in this disease (1-3).

However, in both our human and animal studies, it was observed repeatedly that certain individuals chronically consumed large amounts of salt without developing hypertension whereas others appeared to be singularly sensitive to the effects of NaCl addition or restriction. I will confine my presentation here to observations, made over the past 10 years with rats, which are relevant to this curious variability in response to salt.

For details of the techniques used and the results, some of the earlier papers in this series may be consulted (4-6).

For the first ten years of our studies, unselected 21-day old Sprague-Dawley female rats were obtained from one of several suppliers, and immediately placed on chow containing either about 8% NaCl ("high salt diet") or 0.35% NaCl ("low salt diet"). Systolic blood pressures were measured under highly standardized conditions at one to three month intervals thereafter until a) death of the animal, b) chronic ill-health (usually bronchiectasis, with repeated bouts of pneumonia) or c) the experiment was terminated, ordinarily 12 - 15 months later. Under these conditions it was found that after 6 - 9 months about three-quarters of the animals on high salt intakes would have chronic systolic elevations of 140 mm Hg or more, a level which, since it exceeded by approximately 3 standard deviations the mean systolic pressure of animals on the low salt diet, has been defined as "hypertension". However, approximately one quarter of the salt-fed animals failed to manifest elevated pressures. By contrast, among those that developed some degree of hypertension, there would have been a few that became severely hypertensive

after only 1 - 2 months on salt with death following rapidly thereafter. The spectrum of responses is shown schematically in Fig. 1.

We have analysed in detail the blood pressure responses of 223 consecutive, unselected female rats to either the high or low salt diets, in Figs. 2 - 4. This is, in effect, a retrospective survey of the blood pressure of an unselected population of females subjected to a degree of environmental control impossible in man. In Fig. 2 is shown the frequency distribution of systolic blood pressures among the ¹⁵⁷130 unselected female rats on high salt intakes. The distribution of pressures is compatible with the thesis that blood pressure rises with age and that a so-called "unimodal" curve might best fit the data. The data in Fig. 3, however, suggest that blood pressure need not rise with age, certainly not to a significant degree. Pressures were not significantly different from the 3rd - 4th months through the 13th - 14th months; pressures in excess of 140 mm Hg were uncommon and none in excess of 150 mm was recorded. These 64 animals, in contrast to those in Fig. 2, were maintained on a low salt diet. In Fig. 4, the data from the two preceding graphs have been combined to make direct comparison easier. The changing

distribution of pressures - to higher levels - is clearly made up of only part of this population, namely the salt-fed members. While it would be possible to fit a "unimodal" curve to these combined data, it is evident that this could be misleading if such a curve were interpreted to reflect the response of a single population: while there were some high-salt animals among those with pressures at the lower levels, there were never low-salt animals with pressures among those at the high levels.

From these initial studies, we drew the following tentative conclusions, as applied to rats:

- 1) Among animals maintained on a low salt diet blood pressure levels did not rise significantly with age, per se.
- 2) A high salt intake by a group was reflected in a gradual shift of the median blood pressure to higher levels, with a "unimodal" distribution.
- 3) This effect of a high salt intake on the group response was not necessarily reflected in the response of individuals in that group since some members remained normotensive throughout their lives.
- 4) The variability in individual response among members of the group on

high salt was probably due to variations in genetic constitution.

5) If genetic differences accounted for the variable response to similar salt intakes, by the technique of selective inbreeding two strains should be evolved of which one would develop hypertension from the same salt intake that would be relatively ineffective in raising the blood pressure of the other strain.

The next phase of our work (7 - 8) was concerned with initiating studies to explore the thesis above, namely the role of genetic endowment in the development of hypertension from salt. To this end, two groups of animals were inbred selectively, the general technique for which is outlined schematically in Fig. 5. Briefly, in successive generations we selected for inbreeding on the one hand those animals that developed hypertension most readily from a high salt intake (called the "Sensitive" or "S" strain) and on the other hand those animals that were most insensitive to this effect of salt (the "Resistant" or "R" strain).

After 3 generations of such selective inbreeding, the response of the two strains (by our nomenclature R_3 and S_3 , respectively) to salt was significantly

different, as shown in Fig. 6. All salt-fed animals consumed the same diet from the time of weaning at 21 - 23 days, and in similar amounts; yet, among the Resistant animals no significant elevation of pressures was observed whereas the overwhelming majority of the salt-fed Sensitive rats were hypertensive and many of them severely so. By contrast, the S_3 animals maintained on the control (low salt) diet did not develop hypertension although in subsequent, and presumably more sensitive strains, we have observed a few animals from the "S" strain with hypertension despite having been only on a low salt diet. This initial study suggested that while the S_3 strain had a strong genetic predilection to develop hypertension, a supplementary factor in the environment, in this case a high salt diet, was usually necessary to unmask the trait. Among the R_3 animals, excess dietary salt seemed to have relatively little influence on the blood pressure response. 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 than that of the females ($p < 0.01$). The differences between salt-fed animals in a series recently studied from the 6th and 7th generations of the R and S strains is shown in Fig.

7. The difference in response of the two strains has increased with successive selective inbreeding and is quite marked. Most animals on salt in the S strains now rapidly develop severe hypertension; indeed, we have seen animals in the latest generations that died of fulminating hypertension 3 weeks after beginning a high salt diet and in one study just completed 38 of 41 Sensitive animals were dead with severe hypertension before the end of the 4th month on salt.

We have analysed the frequency distribution of systolic blood pressures among 377 animals of both sexes from these selectively inbred strains (Figs. 8 - 9). Beyond the age of 4 months there were insufficient numbers of blood pressures recorded on healthy survivors among the salt-fed S animals to allow comparison either with the S animals on low salt or with the R strain on high or low salt. Therefore, in contrast with the summaries in Figs. 2 - 4, data on S animals include only ages 2 - 4 months. Furthermore, while the health of R animals was no problem, insufficient numbers were available for comparison at 2 - 4 months of age because we have been making fewer observations on animals from the R strain. In order to avoid an undue dispropor-

tion between the sizes of samples in the S and R populations, 53 of the 164 R animals included in this summary were older than any of the S animals, i.e. they were between 4 and 12 months of age, of which 20 were on low salt and 33 on high salt diets. The inclusion of these blood pressures from the older R animals would tend to underestimate any differences from the 2 - 4 month old S animals if there were a trend toward higher pressures due either to age or salt. Data on both sexes have been combined: this did not change either the results or implications, but made the sample sizes larger.

The four panels in Fig. 8 summarize the frequency distribution of pressures among the Sensitive and Resistant animals on either low or high salt diets. Among the Resistant animals, a high salt intake caused a slight shift toward higher blood pressures, but on the whole the effect was small: 98% of these animals on low salt diets had systolic pressures below 140 mm Hg with none above 160 mm Hg while 84% of those on high salt were below 140 and 1% above 160 mm Hg. On the Sensitive strain, by contrast, the effect of salt was spectacular: whereas about 70% of the animals on low salt had systolic pressures below 140 mm with only 4% above 160 mm Hg, among the animals on high salt only 3% had pressures below 140 mm Hg while 94% were above 160 mm Hg.

Stated another way, in the Sensitive strain, a high salt intake reduced the frequency of pressures below 140 mm Hg to less than 1/20th of what it would have been on low salt and increased the frequency of pressures above 160 mm Hg by more than 20-fold. To each of these four frequency distributions, it would be possible to fit a "unimodal" curve. Yet the disparity in distribution between the two extremes, for instance, (i.e. Resistant on low salt versus Sensitive on high salt) again suggests caution in interpreting unimodal fits to the distribution of blood pressures among general populations.

Fig. 9 is a composite of the data in Fig. 8. Here, it might be possible to fit a "bimodal" curve to the distribution of pressures, with one peak at 120 - 140, and the other at 180 - 200 mm Hg. However, from what is evidently the diverse composition of the populations under the curve with a peak at 120 - 140 mm Hg, caution in making this interpretation also is suggested. For, relative only to the two variables under discussion here, namely genetic endowment and salt intake, it is clear that in a given population depending upon a) relative numbers in each sub-group within the general population under study, b) difference in average salt intakes, and c) genetic sensitivity

to salt, frequency distribution patterns might emerge that could be fitted by one, two, or even more, curves.

Although the pertinence of these animal studies to the human experience must always remain to some degree speculative the ensuing discussion is based on the assumption, so useful in medical research even if occasionally misleading, that controlled experiments in animals are relevant to man.

Among our unselected rats on low salt diets aging per se did not appear to be associated with a significant progression of systolic blood pressure toward higher levels. These animals appeared to be normal in all other respects including growth and development, appearance and behavior, fertility, litter size and birth weight of offspring as well as plasma electrolytes, renal function, and anatomical-histological studies. For these reasons, we have assumed the animals were normal and that failure to show a rise in pressure was not a manifestation of ill health associated with the low salt regimen. The addition of a high salt intake to the regimen of siblings of these animals led to a gradual increase in average blood pressure for the group: if such a salt-fed group were viewed without knowledge of, or reference to, salt intake,

blood pressure would appear to rise with age.

Aging also appeared to have little influence on the blood pressure of our selectively inbred Resistant animals although when they were on a high salt intake there was a statistically insignificant shift to slightly higher average levels and an occasional significant individual increase. In the Sensitive animals, the data are more equivocal: among the 103 Sensitive animals on the low salt regimen, about 30% had systolic pressures in excess of 140 mm Hg when they were between 2 - 4 months of age including 4 animals with pressures of 160 mm Hg or more (160, 168, 168, and 180, respectively). These last 4 pressures at this early age among salt-fed Sensitive animals ordinarily would carry an ominous prognosis, namely rapidly progressing hypertension and death usually within 2 months. We have had the opportunity to follow for long periods only a few of the Sensitive animals that have developed hypertension on low salt but the picture emerging may be somewhat different from similar animals on high salt in that the pressures do not appear to be rising progressively and the animals tolerate the elevation remarkably well. There are several possible explanations for these pressure elevations in Sensitive animals on low salt other than invoking the somewhat

enigmatic process of "aging": 1) Salt is unnecessary to precipitate hypertension in some of these Sensitive animals; 2) animals in this strain have become so responsive to dietary salt that the amount contained in our so-called low-salt diet (0.35% NaCl) is sufficient to induce the hypertensive process (we are currently exploring the latter possibility with a special diet containing only 0.07% NaCl); or 3) other, unknown environmental factors are operating to produce hypertension in some, but not most, animals.

When salt is added to the dietary regimen of these Sensitive animals a highly significant and progressive increase in blood pressure occurs so that in such rats, blood pressure again would appear to rise with age, although the rate at which it increased could be markedly speeded by increasing, or slowed by decreasing, the average daily salt allowance.

We interpret these data on rats as suggesting that age per se has little, or at most modest, effect on blood pressure: the influence of age is apparent rather than real and is actually a result of the longer time over which noxious environmental factors exert their influence on blood pressure in individuals with varying degrees of genetic susceptibility. This interpretation

is compatible with the working hypothesis proposed for man by Pickering (9), namely that the rise of blood pressure with age is a manifestation of the individual's response to environmental factors.

It was mentioned earlier that analysis of the influence on blood pressure of two factors has been initiated in the present studies. Specifically, evaluation of the contributions which genetic inheritance and salt intake can make in raising blood pressure has been attempted. From the limited information presently available it is difficult to assess precisely the relative roles of these two but it seems likely that the genetic predilection is essential and that salt functions to initiate the hypertensive process. Salt could function in such a system much as various carcinogens do in initiating cancer experimentally in certain strains of predisposed mice and not in others (10).

From all of the foregoing, we suggest caution in using frequency distribution patterns of blood pressure to elucidate the nature of essential hypertension in man. We suspect that bias of the investigator is much less important than bias of the population sample in determining the pattern of distribution of pressures. Relative to our rat studies for instance, by proper

selection of strain and appropriate modifications of salt intake, virtually any pattern of distribution desired might be obtained.

If these studies with rats are relevant to man, particularly as to the capacity of salt to unmask the hypertensive trait, they suggest that similar average salt intakes in genetically different individuals need not result in similar elevations of blood pressure. Therefore, given the usual genetic heterogeneity of man, a wide spectrum of blood pressure responses might result in a group, even one in which the individual members had the (unlikely) possibility of equal salt intakes. At the same time, however, in two relatively heterogeneous groups on very different average salt intakes, it would be expected that the group eating more salt would also evince significantly more hypertension.

In conclusion, let me say that the evidence relating salt intake to human hypertension is, and probably always will remain, circumstantial. The evidence relating cigarette smoking to bronchogenic carcinoma in man is circumstantial and when the same sequence is ultimately duplicated in animals, the circumstantiality that is involved in the epidemiology of the human disease will not

have been obviated. For this will remain an animal experiment and, like the present situation with salt and experimental hypertension, its relevance to the human counterpart will carry more or less weight, depending upon one's prejudices.

REFERENCES

1.

1. Dahl, L.K., and Love, R.A., Evidence for relationship between sodium (chloride) intake and human essential hypertension, A.M.A. Arch. Int. Med., 1954, 94, 525.
2. Dahl, L.K., Salt intake and salt need, New England J. Med., 1958, 258, 1152, 1205.
3. Dahl, L.K. Possible role of salt intake in the development of essential hypertension. In: Essential Hypertension. An International Symposium, p. 53. Edited by K. D. Bock and P. T. Cottier. Berlin 1960. Springer-Verlag.
4. Dahl, L.K., Effects of chronic excess salt feeding. Elevation of plasma cholesterol in rats and dogs, J. Exp. Med. 1960, 112, 638.
5. 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.
6. Dahl, L.K., Effects of chronic excess salt feeding: induction of self-sustaining hypertension in rats, J. Exp. Med., 1961, 114, 231.
7. Dahl, L.K., Heine, M., and Tassinari, L., Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion, Nature, 1962, 194, 480.
8. Dahl, L.K., Heine, M., and Tassinari, L., Effects of chronic excess salt ingestion: Evidence that genetic factors play an important role in susceptibility to experimental hypertension, J. Exp. Med., 1962, 115, 1173.
9. Pickering, G., The Nature of Essential Hypertension. London, J. and A. Churchill, Ltd., 1961, p. 60.

References (Continued)

2.

10. Wooley, G. W., Genetics of Neoplasia. Chapter 11, in The Physiology of Cancer. F. Homburger and W. H. Fishman, Eds. Paul B. Hoeber, Inc., New York City, 1953, p. 171.

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LEGEND

FIGURE 1:

Schematic representation of systolic blood pressure responses of rats to high salt intakes. Note that some never respond (A) while others (F) are dead within a few months with fulminating hypertension. (from, Dahl, L.K., Excessive salt intake and hypertension: a dietary and genetic interplay. Brookhaven Lecture Series No. 12, BNL 733 (T-263), 13 Dec., 1961. Reprinted by permission of the Editors).

FIGURE 1

LEGEND

FIGURE 2:

All rats were from the Sprague-Dawley strain and had been started on high salt diets at 21 days of age. Blood pressures were not measured in all rats every 2 months. The two columns on far right indicate frequency distribution above and below a systolic pressure of 140 mm Hg.

FIGURE 2

LEGEND

FIGURE 3:

Animals were siblings of those in Figure 2, but maintained
on low salt diet throughout their lives.

FIGURE 3

LEGEND

FIGURE 4:

Combination of data from Figures 2 and 3. See appropriate legends.

FIGURE 4

LEGEND

FIGURE 5:

Schematic representation of selective inbreeding from successive generations of rats. Animals most resistant to salt were inbred, as were those most sensitive to salt. 2 strains resulted, differing widely in their tendency to develop hypertension from excess salt feeding. (from, Dahl, Lewis K. and Schackow, Eckart, Effects of chronic excess salt ingestion: experimental hypertension in the rat. Canadian Medical Association Journal, 90: 155, 1964. Reprinted by permission of the Editors).

LEGEND

FIGURE 6:

Blood pressures of R_3 (i.e., 3rd generation of rats selectively inbred for their Resistance to developing hypertension from high salt intake) animals were recorded after 6 months, whereas those of S_3 (i.e., 3rd generation of rats selectively inbred for their Sensitivity to salt as manifested by their proneness to develop hypertension from a high salt intake) animals were observed after only 3 months on the same high salt diet. Therefore, differences in response of the two strains will be under rather than over-estimated.

† = animal died before 3rd month; B.P. shown was recorded after only 1 or 2 months on regimen.

The difference between the average blood pressures of the groups of S_3 animals on and off salt, respectively, was significant statistically ($p < 0.01$). No such difference was present among the groups of R_3 animals. On any similar regimen, the males had higher average pressures than the females ($p < 0.01$).

(From L. K. Dahl, M. Heine, and L. Tassinari, J. Exp. Med., 115: 1173 (1962); Reprinted with permission of the Editors).

EVIDENCE OF GENETIC VARIATION IN SUSCEPTIBILITY TO HIGH SALT DIET

FIGURE 6

LEGEND

FIGURE 7:

All animals were weanlings when salt-feeding began. The animals were from the 6th and 7th generations of the R and S strains, respectively. The difference in the average blood pressure response of the two groups was significantly different ($p < 0.01$). (From, Dahl, Lewis K. and Schackow, Eckart, Effects of chronic excess salt ingestion: experimental hypertension in the rat. Canadian Medical Association Journal, 90: 155, 1964. Reprinted by permission of the Editors.)

EFFECT OF GENETIC FACTORS ON DEVELOPMENT OF EXPERIMENTAL HYPERTENSION IN RATS FROM A HIGH SALT DIET FOR 2 MONTHS

FIGURE 7

LEGEND

FIGURE 8:

Animals were from the selectively inbred strains 5th - 7th generation. The "Resistant" strain was inbred to manifest little or no increase in blood pressure to the same high salt diet from which the "Sensitive" strain readily developed hypertension. Two columns on far right as in Legend, Fig. 2. The inclusion of R animals between 4 - 12 months of age would tend to underestimate differences from the 2 - 4 month old S animals if age or salt tended to raise the blood pressure.

See text.

FIGURE 8

LEGEND

FIGURE 9:

Composite of data from Figure 8. The ratio of males to females in the respective groups was as follows: R-, 1.21; R+, 1.75; S-, 1.49; S+, 1.39; R- and R+, 1.45; S- and S+, 1.42; all animals, 1.43.

FIGURE 9