SESSION 1

Chairman: Professor R. F. Farquharson

Effects of Chronic Excess Salt Ingestion: Experimental Hypertension in the Rat

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AT THE International Symposium on Essential Hypertension held in Berne in 1960, the role of dietary salt (sodium chloride) as an etiological factor in human hypertension was discussed.[1] It would be redundant to repeat the evidence for this again. For those unfamiliar with the general thesis, let it suffice to say that, experimentally, chronic excess salt ingestion can induce hypertension in several species of animals and that, among five groups of peoples studied in various parts of the world, the prevalence of hypertension rose in a parallel fashion with the average salt consumption.[2]

We were specifically requested by our host, Dr. Genest, to report original and even preliminary work at this Symposium. Hence, we will limit ourselves to a general discussion of some of our reported[3-8] and ongoing experiments with hypertension induced in rats by salt-feeding. The superb review by Tobian[9] as well as a more recent one[10] might be helpful to those who are moved to explore the subject further.

General Observations

During the last 15 years, we have observed more than 4000 rats to which salt (sodium chloride) has been administered chronically, either in the drinking fluid or in the food. We have used only the second method in the last 10 years.

The appearance, behaviour and development of animals chronically fed excess salt ordinarily does not differ from that of control animals. The animals may not grow quite as fast as normally during the first few months, but thereafter the differences are not significant. The onset of hypertension is not heralded by other discernible clinical effects, unless a malignant or "accelerated" phase develops, at which time food intake decreases and weight loss appears. These animals begin to look frankly sick, with decreased motor activity, shivering, poor care of the coat, and ruffled fur.

The pathological changes associated with chronic salt-feeding in rats have been well described by Koletsky,[11] and our own observations are in accord with his. We are now in the process of reviewing the pathological changes developing in animals in which the hypertensive process develops swiftly, leading often to death within a few weeks. Preliminary observations suggest that early vascular changes will be found in these animals with fulminating disease, but this would not invalidate the results of the chronic experiments reported by Koletsky[11] and Meneely *et al.*[12]

No characteristic histological lesion has been observed in the cardiovascular-renal system early in the course of mild to moderate hypertension. With increasing chronicity or severity of the hypertension, changes become manifest primarily in the heart, the kidneys and the blood vessels. The kidneys and heart become enlarged and the wall of the left ventricle becomes thickened in particular. Focal fibrotic areas may be seen in the myocardium which, in the human being, might have been ascribed to old healed infarcts. The most commonly observed renal change is dilatation of the tubules. Although atherosclerosis has not been seen (even in coronary, renal, and cerebral vessels), periarteritis nodosa is commonly observed in large and small arteries alike. While this is seen occasionally in control animals as well, arteritis is far more frequent and widespread in salt-fed hypertensive animals. Death ordinarily occurs from bronchiectatic pneumonia and, much less commonly, from uremia. The pneumonia is a well-known, well-described entity in rats,[13] since it is the scourge of most long-term studies with these animals. It is the same disease in animals with and without hypertension, but the hypertensive animals seem to resist its ravages much less well.

Cardiac failure has not been seen; the nephrotic syndrome described by Meneely *et al.*[12] has been seen only rarely; only two animals died of what looked like a cerebrovascular accident—in only one of which was there microscopic evidence of cerebral hemorrhage, without identification of the primary lesion.

Blood Pressure Response to Salt

Some common blood pressure responses to chronic salt-feeding in rats are summarized in Fig. 1. It should be noted that the response ranges from none (line A) to fulminating hypertension and rapid death (line F). The hypertension may develop at different intervals after the onset of the salt-feeding. Although the elevation in pressure is usually progressive, it may plateau at a modest level. Once hypertension has appeared, it rarely, if ever, disappears if salt-feeding is continued.

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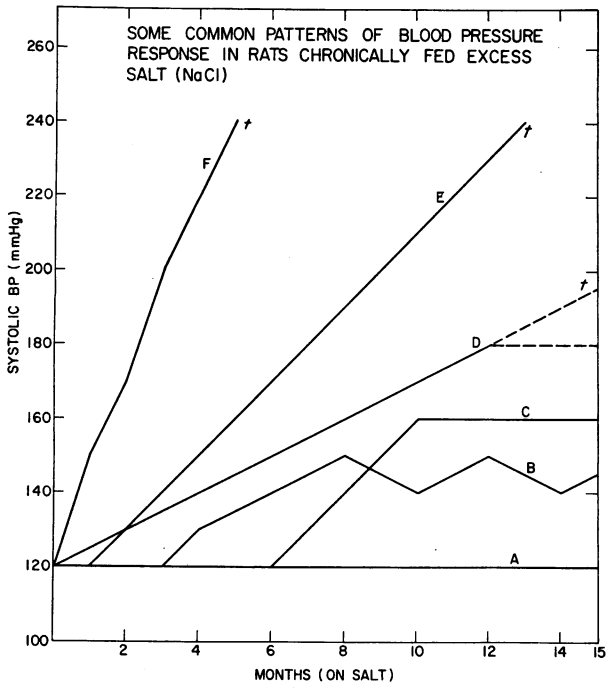

Fig. 1.—Schematic representation of systolic blood pressure responses of rats to high salt intakes. Note that some never respond (A), while others (F) are dead within a few months with fulminating hypertension. (From Dahl, L. K., Excessive salt intake and hypertension: a dietary and genetic interplay. Brookhaven Lecture Series No. 12, BNL 733 (T-263), 13 December, 1961. Reprinted by permission of the Editors.)

There are several biological factors that modify this response to salt, including: (a) the age at which salt-feeding is begun, (b) the sex of the animal, (c) the average daily amount of salt consumed, (d) the duration of salt-feeding, and (e) variations in genetic sensitivity to salt.

(a) *Age.*—In general, the earlier salt-feeding is started in the life of an animal, the more frequent and severe the hypertension. Our female rats with litters are normally maintained on a diet low in sodium. Therefore the offspring receive no added salt in the diet until the time of weaning, which is ordinarily at 21-23 days of life. Prior to weaning, some, possibly all, of the young rats nibble at the diet that is available *ad lib.* to their mother, but the quantity of food and the age at which such consumption begins have not been measured as yet. However, some experiments are being carried out in which the nursing mother is maintained on a high salt intake, thereby making a high salt diet *available* to her litter: thus far the evidence is inconclusive as to whether these young rats subsequently develop hypertension more frequently or of greater severity than do the offspring of mothers maintained on the usual low sodium diet.

Another experiment is now in progress to assess the consequences of delaying salt-feeding at various periods up to three months after weaning; it is clear already that much less hypertension develops when salt-feeding is delayed. This accounts in part for the failure of some investigators to observe experimental hypertension from salt-feeding.

(b) *Sex.*—Males develop hypertension more frequently than females and the disease generally runs a somewhat more rapid course in males. In contrast with Thomas' studies in human beings,[14] our preliminary studies in rats indicate that when only one mate has hypertension, the hypertensive male will have more hypertensive offspring than will the hypertensive female. In conformity with Thomas' studies. we have suggestive evidence that, in such a mating, offspring of the same sex as the hypertensive parent will develop hypertension more frequently than will the offspring of the opposite sex. Our calculations suggest that we are dealing with a partially sex-linked trait and that multiple genes are involved.

(c) *Amount of salt consumed.*—As one might expect, when the average daily salt consumption is increased, the incidence and severity of elevated blood pressure increase. This was first documented clearly by Meneely *et al.*[12] and needs no further amplification at this time.

(d) *Duration of salt-feeding.*—In general, the longer a rat is fed salt, the more likely it is that the animal will develop hypertension. However, animals that have remained normotensive for approximately six months on the salt regimen, rarely develop hypertension thereafter. We are now studying the effect of permanently discontinuing the high salt intake after only two and six weeks, respectively, on the regimen. This study is still going on but the results are shaping up clearly: many animals will develop permanent hypertension after six weeks on salt, and some after only two. Thus, while long-term ingestion of salt will significantly enhance the frequency of hypertension in a *group,* even short-term ingestion can induce the disease in *individuals.*

(e) *Variations in genetic sensitivity to salt.*—Discussion of this important issue will be delayed until later in this paper.

SELF-SUSTAINING HYPERTENSION

Our experience with rats indicates that the hypertension developing after salt-feeding is usually permanent.[5] This is important from the standpoint of whether or not this form of experimental hypertension resembles human hypertension. For, if the hypertension vanished after the added dietary salt was discontinued, our laboratory model would differ significantly from human essential hypertension. In the latter disease, it has been the general clinical experience that, among patients placed on salt restriction one to four months, about two-thirds fail to respond with a significant fall in blood pressure. We recently studied the response to salt restriction of 35 female rats that had become hypertensive during a year of continuous excess salt feeding.[5] This salt-induced experimental hypertension resembled the human variety in its response to sodium restriction: after four months of drastically reduced sodium intake, two-thirds (23 of the

35 animals) had shown no significant blood pressure response. Therefore, in the rat at least, salt-feeding can induce a self-sustaining hypertension refractory to subsequent salt restriction. This study suggests that the lack of response to salt restriction does not rule out an etiological relationship between salt intake and the development of hypertension.

GENETIC STUDIES

There is no need to document the evidence which indicates that human essential hypertension is a familial disease. It is pertinent to point out, however, that a *familial* disease is not necessarily a *hereditary* one: as we have noted before—"the observation that hypertension is more common among blood relatives of hypertensives than among blood relatives of non-hypertensives does not establish that this difference is due to hereditary factors. It *may* be, but it also could be due to common factors in the environment or to an interaction of shared hereditary and environmental patterns."[10] It will be recalled from Fig. 1 that, among rats all of which were exposed to the same environment including a high salt diet, some never developed hypertension whereas a few became severely hypertensive within a month or two after starting the diet. This suggested that these variations in sensitivity to salt were controlled by genetic factors. It was postulated that, if this were true, by using the technique of selective inbreeding, two strains of rats could be evolved differing significantly in their response to salt. About three years ago we undertook to test this postulate, as a result of which two strains have, indeed, been established that show strikingly different responses to salt.

For precise details, the original papers may be consulted[6, 7] but in Fig. 2 a schematic summary of the technique used for selective inbreeding is shown. An unselected (U_1) group of Sprague-Dawley rats, received as weanlings from our supplier, was used initially. Among the animals in this group, those that responded to the regimen with the highest (the *Sensitive* or S strain) and lowest (*Resistant* or R strain) pressures, respectively, were selected for further breeding, and the remaining animals were discarded. The *sensitive* animals so selected were mated and their offspring were designated as the S_1 generation; the offspring from the first mating of *resistant* animals were designated as the R_1 generation. From these S_1 and R_1 animals, and similarly in succeeding generations, only those that responded to the regimen with the highest and lowest pressures, respectively, were selected for breeding the two strains.

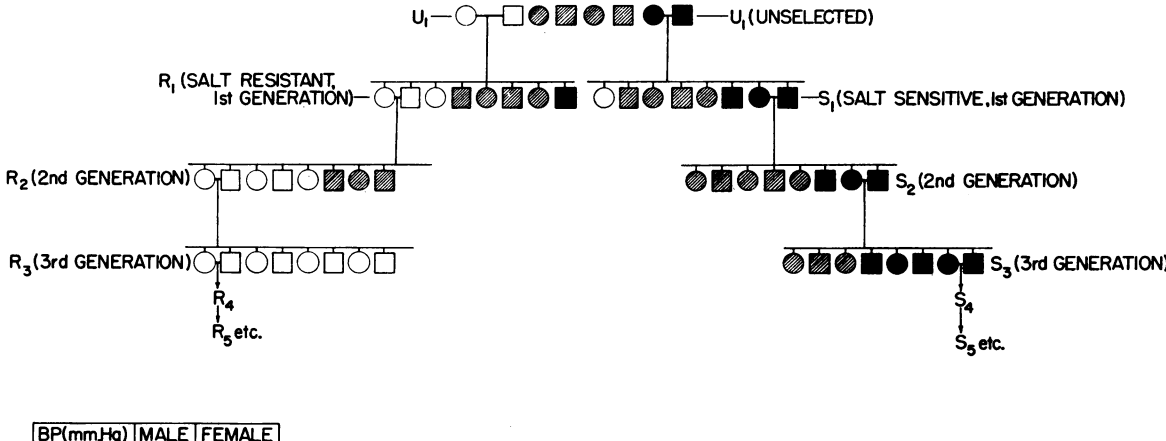

Fig. 2.—Schematic representation of selective inbreeding from successive generations of rats. Animals most *resistant* to salt were inbred, as were those most *sensitive* to salt. Two strains resulted, differing widely in their tendency to develop hypertension from excess salt feeding.

After three generations of such selective inbreeding, the response of the two strains to salt was significantly different, as shown in Fig. 3. All salt-fed animals consumed the same diet and in similar amounts; yet, among such R_3 animals, no significant elevation of pressures was observed whereas the overwhelming majority of the S_3 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. This suggested that, while the S_3 strain had a strong genetic predilection to develop hypertension, the addition of a high salt diet was necessary to unmask the trait. Among the R_3 animals, excess dietary salt seemed to have 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 small series recently studied from

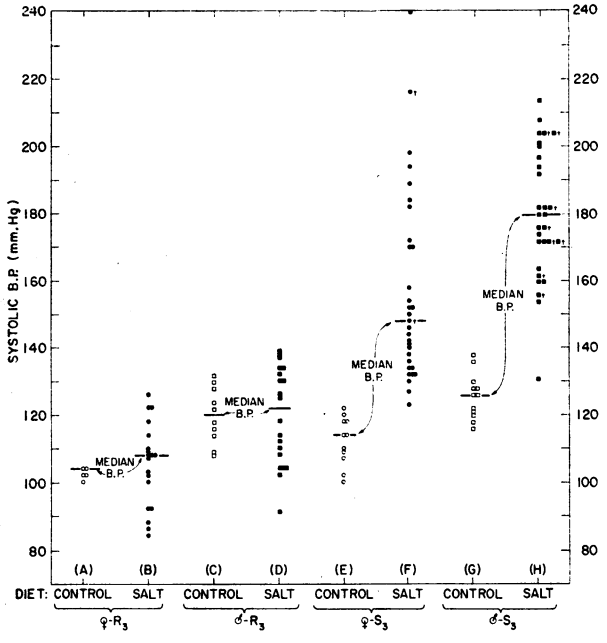

Fig. 3.—Evidence of genetic variation in susceptibility to high salt diet. Blood pressures of R_8 (i.e. third generation of rats selectively inbred for their *resistance* to developing hypertension from high salt intake) animals were recorded after six months, whereas those of S_3 (i.e. third generation 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 three 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 third month; blood pressure shown was recorded after only one or two 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 Dahl, L. K., Heine, M. and Tassinari, L.[7]: reprinted with permission of the Editors.)

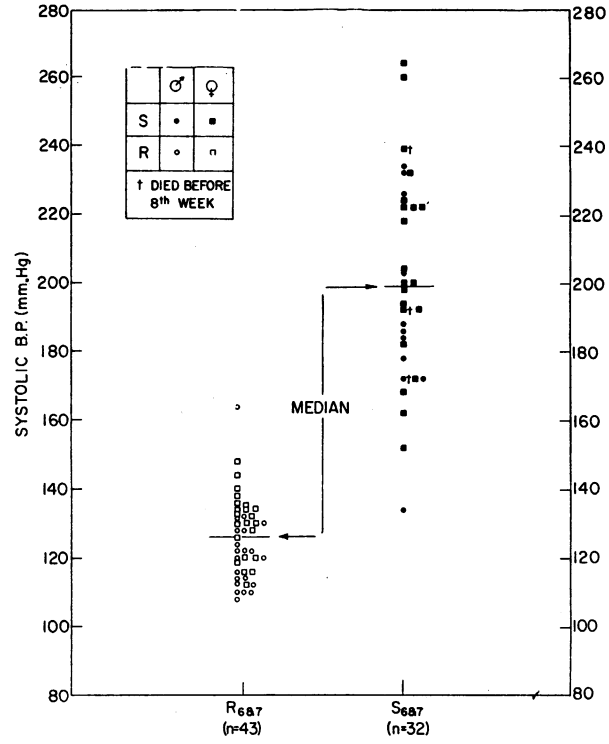

Fig. 4.—Effect of genetic factors on development of experimental hypertension in rats from a high salt diet for two months. All animals were weanlings when salt-feeding began. The animals were from the sixth and seventh generations of the R and S strains, respectively. The difference in the average blood pressure response of the two groups was significant ($p < 0.01$).

the latest (sixth and seventh) generations of the R and S strains are shown in Fig. 4. The difference in response of the two strains is quite impressive. Most animals in the S strains now rapidly develop severe hypertension; indeed, we have seen animals in these latest generations that died of fulminating hypertension three weeks after beginning a high salt diet; between one-quarter and one-half are dead within three months.

We have suggested that similar genetic factors may operate in man,[6,7] and we have brought forward evidence which suggests that excess salt consumption plays an etiological role in human essential hypertension.[1] Yet it was observed repeatedly that some individuals remained normotensive despite the fact that they were chronically consuming large amounts of salt. If our experiments with rats are pertinent to the human experience—and we believe they are—one would expect to find a few *individuals* on high average salt intakes who did *not* develop hypertension as well as a few *individuals* on relatively moderate salt intakes who *did* develop hypertension. However, because of the genetic heterogeneity characteristic of man, it would be expected that the probability of hypertension developing in a *group* would increase as

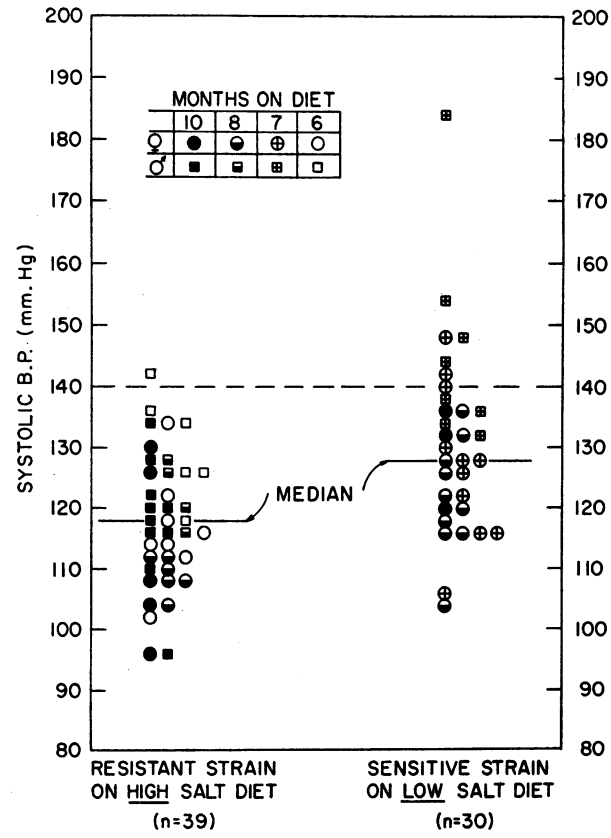

Fig. 5.—Systolic blood pressure of salt-resistant strain after six to 10 months on *high salt* diet and of salt-sensitive strain after seven to 10 months on *low salt* diet. All animals were 21-day-old weanlings at the beginning of the experiment.

the average daily salt consumption of individuals in the *group* increased. This seems to be true.[1]

It is pertinent to ask whether the resistant strain of rats will eventually develop hypertension *with* continued salt-feeding, and whether the *sensitive* strain will eventually develop hypertension *without* salt-feeding. During the past year we have had these problems under study and, while the results are still incomplete, preliminary answers to both of the above questions may be stated: hypertension will occasionally develop (Fig. 5) under both conditions. The observations on which Fig. 5 is based were made on animals in the fifth and sixth generations six to 10 months after weaning. While no significant hypertension is present among the *resistant* animals, it would not be surprising if it appeared occasionally after salt-feeding, since genetic homogeneity can hardly have been achieved as yet. The occasional appearance of hypertension in the *sensitive* strain, even on a low salt diet, may have important implications for man. The hypertension observed in these sensitive animals has been relatively mild, slowly evolving, and no deaths have occurred. This is in sharp contrast to their salt-fed siblings, most of which were dead before they had been six months on the high salt regimen. Nonetheless, the evidence summarized in Fig. 5 indicates that, given sufficient time, a high salt intake may not be necessary to unmask the hypertension in a rat with the appropriate genetic background. We suspect that animals which develop mild to moderate hypertension on a low salt diet would run an inordinately rapid course to death had they consumed a high salt diet, i.e. that they would present the malignant or "accelerated" phase which we are now seeing with such increasing frequency in our animals.

Prior to working with the resistant and sensitive strains of rats, we had observed that when stock animals were fed diets high in sodium about one-fourth failed to develop hypertension (Fig. 1). Rather similar results had been obtained by others using unilateral renal artery compression to induce experimental hypertension in the rat.[15, 16] In the light of our experience it was suspected that variations in genetic susceptibility were influencing the development of experimental renal hypertension as well. Study of this was made with the resistant and sensitive strains of rats,[8] using the technique of Wilson and Byrom.[15] This technique consists of applying an annealed silver ribbon clip to one renal artery, thereby partially occluding blood flow to one kidney, leaving the other kidney untouched. The operation was carried out when the animals weighed approximately 150-200 g.; all animals were maintained throughout on the same low sodium diet (0.15% by analysis) that had been used from the time of weaning. Measurements of blood pressure were made every two weeks for 12 weeks, at which time it was apparent that the pressure had become stabilized. The results, summarized graphically in Fig. 6, show the striking differences

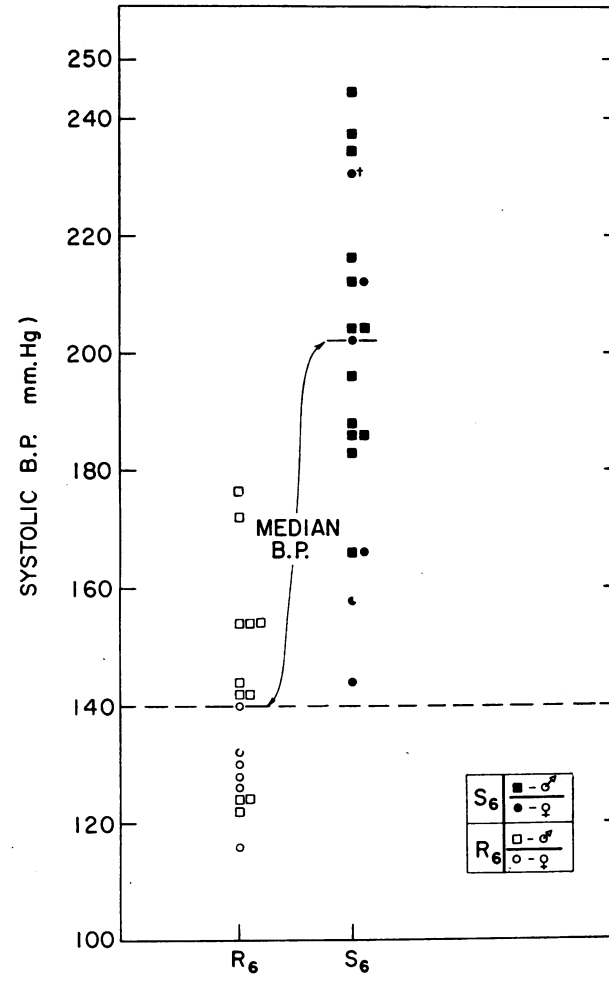

Fig. 6.—Effect of genetic factors on development of renal hypertension in rats, 12 weeks after operation. The technique of Wilson and Byrom[15] was used to induce renal hypertension. Animals weighed 150-200 g. at time of operation and had been on, and were continued on, a low sodium diet throughout. The difference in the average blood pressures of the two groups was significant statistically ($p < 0.01$). (From Dahl, L. K., Heine, M. and Tassinari, L.:[8] used with permission of the Editors.)

in response between resistant and sensitive strains: the genetic substrate clearly influences the development of this form of experimental hypertension just as it influenced the development of hypertension in salt-fed animals. Similar results were obtained in salt-fed animals to which desoxycorticosterone (DOCA) was also administered.[8] Thus, the genetic background critically modifies the response to procedures used to induce at least three "varieties" of experimental hypertension. We suspect that this will be true for other, and possibly all, experimental methods that will produce hypertension.

Since it seems reasonable to suppose that similar interactions are involved in the hypertensive process of man, the following tentative working hypothesis is proposed: chronic hypertension may develop in genetically susceptible individuals; however, non-genetic factors ordinarily will determine whether or not actual hypertension will appear, and possibly the rate at which it develops as well; in "essential" hypertension of man, salt is the commonest precipitating factor, but this does not

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exclude other factors; in "renal" hypertension this process is initiated somehow by the kidney; similar examples might be invoked for other "forms" of hypertension. We plan to continue testing this working hypothesis.

SUMMARY AND CONCLUSIONS

Chronic excess salt ingestion will induce permanent hypertension in rats. Several biological factors modify the response to salt including: (a) the age at which salt-feeding is begun; (b) sex; (c) average daily amount of salt consumed; (d) duration of salt-feeding; and (e) variations in genetic sensitivity to salt.

The importance of the genetic factor(s) was demonstrated by the technique of selective inbreeding, with which two strains of rats were developed, one of which was very prone, the other very resistant, to the development of hypertension after chronic excess salt-feeding. Using these two strains of rats, similar marked differences in blood pressure response were observed both after a manipulation used to induce renal hypertension as well as after administration of salt plus desoxycorticosterone acetate (DOCA).

The genetic background critically controls the response to factors used to induce three "varieties" of experimental hypertension. Therefore, we suspect that this genetic-environmental interaction will prove true for other, possibly all, experimental models of the disease. Similar interactions probably operate in the hypertensive process of man.

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Sodium, Renal Arterial Distension and the Juxtaglomerular Apparatus

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SEVERAL lines of evidence indicate that the renin in a kidney is located in the juxtaglomerular apparatus. Therefore some consideration of the juxtaglomerular body is appropriate for this Symposium. In mammals, one part of the apparatus consists of cells with secretory granules in their cytoplasm. When stained with the Bowie stain, one can see that these cells actually lie in the medial layer of the afferent glomerular arteriole. Thus, if the wall of the afferent arteriole is distended, these cells will also be distended. Conversely, if the wall is underdistended, the cells will undergo a reduced stretch. Their anatomical position may have important implications. When one sees an abundance of juxtaglomerular granules in a section, it is usually a sign of hypersecretion. Conversely, when one sees few juxtaglomerular granules, it signifies a slow rate of secretion. Furthermore, various situations which would be expected to reduce the distension of the renal arterial bed tend to increase the granularity of these cells. And situations which increase the distension of the renal arterial bed tend to decrease their granularity. Thus, a diminished stretch of the cells appears to promote hypersecretion and an increased stretch is accompanied by a low rate of secretion. A few examples of underdistension of the renal arterial bed could be as follows:

First, adrenal insufficiency lowers blood volume and arterial pressure and increases the number of juxtaglomerular granules. The renal artery bed would tend to be underdistended in this situation.

Second, narrowing one renal artery decreases the distension of the arterial bed downstream from the constriction, especially during systole. This is often associated with an increased number of juxtaglomerular granules.

Third, if an ischemic kidney is removed in a rat with unilateral hypertension, the blood pressure comes down to normal and the extracellular fluid volume is probably somewhat less expanded. These effects should decrease the distension of the renal arterial bed. Concomitantly, the juxtaglomerular granules in the remaining kidney rapidly increase toward a normal abundance.

Fourth, many conditions associated with sodium retention and edema formation are also associated with increased juxtaglomerular granularity. Nephrotic edema in the rat, cirrhosis with a large amount of ascites in man, sodium retention following vena caval narrowing in the dog, congestive heart failure with edema in man, congestive heart failure in the dog due to an arteriovenous fistula, all of these are associated with an overabundance of juxta-

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