15. McDonald, T. P., Odell, T. T., Jr., Gosslee, D. G., Proc. Soc. Exp. Biol. and Med., 1964, v115, 684.

16. Gorstein, F., Carroll, H. J., unpublished data.

17. Aster, R. H., Jandl, J. H., J. Clin. Invest., 1964, v43, 843.

18. Spaet, T. H., ibid., 1965, v44, 1099.

19. Salzman, E. W., Chambers, D. A., Neri, L. L., Fed. Proc., 1965, v24, 260.

20. Gaarder, A., Laland, S., Nature, 1964, v202, 909.

Received April 28, 1965. P.S.E.B.M., 1965, v120.

Voluntary Sodium Chloride Intake of Two Strains of Rats with Opposite Genetic Susceptibility to Experimental Hypertension.*(30517)

GEORGE WOLF, LEWIS K. DAHL AND NEAL E. MILLER

Department of Anatomy, School of Medicine, Yale University; Medical Department, Brookhaven National Laboratory; and Department of Psychology, Yale University

Chronic excess salt (NaCl) ingestion will induce permanent hypertension in rats(1) and has been implicated as an etiological factor in human essential hypertension(2,3). However, it is not known to what extent innate differences in salt appetite, if such differences indeed exist, are related to the development of experimental or human hypertension. The present study is concerned with possible relationships between salt appetite and genetic susceptibility to hypertension in rats. A number of investigators have reported relationships between various forms of experimental hypertension and salt appetite. In rats, desoxycorticosterone (DOC) hypertension is accompanied by enhanced voluntary intake of sodium salts(4,5) and there is suggestive evidence that adrenal regeneration hypertension may be associated with enhanced sodium appetite in this species(6). On the other hand, rats with renal(7) or with metacorticoid(5) hypertension manifest relative aversion for sodium salts. Fregly (8-10) has studied thoroughly the NaCl appetite of rats with renal hypertension and has concluded that the aversion of renal hypertensive rats is not specific for NaCl but includes non-sodium salts as well.

Recently, 2 strains of rats have been evolved by selective inbreeding, one of which rapidly develops severe hypertension with various experimental techniques which are almost ineffective in the other strain. While the 2 strains were selected initially on the basis of their respective capacities to develop hypertension from NaCl ingestion(11), it was observed subsequently that this difference in potential was demonstrable by means of other techniques as well; the strain genetically predisposed to develop NaCl hypertension had a similar predisposition to develop hypertension after DOC acetate plus NaCl, as well as after unilateral renal artery compression without NaCl(12). The other strain proved much more resistant to developing hypertension using these techniques. In recent unpublished work a similar disparity in the tendency to develop hypertension has been observed with cortisone and with adrenal enucleation in these two strains. The divergent response to salt in these strains suggested that they might manifest additional disparities. In the present study it was found that rats from the strain with a genetic predisposition to hypertension voluntarily ingested significantly *less* NaCl (as saline) than did the ones with a genetic resistance to developing hypertension.

Method. Subjects. Forty-six female rats between 1 and 2 months of age and weighing 100 to 150 g were used. Twenty-three animals were derived from the so-called *Sensitive* strain, *i.e.,* those with a strong genetic predisposition to hypertension, and 23 were derived from the *Resistant* strain, *i.e.,* those with genetic resistance to developing hyper-

* Work in this study was supported in part by Grant MH02949 from Nat. Inst. of Mental Health, USPHS, and by the U. S. Atomic Energy Commission.

tension(8,9). They had been kept on low salt chow (0.38% NaCl) and tap water *ad lib.* from weaning until the beginning of these experiments.

Testing procedure. The standard "two bottle" self-selection procedure was used. Distilled water and a saline solution were available *ad lib* in inverted graduated test tubes with drinking nozzles which protruded through the fronts of the cages. Each day fluid consumption was measured, the test tubes were refilled, and the positions of water and saline were reversed. The rats were fed standard Purina Chow (0.50-0.75% NaCl) *ad lib* during the experiment.

Spontaneous saline intake. Twelve Sensitive and 12 Resistant rats were placed in pairs in cages and given .15 M saline and water for 6 days as described above. An additional 6 Sensitive and 6 Resistant rats were placed in individual cages and given .25 M saline and water. The 2 concentrations were used because while isotonic saline appears to be palatable to normal rats, they develop an aversion to more hypertonic solutions. Thus, it was possible to determine whether a difference in intake between the 2 strains was independent of the palatability of the solution. The rats given the .15 M solution were run in pairs because greater variance of intake was expected. Later it was found that this was unnecessary and all rats were run individually in the following experiments.

Saline intake after adrenalectomy. Ten Sensitive and 10 Resistant rats were adrenalectomized and given the choice of .25 M saline or water for 12 days. Five rats in each group had been used previously in the first experiment and the remaining 5 of each group were experimentally naive.

Saline intake after DOC. Six intact rats from each strain were given free choice to select either .15 M saline or water and were injected subcutaneously each day for 6 consecutive days with 2.5 mg of desoxycorticosterone acetate (DOCA)† in oil. Fluid intake was measured for an additional 6 days following termination of DOCA treatment. These 12 rats had all previously been used in the first experiment (Spontaneous Saline intake).

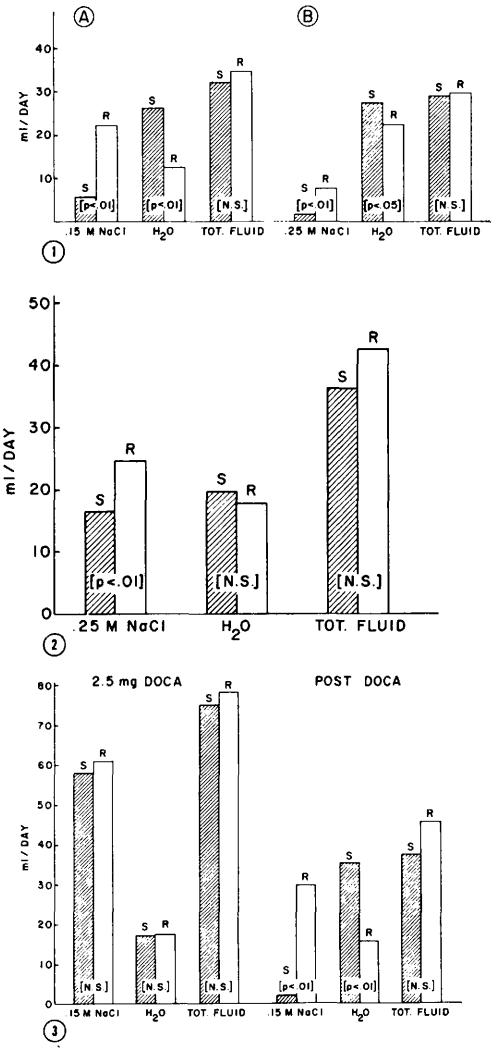

FIG. 1. Mean daily saline, water, and total fluid intake of intact, untreated rats during last 4 days of 6-day period. Data from 6 pairs of Sensitive (S) and 6 pairs of Resistant (R) rats given .15 M saline shown in part "A." Data from 6 Sensitive and 6 Resistant rats given .25 M saline shown in part "B."

FIG. 2. Mean daily .25 M saline, water, and total fluid intake of 10 Sensitive and 10 Resistant adrenalectomized rats during last 10 days of 12-day period.

FIG. 3. Mean daily .15 M saline, water, and total fluid intake of 6 Sensitive and 6 Resistant rats during last 4 days of 6-day injection period (2.5 mg DOCA/day) and during last 4 days of immediately following 6-day period.

Results. Fig. 1 shows the average daily

† Desoxycorticosterone was generously supplied by Dr. Robert Gaunt, CIBA Pharmaceutical Co.

saline, water, and total fluid intakes of the untreated groups of animals. While total fluid intakes were not significantly different in the 2 strains, rats from the Sensitive strain ingested significantly less saline ($p<0.01$ for either 0.15 or 0.25 M saline) and more water ($p<0.01$ and $p<0.05$ for groups given 0.15 M and 0.25 M saline, respectively) than did animals from the Resistant strain. The above and all following statistical analyses are by the Mann-Whitney test and do not include the scores of the first 2 days of observation during which time variability of fluid intake was very high in both strains.

Fig. 2 summarizes the data obtained on adrenalectomized rats from both strains given a choice of 0.25 M saline or water. Those from the Sensitive strain continued to ingest less saline than did those from the Resistant strain ($p<0.01$), although, in accordance with the original observation by Richter(13), the completely adrenalectomized animals manifested an increased appetite for the saline solution. There were no significant differences in water or total fluid intake of the 2 strains under these conditions.

It can be seen from Fig. 3 that daily injection of 2.5 mg of DOCA in intact animals effected the following striking changes: (1) total fluid intake by both strains was more than doubled (from a normal average of about 35 ml/day to about 75 ml/day during DOCA treatment); (2) this increment in fluid consumption was due entirely to a marked increase in the intake of 0.15 M saline (in comparison to the corresponding groups of Fig. 1A, Sensitive animals manifested a 10-fold increase while Resistant animals manifested a 3-fold increase); (3) the net effect of the foregoing was to eliminate the differences in saline and water intake between the two strains. After termination of DOCA injection, 0.15 M saline intake decreased sharply and the normal pattern reappeared.

Discussion. The present results show that when they were given a free choice of either saline solution or water to drink, rats from a strain with a strong genetic predisposition to develop hypertension ingested significantly less saline than did those from a strain genetically resistant to the development of hypertension. Adrenalectomy did not eliminate this difference in saline intake of the 2 strains, but addition of DOC to the regimen completely abolished it. The latter result is somewhat difficult to interpret because of the possibility that a "ceiling" was reached which precluded greater saline intake in the Resistant strain.

The explanation for the difference in salt appetite in the 2 strains is not readily apparent from this or other studies. That some hypertensive rats have a relative aversion for NaCl solutions seems well established, as noted earlier(5,7-10), although there is evidence to suggest that this is not specific for NaCl but may be part of a general salt aversion(10). It is, perhaps, a semantic question whether or not the animals in this experiment from the Sensitive strain are called hypertensive. By some criteria they would not be so classified: 1) the polydipsia which characterizes hypertensive rats(7,14) clearly was not present; in fact, their total fluid intake consistently tended to be slightly lower than that of rats from the Resistant strain; 2) in some unpublished studies, now in preparation, no difference in natruresis was observed to follow NaCl loading in animals of this age from both strains; 3) in the study of Tosteson *et al*(4) rats with renal hypertension which also received DOCA continued to consume less NaCl solution than did the controls. In the individual rats discussed here, blood pressures were not measured. However, our experience suggests that as a group, animals of this age from the Sensitive strain would average about 10-20 mm Hg higher basal systolic pressures than would similar animals from the Resistant strain (*i.e., ca.* 115-125 *vs* 105 mm Hg, respectively). Since the range of blood pressures from "normal" to "high" is a continuum, the Sensitive rats with higher basal pressures would be "hypertensive" relative to the Resistant rats. Furthermore, the genetic propensity for hypertension is now so great among rats from the Sensitive strain that every animal will *predictably* develop hypertension from any of several experimental manipulations. In their study of self-selection of salt solutions by rats

Abrams *et al*(7) observed that, among control animals, those with the highest blood pressures had saline intakes below those of controls with lesser pressures. They suggested that spontaneously developing hypertension in rats might also be accompanied by a lessened appetite for NaCl. If the animals used in our studies from the Sensitive strain were considered to be in the earliest phase of hypertension, *i.e.*, the so-called "pre-hypertensive" stage, their relative aversion for saline might be compatible with the interpretation mentioned above(7).

The mechanism of the disparity in salt appetite between the 2 strains remains obscure. It cannot be attributed primarily to differences in adrenocortical function since it persisted after adrenalectomy. Neither can it be attributed to less efficient excretion of sodium by the Sensitive strain, since unpublished studies have failed to reveal differences in rats from the same 2 strains in a) capacity to excrete sodium after a sodium chloride load, b) total carcass sodium or c) total exchangeable sodium.

It may be that the differences in both vascular reactivity and sodium appetite in the 2 strains are due to genetically determined differences in hypothalamic function. Ample evidence indicates that taste sensitivities(15) and preferences(16) can be transmitted on a genetic basis. There is a large body of data documenting the role of the hypothalamus in taste preferences and in regulation of nutrient intake(17), and destruction or stimulation of various hypothalamic structures has been shown to modify salt intake profoundly(18, 19,20). On the other hand, the hypothalamus has long been known to play a role in the elevation of blood pressure(21,22), and it is conceivable that functionally or structurally related areas of the hypothalamus are responsible for some degree of control of both salt appetite and blood pressure. Thus the differences between the 2 strains of rats might depend upon genetically determined variations in hypothalamic function such that salt appetite is decreased in association with increased vascular reactivity in the Sensitive rats and *vice versa* in the resistant ones.

Summary. Given a free choice of either saline solution or water to drink, rats from a strain with a strong genetic predisposition to hypertension ingested less saline solution than did those from a strain genetically resistant to developing hypertension. Administration of DOC abolished this difference, but adrenalectomy did not. Studies by others have shown that in some forms of experimental hypertension, rats have a relative aversion for saline. Although it is highly unlikely that frank hypertension was present in any animals used in these studies, animals from the strain predisposed to hypertension are known to have basal pressures 10-20 mm Hg higher than in the other strain. If such relative elevations are considered to be evidence of the "pre-hypertensive" phase, the aversion for saline might be taken as early evidence of developing hypertension(7). Some speculations concerning genetic relations among saline appetite, blood pressure and hypothalamic function were given.

1. Dahl, L. K., J. Exp. Med., 1961, v114, 231.

2. Dahl, L. K., Love, R. A., A.M.A. Arch. Int. Med., 1954, v94, 525.

3. Dahl, L. K., in Essential Hypertension, An International Symposium, P. T. Cottier & K. D. Bock, eds., Bern (Springer-Verlag, Heidelberg) 1960, p53.

4. Tosteson, D. C., DeFriez, A. I. C., Abrams, M., Gottschalk, C. W., Landis, E. M., Am. J. Physiol., 1951, v164, 369.

5. Green, D. M., Saunders, F. J., Van Arman, C. G., Calvin, L. D., Sturtevant, F. M., ibid., 1952, v170, 486.

6. Takeda, R., Morimoto, S., Miyabo, S., Murakami, M., Endocrinol., Japan, 1964, v7, 19.

7. Abrams, M., DeFriez, A. I. C., Tosteson, D. C., Landis, E. M., Am. J. Physiol., 1949, v156, 233.

8. Fregly, M. J., ibid., 1956, v187, 288.

9. ———, ibid., 1958, v195, 645.

10. ———, ibid., 1959, v196, 1326.

11. Dahl, L. K., Heine, M., Tassinari, L., J. Exp. Med., 1962, v115, 1173.

12. ———, ibid., 1963, v118, 605.

13. Richter, C. P., Am. J. Physiol., 1936, v115, 155.

14. Oster, K. A., Martinez, O., J. Exp. Med., 1943, v78, 477.

15. Fox, A. L., Proc. Nat. Acad. Sci., 1932, v18, 115.

16. Nachman, J., J. Comp. & Physiol. Psychol., 1959, v52, 451.

17. Teitelbaum, P., Epstein, A. N., in Olfaction and Taste, Proc. 1st International Symposium, Stockholm, Y. Zotterman, ed., Pergamon Press Ltd., London, 1963, 347.

18. Covran, M. R., Antunes-Rodrigues, J., Am. J. Physiol., 1963, v205, 922.

19. Wolf, G., J. Comp. & Physiol. Psychol., 1964, v58, 396.

20. Andersson, B., Jewell, P. A., Larsson, P., in Ciba Fndn. Symp. on the Neurological Basis of Behavior. Ciba, London, 1958, 76.

21. Scherrer, H. F., Friedman, S. M., Acta Endocrinol., 1958, v27, 89.

22. Bard, P., Physiol. Rev., Suppl. 4, 1960, v40, 3.

Received July 12, 1965. P.S.E.B.M., 1965, v120.

Effect of Chlorpromazine on DNA Synthesis in a Cell Free System.* (30518)

ANTHONY V. PISCIOTTA, ANTHONY P. ZIEBERT† AND JEAN E. HINZ

Department of Medicine, Marquette University School of Medicine, and Blood Research Laboratory, Milwaukee County General Hospital, Milwaukee, Wisconsin

Chlorpromazine (CPZ) is known to suppress a wide variety of enzyme activities(1-8) including DNA synthesis(9-11) in intact cells and animals. Rosenberg(9) observed that administration of CPZ to rats with ascites hepatoma results in diminished incorporation of P^{32} into the nuclei of tumor cells. Also, radioautography demonstrated that CPZ inhibits *in vitro* incorporation of H^3 thymidine into human marrow cells engaged in DNA synthesis, followed by delay of mitosis(11,12).

Since CPZ affects cell permeability(13,14) as well as many other biologic activities not necessarily involved with enzymes(15-17) its effect on DNA synthesis in a cell free system was studied. It is felt that a cell free system avoids some of the variables imposed by a cell membrane and the numerous chemical reactions that simultaneously occur in an intact cell or organism. Our approach uses a multi-enzyme source from regenerating rat liver which can promote DNA synthesis.

Material and methods. Table I lists the components of the incubation mixture employed in these experiments; methods were adopted from those recommended by Bollum and Potter(18) and by Main and Walwick (19-21). Primer DNA‡ was obtained from calf thymus.

TABLE I. DNA Synthesis in a Cell Free System(20).

Tris-HCl, pH 7.7	20	μmoles
$MgCl_2$	3	mμmoles
Glucose	25	μmoles
ATP (K salt)*	.5	"
DPN*	1.25	"
dAMP*	25	mμmoles
dGMP*	25	"
dCMP*	25	"
Tritiated thymidine* (Sp. act. = 3 c/mM)	33	"
Protein enzyme†	1-2	mg
DNA	200	"
Test drug (Added either to incubation mix less enzyme or to enzyme)	.2	μmole

(Total volume 0.5 ml) Incubate 1-4 hr at 37°C.

* Source of reagents: ATP (K salt) Pabst Laboratories, Trace ADP; DPN, Pabst; H^3 thymidine, Schwarz, radiochemical purity 99%; dAMP, Calbiochem; dGMP, Calbiochem; dCMP, Calbiochem; all other reagents, chromatographically homogenous.

† Protein enzyme derived from regenerating rat liver homogenate, prepared 24 hr after partial hepatectomy.

* Supported by Research Grant CA-05524-05 from Nat. Cancer Inst., N.I.H., U.S.P.H.S., Bethesda, Md.; the Saul Cooper, Robert Uihlein, Charles F. Vogel, Douglass Van Dyke and Philipp I. Hunkel funds; and grants from Smith, Kline & French Laboratories, Parke Davis Pharmaceutical Co., Eli Lilly Co., & Sandoz Pharmaceutical Co.

† Trainee in Hematology, Training Grant T1 AM-5162-06 from Division of Arthritis and Metab. Dis., N.I.H., U.S.P.H.S.

‡ Worthington Biochemical Corp., Freehold, N. J.

The DNA synthesizing system was prepared from Holtzman strain rats weighing 125-160 g. Seventy-five per cent of the liver was removed under ether anesthesia and the