right angles to the surface (1.5×10^{14}) assuming saturation of surfaces in both instances.

Comment. Since radioactivity in cell and blood vessel digests was confined to the dextran fraction, it can be assumed that the label was not exchanged. Hence, the experiments show that dextran adheres to circulating blood cells *in vivo* and also on luminal arterial surfaces: in the latter it concentrates at sites of injury (intimectomy) possibly because injury increases surface area. The mechanism by which dextran impairs clotting may therefore be 2-fold, involving changes in properties both of circulating formed elements, such as platelets, and of luminal aspects of vessel walls.

The molecular arrangement which dextran seems to assume on the luminal surface of a vessel is consistent with physical (hydrogen) rather than with chemical bonding. The radial molecular arrangement might direct the terminal-free aldehyde groups of dextran towards the lumen, bending the molecule to the surface. This molecular coating may sterically prevent the achievement of the critical concentration of reactants necessary for clotting or may insulate or counter transmural positive thrombogenic potentials(16) which initiate clotting.

Summary. Infused C-14 dextran adheres *in vivo* to circulating blood cells and also to intimal surfaces of arteries, and in the latter to a greater extent at sites of recent injury. Coating of circulating cells, as well as the intima by dextran molecules (apparently radially arranged on blood vessel surfaces) may participate in the antihemostatic effects of dextran, especially at sites of high concentration consequent on vascular damage.

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1. Heckel, G. P., Erickson, C. C., Yuile, C. L., Knutti, R. E., *J. Exp. Med.,* 1938, v67, 345.

2. Hueper, W. C., Landsberg, J. W., Eskridge, L. C., *J. Pharm. and Exp. Therap.,* 1940, v70, 201.

3. Horvath, S. M., Hamilton, L. H., Spurr, G. B., Allbaugh, E. B., Hutt, B. K., *J. Applied Phys.,* 1955, v7, 614.

4. Carbone, J. V., Furth, F. W., Scott, R., Jr., Crosby, W. H., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 101.

5. Jacobaeus, Ulf., *Acta Med. Scand.,* 1957, v157, Suppl. 322.

6. Bergentz, S. E., Eiken, O., Nilsson, I. M., *Thrombosis at Diathesis Haemorrhagica,* 1961, v6, 15.

7. Adelson, E., Rheingold, J. J., Parker, O., Buenaventura, A., Crosby, W. H., *Blood,* 1961, v17, 267.

8. Bloom, W. L., Fowler, N. O., Ward, J. A., Franch, R. H., *J. Surg. Research,* 1963, v3, 152.

9. Bryant, M. F., Bloom, W. L., Brewer, S. S., *J. Med. Assn. of Georgia,* 1961, v50, 580.

10. Bryant, M. F., Brewer, S. S., Bloom, W. L., *Am. Surgeon,* 1963, v29, 256.

11. ———, *Clinical Research,* 1962, v10, 49.

12. Brewer, S. S., Bloom, W. L., Bryant, M. F., *ibid.,* 1963, v11, 35.

13. Brewer, S. S., *Bull. Fulton County Med. Soc.,* March, 1963.

14. Rothman, S., Adelson, E., Schwebel, A., Langdell, R. D., *Vox Sanguinis,* 1957, v2, 104.

15. Ross, S. W., Ebert, R. V., *J. Clin. Invest.,* 1959, v38, 155.

16. Sawyer, P. N., Pate, J. W., *Am. J. Physiol.,* 1953, v175, 103.

17. Sawyer, P. N., Pate, J. W., Weldon, C. S., *ibid.,* 1953, v175, 108.

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Effect of Hypertonic Sodium Nitrate Solutions on Blood Pressure of Rats.* (28921)

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It is well known that excessive ingestion of sodium chloride is accompanied by elevation of blood pressure of both rats and man (1-4). It makes little difference whether sodium chloride is ingested by way of food or drinking water or whether adrenal glands are

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present or absent(3,5,6). The relative contributions of the two ions, sodium and chloride, to the elevation of blood pressure under conditions of salt-induced hypertension are not clearly understood. The studies of Martini and Kaiser(7) and Kaiser(8), in which elevation of blood pressure was induced by excessive ingestion of salt, suggest that both the sodium and chloride ions are required for a pressor response in rats and humans. In another type of experimental hypertension induced by administration of desoxycorticosterone acetate in excess to rats, Selye *et al*(9) observed that the sodium, but not the chloride, ion is important in development of hypertension and cardiovascular lesions.

Preliminary studies not reported here showed rats to be tolerant to administration of hypertonic sodium nitrate solution as their sole drinking fluid. The present study was therefore designed to test cation specificity in development of "salt"-induced hypertension by utilizing hypertonic sodium nitrate instead of sodium chloride as the sole drinking fluid.

Methods. Two separate experiments were performed. Each used male Holtzman rats and was carried out in an identical fashion with the exception that the second experiment utilized a higher concentration of sodium nitrate that the first and the rats were 8 weeks younger at beginning of the experiment. The experimental procedure described below therefore applies to both experiments.

All rats were kept 3 to a cage in a windowless room maintained at 26 ± 1°C and illuminated from 8 AM to 6 PM. All rats were given Purina Laboratory Checkers to eat *ad libitum* and either tap water (control rats) or sodium nitrate solution (experimental rats) to drink.

Experiment 1. At the end of a 6-week control period the rats were divided arbitrarily into 2 groups containing 8 rats each. Group 1 served as controls and continued to receive water as drinking fluid while Group 2 received 0.15 M $NaNO_3$ solution to drink. At the end of the 19th week, Group 2 was given 0.175 M $NaNO_3$ solution in place of 0.15 M. Blood pressures were measured weekly by the microphonic manometer technique, without anesthesia(10). Body weight of each rat was also measured weekly.

At the end of the 32nd week of experiment, 0.5 ml blood was withdrawn anaerobically from the tail vein and its pH measured by means of a capillary electrode coupled to a Metrohm pH Meter. At the end of the 33rd week of experiment all rats were killed by decapitation. Blood was collected without anticoagulant, allowed to clot and the serum separated. Serum was analyzed for chloride concentration by the Cotlove chloridometer (11) and for sodium and potassium by flame photometry using lithium as the internal standard. At death, the heart, kidneys, testes, adrenals, thyroid, thymus, seminal vesicles and prostate were removed, trimmed of fat and connective tissue and weighed on a torsion balance.

Experiment 2. Eight control and 8 experimental male rats were used. The objective was to utilize a higher concentration of $NaNO_3$ in the drinking water than had been used in the first experiment.

Blood pressure and body weight were measured for 2 weeks. At the end of this control period the experimental group was given 0.15 M $NaNO_3$ solution in place of water. The control group continued to receive tap water as drinking fluid throughout the experiment. At the end of the 3rd week, the concentration of $NaNO_3$ in the drinking water of experimental rats was increased to 0.175 M while a further increase to 0.25 M $NaNO_3$ was effected at the end of the 9th week of experiment. At weekly intervals during the 28-week experiment systolic blood pressure and body weight were measured.

Results. Results of the first experiment are shown in Fig. 1. Mean systolic blood pressure of the $NaNO_3$-treated group was slightly, but not significantly, greater than that of controls during the 20 weeks 0.15 M $NaNO_3$ solution was given as the sole drinking fluid (Fig. 1A). Increasing the concentration of $NaNO_3$ solution to 0.175 M was accompanied by an elevation of mean systolic blood pressure significantly ($P<.05$) above that of controls during the last 13 weeks of the experiment.

Administration of $NaNO_3$ solution resulted

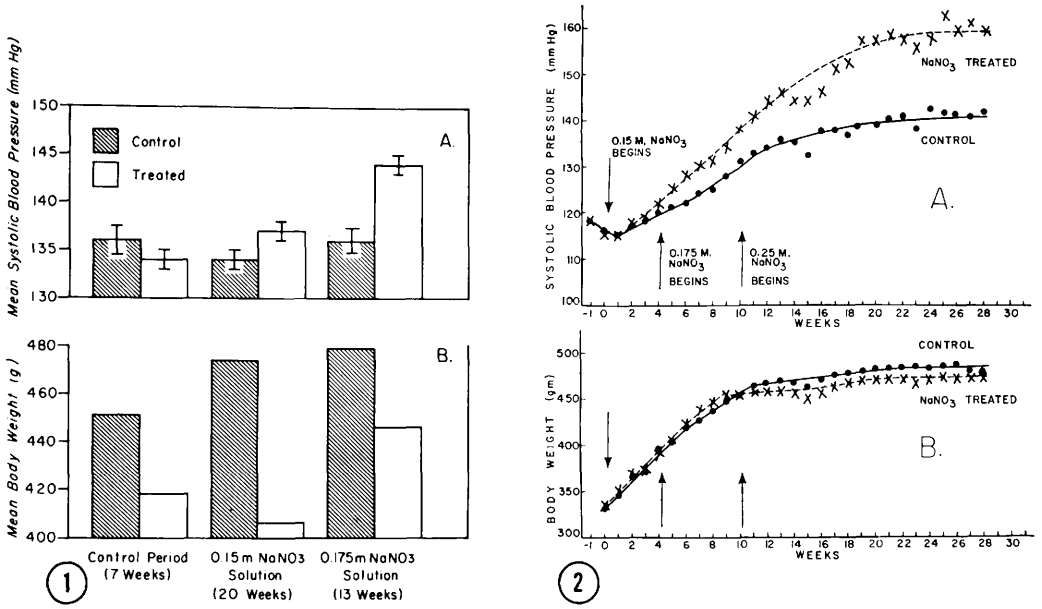

FIG. 1. A. Mean systolic blood pressures of 8 control (cross hatched rectangle) and 8 $NaNO_3$-treated (open rectangle) rats during control and treatment periods. Duration of treatment and concentration of $NaNO_3$ given as sole drinking fluid are shown at bottom of figure. Standard error ± 1 is set off at each mean. B. Mean body weight of the same rats as in part A is shown for control and treatment periods.

FIG. 2. A. Mean systolic pressure of 8 control (●) and 8 $NaNO_3$-treated (×) rats throughout the experiment. Three different concentrations of $NaNO_3$ solution were given as the sole drinking fluid beginning at times indicated by arrows on graph. Controls received tap water to drink. B. Mean body weights of control and $NaNO_3$-treated rats during experiment.

in an initial loss of body weight accompanied by a gradual increase nearly to the weight of control rats by the end of the experiment. This occurred in spite of an increase in the $NaNO_3$ concentration beginning week 21 of the experiment (Fig. 1B).

Rats given $NaNO_3$ solution to drink had lower serum concentrations of sodium and chloride than controls (Table I). However, the differences between means of control and treated rats are not significant ($P>.05$). Blood pH and serum potassium concentration tend to be higher in the $NaNO_3$-treated rats than in controls. Thus, the treated rats tended to be alkalotic by comparison with controls.

Organ to body weight ratios for control and treated rats are given in Table II, Exp. 1. No significant differences were observed between organ weight ratios of any of the organs of control and treated rats.

Three treated rats died during the experiment. One rat died during each of weeks 15, 19 and 22. Causes of death were undetermined.

Results of the second experiment indicate that administration of $NaNO_3$ in the drinking fluid is accompanied by an elevation of

TABLE I. Comparison of Serum Electrolyte Concentrations of Treated with Control Rats in Experiment 1.

Group	No. of rats	Serum concentration (meq/l) of: Sodium	Potassium	Chloride	Blood pH
Control	8	137.0 ± 1.1*	6.51 ± .35	92.0 ± 1.5	7.46 ± .02
$NaNO_3$-treated	5	132.2 ± 3.8	9.37 ± 1.06†	88.9 ± 1.7	7.52 ± .02

* ± 1 standard error of mean. † Significantly different from control ($P<.05$).

TABLE II. Comparison of Organ Weight/Body Weight Ratios of Sodium Nitrate-Treated and Control Rats.

	Group	Mean body wt (g)	No. of rats	Organ to body weight ratio (mg/100 g BW)						
				Heart	Kidneys	Testes	Adrenals	Thyroid	Thymus	S. vesicles	Prostate
Exp 1	Control	481	8	299.7 ±11.8*	736.7 ± 26.1	677.0 ± 35.0	11.5 ± .9	5.7 ± .2	60.3 ± 9.0	151.2 ± 8.4	117.2 ± 5.3
	$NaNO_3$-treated	441	5	298.2 ± 2.6	754.9 ± 38.3	767.7 ± 21.4	10.3 ± .5	6.6 ± .5	35.2 ± 5.3	184.3 ±11.1	133.5 ±19.8
Exp 2	Control	476	8	290.1 ±11.4	669.5 ± 27.9	700.9 ± 45.9	11.8 ± .8	5.8 ± .3	65.1 ± 3.7	155.2 ± 7.9	146.4 ± 9.8
	$NaNO_3$-treated	456	5	289.7 ± 9.2	948.0 ±110.9	745.9 ± 20.9	12.1 ±1.7	7.1 ± .3†	53.6 ± 3.5	156.2 ± 9.3	123.3 ± 6.2

* 1 standard error of mean.
† Significantly different from controls ($p < .01$).

blood pressure to a level significantly above that of controls (Fig. 2A). The rise in pressure is detectable within one week after receiving 0.175 M $NaNO_3$ to drink and significantly different ($P < .05$) from control levels by the second week of administration. Blood pressure of control rats rose with time from initial levels of approximately 119 mm Hg at the beginning of experiment to 140 mm Hg at the end of the 28th week of experiment. This gradual rise in blood pressure of control rats with time is attributed to ageing of the rats and has been observed previously(12). Administration of $NaNO_3$ solution as the sole drinking fluid enhanced blood pressure rise of the experimental group.

Body weight was affected by treatment with $NaNO_3$ in that treated rats gained slightly less weight (12 g) than controls during the experiment (Fig. 2B).

Three treated rats died suddenly during the 14th week of the experiment and within one day of each other. Cause of death in each case was not determined.

Mean organ to body weight ratios of the organs removed at autopsy from rats of each group are shown in Table II, Exp. 2. The only significant difference observable between the two groups is the larger thyroid weight of the treated group.

Statistical comparison of the data was made by means of the "t" test for the 95% confidence limit(13).

Discussion. Excessive ingestion of sodium nitrate by rats is accompanied by an elevation of systolic blood pressure. This response is dependent on the concentration of sodium nitrate used. The threshold concentration for elevation of blood pressure lies between 0.15 and 0.175 M. Rise in systolic blood pressure of rats treated with the former concentration is not significant even after 20 weeks of treatment (Fig. 1A). In contrast, a rise of systolic blood pressure is detectable within a week after administration of 0.175 M sodium nitrate solution (Fig. 2A). Apparently both concentration and duration of treatment are important factors in elevating blood pressure.

Elevation of blood pressure is not accompanied by cardiac hypertrophy. This is not

surprising since systolic blood pressure rose to maximal levels of only 150 to 160 mm Hg with treatment. A previous study, concerned with the relationship between systolic blood pressure and organ weights in a large number of rats, revealed that the first significant increase in heart weight to body weight ratio occurs at systolic blood pressures ranging from 160 to 169 mm Hg(14).

The experiments reported here offer no direct explanation for the elevation of blood pressure by hypertonic $NaNO_3$ solutions although some clues are provided. Mean serum chloride concentration is lower on the average than control values while blood pH is higher (Table I). Apparently the $NaNO_3$-treated rats are alkalotic by comparison with controls. Similar results have been reported by Hiatt(15) for dogs given $NaNO_3$. Whether the alkalosis contributed to the elevation of blood pressure of the treated rats requires further study.

A further clue concerns the enlarged thyroid glands observed in $NaNO_3$-treated rats (Table II). Bloomfield *et al*(16) studied thyroid function of KNO_3-treated rats fed this salt in the diet for 5.5 weeks. They report an initial decrease in uptake of radioactive iodide followed after 2 weeks by return to normal values. By the end of 5.5 weeks, the KNO_3-treated rats had a greater protein bound iodide concentration than controls. The authors did not study metabolic rate, although the elevated protein bound iodide concentration of serum infers that metabolic rate was elevated. If this was the case, it may be postulated that the elevation of blood pressure of the $NaNO_3$-treated rats is associated with increased thyroidal activity(17).

Administration of either hypertonic $NaNO_3$ or NaCl solution as the sole drinking fluid results in a significant elevation of blood pressure(3,5). These studies, considered together, suggest that the cation is more important than the anion in elevation of blood pressure. However, these results must be interpreted cautiously since it has been shown that an elevation of blood pressure also occurs in rats given .15 to .30 M potassium nitrate as their sole drinking fluid(18). Hence these results, considered with those reported here, could be interpreted as indicating that the anion is more important than the cation in elevation of blood pressure. Others have also shown that either potassium or lithium chloride may be substituted for NaCl as a sensitizing agent in development of desoxycorticosterone acetate (DCA)-induced(19, 20) hypertension in rats. In contrast, "acidifying" salts (ammonium sulfate, ammonium chloride, ammonium nitrate and calcium chloride) actually protect rats against renal and cardiovascular lesions normally occurring with DCA overdosage(9). To complicate further the problem of anion and cation specificity for elevation of blood pressure of rats it has been shown recently that dietary administration of very small amounts of cadmium(21) or intraperitoneal injection of lithium chloride (4 mg/day)(22) induce hypertension in otherwise untreated rats. No theory completely unifies the mechanisms of action of the different ions in elevation of blood pressure although the possibilities exist that hypertonicity *per se,* as well as the nephrotoxic action of at least some of the cations, represent common denominators among them(19,21). The possibility that changes in acid-base balance induced by these salts contribute to elevation of blood pressure must also be taken into account.

In contrast, it seems clearer that drastic dietary restriction of sodium ion *lowers* blood pressure of both experimental animals and humans with established hypertension(23). In addition, treatment with either natriuretic agents or cationic exchange resins is known to lower blood pressure of hypertensive patients(24). Hence, depletion of chloride ion seems less important than depletion of sodium ion for *reduction* of blood pressure.

Further study is needed to elucidate common factors and suggest roles for the actions of salts both in elevation and reduction of blood pressure of rats.

Summary. Administration of $NaNO_3$ solution to rats as their sole drinking fluid is accompanied by a significant elevation of systolic blood pressure. The threshold concentration for elevation of blood pressure lies between 0.15 and 0.175 M. Both concentration of $NaNO_3$ solution and duration of treat-

ment appear to be important factors in elevating blood pressure. Administration of $NaNO_3$ is also accompanied by an increase in thyroid to body weight ratio.

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1. Meneely, G. R., Tucker, R. G., Darby, W. J., Auerbach, S. H., *J. Exp. Med.,* 1953, v98, 71.
2. ———, *Ann. Int. Med.,* 1953, v39, 991.
3. Sapirstein, L. A., Brandt, W. L., Drury, D. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 82.
4. McDonough, J., Wilhelmj, C. M., *Am. J. Dig. Dis.,* 1954, v21, 180.
5. Fregly, M. J., *Endocrinology,* 1960, v66, 240.
6. Wilgram, G. F., Ingle, D. J., *Am. J. Cardiol.,* 1961, v8, 582.
7. Martini, P., Kaiser, K., in *Hypertension, Humoral and Neurogenic Factors,* ed. by Wolstenholme, G. E. W., Cameron, M. P., Etherington, J., Little, Brown & Co., Boston, 1953, p272.
8. Kaiser, K., *German Med. Monthly,* 1959, v4, 93.
9. Selye, H., Mintzberg, J., Rowley, E. M., *J. Pharm. Exp. Therap.,* 1945, v85, 42.
10. Friedman, M., Freed, S. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 670.
11. Cotlove, E., Trantham, H. V., Bowman, R. L., *J. Lab. Clin. Med.,* 1958, v51, 461.
12. Fregly, M. J., *Am. J. Physiol.,* 1955, v182, 139.
13. Fisher, R. A., *Statistical Methods for Research Workers,* 10th ed., Hafner, New York, 1948, p114.
14. Fregly, M. J., *Am. J. Physiol.,* 1962, v202, 967.
15. Hiatt, E. P., *ibid.,* 1940, v129, 597.
16. Bloomfield, R. A., Welsch, C. W., Garner, G. B., Muhrer, M. E., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 288.
17. Tepperman, J., *Metabolic and Endocrine Physiology,* Yearbook Medical Pub. Inc., Chicago, 1962, p97.
18. Fregly, M. J., *Am. J. Cardiol.,* 1961, v8, 732.
19. Hall, C. E., Hall, O., *ibid.,* 1961, v8, 719.
20. Rosenman, R. H., Freed, S. C., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 77.
21. Schroeder, H. A., *Am. J. Physiol.,* 1962, v202, 515.
22. Bach, I., Braun, S., Gati, T., Sos, J., Udvardy, A., *Nature,* 1961, v192, 322.
23. Grollman, A., Harrison, T. R., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 53.
24. Helmer, O., in *Hypertension, Humoral and Neurogenic Factors,* ed. by Wolstenholme, G. E. W., Cameron, M. P., Etherington, J., Little, Brown & Co., Boston, 1955, p279.

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Phytic Acid Studies.* (28922)

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Phytic acid has interested analytical chemists, biochemists, and nutritionists for several years. Brown *et al*(1) recently supported Neuberg's proposed structure and concluded that of the 18 acid hydrogens in the Neuberg model, only 12 were available for titration in aqueous solution.

Chicks fed purified diets containing sesame meal(2,3) or isolated soybean protein(4) as the sole source of protein developed zinc deficiency symptoms, although the diet contained more than the optimum level of zinc. Autoclaving the protein source or addition of EDTA (ethylenediamine tetraacetic acid) to the diet prevented the deficiency symptoms. O'Dell and Savage(5) observed that phytic acid formed complexes with isolated soybean protein and casein. When this complex was fed in the diet of chicks as a source of protein, zinc was found to be far less available for growth.

The work reported here was undertaken to estimate qualitatively the complex formation between metal ions and phytic acid using the "pH drop method"(6,7). The effect of feeding phytic acid on utilization of dietary zinc by chicks has been studied.

Methods. Phytic acid,§ 4.48 $\times$ 10^{-3} M,

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