that at plasma concentrations below 0.002 no agglutination would occur.

Discussion. It is probable that formaldehyde modified the cell surfaces even though they could still be agglutinated once the free agent was removed since the second "gelation" stage was never observed in these stabilized systems. The effect on the plasma factor was more drastic since even after dialysis, the formaldehyde-treated plasma could not agglutinate the resuspended cells. Formaldehyde also very effectively prevents the interaction of fibrinogen to form fibrin (5). Inasmuch as heterologous plasma did not cause agglutination it would appear that a special "factor" was involved rather than a non-specific effect of, say, plasma protein.

The quantitative resistance of this cellular activity to drying is of interest but is in accord with the general durability of agglutinating systems. The very high temperature coefficient of the reaction is perhaps surprising. Much lower values have been observed for the polymerization of both vertebrate and invertebrate fibrinogens(6).

Summary. Stable cell suspensions were prepared from *Limulus* blood using 0.05-0.10% formaldehyde. Washed cells could be agglutinated by as little as 0.01 volume of fresh homologous plasma. The initial stages of this reaction showed moderate concentration dependence and very strong temperature dependence.

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Evidence for an Increased Intake of Sodium in Hypertension Based on Urinary Excretion of Sodium.*† (22846)

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In other papers, evidence has been presented which indicated that salt (*i.e.* sodium) intake played a primary role in the pathogenesis of human essential hypertension(1-2). Since this thesis was developed by semi-quantitative means, confirmation by quantitative technics seemed pertinent and forms the subject of the present report. Beginning in 1953, every member of the Brookhaven National Laboratory staff who reported to the staff clinic for annual physical examination by Dr. Robert A. Love was questioned as to his salt intake and classified into one of 3 groups as follows: 1) *Low intake*—this individual did not add, and had never added, salt to his food at table; 2) *Average intake*—this individual added salt to his food if *after* tasting, it was insufficiently salty; and 3) *High intake*—this subject routinely added salt to foods customarily salted, but did so *before* tasting them. The defects in this type of classification were recognized, but the author thought this was a method by which a large series could be studied readily and the implications of a low versus a high salt intake tested. The other papers may be consulted for further details but in summary the following results were obtained: Among the 1346 adults who were classified according to the categories listed above, there were 135, 630 and 581 in

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TABLE I. Average Daily Urine Sodium Values in 28 Ambulatory Male Subjects.

"Low" NaCl intake				"Avg" NaCl intake				"High" NaCl intake			
Age	Days urine collected	mEq Na per day (avg)	Hyper-tension	Age	Days urine collected	mEq Na per day (avg)	Hyper-tension	Age	Days urine collected	mEq Na per day (avg)	Hyper-tension
48	9	197	+	33	7	210	0	45	7	244	+
40	6	173	0	69	7	202	+	25	7	236	0
33	7	171	0	37	8	191	0	33	7	236	+
43	7	158	0	33	28	181	0	42	7	227	+
39	7	157	0	40	38	180	0	36	7	202	+
49	7	140	0	56	7	179	+	31	7	195	0
55	13	126	0	35	8	134	0	53	7	192	+
36	7	125	0	29	7	131	0	27	7	185	0
				43	7	116	0	45	7	184	0
								45	6	160	+
								28	7	156	0

the Low, Average and High intake groups respectively. The 105 hypertensives in this series were distributed as follows: Low intake, 1; Average intake, 43; High intake, 61. Tested by the chi-square method, the probability of this distribution occurring by chance was found to be less than one in a thousand ($\chi^2 = 16.1$, $p<.001$). Individuals classified in the Low NaCl intake group had significantly less hypertension ($p<.01$) while those on a High intake had significantly more hypertension ($p<.01$) than would have been predicted by chance alone.

The current study had as its goal the quantitation of urinary sodium among individuals with and without hypertension in the several salt-intake groups described above. In prolonged metabolic studies on ambulatory individuals both normal and hypertensive, the author has found the urinary sodium to be a highly reliable measure of the sodium intake. Therefore, while urinary excretion of sodium was measured, under the conditions of the current study it seems reasonable to assume that this accurately reflected the actual intake of this ion.

Procedure. Selection of individuals was based on: 1) Sex—only males were tested because of greater ease of collecting samples, lack of need to allow for pre-menstrual Na retention, and because analysis of data had indicated that statistical significance was unaffected by elimination of females; 2) Presence or absence of hypertension; 3) Availability and willingness to participate for approximately one week. All subjects were in essentially sedentary occupations at the Laboratory. Most of these people were unaware of the nature of the study, but included in the series are 5 colleagues from Brookhaven Medical Department who knew the general problem under investigation: all 5 denied any conscious deviation from their normal use of salt at table. Two others, however, stated that they had decreased their salt intakes consciously and therefore have been omitted. Complete 24-hour urine collections were made for 6-38 days (median 7 days; average 9.1 days) and in almost all instances, the 24-hour collections were made on consecutive days. Each collection unit contained a detailed set of directions on procedure to be followed; in case a sample was lost, the subject was instructed to discard the collection and resume on a full 24-hour basis. Each individual was told not to deviate from his normal behavior pattern and only in event of illness was the test to be abandoned. Urine was voided directly into chemically clean capped bottles. After measurement of volume from each 24-hour specimen an aliquot was removed for subsequent sodium analysis. Sodium concentration was measured in duplicate on a Baird Model DB2 flame photometer using internal lithium standard. From the 24-hour urine volume and concentration of sodium, the 24-hour urine sodium output was calculated. Since stool sodium amounts to only about 3 mEq/d(3), in the absence of unusual extrarenal losses, Na retention, or advanced renal disease, the urine Na output reflects accurately the intake of this ion(4). Because

TABLE II. Statistical Summary of Data.

By Student's t test, the mean ages in the groups did not differ significantly. By the same test, the mean level of sodium in the urine differed significantly among the following groups:
Low *vs* high $p < .01$
Low *vs* high (non-hypertensives) $p < .02 > .01$
Non-hypertensives (all groups) *vs* hypertensives (all groups) $p < .01$

	"Low" NaCl group	"Avg" NaCl group	"High" NaCl group	Total
Subjects	8	9	11	28
Age—yr*	42.9 ± 7.4	41.7 ± 10.6	37.3 ± 9.2	40.3 ± 10.6
Hypertension	1	2	6	9
Urine Na—mEq/day*				
(a) All subjects	155.9 ± 25.2	169.3 ± 33.7	201.5 ± 30.5	178.1 ± 35.2
(b) Non-hypertensives	150.0 ± 19.5	163.2 ± 35.7	191.2 ± 28.9	165.7 ± 32.0
(c) Hypertensives	197	191	210	204.3 ± 27.3

* Mean and S.D.

all studies reported here were done during cool or cold weather, it is unlikely that sweat losses were significant. There were no clinical grounds for expecting abnormal Na retention. There was no evidence of renal disease in the subjects except for mild albuminuria in some members with hypertension. Blood pressure measurements were made with mercury sphygmomanometer while patient sat quietly in upright position with arm resting on examiner's desk. Systolic and diastolic levels were assumed to be represented by the first and fourth auscultatory phases, as recommended by a Committee of the American Heart Association(5). Hypertension was defined as a level of at least 140 mm of mercury systolic in combination with a pressure of at least 90 mm diastolic.

Results. The data are summarized in Tables I and II. The most significant points to be noted are:

1) Groups of individuals who had been previously classified as having "low" salt intakes showed significantly lower ($p < .01$) average levels of urinary sodium excretion than individuals classified as having "high" salt intakes.

2) Individuals previously classified as having "average" salt intakes were presently found, as expected(1), to range throughout the low-average-high spectrum of sodium excretion. Statistically this group did not differ significantly from the other 2 groups.

3) The single individual in the "low" intake group who had hypertension, excreted sodium in amounts equal to members of the "high" salt-intake group rather than to his non-hypertensive companions in the "low" intake group.

4) When the 28 subjects were reclassified into 2 groups according to presence or absence of hypertension, it was found that the group with hypertension had significantly greater ($p < .01$) Na excretion than did those without hypertension.

Discussion. In view of the sedentary occupations of these subjects differences in activity among members of 3 groups with corresponding variations in sodium loss through sweat, could hardly account for these findings. There is no evidence that hypertensives have a different metabolic rate from non-hypertensives; and a metabolic study reported by Dole *et al.*(6) as well as observations in the author's laboratory reveal no correlation between changes in sodium intake and metabolic rate. Therefore it is improbable that the output of sodium is related to metabolic rate. In rats with experimental renal hypertension, it has been found that if self selection is permitted, a *reduction* of sodium intake usually occurs in association with development of hypertension(7). Aside from obvious differences in the species of animal in that study and the present one, it seems fair to say that human essential hypertension and experimental renal hypertension cannot be regarded as identical diseases(8). Indeed, Castleman and Smithwick have reported(9,10) that among individuals who

underwent bilateral sympathectomy for hypertension, histologic changes were absent or insignificant in biopsied kidneys of about a quarter and nearly the same fraction had only minimal changes.

The correlation between high salt intake (as intake is defined by urinary sodium excretion) and hypertension does not necessarily establish the dependency of one on the other but such a cause-effect relationship must be heavily implicated. More prolonged studies of a larger series of subjects are obviously necessary. Nonetheless, since the quantitative data in this relatively small group are in agreement with those of the 1346 individuals analyzed earlier, it is reasonable to anticipate further confirmation. If an enhanced salt intake is indeed found in hypertensives an important question is whether it is a manifestation, rather than a cause, of the disease. It has been suggested that hypertensives have an increased appetite for salt(11) a possibility which the work reported here cannot exclude. However, for 8 years the author has used drastic Na restriction (40-125 mg/day) under metabolic ward conditions for study and treatment of patients in all stages of this disease: no evidence of salt craving has been noted such as might have been expected if excessive salt appetite were part of the illness. Many of these patients had consumed very large amounts of salt prior to reduction of intake so that this seems relevant to the issue.

Conclusion. Among 28 subjects with and without hypertension, the group with high blood pressure had significantly greater ($p<.01$) sodium intakes (as measured by urinary outputs) than did the group of nonhypertensives. This finding was in agreement with a hypothesis previously proposed, namely, that the level of sodium intake was of primary etiologic importance in the development of essential hypertension.

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