

March 19, 1960

To the Graduate Council:

I am submitting herewith a thesis written by Magill Echols entitled "The Uptake of Sodium Ions by the Tongue." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Psychology.

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THE UPTAKE OF SODIUM IONS BY THE TONGUE

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Doctor of Philosophy

by
Magill Echols
March, 1960

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CHAPTER I

DEVELOPMENT AND STATEMENT OF THE STUDY

The History of Research in Self-selection of Salt

Self-selection is a term which refers to instances in which organisms have been observed to select foods to meet certain of their needs. The general field of self-selection and the experiments in that field have been discussed in Morgan & Stellar (1950) and by Young (1957). It has become apparent that abilities of self-selection are by no means infallible. It is more acceptable to say that there are some food deficiencies to which some organisms respond by selecting foods which reduce the deficiencies. Even this statement must be confined to organisms which have experienced no procedures to develop interfering habits.

For the present study salt deficiency is selected as a specific condition for which organisms have shown abilities of self-selection. The anecdotal accounts of animals traversing great distances to salt licks are compatible with the data of experiments (Babcock, 1905; Richter, 1936). There is ample evidence that the salt deficient animal increases its consumption of salt. For purposes of discussion salt deprivation will include the situation of the adrenalectomized animal for which the salt deficiency is well known (Morgan & Stellar, 1950).

In accounting for the behavior of organisms deprived of salt

two questions become important at the outset:

1. In what way do salt preference thresholds vary during periods of salt deprivation?
2. What processes on or about the tongue are altered during salt deprivation?

While this investigation was directed primarily at the second question, it is pertinent to review the data relating to both questions.

Concerning the first question it is necessary to identify the behavioral criteria by which variations in an organism's preference thresholds are recognized. One of these is the more frequent choice of one sample from two or more unlike samples. In measuring the threshold the samples are made progressively more alike until the organism no longer discriminates between them. The preference threshold is usually reported as the lowest discriminable concentration. There are variations in this method of determining preference thresholds, but they all use the quantity consumed as the criterion, except where human subjects are used. In such cases the individual's statement as to whether he can or cannot taste salt in a sample is the criterion. It is possible to verify the statement by repeated tests using several solutions, the concentrations of which are unknown to the subjects.

Richter (1939) determined the salt preference thresholds of normal and adrenalectomized rats. The latter animals demonstrated a greater sensitivity for salt than did the normal animals. That is,

their salt preference thresholds were well below those demonstrated by normal rats. Fregley (1955) found that sodium-deprived rats had greater sensitivity to salt than did non-deprived rats. These studies indicate that reduced salt preference thresholds are associated with conditions of salt deprivation. Pfaffmann (1957), in a review of the data in this field, stated that the salt-needy animal not only consumes more of the stronger solutions but does so at all concentrations and its preference becomes apparent even at lower concentrations.

Some apparent contradictions are encountered in relation to the second question. The mechanism most logically expected to change with salt privation is the neural excitatory threshold for the salt taste. Pfaffmann (1950) measured neural responses of gustatory fibres associated with NaCl stimuli in normal and adrenalectomized rats. He found no significant difference between the neural response thresholds of the two groups. Carr (1952) measured both neural thresholds and preference thresholds for salt in normal and adrenalectomized rats and confirmed the findings of Richter (1939) and Pfaffmann (1950).

It was concluded that salt deficiency does not alter the sensitivity of the taste receptors but that it does alter preferences. These findings offer a paradox. The data suggest that the organisms have acquired increased salt taste sensitivity during salt deficiency while the mechanism known to be involved in taste sensitivity remains unchanged. Pfaffmann (1952) demonstrated that self-regulation of salt intake depends upon stimulation of the taste receptors. He speculated (1957) that both normal and adrenalectomized animals alike were able

to taste the salt in the same dilution, but that only the latter animals were motivated to ingest it. Yensen (1958) deprived human subjects of salt and found that they became able to consistently identify salt in weaker concentration than they had prior to deprivation, and that non-deprived subjects were unable to make the same identifications.

The question remains open as to what cues enable an organism to adjust its salt consumption to meet its needs.

Statement of the Problem

We have seen a wealth of data demonstrating that salt preference thresholds decrease as salt deprivation continues. Careful studies indicate that these fluctuations cannot be accounted for in terms of changes in neural excitatory thresholds. It is reasonable to assume that something on or about the tongue may change with sodium deprivation. The problem approached in this study was the search for evidence of such a change.

The experimental hypothesis was, "that sodium ions actually penetrate the surface of the tongue according to the sodium deprivation experienced by the animal."

This was basically a search for processes associated with changes in behavior. It was attempted to demonstrate concomitant changes in the drinking of solutions of sodium chloride with the same periods of sodium deprivation.

There was also an attempt to determine the effects of sodium deprivation on the level of serum sodium in rabbits. It is known that the sodium concentration in human serum does not change significantly during sodium deprivation (Walker, Boyd & Asimov, 1952). Everett (1944) discusses the mechanisms believed to be involved in resisting changes in serum sodium concentration. Large quantities of water are excreted while sodium is reabsorbed by the renal tubules. Desoxycorticosterone plays a role in this reabsorption. Mraz* found that the sodium concentration in the serum of rats does not change significantly during sodium deprivation.

*Mraz, F. R. In an unpublished study at the University of Tennessee Atomic Energy Commission Agricultural Research Program, 1957.

CHAPTER II

METHOD OF INVESTIGATION

Principle

In testing the hypothesis that a substance is taken up by the tongue it was necessary to use a substance which could be followed from the tongue to the blood stream. This was possible through the use of radioactive tracers. Since there is a constant interchange of substances between the cells and the blood stream, it was in the blood that the labelled substance was sought.

In this study the uptake of sodium ions through the tongue was investigated. This uptake was studied under varying degrees of sodium deprivation. Concurrently the drinking of a solution of sodium chloride by the animals was studied so that any accompanying changes in sodium consumption could be observed.

An investigation was also made of the effects of sodium deprivation upon the level of serum sodium in the organisms. This was carried out in a separate study.

The possibility that the chloride ion also penetrates the tongue exists. However, it was assumed that such an occurrence is on a very small scale. Beidler* placed sodium cyanide on the tongue of a rat. The taste receptors responded immediately and no irreversible

*In a personal communication to the writer, 1957.

effects occurred. However, when a small lesion was made on the tongue the chemical entered the blood stream and was at once fatal to the animal.

Subjects

Four female New Zealand rabbits (*Oryctolagus cuniculus*) were used. They were litter mates, four months of age at the start of the experiment. They were arbitrarily assigned the numbers 1, 2, 3 and 4. Rabbits were used because of their vigorous neural response to sodium chloride (Beidler, 1955). Also the tongue of the rabbit is sufficiently large to permit drawing it well away from the mouth so that solutions do not reach the throat.

Materials

Sodium chloride labelled with Na^{24} , a gamma emitter, was used in the tests. It was procured from the Oak Ridge National Laboratories. It was available only on Mondays, thereby limiting the tests to Mondays. The isotope has a fifteen hour half-life so that the sample was no longer suitable for experimentation on Tuesday.

The counting instruments consisted of a Nuclear-Chicago DS3 scintillation detector and a Nuclear-Chicago Model 161 scaling unit.

The sodium deficient diet was prepared according to the constituents and amounts shown in Table I. The mixture was ground to the consistency of meal. In preparing the normal diet fed to the control

animals a second portion of the deficient diet was mixed and one-half of one per cent sodium chloride was added.

Serum sodium analysis was made by means of a Coleman Flame Photometer, Model 21, with readings made through a Coleman Spectrophotometer, Model 14.

Experimental Procedure

Two of the animals, #1 and #2, were alternated as control and experimental animals each week for five weeks. Each week the experimental animal was fed the sodium deficient diet and distilled water. When the animal was scheduled to be a control, it was fed the normal diet and distilled water. It also had access to a two per cent solution of sodium chloride. This was to insure normal or adequate sodium chloride for the control animal. On the morning of the seventh day all liquids were removed from all cages six hours prior to the start of the tracer experimentation. This completed the weekly schedule of deprivation.

Following each weekly test the animals were reversed in their experimental-control roles. This provided a self-control design which minimized the effects of individual differences between the animals. The test program was conducted five times on these two animals.

The other two animals, #3 and #4, were tested under other periods of sodium deprivation. One test was conducted following three days of sodium deprivation and two other tests at fourteen days

deprivation. The latter two tests were of the self-control design described above. Table II presents the experimental paradigm.

All solids and liquids provided the animals were provided in excess of their normal intake.

Sodium Uptake Test

The uptake portion of the study deals with the activity of the radioactive tracer placed on the tongue.

Each radioactive sample of sodium chloride was delivered in a solution of approximately one normal hydrochloric acid. The sample measured three millicuries of radioactivity at eight o'clock on the morning of delivery. A typical sample contained one and one-half milligram of sodium with a specific activity of two millicuries per milligram. The sample was evaporated to dryness and dissolved in sufficient sodium hydroxide solution of pH ten to reach a total sodium concentration of 0.40 normal.

The uptake test consisted of administering a dose of the radioactive solution to the tongue of the rabbit, drawing a blood sample and obtaining the radioactivity count of the blood sample. Each test was conducted in the following manner. The animal was anesthetized with ether and secured to an animal board. The mouth was propped open and the tongue drawn outward and held in a fixed position so as to expose the dorsal surface horizontally. A micropipette was used to apply ten lambda (0.01 milliliter) of the radioactive solution to the

right, forward side of the tongue. After a lapse of eight minutes a 0.40 milliliter sample of blood was drawn from the femoral vein of the animal. The process was conducted with each animal tested on a given day. Each week's blood samples were counted between 2:45 and 3:15 P.M. On a given day the samples were counted as closely together in time as possible.

Drinking Test

The drinking test was begun one hour after the uptake test was completed. This provided approximately six hours of water deprivation. The animals were placed in their cages and presented with thirty milliliter samples of a two per cent saline solution in Richter drinking tubes. No other liquids were available to them. One hour later, the amount of drinking of each animal was recorded. This test was conducted each week for a period of five weeks. A further test was conducted on the sixth week under the same sodium deprivation but without water deprivation. Distilled water was available to all animals throughout this sixth test.

At the close of the day of experimentation the animals were reversed in their experimental-control roles.

Certain variations in the radioactive samples were unavoidable as the samples were delivered in terms of total millicuries. The amount of sodium required to provide three millicuries varied with the specific activity of the sodium. However, it was necessary to obtain

the same salinity in all samples. This was accomplished by evaporating the sample to dryness and dissolving it in sufficient liquid to reach a total sodium concentration of 0.40 molar.

Kamen (1957), in a discussion of the factors influential in the active transport of sodium ions, listed pH as an important variable. Higher pH values were associated with increased transport. In the present experiment a reproducible pH was obtained by preparing one supply of a sodium hydroxide solution of pH ten. Each dry radioactive sample was dissolved in the same solution.

The specific activity of each sample was accepted at the value obtained in the redissolved sample. The ten lambda application contained from 0.18 to 0.25 millicurie from week to week. The measured counts of the blood samples were then corrected to the value corresponding to a 0.20 millicurie dose. Two of the samples fell on this value and required no correction.

A further variable was the decay in the radioactivity of the sample. This was met by taking all counts within a few minutes of a specified time. The greatest time difference for these readings was nine minutes. A period of this magnitude is negligible for an isotope with a half-life period of fifteen hours.

The problem of residual radioactivity in the animals from week to week was considered and found to be negligible after seven days had lapsed. This period amounts to eleven half-lives for the sodium isotope. Thus a sample with a count of six thousand disintegrations per minute would retain, after a lapse of eleven half-lives, less than

three counts per minute.

The problem of keeping the technique constant required that the same personnel administer each dose. The radiochemist who prepared the sample also administered each dose. With the animal secured and the tongue clamped as nearly as possible in the same position each time, the dose was applied to the right side of the tongue. The tongue was held in a horizontal position and the dose was placed well forward to avoid having the dose reach the throat. A very small volume dose (ten lambda) was applied to further reduce the possibility of liquid reaching the throat. Ten lambda is approximately one-fifth of a drop.

Precise timing was required in obtaining the blood samples. The isotope remained on the tongue and continued to enter the blood stream. It was necessary that the same period of time elapse in each test before the blood sample was drawn. This time period was eight minutes.

Each sample was counted for two minutes and the average count per minute was computed. This was corrected for laboratory background which varied only slightly from day to day.

Serum Sodium Test

A separate study was conducted to determine the effects of sodium deprivation upon the level of serum sodium in rabbits. Blood samples were drawn from seven female New Zealand rabbits which had

been fed the normal diet for one week. They were then fed the sodium deficient diet for two weeks. At the end of that time additional blood samples were drawn. The animals were then fed the normal diet for one week with access to both distilled water and a two per cent saline solution. A third set of blood samples were drawn at the end of that week.

CHAPTER III

RESULTS

Table III presents the results of the uptake and of the drinking portions of this study. The counts were corrected for laboratory background and for variation from 0.20 millicurie per dose.

Table IV gives the serum concentrations of sodium in the blood samples drawn from animals subjected to two weeks of a sodium deficient diet.

Figure 1 is a graphic representation of the relationship between days of sodium deprivation and the uptake of radioactive sodium, and the parallel drinking of two per cent saline solution.

Figure 2 shows the radioactive counts of blood samples from animals #1 and #2 during the five weeks of testing periods. Figure 3 shows the consumption of saline solution by animals #1 and #2 through six weeks of drinking tests. Figures #1 and #2 best illustrate the relationship between the experimental and control values obtained. Each week the animals exchanged the roles of experimental and control subjects. In every case the experimental animal took up considerably more of the isotope than did the control animal. The data for these animals were amenable to an analysis by the binomial expansion. The difference between the experimental and control data were found to be significant at the five per cent level. The drinking data were more random, but were free of reversals at this level of sodium deprivation.

Analysis of these data by the binomial expansion revealed a difference between the experimental and control data which was significant at the five per cent level.

The remaining two animals were tested at other periods of sodium deprivation. A trial following three days of deprivation gave a radioactive count of the same value which was obtained with no deprivation. There was a small reversal in the drinking of saline solution following three days of sodium deprivation.

Deprivation of sodium for fourteen days yielded uptake counts averaging seventeen per cent above the average for those following seven days deprivation. The corresponding change in the drinking of saline solution was a thirty-seven per cent increase. The data of the sixth week were of the same order as those of the previous weeks.

Table IV shows the values for serum sodium measurements. No significant differences were discernible between the values for the three different conditions when examined by means of the Fisher-Yates Exact Test or the Wilcoxon Matched Pair Rank Test. In these methods the middle column of values was compared separately with the first and third columns.

CHAPTER IV

CONCLUSIONS AND DISCUSSION

Major Findings

The major findings of this study were:

1. In rabbits there was a penetration of the tongue and blood stream by sodium ions placed on the surface of the tongue as sodium chloride.
2. The rate of penetration in subjects which were fed a sodium deficient diet exceeded the rate in subjects fed a normal diet, and increased periods of deprivation were associated with increased rates of penetration.
3. The drinking of a two per cent saline solution was greater in subjects fed a sodium deficient diet than in those fed a normal diet, and increased periods of deprivation were associated with increased consumption of the saline.
4. The level of serum sodium in rabbits showed no significant change following fourteen days maintenance on a sodium deficient diet.

Discussion

These data lend support to the hypothesis that there is an uptake of sodium ions through the tongue and that the uptake increases as deprivation continues. With this support it is possible to point

out a common phenomenon between physiological and psychological responses following sodium deprivation. The former is an increased uptake of sodium ions through the tongue. The latter is an increase in the organism's consumption of a relatively strong solution of sodium chloride.

Young and Falk (1956) investigated salt preferences of rats and found the range of preferred concentrations to be from 0.5 to 0.7 per cent. Carpenter (1956) reported that rabbits showed a preference in the same range and that intake fell off sharply as the concentration increased above that range. Weiner and Stellar (1951) found 0.8 per cent to be the preferred salt concentration for rats while the "aversion point" was at 1.5 per cent. The preference and aversion curves for rats and rabbits followed the same general values (Carpenter, 1956).

The two per cent salt solution used in the present study was selected so as to be well within the range consistently rejected by rabbits but not so strong as to preclude consumption altogether. The six-hour period of water privation was instituted to decrease the likelihood that the rabbits would drink the two per cent saline unless the sodium deprivation were a factor. Young (1956) reported that conditions of thirst greatly reduced the tendency of rats to drink saline at any concentration. A supplementary test was conducted with the rabbits in the present study to determine whether the increased consumption of saline by the experimental animals was to gain water rather than sodium chloride. The findings indicated that the sodium

chloride was the crucial factor.

It should be noted here that the significance of the fact that the experimental animals behaved differently in response to saline solution than did the controls is not dependent upon any theory which might account for the difference. The important point here is that the animals showed behavioral changes associated with sodium deprivation. With this established it is possible to speak of physiological changes concomitant with the behavioral changes.

The manner in which the uptake of sodium ions varied suggests a threshold process. As sodium deprivation progressed, increasing quantities of sodium ions entered the blood stream from the surface of the tongue. Bull (1951) discussed the active transport of sodium ions through certain membranes and pointed out that, other things being equal, the transport is toward the side with the lower concentration at a rate which varies with the difference in concentrations. It is interesting to speculate as to whether such processes are involved in the passage of sodium ions from the surface of the tongue into the blood stream. However, the data of this and of other studies indicate that the concentration of sodium in the blood serum does not vary with sodium deprivation.

We are confronted with the question of how, in the absence of changes in the level of serum sodium, there were changes in the rate of sodium penetration. The data of the present study do not provide the answer to this question, nor is it found in the data of other studies. However, it seems reasonable to speculate that some

intermediate step exists between the application and the uptake of the sodium. The most acceptable possibility seems that the sodium concentration of the taste cells might vary during sodium deprivation and thus alter the rate of sodium ion penetration of the cells. It is emphasized that this is only a speculation, but it carries with it some interesting possibilities. The active transport of sodium ions into the taste cells should generate electrical charges across the cell membranes (Bull, 1951). Since the taste nerve endings are located on the cell membranes (Beidler, 1952), it may be that electrical charges from active transport would stimulate them.

In such a framework we would have two thresholds involved in the receptor system for the salt taste. Pfaffmann (1957) has discussed the likelihood that preference and neural thresholds are based upon entirely different processes. The present discussion of active transport seems compatible with the experimental data.

However, the above speculations go beyond the aims of the present study. The major purpose of this study was the attempt to identify a physiological concomitant for the behavioral changes seen. To the psychologist it is not sufficient to state that organisms know instinctively when to change their intake of a substance. It is necessary to demonstrate that physiological processes are active which may serve as the basis of cues to which the organisms respond. The present study has not sought to specify the mechanics of the uptake of sodium ions. Rather, it has sought evidence of the existence of that phenomenon. The discovery of the evidence raises some questions for

future investigation.

Indications for Future Research

Tracer studies have brought clarification to problems in many fields of biological research. It seems likely that tracers can be useful in preference studies. Substances other than the sodium ion might be taken up by the tongue and this might relate to other behavioral changes. It should be especially interesting to learn whether any of the sweet tasting substances are taken up by the tongue. Since most of these are not in ionic form, it is likely that any uptake would involve entirely different principles. A most fascinating question would be whether the sweet taste is associated with the uptake of both ionized and non-ionized substances.

However, there remains much to be learned of salt preferences by means of tracer studies. An investigation of nerve action potentials in relation to sodium ion uptake, especially with near threshold concentrations, should more clearly define the processes involved. It would be interesting to know the extent to which chloride ions are taken up although this may be quite restricted. A comparison of that datum with those for other anions might help to account for some differences in preferences.

Studies of possible localization of taste modalities on the tongues of animals are possible through the use of radioactive tracers. In short, the information now available in the literature on

preference and taste research should be greatly augmented by tracer studies.

CHAPTER V

SUMMARY

A search was made for evidence of some physiological change on or about the tongue which might be associated with the behavioral changes which occur during sodium deprivation. Sodium chloride labelled with Na^{24} , a gamma emitter, was placed on the tongues of four rabbits which had experienced varying periods of sodium deprivation. Eight minutes after the application of the radioactive tracer, blood was drawn from the femoral veins of the animals and measurements for radioactivity of the blood were made. The degree of radioactivity was found to be greater in the blood of the animals which had experienced longer periods of sodium deprivation.

Concurrently (one hour after the blood extractions) the animals were given access to a relatively strong (two per cent) solution of ordinary sodium chloride. The animals which had experienced the longer periods of sodium deprivation were those which drank the larger quantities of saline. This trend persisted even when the animals had access to both saline solution and distilled water.

In a separate study it was found that the serum sodium level in seven rabbits did not change significantly following periods of sodium deprivation of the longest duration used in the previous portions of this study.

The findings indicate that some process on or about the tongue

is altered during sodium deprivation and that the drinking of saline by the animals is also altered during sodium deprivation.

These data are compatible with many other experimental data in studies of preference behavior and may be of use in dealing with some apparent contradictions in previous findings.

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APPENDIX

TABLE I
SODIUM DEFICIENT DIET

Substance	Per Cent
Yellow corn	42.4
Wheat	25.0
Beet pulp	20.0
Drachett	10.0
Potassium chloride	1.4
Dicalcium phosphate	1.0
Riboflavin	0.10
Vitamin D ₃ (3,000)	0.05
Vitamin A (10,000)	0.05

TABLE II

DAYS OF SODIUM DEPRIVATION

Week	Subjects			
	1	2	3	4
1	7	0	3	
2	0	7	0	14
3	7	0		
4	0	7	14	0
5	7	0		

TABLE III

RADIOACTIVE COUNTS AND CONSUMPTION OF 2% NaCl

Week	Subject	Days Sodium Deprivation	Corrected Counts/min.	Consumption of 2% NaCl Solution in cc.
1	1	7	4812	21
1	2	0	3235	8
1	3	3	3248	0
2	1	0	3080	6
2	2	7	6566	12
2	3	0	3553	1
2	4	14	6935	23
3	1	7	5786	16
3	2	0	4159	3
4	1	0	2692	0
4	2	7	5416	9
4	3	14	6230	20
4	4	0	2756	4
5	1	7	5719	19
5	2	0	3159	1
6	1	0		5
6	2	14		18
6	3	0		7
6	4	14		15

TABLE IV

SERUM SODIUM IN MILLI-EQUIVALENTS PER LITER

Subject	0 Days Deprivation	14 Days Deprivation	0 Days Deprivation
11	141	142	144
12	157	155	155
13	153	150	151
14	153	156	154
15	144	148	150
16	149	145	144
17	154	152	149

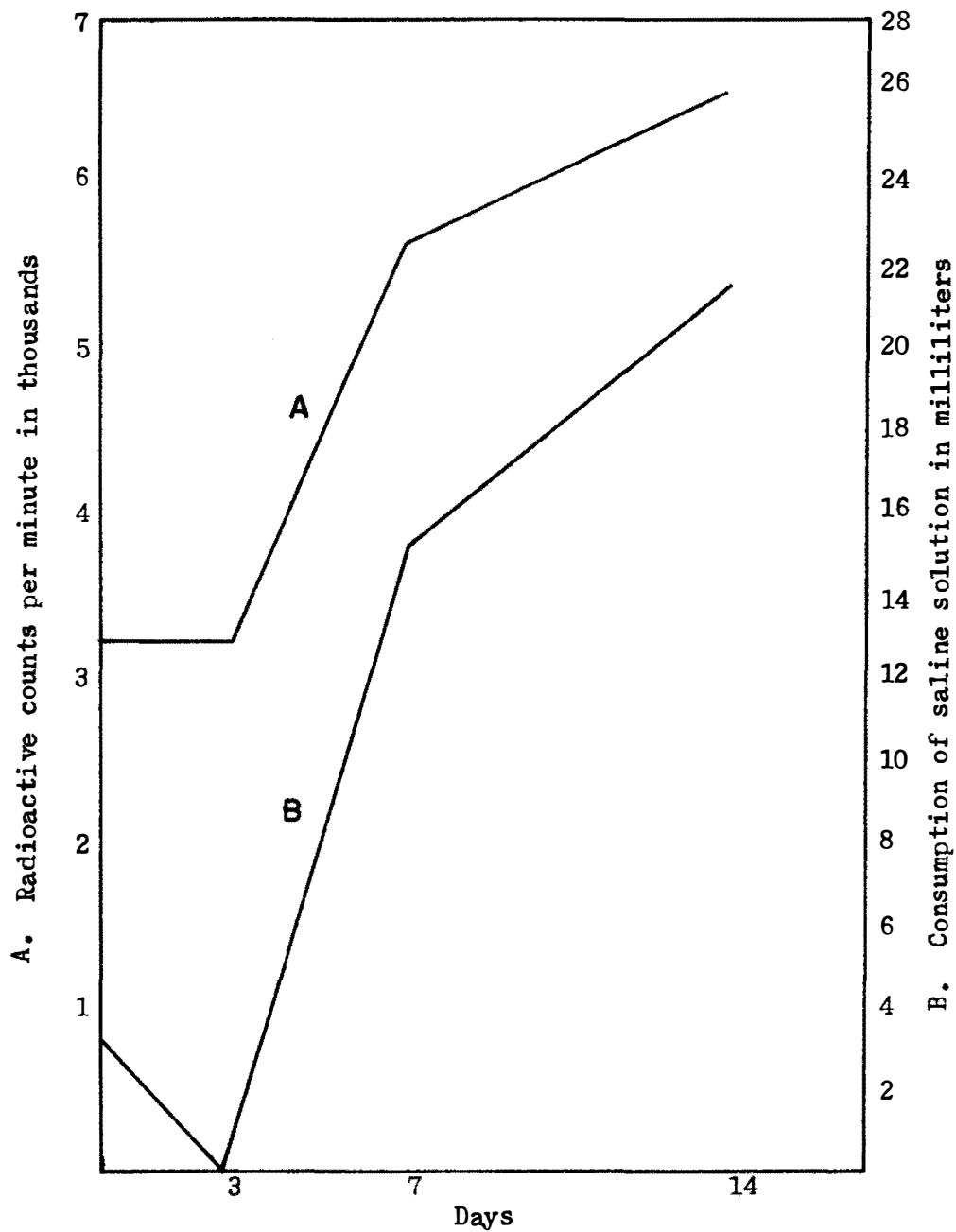


Figure 1. Relationship between days of sodium deprivation and the two dependent variables: A. radioactive counts of the blood samples and B. consumption of saline solution.

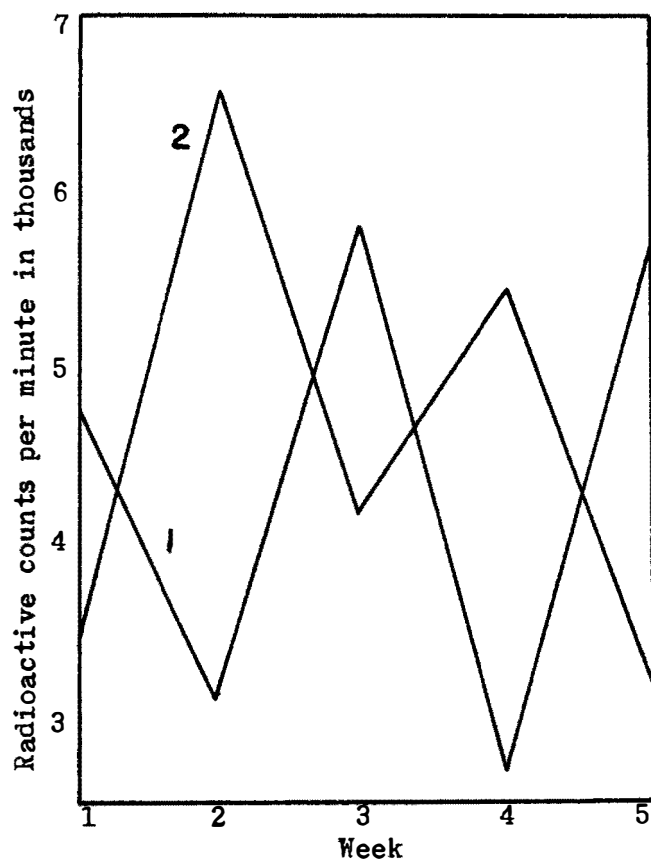


Figure 2. Radioactive counts in thousands for subjects #1 and #2.

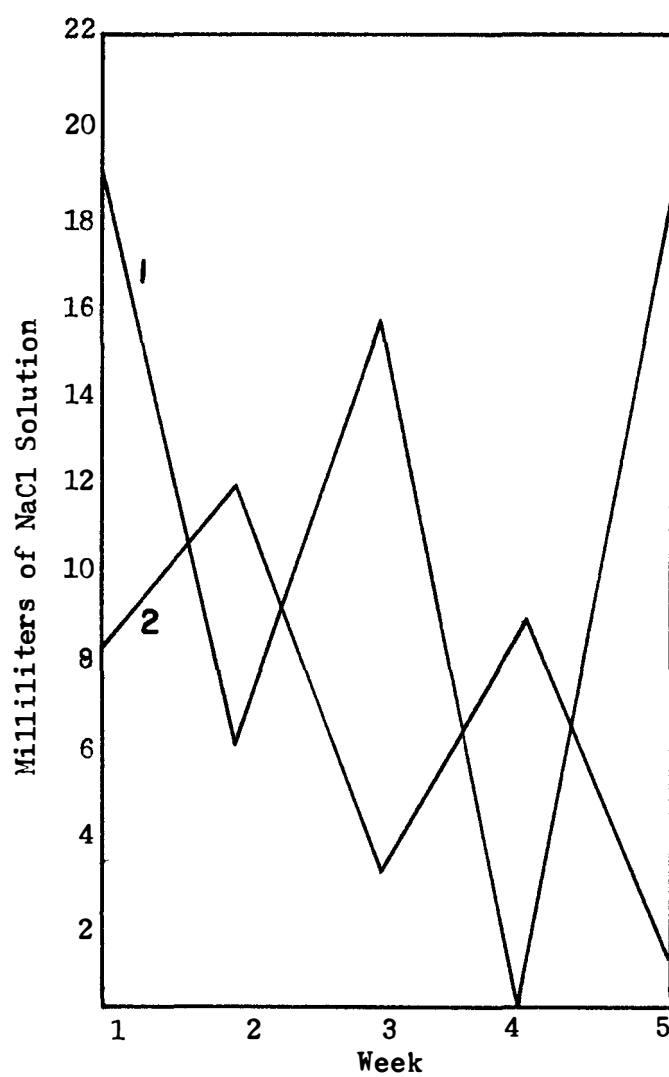


Figure 3. Drinking of NaCl solution in milliliters for subjects #1 and #2.