

TECHNICAL REPORT

71-21-FL

AD 717 667

**THE EFFECTS OF VARYING RATIOS OF MINERALS AND
VITAMINS TO CALORIES CONSUMED DURING
FOOD INTAKE RESTRICTION ON
PHYSICAL CONDITION OF RATS AND MAN**

Information from a rat experiment and literature review

by

D. Tollenaar

October 1970

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760



Food Laboratory

FL-124

This document has been approved for public release and sale;
its distribution is unlimited.

Citation of trade names in this report does not constitute
an official indorsement or approval of the use of such items.

Destroy this report when no longer needed. Do not return it
to the originator.

DISCLAIMER NOTICE

THIS DOCUMENT IS BEST QUALITY PRACTICABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

①

This document has been approved
for public release and sale,
its distribution is unlimited.

AD _____

TECHNICAL REPORT
71-21-FL

THE EFFECTS OF VARYING RATIOS OF MINERALS AND VITAMINS
TO CALORIES CONSUMED DURING FOOD INTAKE RESTRICTION ON
PHYSICAL CONDITION OF RATS AND MAN

INFORMATION FROM A RAT EXPERIMENT AND LITERATURE REVIEW

by

Dirk Tollenaar

Project Reference:
1J061102A71C

October 1970

DDC
RECEIVED
8 1970
RECEIVED
A

Food Laboratory
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts 01760

FOREWORD

The experiment reported here was conducted to document the degree to which physical performance, among other factors, in rats¹ can be affected by supplements of minerals and vitamins to certain low calorie diets already containing these minerals and vitamins. This information is necessary in evaluating assertions relative to alleged military low calorie ration design inadequacies regarding the mineral and vitamin content. The literature review presented intends to provide additional pertinent information regarding mineral and vitamin requirements during restricted caloric intake of mammals.

The work reported was conducted under Project No. 1J061102A71C.

¹ In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council, and by the National Society for Medical Research.

TABLE OF CONTENTS

	<u>Page No.</u>
ABSTRACT	iv
INTRODUCTION	1
METHODS.	2
RESULTS	
a. Running Performance	4
b. Water Consumption	5
c. Weights	5
d. Lifespan	5
DISCUSSION	
a. Food intake restriction and physical performance on different diets.	5
b. Possible deficiencies of minerals and vitamins and effects of administration during caloric deprivation	6
c. Effect of exercise on mineral and vitamin requirements and excretion.	8
d. Weight and water loss during food restriction and effects of nutrient intake.	8
e. General comments.	11
ACKNOWLEDGMENTS.	11
TABLES AND FIGURE.	12
LITERATURE CITED	16

ABSTRACT

In order to determine whether on a restricted caloric intake a higher ratio of minerals and vitamins to calories consumed than normal for ad libitum food intake is desirable, an experiment with rats was conducted and a literature review performed. Male, adult rats received a semipurified diet with two mineral-vitamin levels and two levels and kinds of fat. Under the experimental conditions, at a 40% of average ad libitum food intake, a higher ratio of minerals and vitamins to calories consumed than that normal for ad libitum food intake did not result in a significant difference in treadmill running performance. However, the relatively higher mineral-vitamin level increased water consumption significantly. The rats receiving 15% of calories from corn oil ran significantly longer than the groups receiving 41% of calories from butter. Literature reports indicate that during complete caloric deprivation additional mineral and B-vitamin intake often is beneficial. Extracellular water loss during the first days of caloric restriction has been reported to be positively correlated with the Na content of the diet prior to the period of restriction.

INTRODUCTION

The purpose of the study reported here is specifically to provide information regarding the question whether in mammals during certain restricted caloric intake conditions a need for a higher ratio of minerals and vitamins to calories consumed is indicated than on an unrestricted caloric intake, as determined primarily from exercise endurance. To this end, rats² were employed in the experiment reported, and their treadmill running endurance, lifespan and weight changes were determined with and without mineral-vitamin supplements. Further, it was deemed desirable to determine to which extent the ingestion of the supplements would affect water consumption.

In order to determine exercise endurance, treadmill running rather than swimming was employed because the former probably simulates closer the exercise and metabolism conditions such as encountered in strenuous terrain marches of man. During swimming, especially with relatively short endurance times (1), it is likely that anaerobic metabolism plays a not inconsiderable part in the provision of energy during the exercise. Furthermore, factors like a lower specific gravity of smaller animals than of larger ones of the same strain and age (2) and a negative correlation between specific gravity and total body fat content (3) can be expected to affect endurance to a different degree during swimming than during treadmill running.

Regardless of caloric intake, a minimum absolute need, in many cases at least, seems to exist for an external supply of certain minerals and vitamins, especially potassium and B-vitamins. This has been indicated by the occurrence in many cases of vitamin and mineral deficiency during total starvation (4-10). Vitamin B₆ deficiency has been observed to occur as early as the end of the first week of fasting (7). The symptoms of mineral and vitamin deficiencies occurring during fasting could be prevented or corrected by oral administration of the nutrients involved (4-12). Likewise, a negative Na and K balance during fasting or semi-starvation has been shown to be reduced by, respectively, Na (13, 14) and K (8, 15) administration. Few data are available on mineral and vitamin requirements during a partially restricted caloric intake. Slightly abnormal EEG patterns were observed in all of a group of human subjects after nine days on 400 kcal/day from sucrose plus 14 g of Kool-Aid powder without any mineral or vitamin supplement (11, 12). A group receiving a mineral supplement did not show these abnormalities. However, the ratio of K, Na, Ca, Mg and P to caloric intake for this latter group was considerably higher than would normally occur on an unrestricted, adequate diet (16-20). The K/Na ratio in the total body of rats on a partially restricted

² See footnote 1

caloric intake of a presumably relatively well-balanced diet has been found to be decreased, as compared to controls (21).

Dehydration has also been reported during partial caloric intake restriction (11, 22-25). Such dehydration may under certain conditions be detrimental to exercise performance (23). It has been shown to be reduced by Na administration (14, 25, 26), but could not be corrected by increased water consumption (23, 26, 27).

METHODS

Male, adult, Sprague-Dawley rats, randomly bred, were housed individually, at $24 \pm 1.5^\circ$ and $50 \pm 3\%$ relative humidity, in stainless steel cages with wire-mesh bottoms. Light was provided from 6:30 a.m. to 7:00 p.m. At arrival, these animals were 18 weeks old and weighed 425 ± 35 g (mean \pm SD).

These rats were fed a commercial rat food² and tap water ad libitum for the first five weeks (table 1). After the first week, the animals were subjected to training on a treadmill. The design of this treadmill was a modification of that used by Kimeldorf (28). It consisted of an endless, motor-driven, neoprene-impregnated belt which could be elevated with a hydraulic jack. A rack over this belt contained ten cages in two rows of five, with translucent plastic walls and top, the two rows being in tandem according to the running direction. The top panels contained numerous perforations, and the bottom 9 mm of the front and rear of each cage was open to allow air circulation. Further, the rear of each cage at the inside had a hinged panel attached with a shocking grid which was wired to a constant current shocker with shock scanner³, providing a current of about 3 ma. Between the hinged panel and the rear wall of each cage a micro switch had been installed, wired to a ten channel event recorder⁴ and individual time-delay relays, the latter wired, in turn, to individual lights and one common buzzer. The intensity of exercise (running time, speed, and inclination of the running surface) was gradually increased over a period of six weeks with twice-weekly training periods. The adult Sprague-Dawley rats proved relatively difficult to train in comparison to adult Long-Evans rats used in later experiments. Of the 78 animals used at the start, only 32 of the best performing animals were retained. Following this six-week training period, the rats were run to exhaustion five times during three weeks (table 1), and running times were recorded.

² Purina Laboratory Chow, Ralston Purina Company, St. Louis, Mo.

³ Lehigh Valley Electronics Inc., Fogelsville, Pa.

⁴ Esterline Angus Instrument Co., Inc., Indianapolis, Ind.

After the period on the commercial rat food, the rats were divided into two groups of equal number. Each of these groups received a different amount and composition of fat. These two kinds of fat supply were a level and degree of saturation as commonly used in human diets in the United States, and a low level with a low degree of saturation and relatively high degree of polyunsaturation. Thus, one group received a semipurified diet deriving 14% of the calories from fat in the form of corn oil (low fat group); the other group received an, otherwise, similar diet but having 41% of the calories provided by butter as the fat source (designated henceforth as 'high' fat group). The composition of these diets, including that of the mineral-vitamin mixture provided, was as previously described (1). As in this previous experiment, the diets were kept refrigerated. It has been reported (29) that thiamine stability in wet diets is adversely affected by commercial casein treated with sulfur dioxide. However, under the conditions of dry diets, refrigerated storage, and with the composition of mineral mixture employed, the loss of thiamine per week in any of the food mixtures did not exceed 15% (1). After five weeks on the semipurified diets, the 'high' and the low fat groups were subdivided into two groups⁵ of eight animals each. One group was fed an amount of food (9.0 g/rat/day) which was approximately 40% of the average previous ad libitum consumption. The other group had a similarly restricted caloric intake but in addition received an amount (0.62 g/rat/day) of mineral-vitamin mixture which increased the intake of these minerals and vitamins to approximately that of the rats fed ad libitum. The minerals and the vitamins in the supplements were provided in the same ratio to each other as their mutual ratio in the diets. The period during which the caloric intake was thus restricted lasted for ten days. Thereafter, all groups were subjected to complete caloric deprivation, primarily to determine lifespan. During this latter period, the groups previously receiving the mineral-vitamin supplement continued to receive an amount (1.0 g/rat/day), which maintained the intake of minerals and vitamins at approximately the level consumed by the rats fed ad libitum. The same amount and composition of synthetic sweetening ingredients as previously used was added (1). All animals consumed this sweetened mineral-vitamin mixture completely. The mineral-vitamin mixture was prepared each week and stored refrigerated. All food or mineral-vitamin mixture, provided after 9 a.m., always was consumed before 11:30 a.m. Water was supplied ad libitum. During the period when the semipurified diets were provided, the drinking water used was all-glass distilled. Its resistance always was in excess of 10^6 ohms/cm² x cm at about 23°.

⁵ The following abbreviations will be used henceforth:
LF: low fat, reduced food intake, with corn oil as the fat source;
HF: 'high' fat, reduced food intake, with butter as the fat source;
+MV: plus additional minerals and vitamins.
Bracketed symbols indicate diet prior to complete caloric deprivation.

All running to exhaustion during the preliminary period and during the main phase of the experiment was conducted from about 1 p.m. on, in order to eliminate possible diurnal rhythm effects on performance, and to allow some time after the food intake. Four running endurance tests were performed during the main phase of the experiment, namely, 3, 7, 10 and 14 days after the start of the limited caloric intake. Running speed was 66 fpm.

RESULTS

a. Running performance. During the period on ad libitum intake of the semi-purified diets, the average running times to exhaustion for what were to become the four different groups were: LF⁶, 34.8 ± 6.7 (mean \pm SD); LF + MV, 35.0 ± 4.9 ; HF, 31.2 ± 6.4 ; HF + MV, 31.3 ± 4.1 minutes, respectively. It should be borne in mind that the differentiation between fat intake was started two weeks prior to these preliminary running tests to exhaustion. The average preliminary running performances to exhaustion in kgm⁷ were: LF, 48.3 ± 8.2 ; LF + MV, 48.5 ± 10.4 ; HF, 48.6 ± 8.7 ; HF + MV, 49.2 ± 9.2 . For the period of partial food deprivation, the endurance times and performance in kgm for the rat groups at different times are shown in table 2. Split-plot factorial analysis of variance (30) of the values obtained over all three test periods indicated that the difference in running times between the LF and HF groups was significant ($P < 0.025$). This comparison is probably valid since care had been taken to equalize environmental conditions as closely as possible for all (future) subgroups, and choice of animals for each subgroup had been at random. Some evidence for the closeness of this matching would seem to be offered by the near identical performance results, during the preliminary running tests, between the low fat groups, and also between the two 'high' fat groups. It should be remembered that the LF and HF groups had received both a different level and a different kind of fat. After seven days on the restricted food intake the animals ran significantly longer ($P < 0.05$) than after three days, if in the calculation all diet groups are included. None of the other differences in running performance was significant at the $P < 0.05$ level. After 14 days on the experimental 'diet' regimen, the running times for the (HF) and (HF) + MV⁵ groups, adjusted for the average preliminary times were, respectively, 34.1 ± 15.2 and 34.8 ± 11.6 min. (mean \pm SD). Their performance in kgm, similarly adjusted, was, respectively, 47.9 ± 21.2 and 48.3 ± 16.1 . Each of these groups was comprised of six animals. In the other groups, the number of animals that could perform adequately on the treadmill had become too small at this time to afford a meaningful comparison.

⁶ See footnote 5

⁷ kgm = weight of animal in kg, multiplied by distance elevated during running in m.

b. Water consumption. The water consumption during the first 11 days on the experimental diet regimen is given in table 3. Split-plot factorial analysis of variance of all data over this period indicates that the difference due to mineral-vitamin level was highly significant ($P < 0.01$), and that due to fat level and kind of fat was significant ($P < 0.025$). On the average, during these 11 days, the animals in the LF + MV group drank 11.5% more than those in the LF group, and the rats in the HF + MV group drank 11.6% more than those in the HF group. Further, under the same conditions, the animals in the LF group drank 10.9% more than those in the HF group, and the rats in the LF + MV group drank 10.7% more than those in the HF + MV group. The difference due to mineral-vitamin level tended to be only fractionally higher at the end of the 11-day period than at the beginning. The average difference during the first four days for the LF + MV versus the LF group and for the HF + MV versus the HF group was, respectively, 11.3 and 10.5%; that during the last four days was, respectively, 11.7 and 12.6%.

c. Weights. Average weights for each of the four groups are shown in figure 1. The average weights for the rats fed the 'high' fat diet at all times were higher than those for the animals fed the low fat diet. The higher mineral-vitamin levels and higher water consumption of the groups receiving these during the period of caloric restriction was not reflected in a higher weight retention. Further, the loss of weight of the 'high' fat groups approximately paralleled that of the low fat groups during this period of caloric restriction, notwithstanding the higher daily caloric intake of the former groups.

d. Lifespan. The lifespan, in days, from the start of caloric restriction, for the different groups was as follows (mean \pm SD):

(LF)	14.0 \pm 1.3	(HF)	18.3 \pm 4.3
(LF) + MV	14.3 \pm 1.9	(HF) + MV	17.8 \pm 2.8

Factorial analysis of variance indicated a highly significant ($P < 0.005$) difference due to level and kind of fat.

DISCUSSION

a. Food intake restriction and physical performance on different diets.

Running endurance was selected as the criterion for physical performance in the author's experiment described above. However, it should be realized that various types of physical performance can be affected to a different degree by varying severity and duration of food restriction. For example, a few days of

complete food deprivation causes a far greater decline in coordination than in strength in the human (31). In contrast, prolonged semistarvation produces greater losses in strength than in coordination. Auditory acuity was not found to be decreased by prolonged semistarvation.

Fat turned out to be the only factor significantly affecting running endurance under the conditions of the author's present experiment. It seems likely that the approximately 15% higher weight of the HF group was an important causal factor in the difference in results between the performance expressed in time and that expressed in kgm. One might wish to compare in general the relative usefulness of these two types of measurement, namely, 'unloaded' running times with performance data in kgm as a gauge for running endurance of subjects with a load on their back. The relative values in kgm should then receive a higher weighting, the higher the ratio of external load weight to body weight, and vice versa. Further, energy expenditure per day will affect deterioration of performance with time during food restriction.

The exercise performance data in table 2 for the groups LF, LF + MV, HF, and HF + MV reflect relative weights of food intake of 1.000, 1.069, 1.000, and 1.069, respectively, and relative caloric intakes of 1.00, 1.00, 1.18, and 1.18, respectively.

Increased lifespan for the groups which had received 40% of calories from butter with and without extra minerals and vitamins was probably due to the higher average weight and, presumably, higher energy reserves of the animals in these groups.

b. Possible deficiencies of minerals and vitamins and effects of administration during caloric deprivation.

In fasted obese human subjects not receiving KCl supplements, weakness and lassitude frequently developed after four to six weeks (8). The supplements prevented the occurrence of these symptoms. The urinary K/N ratio for the initial two to three weeks of fasting exceeded that for 'fat-free protoplasm' in such subjects (8, 15, 32). The K/N ratio has been observed to fall in the total body of rats fasted for three to six days (9). On the first day of food deprivation, tissue losses of K in rats were found to be three to eleven times higher than those of Na (26). Consolazio et al. (33) found that on the first day of starvation of human subjects blood K was significantly increased (but was decreased on the fifth and tenth day measured). However, K as a percentage of muscle protein (9) and body protein (21) has been reported to remain unchanged in rats fasted for periods varying from three days to five weeks. Initial K loss during food deprivation is probably to some extent related to glycogen loss (26, 34, 35). Mineral concentration of the plasma during food deprivation with unrestricted water intake, generally, has been found to remain normal (4, 9, 13, 23, 36), although K tended to decrease to a low normal level.

Carbohydrate ingestion during fasting has been shown to reduce K and Na losses (14, 37-40). However, Rogers et al. (22) observed no appreciable effects in this manner from carbohydrate ingestion. It may be that cold diuresis (41) was a dominant factor here. Further, refeeding with glucose after fasting has been found to decrease urinary Ca, Mg and P losses (42). Protein, also, has been found to decrease the urinary Na loss of fasting in man (13, 39), as well as reduce urinary K and Na losses in comparison to those on a low, equicaloric intake of fat (43). But the reduction of Na loss was not apparent until the second day of protein feeding (39), whereas it was seen on the first day of a carbohydrate regimen. Further, the cumulative retention of Na over four days of refeeding was less with protein than with carbohydrate, although by the fourth day the positive balance of Na achieved with protein was comparable to that on the carbohydrate regimen. The feeding of fat alone has been found to have little or no effect on the Na excretion during fasting (38, 40). The aforementioned experiments of Consolazio et al. (11, 12) indicate a minimum external requirement of certain minerals remaining on a low caloric intake from carbohydrate, and prevention of slightly abnormal EEG patterns by mineral intake under these conditions. The plasma Ca concentration of young adult human volunteers was decreased after 24 weeks on an average daily intake of 1570 kcal from a mixed diet (44). The drinking of a KCl solution produced a decrease in the negative K balance of rats, as determined during the first two days of starvation, without increasing the negative Na balance (26). In man, administration of K during starvation also has been found to decrease the net K loss (8, 15), to increase low serum K levels (8, 45), and to prevent or alleviate any symptoms of weakness and lassitude (8). However, abdominal discomfort and nausea were often reported by subjects after ingestion of large doses of rapidly dissolving K salts during the fasting state (8). An intake of Na during complete food deprivation of man and rats has been found to reduce a negative Na balance (14, 40) or to result in a positive balance (13, 26). However, ingestion of Na has been found to increase K excretion (8, 14), with a concomitant withdrawal of intracellular water.

During the period on about 40% of ad libitum caloric intake in the author's present experiment, the rats getting supplements received in these supplements about 128 mg K/kg body weight x day (equivalent to 244 mg KCl/kg x day), and about 83 mg Na/kg body weight x day (equivalent to 211 mg NaCl/kg x day).

Symptoms of vitamin deficiency, especially of the B-vitamins, have been demonstrated during starvation of man (4-7, 10). Symptoms of inadequate vitamin B₆ levels in human subjects have been shown to occur as early as the end of the first week of complete food deprivation (7). Swenseid et al. (10) found no

consistent ratio between the amounts of riboflavin, pantothenic acid and biotin excreted, and N loss. Windmuelleer et al. (46) have observed that during a 24- to 37-hour fast of normal human subjects the riboflavin-to-N ratio in the urine was markedly increased. However, during the first few days of food deprivation, the change in the fecal flora may affect the relative loss of some of the vitamins.

Symptoms of vitamin deficiency during fasting have been shown to be prevented by oral administration of such vitamins (4-7). However, results with riboflavin supplements suggest that the starving human subject can store and utilize supplements like this only ineffectively (10).

Thiamine, riboflavin and vitamin B₆ requirements have been found to be positively correlated with caloric or protein intake (47, 48). However, as indicated above, without caloric intake a 'residual' requirement for certain vitamins remains.

The mineral-vitamin supplement used in the author's experiment, described in this report, increased water consumption by about 12%.

c. Effect of exercise on mineral and vitamin requirements and excretion.

Exercise has been reported to increase the K requirement in rats (49, 50). However, Castenfors (51) did not find urinary K excretion to be significantly changed in human subjects during bicycle ergometer exercise. Ca (52) and P (53) excretion in humans have been found to be increased during muscular activity. Further, the requirement for thiamine has been observed to be increased in human subjects during heavy exercise (54). The same vitamin was found to have a beneficial effect in dogs run to exhaustion (55).

In the author's experiment with rats, described in this report, the average duration of the running involved was about 125 min. in 10 days. At a speed of 66 fpm and average inclination of the treadmill belt of 9°, this amount of exercise is not likely to have increased the requirement of any mineral or vitamin to more than a modest degree. Adult animals were used. The amounts per unit weight of diet of each mineral and vitamin supplied in this and a previous (1) experiment of the author were equal to or higher than the requirements for the growing rat given by the National Research Council (56). One would expect that such levels, apart from possibly changed requirements during caloric restriction, would be adequate in adult rats for the average amount of exercise involved.

d. Weight and water loss during food restriction and effects of nutrient intake.

Extra- and intracellular water and weight loss, especially during the first days of food intake restriction, is a generally observed phenomenon (11, 24-26, 32, 33, 57-60). Part of this initial body weight and water loss is due to loss of

gut contents. Such extracellular water loss, accompanied by a decrease in blood and plasma volume (57-59, 61), has been found in some instances, even on unlimited water intake, to be combined with an increase in hematocrit (23, 59, 62) and blood viscosity (23). Although these effects may be enhanced by cold diuresis (41), an observed increase in hematocrit is not restricted to a cold environment. An increase in hematocrit, accompanied by an increase in blood viscosity, may have a detrimental effect on work performance under certain conditions. That such body water loss is not necessarily due to a voluntary restriction of water intake is indicated by the findings of Millar, Morrison et al. (25, 26) who report that during the first two days of starvation rats drank greatly in excess of the apparent need.

Morrison (60) reports that, after 4 days of starvation of rats, previously fed Purina chow, absolute extracellular space was decreased. However, the thiocyanate space as a percent of lean body mass was unchanged. Similar information has been obtained by others regarding an unchanged ratio of body water to fat-free solids (9) and to total protein (21, 63) in rats after 3 to 6 days of starvation. These results with rats might, at least partially, be explained by a relatively low Na content of the diet prior to food deprivation.

Carbohydrate or protein ingestion has been shown to decrease the Na and body water loss of fasting in man (13, 14, 37-40, 59). In rats undernourished for five weeks an increase at the end of this period of the ratio of body water to total body protein was found (21). Keys et al. (29) also reported an increase in relative plasma and extracellular fluid volume in their semistarvation studies. However, signs of oedema were observed in their semistarved subjects. Human subjects receiving, besides unlimited water, nothing but 100 g of glucose daily, had an extra- and intracellular water loss per 70 kg body weight during six days of semistarvation of 720 and 876 ml in total, respectively, or 5 and 2% of the estimated extra- and intracellular compartment size, as compared to values of 10 and 3% during complete food deprivation (14). During partial food deprivation of man (1010 kcal/day) with unrestricted water intake, the highest rate of water loss was found to occur during the first three days (24).

In the author's experiment with rats, described above, the average weight loss seven days after the start of the food intake restriction for all groups was 8.8% of their weight at the start of the restricted intake period. Ten days after the start of food intake restriction, this average weight loss was 15.1%.

In attempting to relate weight losses to physical performance, however, one should be careful in comparing results, even for the same species, if different diets have been used shortly before or during food deprivation. For example, initial extracellular water and weight loss during food intake restriction has been

shown to depend, among other factors, on NaCl intake during the period shortly before food deprivation. A positive correlation has been found to exist between extracellular water balance, Na balance, and daily change in weight at various caloric intake levels (14, 25, 32, 36-38, 57, 59, 62). Millar and Morrison (26) have reported that in rats body losses of Na and K during the first two days of food deprivation were smaller when the level of Na and K in the preliminary diet was lowered. Similar results in the human regarding Na have been found by Katz et al. (39). Both net total water loss and extracellular water loss were smaller under these conditions. This indicates that the Na intake level before a food deprivation period, through its possible effect on blood hematocrit and blood viscosity subsequently, under certain conditions may be a factor affecting work performance.

In the author's present experiment the Na intake level before the semi-starvation period was relatively low (593 mg as NaCl per 100 g diet; but 466 percent of the Na requirement for the growing rat, according to the National Research Council (56)), as compared to levels frequently used by man (16, 47).

The positive correlation between NaCl intake and extracellular water retention raises the question whether a relative 'overhydration' in man often occurs on an adequate diet relatively high in NaCl, as is frequently used. The increased extracellular fluid volume and increased blood pressure as a result of a relatively high NaCl intake (45, 64-68), and the loss of extracellular fluid in normal human subjects when NaCl is restricted in the presence of quite adequate diets (69) indicate this possibility. If subjects change to a reduced caloric intake with a, usually, concomitant reduction in Na intake, the resulting loss of body water, therefore, does not necessarily imply a hypohydration but can actually mean an adjustment to a more normal hydration state.

During complete food deprivation of man and rats, an intake of Na has been found to reduce a negative Na balance (14, 40) or to result in a positive balance (13, 26), in turn resulting in some reduction of extracellular water loss (14, 25, 26, 36) and weight loss (22). Urinary excretion of Na and weight loss continued at a slightly higher rate after the substitution of an equivalent amount of NaHCO₃ for the NaCl during the fasting period (40). Ingestion of Na, however, has been found to increase K excretion (8, 14), with a concomitant withdrawal of intracellular water. Further, water retention due to additional mineral uptake, primarily Na, may even result in some pitting oedema in man during starvation (36). Water loss in rats and rabbits, deprived of food, was not decreased by drinking a 0.75%, and 0.58% KCl solution, respectively (25, 26, 70). Neither did the KCl solution increase the water intake (possibly because of the bitter taste?) of the rats during the first two days of starvation that measurements were made. Equimolar NaCl solution under the same conditions did increase the water intake, compared to distilled water as drinking fluid.

If NaCl in addition to K-salt is given orally, the former will camouflage to some extent the bitter taste of certain K-salts (71).

Intracellular water loss always takes place during caloric deprivation. Initially, this is reported to be linked primarily to K loss, which, in turn, initially to some extent is probably related to glycogen loss (26, 34, 35). In a later stage, intracellular water and - mineral loss becomes more closely related to tissue and N loss (9, 15, 26).

The reason the supplements did not reduce weight loss during the caloric restriction phase in the author's experiment, described in this paper, is probably because of the combined effect of the relatively low Na intake prior to the period of food deprivation, and that of carbohydrate and protein intake during that period. A decreased rate of weight loss on these supplements was only noticeable during the first week on a one-third of ad libitum caloric intake in a previous, partially comparable, experiment (1).

e. General comments.

The need during caloric intake restriction for higher than 'normal' concentration levels (i.e., as used in an ad libitum fed diet, and adequate according to recommendations by the National Research Council (56) on this basis) of minerals and vitamins in the food, providing more than a certain minimum absolute amount, has not been indicated under the conditions of the author's experiment described above. Such a need, actually, has been contraindicated under the conditions of a previous experiment by the author. Further, the mineral-vitamin supplement increased water consumption.

During complete food deprivation, at least in man, it seems, from the reports mentioned, that a good case can be made for the gradual supply of K, some Na in certain cases, and of B-vitamins.

ACKNOWLEDGMENTS

The author wishes to thank J. W. Ratteree for his valuable technical assistance, and Dr. W. K. Calhoun for his support which has helped to make the present work possible.

TABLE 1
Feeding and treadmill running schedule

Feeding		Treadmill running	
Diet ¹	Days from beginning	Intensity ¹	Days from beginning
Commercial food	1 through 35	Training, twice weekly	8 through 46
Semipurified diets, ad libitum	36 through 70	Running to exhaustion	50, 53, 57, 60, 67, 74, 78, 81, and 85
Semipurified diets, restricted intake	71 through 80		
Complete caloric deprivation	from day 81 on		

¹ Explanation of diets and treadmill running in text.

TABLE 2

Effect of different diet regimens on running performance
at different times during the diet treatments

Period on restricted food intake	Diet groups ¹			
	LF	LF + MV	HF	HF + MV
Running times, min.				
3 days	40.7± 8.2 ²	43.2±13.8	36.4± 8.3	37.0±13.4
7 days	45.5± 6.4	45.8± 8.0	41.2± 9.3	39.1±11.2
10 days	44.4±11.2	42.6±14.8	37.0±11.4	45.9± 9.5
Running performance, kgm				
3 days	61.1±12.7	66.4±20.3	63.2±12.9	63.3±23.8
7 days	64.4± 8.6	65.6±11.5	67.8±14.8	63.5±20.5
10 days	58.2±13.3	57.5±19.3	57.7±18.8	69.1±15.1

¹ LF = low fat, corn oil as fat source; HF = 'high' fat, butter as fat source;
+ MV = plus additional minerals and vitamins

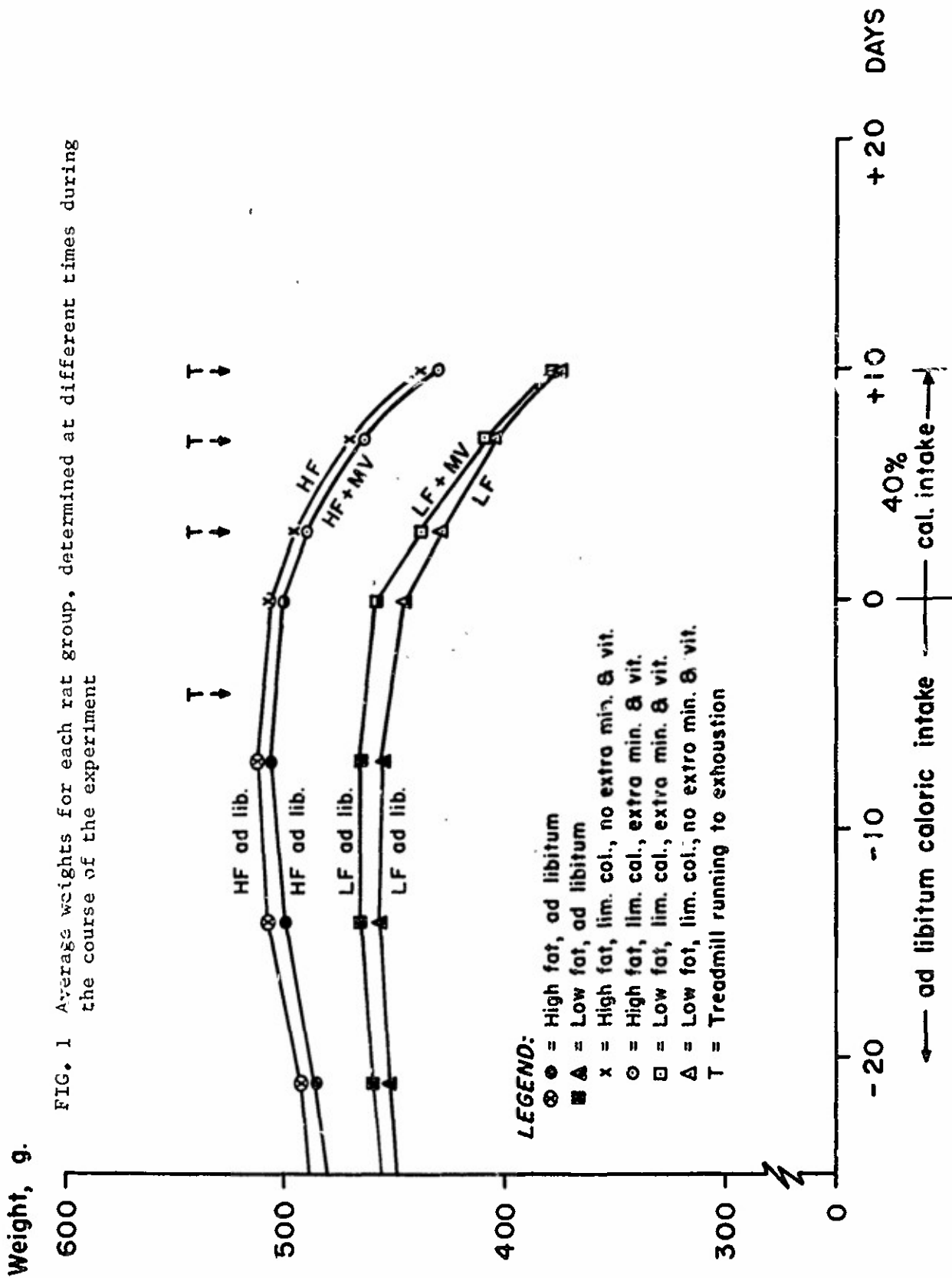
² Mean + SD. The performance values for the LF and LF + MV groups were corrected for the (very slight) difference in average performance between these, future, groups as found during the preliminary running tests. The same was done between the HF and HF + MV groups.

TABLE 3
 Effect of different diet regimens on water consumption
 at different times during the diet treatment

Period on restricted food intake	Diet groups ¹			
	LF	LF + MV	HF	HF + MV
	Water consumption, ml/ratxday			
2 days	28.7± 9.1 ²	33.4± 7.1	25.7± 4.8	26.8± 4.5
4 days	29.2± 8.7	32.2±10.7	26.7± 8.5	28.2± 7.3
7 days	26.0±10.0	29.7± 9.5	25.6± 9.0	30.2± 7.7
9 days	30.6± 8.1	34.7± 7.1	27.6± 6.0	35.6± 9.6
11 days	30.0± 6.5	36.0± 8.8	27.6± 7.8	33.7± 6.3

¹ LF = low fat, corn oil as fat source; HF = 'high' fat, butter as fat source;
 + MV = plus additional minerals and vitamins.

² Mean ± SD.



LITERATURE CITED

1. Tollenaar, D. 1966. Effects of mineral and vitamin supplementation on swimming times and other parameters related to performance of rats on a low calorie regimen. *J. Nutr.*, 90: 441.
2. Scheer, B. I., S. Dorst, J. F. Codie and D. F. Soule. 1947. Physical capacity of rats in relation to energy and fat content of the diet. *Amer. J. Physiol.*, 149: 194.
3. Rathbun, E. N., and N. Pace. 1945. Studies of body composition. I. The determination of total body fat by means of the body specific gravity. *J. Biol. Chem.*, 158: 667.
4. Drenick, E. J., M. E. Swendseid, W. H. Blahd and S. G. Tuttle. 1964. Prolonged starvation as treatment for severe obesity. *J. Amer. Med. Assn.*, 187: 100.
5. Scheck, J., H. Spencer, I. Lewin and J. Samachson. 1966. Mineral and protein losses during starvation. *J. Amer. Diet. Assn.*, 49: 211.
6. Drenick, E. J., E. Vinyard and M. E. Swendseid. 1969. Vitamin B₆ requirements in starving obese males. *Amer. J. Clin. Nutr.*, 22: 70.
7. Vinyard, E., C. B. Joven, M. D. Swendseid and E. J. Drenick. 1967. Vitamin B₆ nutriture studied in obese subjects during 8 weeks of starvation. *Amer. J. Clin. Nutr.*, 20: 317.
8. Drenick, E. J., W. H. Blahd, F. R. Singer and M. Lederer. 1966. Body potassium content in obese subjects and potassium depletion during prolonged fasting. *Amer. J. Clin. Nutr.*, 18: 278.
9. Huth, E. J. and J. R. Elkinton. 1959. Effect of acute fasting in the rat on water and electrolyte content of serum and muscle and on total body composition. *Amer. J. Physiol.*, 196: 299.
10. Swendseid, M. E., G. Schick, E. Vinyard and E. J. Drenick. 1965. Vitamin excretion studies in starving obese subjects. Some possible interpretations for vitamin nutriture. *Amer. J. Clin. Nutr.*, 17: 272.
11. Consolazio, C. F., L. O. Matoush, H. L. Johnson, H. J. Krzywicki, G. J. Isaac and N. F. Witt. 1968. Metabolic aspects of calorie restriction: hypo-hydration effects on body weight and blood parameters. *Amer. J. Clin. Nutr.*, 21: 793.

12. Consolazio, C. F., L. O. Matoush, H. L. Johnson, H. J. Krzywicki, C. J. Isaac and N. F. Witt. 1968. Metabolic aspects of calorie restriction: nitrogen and mineral balances and vitamin excretion. *Amer. J. Clin. Nutr.*, 21: 803.
13. Hamwi, G. J., M. C. Mitchell, R. C. Wieland, F. A. Kruger and S. S. Schachner. 1967. Sodium and potassium metabolism during starvation. *Amer. J. Clin. Nutr.*, 20: 897.
14. Gamble, J. L. 1947. Physiological information from studies on the life raft ration. *The Harvey Lectures Series*, XLII, 1946-1947, pages 247-273.
15. Birkenhäger, J. C., A. Haak and J. C. Ackers. 1968. Changes in body composition during treatment of obesity by intermittent starvation. *Metab.*, 17: 391.
16. Dahl, L. K. 1958. Salt intake and salt need. *N. Engl. J. Med.*, 258: 1122, 1205.
17. Wilde, W. S. 1962. Potassium, in: Comar, C. L. and F. Bronner (eds.) *Mineral Metabolism*, vol. 2, part B, p. 73. Academic Press, New York.
18. Manalo, R., R. E. Flora and J. E. Jones. 1967. A simple method for estimating dietary magnesium. *Amer. J. Clin. Nutr.*, 20: 627.
19. Watt, B. K. and A. L. Merrill. 1963. *Composition of Foods*. Agric. Handb. No. 8. U. S. Dept. Agric., Washington, D. C.
20. Sherman, H. C. 1947. *Calcium and Phosphorus in Foods and Nutrition*. Columbia Univ. Press, New York.
21. Widdowson, E. M. and R. A. McCance. 1956. The effects of chronic undernutrition and of total starvation on growing and adult rats. *Brit. J. Nutr.*, 10: 363.
22. Rogers, T. A., J. A. Setliff and A. C. Buck. 1965. Ameliorative measures in fasting, subarctic survival situations. Tech. Docum. Rep. AAL-TR-65-10, Arctic Aeromed. Lab., Alaska.
23. Rogers, T. A., J. A. Setliff and J. C. Klopping. 1964. Energy cost, fluid and electrolyte balance in subarctic survival situations. *J. Appl. Physiol.*, 19: 1; 1963, Tech. Docum. Rep. AAL-TDR-63-16, Arctic Aeromed. Lab., Alaska.
24. Keys, A., H. L. Taylor and J. Brzezek. 1958. Nutrition and performance capacity. Contract DA44-109-QM-1526, Rep. 13. Clearing House (CFSTI), Springfield, Va.

25. Morrison, S. D., G. Mackay, E. Hurlbrink, J. W. Wier, M. S. Nick and F. K. Millar. 1967. The water exchange and polyuria of rats deprived of food. *Quart. J. Exp. Physiol.*, 52: 51.
26. Millar, F. K., and S. D. Morrison. 1968. Relation of tissue electrolyte losses to the relative polydipsia of early starvation in rats. *J. Nutr.*, 94: 211.
27. Consolazio, G. F., L. O. Matoush, H. L. Johnson, R. A. Nelson and H. J. Krzywicki. 1967. Metabolic aspects of acute starvation in normal humans (10 days). *Amer. J. Clin. Nutr.*, 20: 672.
28. Kimeldorf, D. J. 1961. The measurement of performance in small laboratory animals. In: *Performance Capacity. Symp. QM Food & Cont. Inst. Armed Forces*: 99.
29. Joslyn, M. A. and J. Leichter. 1968. Thiamine instability in experimental wet diets containing commercial casein with sulfur dioxide. *J. Nutr.*, 96: 89.
30. Steel, R. G. D. and J. H. Torrie. 1960. *Principles and Procedures of Statistics with Special Reference to the Biological Sciences.* McGraw-Hill Book Co., New York.
31. Keys, A. 1946. Nutrition and capacity for work. *Occup. Med.*, 2: 536.
32. Smith, R. and E. J. Drenick. 1966. Changes in body water and sodium during prolonged starvation for extreme obesity. *Clin. Sci.*, 31: 437.
33. Consolazio, G. F., L. O. Matoush, H. L. Johnson, R. A. Nelson and H. J. Krzywicki. 1967. Metabolic aspects of acute starvation in normal humans (10 days). *Amer. J. Clin. Nutr.*, 20: 672.
34. Fenn, W. O. 1939. The deposition of potassium and phosphate with glycogen in rat livers. *J. Biol. Chem.*, 128: 297.
35. Ahlborg, B., J. Bergström, L. -G. Ekelund and E. Hultman. 1967. Muscle glycogen and muscle electrolytes during prolonged physical exercise. *Acta Physiol. Scand.*, 70: 129.
36. Garnett, E. S., J. Ford, P. L. Golding, R. J. Mardell and A. E. Whyman. 1968. The mobilization of osmotically inactive sodium during total starvation in man. *Clin. Sci.*, 35: 93.

37. Russell, C. F. M. 1962. The effect of diets of different composition on weight loss, water and sodium balance in obese patients. *Clin. Sci.*, 22: 269.
38. Bloom, W. L. 1962. Inhibition of salt excretion by carbohydrate. *Arch. Intern. Med.*, 40: 26.
39. Katz, A. I., D. R. Hollingsworth and F. H. Epstein. 1968. Influence of carbohydrate and protein on sodium excretion during fasting and refeeding. *J. Lab. Clin. Med.*, 72: 93.
40. Haag, B. L., M. M. Reidenberg, C. R. Shuman and B. J. Channick. 1967. Aldosterone, 17-hydroxycorticosteroid, 17-ketosteroid, and fluid and electrolyte responses to starvation and selective refeeding. *Amer. J. Med. Sci.*, 254: 652.
41. Bazett, H. C., F. W. Sunderman, J. Doupe and J. Scott. 1940. Climatic effects on the volume and composition of blood in man. *Amer. J. Physiol.*, 129: 69.
42. Brickman, A. S., E. J. Drenick, I. F. Hunt and J. W. Coburn. 1969. Effect of carbohydrate refeeding on renal divalent ion excretion in fasting humans. *Amer. J. Clin. Nutr.*, 22: 669.
43. Bell, J. D., S. Margen and D. H. Calloway. 1969. Ketosis, weight loss, uric acid, and nitrogen balance in obese women fed single nutrients at low calorie levels. *Metab.*, 18: 193.
44. Keys, A., J. Brozek, A. Henschel, O. Mickelsen and H. L. Taylor. 1950. *The Biology of Human Starvation. Vol. I.* The Univ. of Minnesota Press, Minneapolis.
45. Anonymous. 1962. Sodium/potassium ratios and essential hypertension. *Nutr. Rev.*, 20: 195.
46. Windmueller, H. C., A. A. Anderson and O. Mickelsen. 1964. Elevated riboflavin levels in urine of fasting human subjects. *Amer. J. Clin. Nutr.*, 15: 73.
47. Recommended Dietary Allowances, Seventh Edition. NAS-NRC publication 1694, Washington, D. C.
48. Present Knowledge in Nutrition, Third Edition. The Nutrition Foundation, Inc., New York, N. Y., 1967.

49. Mitolo, M., D. Leone and E. Vitellio. 1966. Potassium and physical exercise. *Bull. Soc. Ital. Biol. Sper.*, 42: 881 (cited in *Chem. Abstr.*, 66: 74677k, 1967).
50. Mitolo, M. and D. Leone. 1967. Potassium and physical exercise. *Bull. Soc. Ital. Biol. Sper.*, 43: 807 (cited in *Chem. Abstr.*, 68: 20306m, 1968).
51. Castenfors, J. 1967. Renal clearances and urinary Na and K excretion during supine exercise in normal subjects. *Acta Physiol. Scand.*, 70: 207.
52. Contea, I., A. Rujinski, S. Dumitrache and P. Sutescu. 1965. Calcium balance in humans during muscular activity. *Studii Cercetari Biochim.*, 8: 415 (cited in *Chem. Abstr.*, 64: 16359b, 1966).
53. Aurell, M., M. Carlsson, C. Crimby and B. Hood. 1967. Plasma concentration and urinary excretion of certain electrolytes during supine work. *J. Appl. Physiol.*, 22: 633.
54. Yakovlev, N. N. 1958. Vitamin C requirements. *Ernaehrungsforsch.*, 3: 446.
55. Cotugno, V. 1963. Mechanism of antifatigue action of NaCl, eventually associated with vitamins. *Riv. Biol.*, 56: 101 (cited in *Chem. Abstr.*, 59: 10526d, 1963).
56. National Academy of Sciences, Committee on Animal Nutrition. 1962. Nutrient requirements of laboratory animals, publ. 990. National Academy of Sciences - National Research Council, Washington, D. C.
57. Rapoport, A., C. L. A. From and H. Husdan. 1965. Metabolic studies in prolonged fasting. *Metab.*, 14: 31.
58. Bloom, W. L., C. Azar and E. C. Smith, Jr. 1966. Changes in heart and plasma volume during fasting. *Metab.*, 15: 409.
59. Taylor, H. L., A. Henschel, O. Mickelsen and A. Keys. 1954. Some effects of acute starvation with hard work on body weight, body fluids and metabolism. *J. Appl. Physiol.*, 6: 613.
60. Morrison, A. B. 1961. Inulin and thiocyanate spaces of rat in starvation and undernutrition. *Amer. J. Physiol.*, 201: 329.

61. Krzywicki, H. J., C. F. Consolazio, L. O. Matoush and H. L. Johnson. 1968. Metabolic aspects of acute starvation. Body composition changes. *Amer. J. Clin. Nutr.*, 21: 87.
62. Gamble, J. L., G. S. Ross and F. F. Tisdall. 1923. The metabolism of fixed base during fasting. *J. Biol. Chem.*, 57: 633.
63. Hagan, S. N. and R. O. Scow. 1957. Effect of fasting on muscle protein and fat in young rats of different ages. *Amer. J. Physiol.*, 188: 91.
64. Fuengers, A., K. Kaiser and P. Martini. 1958. The effect of sodium chloride upon essential hypertension. *Deut. Arch. Klin. Med.*, 204: 603.
65. Dahl, L. K., K. D. Knudsen, M. A. Heine and G. J. Leitl. 1968. Effects of chronic excess salt ingestion. Modification of experimental hypertension in the rat by variations in the diet. *Circ. Res.*, 22: 11.
66. Dahl, L. K. 1961. Effects of chronic excess salt feeding. ~~Induction~~ Induction of self-sustaining hypertension in rats. *J. Exp. Med.*, 114: 231.
67. Haight, A. S. and J. M. Weller. 1962. Electrolytes and blood pressure of rats drinking NaCl solutions. *Amer. J. Physiol.*, 202: 1144.
68. Koletsky, S. 1959. Role of salt and renal mass in experimental hypertension. *Amer. Med. Assn. Arch. Pathol.*, 68: 11.
69. Chapman, C. B., T. Gibbons and A. Henschel. 1950. The effect of the rice-fruit diet on the composition of the body. *New Engl. J. Med.*, 243: 899.
70. Cizek, L. J. 1961. Relationship between food and water ingestion in the rabbit. *Amer. J. Physiol.*, 201: 557.
71. Frank, R. L. and O. Mickelsen. 1969. Sodium-potassium chloride mixtures as table salt. *Amer. J. Clin. Nutr.*, 22: 464.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) US Army Natick Laboratories Natick, Massachusetts 01760		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	
		2b. GROUP	
3. REPORT TITLE The Effects of Varying Ratios of Minerals and Vitamins to Calories Consumed During Food Intake Restriction on Physical Conditions of Rats and Man. Information from a Rat Experiment and Literature Review			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)			
5. AUTHOR(S) (First name, middle initial, last name) D. Tollenaar			
6. REPORT DATE October 1970		7a. TOTAL NO. OF PAGES 27	7b. NO. OF PAGES 71
8a. CONTRACT OR GRANT NO.		9a. ORIGINATOR'S REPORT NUMBER(S) 71-21-FL	
b. PROJECT NO. 1J061102A71C		9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
c.			
d.			
10. DISTRIBUTION STATEMENT This document has been approved for public release and sale; its distribution is unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY US Army Natick Laboratories Natick, Massachusetts 01760	
13. ABSTRACT In order to determine whether on a restricted caloric intake a higher ratio of minerals and vitamins to calories consumed than normal for <u>ad libitum</u> food intake is desirable, an experiment with rats was conducted and a literature review performed. Male, adult rats received a semipurified diet with two mineral-vitamin levels and two levels and kinds of fat. Under the experimental conditions, at a 40% of average <u>ad libitum</u> food intake, a higher ratio of minerals and vitamins to calories consumed than that normal for <u>ad libitum</u> food intake did not result in a significant difference in treadmill running performance. However, the relatively higher mineral-vitamin level increased water consumption significantly. The rats receiving 15% of calories from corn oil ran significantly longer than the groups receiving 41% of calories from butter. Literature reports indicate that during complete caloric deprivation additional mineral and B-vitamin intake often is beneficial. Extracellular water loss during the first days of caloric restriction has been reported to be positively correlated with the Na content of the diet prior to the period of restriction.			

DD FORM 1473

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED

Security Classification

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Vitamins						
Minerals						
Exercise						
Endurance						
Inanition						
Starvation						
Fasting						
Performance						
Water Consumption						
Fat						

UNCLASSIFIED

Security Classification