MEDICAL EVALUATION OF NUTRITIONAL STATUS[1]

XIV. NEUROMUSCULAR RESPONSE TO GALVANIC CURRENT AS A GUIDE TO THE ADEQUACY OF THE CALCIUM NUTRITION OF ADOLESCENTS

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DIET studies (1, 2) have shown that the calcium content of the American diet is very frequently less than the amount needed to provide adequately for bodily requirements for this essential food element. Although it is particularly desirable that calcium deficiency in the body should be discovered in its early or mild state before signs of gross change have become manifest, no reliable, feasible, and routine method of detection has been demonstrated. Sherman (3) has epitomized the problem in these words: "Growing children whose height, weight, and appearance are normal may have a calcium-poor condition of body which even the best physical examination cannot reveal, but which is revealed by the chemical evidence of the calcium balance experiment . . ." Such a procedure is not, however, well adapted to the routine examination of large population groups.

Fully developed clinical manifestations of calcium deficiency or disturbance appear in the nerves, blood, and bone. There has also been a definite trend toward the detection of slight changes in these tissues characteristic of deficiency. Appropriate methods for this purpose have been used diagnostically in clinical practice, but no thorough test has been made of their applicability in surveys of the nutritional status of the population.

Following its development by Erb (4), clinical medicine adopted

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the method of detecting hyperirritability of the nerves by observation of the neuromuscular response to galvanic stimuli. Erb described the application of the method to adults; Escherich (5) introduced and Thiemich (6) and Mann (7) perfected its use with children. Soon it became limited in its application almost entirely to the diagnosis of tetany in infants. Long before the cause of tetany was known, Erb differentiated the fully developed condition, with its easily perceptible clinical signs, from the state which was demonstrable only by neuromuscular response elicited by very mild electrical or mechanical stimuli. The latter state he characterized as latent.

In studying the effects of ions on nerve-muscle preparations, Loeb (8, 9) showed that calcium decreased the irritability of these tissues, and that inadequacy of calcium increased their sensitivity. Studying these effects on nerves, Mathews (10) later substantiated Loeb's findings. After MacCallum and Voegtlin (11) had demonstrated that the tetany resulting from parathyroidectomy was attributable to lowered calcium concentration in the blood, Howland and Marriott (12) showed that infantile tetany and spasmophilia were characterized by a similar mechanism. Later Hess and his coworkers (13) induced tetany in rats merely by a swift change from a rickets-producing diet, high in calcium and low in phosphorus, to a diet of normal Ca:P ratio. The tetany was accompanied by a decline in serum calcium which was, however, maintained for only a few days. Recently Greenberg, *et al.* (14) have shown that reduction of the serum calcium, if produced slowly on a dietary basis, is insufficient to cause tetany in the rat.

Meanwhile, the critical levels of neuromuscular reactions in the galvanic test had been shown to vary with age. Furthermore, irregular and uninterpretable results at puberty sharply limited the application of the test. Confidence in its reliability was greatly shaken. With the development of the method for determining calcium concentration in the blood and the demonstration of hypocalcemia as the primary disturbance in infantile tetany, the electrical techniques were largely superseded by the chemical method.

It soon became clear that calcium metabolism is affected by many factors operating through numerous and complex mechanisms. It was found that tetany resulted from alkalosis of various origins (hyperventilation, persistent vomiting, and ingestion of excessive alkalies) and that it might not be accompanied by any disturbance in the total calcium level of the blood (15, 16). Although some investigators maintained that alkalosis produced tetany directly, others asserted that it operated by disturbing the total calcium-ionized calcium equilibrium with lowering of the ionic calcium concentration. By the latter school, it was believed that tetany would appear with a normal concentration of total calcium in the blood when the ionic calcium concentration was lowered, and that diminished concentration of ionic calcium was an important cause of tetany. Studies on the concentration of diffusible calcium in tetany tended to confirm this hypothesis. But it remained a question whether diffusible calcium represented ionic calcium. Attempts to determine the concentration of ionic calcium were indirect until 1932, when McLean and Hastings (17, 18) introduced a biological method through which they showed that the concentration of ionic calcium is indeed diminished in tetany.

Unfortunately, there is no easy method for determining ionic-calcium concentration directly in routine surveys. The concentration of ionic calcium already had been shown to be influenced by the concentration of HCO_3^-, PO_4^{---}, and HPO_4^{--} ions when McLean and Hastings (18) found that it was to a much greater extent controlled by the concentration of protein in the serum (19). They have prepared a nomogram on which values for the concentrations of total calcium and total protein yield values for the concentration of ionic calcium (20).

Since calcium is likewise an important constituent of bone, disturbances in calcium economy are reflected in the osseous system.

Depending on circumstances, the result is rickets, osteomalacia, or osteoporosis. One form of rickets, the least common, is characterized by a high or normal concentration of phosphorus and a low concentration of calcium in the blood. Characteristic gross and histologic changes occur in the bone. Osteomalacia is regarded as adult rickets. In generalized osteoporosis, there is rarefaction or deficient calcification of the osseous tissue. Attempts have been made to determine calcium deficiency in the skeleton by a study of bone density from roentgenograms (21, 22).

When the Medical Evaluation of Nutrition Study was planned by the cooperating agencies, the lack of a simple yet sensitive test for calcium nutrition received considerable attention. It was decided to include irritability to galvanic current among the test procedures in order to evaluate it as a guide to the calcium nutrition of groups of subjects. Certain difficulties were recognized. Determinations of ionic calcium were not to be made, and the absence of any real relationship between *total* serum calcium and the galvanic response would not preclude the possibility that the galvanic reaction did depend upon the concentration of *ionic* calcium. Also, any relationship between total serum calcium and galvanic irritability might well hold only within a restricted region of calcium values, e.g., those indicative of marked deficiency. Above some critical value they might be entirely unrelated. Furthermore, evidence that neuromuscular irritability was independent of serum calcium would not necessarily disprove its value as an index to calcium nutrition, for the intricate mechanism serving to stabilize the calcium content of body fluids is such that decalcification of the skeleton may occur without change in the level of serum calcium (16).

It seemed clear that a rigorous experiment, devised to evaluate the galvanic test as a means of detecting mild calcium deficiency, would not be feasible in a survey of school children. Although the Study plan could not provide for such an experiment, it was felt that any truly useful bond between calcium nutrition and neuromuscular irritability should be evident in an extensive survey among adolescents diverging widely with respect to the adequacy of their calcium nutrition.

A problem of major importance arises from the lack of any valid information on the basis of which subjects might be arrayed according to their calcium nutrition. At best, the available data have only partial or presumptive value as indicators of calcium nutrition; none provides an accurate measure, and each may reflect a different aspect of calcium nutrition. The Study observations include a careful two-day diet history obtained in an interview with the subject, a calcium determination [following Clark and Collip's modification (23) of the Tisdall method] on the blood serum, and roentgenograms of hand and wrist, elbow, and hip. The diet data were processed by means of standard tables to yield a daily average calcium intake in grams. Information of this type is necessarily approximate and ignores the variation among foods with respect to the availability of the calcium to the organism. However, since foods of high calcium content, especially milk, tend to be consumed either habitually or little at all, the diet histories should suffice to classify the subjects into broad groups of differential calcium intake. The serum calcium of a given healthy individual does not usually fluctuate greatly over short periods of time, and the method which was followed may be relied upon to provide accurate determinations. However, the total serum calcium is of only doubtful value as an index to calcium nutrition.

The roentgenograms in the Study have been utilized to classify pupils for skeletal age and do not afford data on bone density which has been suggested as a useful criterion of calcium nutrition. The relation between calcium nutrition and skeletal development and maturation is not established, but it seems probable that a deficiency of calcium does affect skeletal growth. Therefore, the skeletal age, estimated according to Todd's method (21) and taken in relation to chronological age, is used as indirect, but by no means sure, evidence on calcium nutrition. However, skeletal development depends upon so many environmental and physiological factors other than calcium nutrition that any connection between them may be obscured in a particular case. Certainly an adequate supply of calcium is but one factor in growth and development and does not itself control the growth process. Retarded skeletal development may not indicate deficient calcium nutrition, therefore, unless the meaning of the latter be extended to the entire metabolic process. On the other hand, the relationship should be strong enough to be perceptible in a statistical analysis of a large number of persons of different diet and mode of life.

Procedure and Apparatus

For the present Study an apparatus (Figure 1) was constructed to provide both "make" (closing) and "break" (opening) galvanic shocks of graduated intensity. The circuit was designed to avoid the usual difficulty of unpleasant or painful *closing* contractions before obtaining effective *opening* contractions. This is accomplished with the break-shock circuit by a presetting at a certain level of intensity which operates to cut off the rapidly rising current at the present level. The apparatus provides shocks up to 5 milliamperes in intensity, the readings being to the nearest ma. The discharge of a condenser is utilized to facilitate the location of motor points. Both anode and cathode may be employed as stimulating electrodes.

Since neuromuscular irritability is presumably fairly constant throughout the neuromuscular system of a particular subject at any one time, the choice of the nerve for stimulation is largely a matter of convenience. In the present Study the ulnar nerve was chosen. Three different levels of contraction were established:

(1) the first visible muscular twitch;
(2) a visible twitch of a finger; and
(3) a visible twitch of the entire hand.

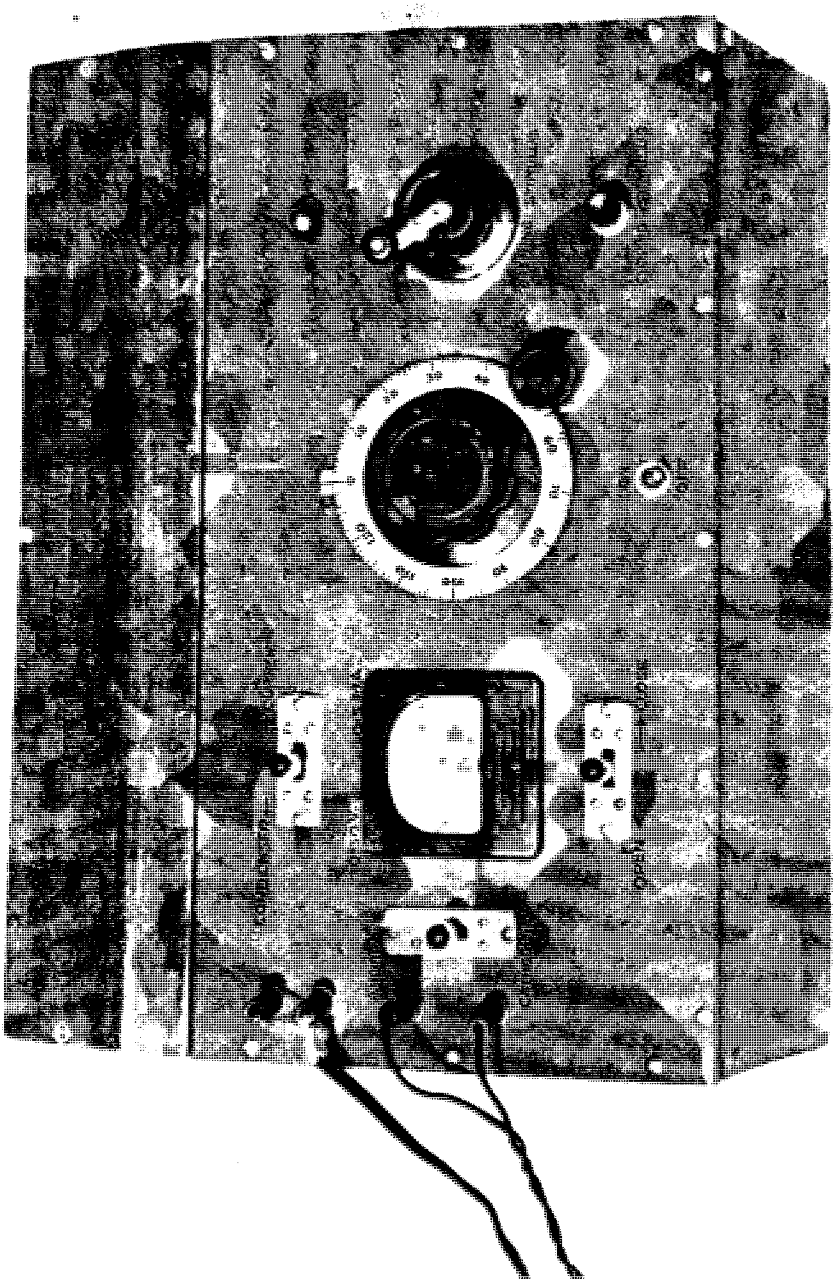


Fig 1. Panel of galvanic apparatus.

Fig. 2. Subject with electrodes adjusted.

Each subject was tested for both arms, and about twelve different readings were made on each arm.

The instrument was operated by two technicians, one handling the control panel and another taking care of the subject and observing his response to stimulation. The instructions for preparing the subject follow:

> The subject is seated on a high stool facing the instrument. The upper outer aspect of his right arm is briskly scrubbed with a cotton sponge soaked in alcohol. The same area is briskly rubbed with electrode jelly.
>
> A small amount of jelly is now rubbed over the arm, and the indifferent (plate) electrode is fastened snugly by means of the elastic strap. The same procedure of rubbing with alcohol and electrode jelly is followed for the area of the ulnar supracondylar notch of the elbow.
>
> The leather sling which hangs from an adjustable standard is now brought into position so that the forearm may be placed in it, and the height of the standard adjusted so that the forearm is horizontal and swings relaxed across the front of the body. (*See* Figure 2.)
>
> The stimulating (round-headed) electrode is now firmly applied to the bony notch felt in the ulnar region of the elbow and held by hand during the preliminary part of the test.

The motor point is then located by means of the condenser discharge, and the voltage required for the threshold response is recorded. With the cathode as the stimulating electrode, and the stimulus applied by closing the circuit, the least amount of current required to provoke each type of contraction is then determined. Similar values are then obtained by reversing polarity to make the anode the stimulating electrode. The shock is then applied by opening the circuit, first with the anode as the source of stimulation, and then the cathode. Having completed the tests on the right arm, the technicians prepare the left arm and proceed as before.

The observer was specially instructed in the technique of detecting the three levels of response. Although an effort was made to utilize a single pair of technicians as long as possible, personnel turnover on the Study was such that the final bulk of observations reflects the work of many different pairs. This fact may explain some of the temporal variability in results discussed below.

The test was administered in many ways in the thought that its most suitable form could be readily selected if it proved useful in the detection of mild states of calcium deficiency. In addition to this multiplicity of form, the technique differs from previous work in two other respects. The more important of these is the use of three thresholds of response in place of the more common reliance upon the first perceptible muscular twitch. The other concerns the selection of the nerve to be stimulated. Most previous work has utilized either the peroneal or the median nerve.

EXTRANEOUS SOURCES OF VARIATION IN THE GALVANIC RESPONSE

The Subjects. The subjects examined during the course of the Study were pupils of the Fieldston Ethical Culture School and of the Seward Park High School, a private and a public high school drawing from very different income levels. Unless otherwise stated in connection with a particular point, this report is confined to subjects whose cultural background is Jewish. The galvanic tests were begun at Seward Park High School about April 1, 1939, and continued until the end of June. They were resumed about November 1 and completed during the middle of March, 1940. All the Fieldston pupils were tested between March 1 and April 15, 1940. Since the physical examination, blood tests, and other studies of any given pupil were all administered within a short period of time, the date of the galvanic test may be accepted as the date when any other information was obtained.

Age and Sex. In order to sharpen the comparison of the galvanic response with the various factors which may have a bearing upon calcium nutrition, the galvanic readings were explored for important sources of variation which might be controlled. Two which may be of general interest are age and sex. Significant age-trends have been recognized since Holmes' 1916 report (24) giving average values from birth to age 13. His age curves are drawn in Figure 3 together with the Study curves differentiated as to sex and variety of galvanic test. Holmes' curves explain why the age-trends in the Study data should be statistically insignificant. In

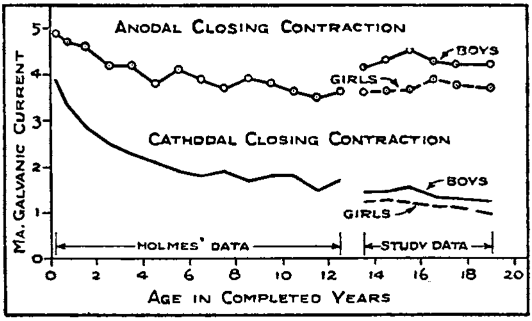


Fig. 3. Age variation in amount of galvanic current required to provoke anodal closing and cathodal closing contractions, comparison of Holmes' and Study data.

parallel tests the agreement between his values and those reported here is reasonably close. The average level in Figure 3 for cathodal closing contraction (least perceptible twitch) lies between the averages of Stinzing (25) for *n. ulnaris I* and *n. ulnaris II* in adults of both sexes.

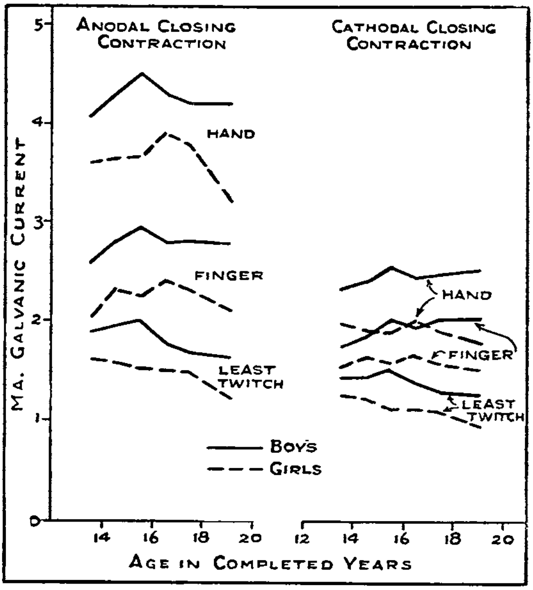


Fig. 4. Variation in amounts of galvanic current required to provoke anodal closing and cathodal closing contractions, by age, sex, and type of contraction.

Consistent sex differentials stand out in Figures 3 and 4 and in all other varieties of the test as administered in the Study, although the literature on the electrical diagnosis of tetany makes no point of sex differences. The margin is small enough to be overlooked in anything but a fairly large series of observations. No explanation of the finding is ventured here. Table 1 compares boys and girls of each school with respect to the

Ma. Galvanic Current	Fieldston		Seward Park	
	Boys	Girls	Boys	Girls
Total	100.0	100.0	100.0	100.0
0–0.4	.5	—	—	—
0.5–0.9	—	—	.1	—
1.0–1.4	—	—	.1	.1
1.5–1.9	—	.6	—	.4
2.0–2.4	.6	3.6	.1	—
2.5–2.9	.5	—	.5	.7
3.0–3.4	—	5.4	.3	1.1
3.5–3.9	1.7	6.6	.8	1.3
4.0–4.4	4.5	4.8	.8	2.0
4.5–4.9	8.4	19.7	1.1	2.0
5.0 or More	83.8	59.3	96.2	92.4
Number of Cases	179	167	761	715

Table 1. Distribution of subjects by amount of galvanic current required to provoke a cathodal opening contraction, by sex and school.

stimulus required to produce a cathodal opening contraction (least perceptible twitch). The excess of low values among the girls is readily apparent and very significant in the statistical sense.

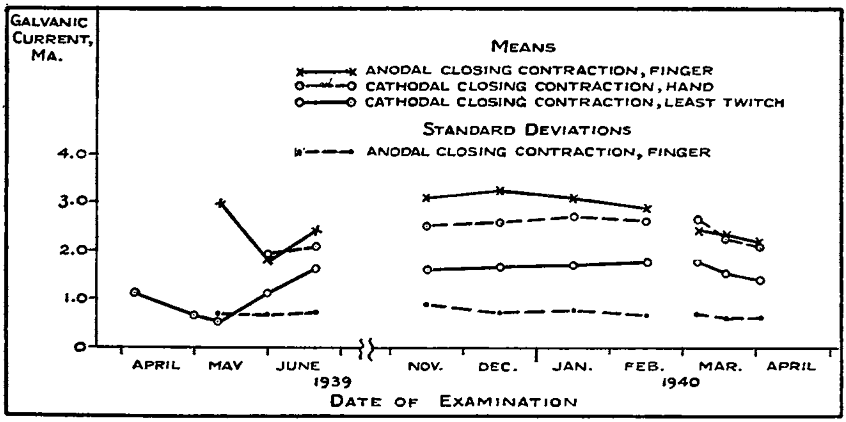
Varieties of Galvanic Test. Different ways of administering the galvanic test yield somewhat different results, each variety having its characteristic level. No attempt has been made to select from among those administered a "best" or most suitable test, since the analysis which follows reaches entirely negative conclusions. However, their very multiplicity makes some selection imperative.

Parallel tests on right and left arms give the same results and may be regarded as interchangeable. The test based on the condenser discharge correlates poorly with other forms of the test. Since anodal opening contractions were seldom obtained with as little as 5 ma. of current, this test fails to differentiate subjects sufficiently for statistical purposes. This is somewhat less true of the cathodal opening contractions and this variety of the galvanic test was retained for further work. Among the others, good correlation was obtained for the anodal-cathodal comparison on the basis of the least perceptible twitch, for the twitch-hand comparison using the cathode as the stimulating electrode, and for the hand-finger comparison on the basis of the cathode. Poor correlation was found for three twitch-finger comparisons, and for a twitch (anode)-hand (cathode) comparison. Probably good correlation would also obtain for the finger-hand comparison on the basis of the anode, but too many of the hand values were 5.0 or more. The forms finally chosen are:

Right arm, least twitch, cathodal opening stimulus
Right arm, least twitch, cathodal closing stimulus
Right arm, finger twitch, anodal closing stimulus
Right arm, hand twitch, cathodal closing stimulus

Time. The galvanic readings display a marked variation with time which cannot be satisfactorily explained on the basis of the Study observations. In order to increase the rigor of the test of the hypothesis which forms the subject of this paper, however, this significant but extraneous variation should be controlled by statistical means. For each of three galvanic tests, Figure 5 presents the mean values obtained for boys at different periods, and also the standard deviations for one of these. For the anodal closing contraction, Table 2 presents the mean and standard deviation for each

Fig. 5. Temporal variation in amount of current required to provoke contractions among boys.



time period by sex and by school. The values for the girls display similar heterogeneity. Statistical comparisons leave little room for belief that the results of the several periods differ only by chance. The principal source of heterogeneity is the jump from the spring, 1939, levels to the fall, 1939, levels. The middle period of fall and winter is one of fairly homogeneous values.

Table 2. Number of subjects and mean and standard deviation of amount of anodal closing current required to provoke finger contraction, by school, sex, and date of test.

Date of Test	Boys			Girls		
	Number of Cases	Mean (Ma.)	Standard Deviation (Ma.)	Number of Cases	Mean (Ma.)	Standard Deviation (Ma.)
	SEWARD PARK					
Total[1]	621	2.92	.868	602	2.32	.730
1939						
May 6–20	83	2.97	.691	86	2.48	.684
May 21–June 10	55	1.78	.642	56	1.51	.508
June 11–30	60	2.42	.716	83	2.04	.591
Nov. 1–30	65	3.12	.902	83	2.64	.862
Dec. 1–31	103	3.33	.749	94	2.51	.705
1940						
Jan. 1–31	194	3.09	.789	159	2.38	.619
Feb. 1–29	61	2.89	.700	41	2.32	.565
	FIELDSTON					
Total[1]	179	2.30	.665	167	2.10	.659
1940						
Feb. 29–Mar. 11	29	2.48	.713	78	2.30	.700
Mar. 12–25	54	2.34	.646	68	1.97	.597
Mar. 26–Apr. 8	96	2.21	.658	21	1.78	.460

[1] Standard deviations shown on total lines were obtained directly from Σx and Σx^2 for all cases, not from weighted means of the variances for different periods.

Variation of such magnitude could seriously interfere with the statistical investigation of the problem in hand, especially if the possible indicators of calcium nutrition also displayed temporal variation. That it might reflect the operation of truly seasonal factors is suggested by the decline in the late spring. It might also represent such changes in technical skill as make up the learning curve for the technique, or differential skill among technicians, e.g., in placing the electrodes and in observing the contractions. Errors in the placement of the electrode should produce high values. Similar reasoning applies to the observation of contractions, unless one assumes that the technician is as likely to report a nonexistent contraction as to fail to report one which actually occurs. When the technicians were learning, accordingly, high values would be expected. However, statistical analysis of the observational data does not support this interpretation. Moreover, the two pairs of technicians who made most of the early observations agree fairly well in their average values at the same periods. The available information is insufficient to determine whether the temporal variation has a physiological basis or whether it merely resulted from technical variation beyond control.[2]

The question of experimental error in the galvanic determinations has some bearing on the importance of technical variation during the learning process and also among different operators. Truly random errors, of course, would merely inflate the variances without disturbing the mean values. When parallel right and left readings were processed as independent duplicates, the resulting estimates of the error of measurement differed reliably among different technicians, but gave no further clue to the temporal varia-

[2] The pattern of seasonal variation shown by these data is consistent with one frequently observed for nutritional deficiencies, that is, poorer nutritional status in the late spring than in fall and winter. However, if the lower mean threshold for galvanic response in the spring derived from the nutritional status, one would expect a higher standard deviation for threshold values at this time, since presumably only some children would be affected. Contrary to this expectation, the standard deviation also decreased slightly, indicating a general shift to lower threshold values.

tion. On the assumption that the right and left values should correlate perfectly, and that the differences between them are randomly distributed about a mean of zero and are independent of the "true" values, the error variance for the cathode closing variety of the galvanic test is as large as the true variance. Hence, the variance of a set of measurements subject to this degree of error might be about twice what it should be. Although this estimate is necessarily approximate, error is certainly so large a part of any observed variability that no useful clinical purpose can be served by a single galvanic determination on a subject.

The large experimental error reduces the power of the observational data to reveal any correlation which might exist between neuromuscular irritability and calcium nutrition. If both the galvanic reaction and a particular indicator of calcium nutrition involved an experimental error sufficient to double each of the true variances, the highest possible estimate of any true correlation coefficient r would be $r' = r/2$. However, if a large number of observations revealed no significant correlation at all ($r' = 0$), it would be reasonable to assume that r is 0. Similarly, a significant coefficient of, say, $r' = .3$ might be regarded as a lower limit upon the probable value of r. Large as the experimental error undoubtedly is, therefore, it by no means disqualifies the observations for the task in hand.

If any set of galvanic observations made within a short time-interval is subject to some constant bias, and if the bias varies among periods, precision is lost by combining data obtained at different times. Although the problem of temporal variation in the Study data cannot be entirely resolved, it is readily circumvented if attention is confined to fairly short periods of time, when the galvanic observations tend to be fairly homogeneous. A constant error pervading all the readings of a period, it can be shown, would not affect the estimate of the correlation between the galvanic reading and another factor. In the main argument which follows, therefore, the relationship between the galvanic response and each of the possible indicators of calcium nutrition will be sought for fairly short time-intervals.

Galvanic Response and Calcium Intake

Wiehl (26) found that the diet histories of the Seward Park pupils frequently revealed deficient calcium intake. Seventy-two per cent reported an average daily calcium intake below the level recommended by the Committee on Food and Nutrition of the National Research Council, and 28 per cent reported an average intake of less than two-thirds of the recommended allowance. For a discussion of the collection and interpretation of the diet data the reader is referred to Wiehl's paper (26).

Complete distributions of pupils according to the estimated weight of their daily average calcium intake are shown in Table 3 for each school-sex group. The open character of the terminal groups and the evident skewness of the underlying distributions

Table 3. Distribution of subjects by reported daily average calcium intake, by school and sex.

Calcium Intake Gm. Daily	Fieldston		Seward Park	
	Boys	Girls	Boys	Girls
Total	100.0	100.0	100.0	100.0
Less Than .30	0	.6	1.4	4.0
.30– .39	.6	3.6	1.8	5.5
.40– .49	.5	6.0	3.3	6.9
.50– .59	.6	3.6	5.0	6.9
.60– .69	1.1	3.0	6.5	10.6
.70– .79	4.5	7.1	8.6	11.5
.80– .89	4.5	8.3	8.7	10.2
.90– .99	5.0	8.3	7.8	9.2
1.00–1.09	8.9	9.5	8.0	9.0
1.10–1.19	5.6	13.7	9.1	8.6
1.20–1.29	9.5	7.1	6.6	6.1
1.30 or More	59.2	29.2	33.2	11.5
Number of Cases	179	168	768	724

forbid reliance upon normal correlation theory. This is also true of the readings for the cathodal opening contraction (least perceptible twitch) as noted in conjunction with Table 1. The amounts of current required to provoke contractions under the cathodal closing stimulus (least perceptible twitch), the anodal closing stimulus for the finger, and the cathodal closing stimulus for the hand, on the contrary, are distributed in fairly normal fashion. The relationship between calcium intake and galvanic response, therefore, may be tested by the method of the contingency-table when the cathodal opening variety of the galvanic test is under consideration, and by analysis of variance when the other three galvanic tests are under scrutiny.

Each school-sex group was subdivided by date of test, and the resulting school-sex-time subgroups were analyzed individually. Table 4 gives the analysis for one such subgroup, the Seward Park girls tested in May and June, 1939. Although about a third of the 222 subjects reported an average daily calcium intake of less than .70 gm., there is no evidence that a disproportionate number of them required only small amounts of galvanic current to provoke the cathodal opening contraction. The numbers in parenthesis there

Table 4. Relation between calcium intake and galvanic response, cathodal opening contraction for Seward Park girls tested May and June, 1939.

Calcium Intake in Gm.	Ma. Galvanic Current		Total
	Under 5.0	5.0 or More	
Total	46	176	222
Under .70	16 (14.50)*	54 (55.50)	70
.70– .99	13 (13.06)	50 (49.94)	63
1.00–1.29	9 (10.98)	44 (42.02)	53
1.30 or More	8 (7.46)	28 (28.54)	36

$n=3$, $X^2=.695$, $.80 < P < .90$

* Values in parentheses are expected values calculated under assumption of independence.

are those "expected" on the assumption that the two factors are unrelated. Observed and expected values at least as divergent as these would occur by chance 80 to 90 per cent of the time under repeated sampling. Table 4 is typical of the tests which were made, as may be seen from the summary in Table 5. Statistical tests for intervals of even shorter length than those shown in the table were made, with similar results. It is quite clear, therefore, that if the reported calcium intake and the observed cathodal opening readings be accepted as valid, the two factors must be regarded as independent. Moreover, parallel analyses on the basis of the *anodal* opening stimulus gave essentially the same results.

Table 5. Summary of results of statistical tests of relationship between reported calcium intake and response to cathodal opening stimulus, by school and sex.

Sex	Date of Tests	Number of Classes		Computed Value of X^2	P[1]
		Ca.	Galvanic		
	FIELDSTON				
	1940				
Girls	Feb. 29–Apr. 8	4	3	8.39	.20
Boys	Feb. 29–Apr. 8	2	3	.11	>.95
	SEWARD PARK[2]				
	1939				
Girls	Mar. 21–May 10	2	2	*	.18
Girls	May 11–June 30	4	2	.70	.80–.90
Boys	Mar. 21–May 10	2	2	*	>.05
Boys	May 11–June 30	2	2	<.45	>.50

[1] P gives the probability with which differences equal to or exceeding those observed might arise through chance.

[2] No perceptible twitching was observed to occur under the cathodal opening stimulus applied to any Seward Park pupils tested from November, 1939 to March, 1940. As recorded, therefore, the data provide no evidence of association for this period also.

* On the basis of an exact test for four-fold tables.

In order to study calcium intake in relation to the other three galvanic tests, each school-sex-time subgroup was further divided into four parts differing with respect to calcium intake. The class intervals employed for calcium intake are those of Table 4. If the neuromuscular response actually does depend upon calcium intake, these four groups should differ significantly with respect to the amounts of current required to provoke a muscular contraction, and a low calcium intake should be accompanied by a low average galvanic value. Inspection of each set of means does not support this expectation, and analysis of the variance within and among intake classes reveals nothing beyond chance differences among them. The salient features of the analysis are set forth in Table 6 for the cathodal closing contraction (least perceptible twitch). Similar tables for the other two tests have been omitted for lack of space, but they permit the same conclusion.

On each horizontal line for a particular school-sex-time subgroup, Table 6 gives the set of means for the amount of current required to provoke the cathodal closing contraction, the degrees of freedom corresponding to the greater and to the lesser mean square, the variance ratio (usually the variance among classes divided by the variance within classes), and the probability judgment corresponding to the *F* ratio considered in relation to the degrees of freedom.

For the range of calcium intake characteristic of the Study subjects, the observations do not support the view that the galvanic response correlates with reported calcium intake.[8] It must be borne in mind that the subjects were on their usual, every-day diets and that the diet record was for only a two-day period. However frequently their average intake may have fallen below the recommended allowances for calcium, it may never have reached a level sufficiently low for a sufficiently long period for the reaction to

[8] The results of the galvanic test also were studied in relation to the calcium intake per kilogram of body weight. This analysis also gave no evidence of significant differences for the galvanic response among groups of children whose reported calcium intake per kg. was at different levels.

Table 6. Summary of results of statistical tests of relationship between reported calcium intake and cathodal current required to provoke perceptible contraction, by school, sex, and date of test.

Date of Test	Mean Ma. of Current by Calcium Intake, in Gm.				Degrees of Freedom		Variance Ratio (F)	P[1]
	Under .70	.70–.99	1.00–1.29	1.30 and Over	n1	n2		
1940	GIRLS, FIELDSTON							
Feb. 29–Mar. 11	1.39	1.56	1.32	1.50	3	74	1.34	>.05
Mar. 12–25	1.06	1.28	1.20	1.15	64	3	1.45	>.05
Mar. 26–Apr. 8	1.42		1.12		1	19	3.32	>.05
	BOYS, FIELDSTON							
Mar. 12–25	1.57		1.41	1.57	51	2	1.67	>.05
Mar. 26–Apr. 8	1.44		1.38	1.45	93	2	2.86	>.05
1939	GIRLS, SEWARD PARK							
Mar. 21–Apr. 20	.94	.89	.92	.74	71	3	2.75	>.05
Apr. 21–May 5	.57	.54	.41	.60	50	3	1.28	>.05
May 6–20	.41	.52	.52	.53	3	80	1.25	>.05
May 21–June 10	.88	.90	.93	.85	51	3	6.79	>.05
June 11–30	1.49	1.37	1.33	1.53	78	3	1.16	>.05
Nov. 1–30	1.44	1.39	1.30	1.20	3	60	1.54	>.05
Dec. 1–31	1.25	1.31	1.24	1.11	90	3	1.28	>.05
1940								
Jan. 1–31	1.41	1.38	1.22	1.27	3	152	2.10	>.05
Feb. 1–29	1.36	1.34	1.58		2	38	1.61	>.05
1939	BOYS, SEWARD PARK							
Mar. 21–Apr. 20	1.01	1.08	.92	1.21	3	89	1.54	>.05
Apr. 21–May 5	.67	.69	.63	.57	52	3	3.38	>.05
May 6–20	.52	.60	.53	.57	78	3	4.79	>.05
May 21–June 10	1.11	1.06		1.10	52	2	14.71	>.05
June 11–30	1.60	1.53	1.55	1.76	55	3	1.41	>.05
Nov. 1–30	1.89	1.34	1.71	1.52	3	44	1.87	>.05
Dec. 1–31	1.79	1.64	1.67	1.63	98	3	1.72	>.05
1940								
Jan. 1–31	1.86	1.73	1.81	1.68	3	185	1.05	>.05
Feb. 1–29	1.81	1.61	1.94	1.74	57	3	1.07	>.05

[1] Probability of obtaining a set of means equally or more divergent by chance.

galvanic stimuli to be affected. Subjects fed on experimental diets having a very low calcium content might manifest a disturbance of galvanic reaction not seen in the Study subjects. Even experimental verification of such a view would not alter the fact that no relationship is demonstrable within the extensive range of the reported calcium consumption of the Study subjects. Insofar as the reported absolute intake of calcium has any value as a sign of calcium nutrition, therefore, the galvanic test has no diagnostic value for subjects like those studied here.

Mention should be made of the experimental results obtained by Sjollema and Seekles (27) with rabbits. They varied the mineral content of experimental diets in order to provoke galvanic responses symptomatic of tetany in latent or clinically evident form. Although their observations are too few to permit precise conclusions, on diets having Ca./P ratios of between 1:2.25 and 1:4.5 their animals had normal galvanic reactions. Only when the ratio reached or exceeded 1:5.6 was it possible to diagnose tetany (latent or active) in their animals. In the normal human diet calcium and phosphorus tend to occur together in the same foods, and ratios of the order of 1:5.6 are rather unlikely. The same authors also report normal galvanic reactions for animals in a state of starvation.

Galvanic Response and Serum Calcium

Since the Study observations on serum calcium vary from one period to another, it is essential that any apparent relationship between serum calcium and galvanic response be independent of time. Hence, in this section also the analysis utilizes the individual school-sex-time groups as the basic units for which a relationship is to be sought. Since the serum calcium values are distributed in approximately normal fashion, normal correlation theory may be relied upon for the detection and measurement of any relationship with any but the cathodal opening variety of the galvanic test.

The distribution of values for the cathodal opening contraction

Serum Calcium Mg. Per Cent	Ma. Current Required for Cathodal Opening Contraction							
	2.0–2.4	2.5–2.9	3.0–3.4	3.5–3.9	4.0–4.4	4.5–4.9	5.0 or More	All
Total	1	1		2	5	8	65	82
10.0–10.4	1				3	2	17	23
10.5–10.9		1		1	1	6	35	44
11.0–11.4					1		13	14
11.5–11.9				1				1

As a 3 x 2 contingency table, n=2, $X^2=.90$, and $.50 < P < .70$.

Table 7. Relationship between serum calcium and galvanic response, Fieldston boys, cathodal opening contraction.

presents a special problem, but the method of the contingency table provides a convenient test of the association between serum calcium and the current required to provoke the cathodal opening contraction. The observations summarized in Table 7 are more or less typical of those available for study, except that the serum calcium values shown there are higher than those for Seward Park. The Fieldston boys examined in March and April, 1940, fail to support the position that neuromuscular irritability depends on serum calcium. All the statistical tests of this character are summarized in Table 8.

The serum calcium values for Seward Park pupils are somewhat lower, on the average, than those shown in Table 7 for Fieldston boys. The difference is thought to reflect either technical or seasonal variation. With but one exception, however, the probabilities of Table 8 suggest no reason to reject the position that the neuromuscular response to cathodal opening current is actually *independent* of serum calcium. The exception concerns Seward Park boys examined in April and May, 1939. The number having low (under 5.0 ma.) galvanic values among all those of stated serum calcium is: one among eight at the 9.5-9.9 mg. per cent level; two among thirty-seven at the 10.0-10.4 mg. per cent level; four among fifty-four at the 10.5-10.9 mg. per cent level; and none among sixty-seven at or above 11.0 mg. per cent. Dividing the group at 11.0 mg. per cent makes a four-fold table with a slight suggestion of heterogeneity. Although the association is of the expected character, low galvanic values appearing with low serum calcium values, the probability of .07 fails to reach the .05 level arbitrarily used here, and no other portion of the statistical analysis reflects a similar pattern.

None of the other three galvanic tests furnishes values which are correlated with serum calcium, as may be seen from Table 9. The linear correlation coefficient (r) was computed for each school-sex-time group of sufficient size (the number of subjects ranges from 15 to 92). Taken individually, four of the fifty-six coefficients are

Table 8. Summary of analysis of relationship between serum calcium and cathodal opening contraction, by school, sex, and date of test.

Sex	Date of Test	Number of Classes		X^2	P[1]
		Ca.	Galvanic		
	FIELDSTON				
	1940				
Girls	Feb. 29–Apr. 8	2	3	1.31	.50–.70
Boys	Feb. 29–Apr. 8	3	2	.90	.50–.70
	SEWARD PARK[2]				
	1939				
Girls	Apr. 21–May 10	2	2	*	.25
Girls	May 11–June 30	3	2	.33	.80–.90
Boys	Apr. 21–May 10	2	2	*	.07
Boys	May 11–June 30	4	2	.75	>.80

[1] Chance of obtaining a set equally or more heterogeneous, under hypothesis of independence.

[2] All Seward Park pupils examined from November, 1939 to March, 1940 required at least 5 ma. for cathodal opening contraction.

* Exact test for four-fold table employed in lieu of chi-square approximation.

statistically significant at the $P \leq .05$ level; but, if the underlying r's were truly *o*, one would expect to attach significance (erroneously) to three sample coefficients if the .05 criterion were employed, and the excess of one is unimpressive. All three distributions evidently center about *o*. For the cathodal closing contraction (least perceptible twitch) a statistical test of homogeneity shows that a set of coefficients equally or more divergent than that observed should occur once in ten trials even if one repeatedly sampled the same normal bivariate distribution having a given value of r, the population coefficient of correlation. The distribution of r' for the finger test (anodal closing) is more variable than would be expected from the operation of purely chance factors ($.05 > P > .02$) but that for the hand test (cathodal closing) is homogeneous ($.70 > P > .50$). However, the frequency distributions suffice to show that there is no reason to assume any real correlation between serum calcium and any of the three galvanic tests.

The absence of a significant relationship between serum calcium and galvanic response does not prove the galvanic test useless as a guide to calcium nutrition. It was noted above that serum calcium probably does not register the state of calcium nutrition enjoyed by

Table 9. Distributions of coefficients of correlation between serum calcium and galvanic response for three varieties of galvanic test.

Value of r[1]	Cathodal Closing (Least Twitch)	Anodal Closing (Finger)	Cathodal Closing (Hand)
Total	22	18	16
−.44 to −.35	1	1	—
−.34 to −.25	1	2	2
−.24 to −.15	5	2	1
−.14 to −.05	5	3	3
−.04 to +.05	3	5	4
+.06 to +.15	6	2	5
+.16 to +.25	0	3	1
+.26 to +.35	1	—	—

[1] Each r' represents the experience of a particular school-sex-time group. The number in each such group varies from 15 to 92.

the organism unless very marked and clinically apparent symptoms are present. It is of interest, therefore, to note that calcium intake and serum calcium are entirely unrelated for the subjects in this Study. Sixteen school-sex-time groups were studied for variations in average serum calcium corresponding to differential calcium intake, but no positive relation between the two factors was disclosed.

Galvanic Response and Skeletal Age

For adolescents of the same chronological age, skeletal age is in some ways more closely associated with calcium nutrition than any other single measurement provided by the Study. Schmidt (28) has described how roentgenograms of hand and wrist, elbow, and hip were obtained for each subject and assessed against the Western Reserve University standards of Todd and his associates. The average of the three skeletal age equivalents was employed in the statistical analysis as the best estimate of general skeletal development.

Both Fieldston and Seward Park pupils are, on the average, above the Western Reserve standards for each chronological age. Although not without interest, this fact does not affect the argument here. Whether a particular group of subjects is above or below the average for a given age is a purely statistical question which can be reliably answered only on the basis of truly representative standards (averages). Either Todd's standards of skeletal age for each chronological age may not be truly representative, or even the relatively impoverished Seward Park pupils are above average in their skeletal development. Most of the Jewish children in the school partook of a relatively orthodox Jewish diet, and their consumption of dairy foods may have secured for them a better than average supply of calcium. However, the evidence of the diet histories (26) strongly suggests that their average calcium intake was frequently well below recommended allowances. Moreover, the precise chronological age equivalent of a given degree of skeletal maturity is beside the point. What matters is that the x-rays make it possible to array any set of subjects of comparable chronological age according to the degree of skeletal maturity. If pupils of retarded skeletal maturity tend also to be deficient in their calcium nutrition, and if the galvanic response is a measure of calcium deficiency, then galvanic hyperirritability should be at least noticeably frequent among subjects whose skeletal development falls short of the average for their chronological age.

Although skeletal maturation is controlled only in part by the available supply of calcium, the relationship should be sufficiently close to invest skeletal age with some value as a guide to calcium nutrition, especially for the deficiency range. The retarded skeletal development of a particular subject may or may not represent inadequate calcium nutrition, but the relationship should in general be strong enough to provide a statistical basis for exploring the association between calcium nutrition and galvanic response. If this postulate be accepted, then any relationship between galvanic response and calcium nutrition would be indeed tenuous if it failed

Table 10. Relation between skeletal maturity and response to cathodal opening stimulus, for Fieldston girls of all ages.

MA. Galvanic Current	Skeletal Age[1]			
	Low	Moderate	Advanced	Total
Total	41	97	28	166
Less Than 4.50	11 (8.65)*	20 (20.45)	4 (5.90)	35
4.50–4.99	8 (8.15)	16 (19.28)	9 (5.57)	33
5.00 or More	22 (24.20)	61 (57.27)	15 (16.53)	98

$n=4$, $X^2=4.52$, $.30 < P < .50$

[1] Taken in relation to chronological age. The distribution for each chronological age group was separated into three parts, and like parts for the different chronological age groups were summed.

* Values in parentheses are expected values calculated under assumption of independence.

to appear in so extensive an analysis of skeletal maturity and galvanic response as this Study furnishes.

The insignificant correlation between galvanic irritability and both calcium intake and serum calcium is reinforced by the apparent independence of skeletal age and galvanic response. Each school-sex-age group was sorted by skeletal age against each of the galvanic tests. The Study observations on the amount of current required to provoke the cathodal opening contraction are illustrated in Table 10 with the results obtained for the Fieldston girls.

Table 11. Summary of analysis of relation between skeletal age and galvanic response, by sex, school, and age.

Sex	Age	Number of Classes		X^2	P[1]
		Skeletal Age	Current		
		FIELDSTON			
Boys	All[2]	3	2	<1.3	>.50
Girls	All[2]	3	3	4.5	.30–.50
		SEWARD PARK			
Boys	14	2	2	*	.16
	15	2	2	*	>.26
	16	2	2	<3.8	>.05
	17	2	2	*	>.12
	18	2	2	*	>.19
	All[2]	3	2	1.2	.50–.70
Girls	14	2	2	*	>.30
	15	2	2	<3.8	>.05
	16	2	2	2.1	.10–.20
	17	2	2	*	>.29
	18	2	2	*	.11
	All[2]	3	2	<1.6	>.30

[1] Chance of obtaining a set equally or more heterogeneous, under hypothesis of independence.
[2] Combined as described in footnote 1, Table 10.
* An exact test was made.

Three degrees of skeletal maturity were established at each age, and the data for the several ages were then combined to yield the 3 x 3 contingency table shown. The expected values (in parentheses) are close to those observed, and the chi-square value of 4.52 indicates a probability range of .30 to .50. In other words, there is no evidence whatever that retarded skeletal growth is associated with galvanic irritability as measured by the cathodal opening contraction. The information about single-year age groups is essentially similar to that shown for all the Fieldston boys and girls in Table 11. None of the tests on any of the Seward Park pupils also shown there suggests any reason for assuming the existence of a

Table 12. Summary of evidence on relation between skeletal age and galvanic response among Seward Park pupils, by sex, chronological age, and variety of galvanic test.

Age	Cathodal Closing Contraction (Twitch)			Anodal Closing Contraction (Finger)			Cathodal Closing Contraction (Hand)		
	Number of Pupils	Value of $\acute{r}$	P[1]	Number of Pupils	Value of $\acute{r}$	P[1]	Number of Pupils	Value of $\acute{r}$	P[1]
	BOYS								
13	22	+.249	>.10	20	+.151	>.10	19	+.238	>.10
14	90	−.002	>.10	82	+.151	>.10	76	+.065	>.10
15	180	+.151	.04	164	+.166	.03	148	+.244	<.01
16	213	−.059	>.10	186	+.059	>.10	159	−.004	>.10
17	151	+.014	>.10	114	−.135	>.10	85	−.019	>.10
18	40	+.182	>.10	30	+.094	>.10	27	+.007	>.10
	GIRLS								
14	91	+.037	>.10	86	+.020	>.10	76	−.071	>.10
16	202	+.045	>.10	175	+.034	>.10	153	+.001	>.10
18	29	+.047	>.10	28	+.043	>.10	20	+.055	>.10

[1] *P* gives the probability with which the degree of relationship observed might arise through chance.

real relationship between cathodal opening contraction and skeletal maturity.

The method of correlation analysis was followed for the other three galvanic tests, with the results summarized in Table 12 for various school-sex-age groups. There was considerable variation in skeletal age among subjects of a given chronological age, and of course the current required to provoke contractions varied greatly among subjects. Nevertheless, correlation analysis furnishes little reason to believe that galvanic irritability and retarded skeletal maturation have anything more than a chance association. The observations on the Fieldston pupils are omitted on the view that the experience of the Seward Park pupils provides a more cogent test of the value of the galvanic test, and only certain age groups among the Seward girls were studied. The correlation coefficients shown in Table 12 are both low and statistically insignificant in all but three instances. These were all obtained for the Seward Park boys aged 15 and are +.151, +.166, and +.244 for the cathodal closing (least twitch), anodal closing (finger), and cathodal closing (hand) tests, respectively. If the true coefficients were zero, the likelihood of obtaining such coefficients by chance would be rather small, as the tabled probability values indicate. However, the coefficients themselves are not large and, in view of their exceptional nature, they hardly constitute weighty evidence of a real relationship between galvanic response and skeletal maturity.

Conclusion

Three possible indicators of calcium nutrition were studied for their relationship to the neuromuscular response to galvanic stimulation in order to assess the value of the galvanic test as a possible means of detecting mild states of calcium deficiency in the body. Several varieties of the galvanic test were studied and the results of each were individually compared with determinations of total serum calcium, calcium intake, and relative skeletal maturity. Some extraneous sources of variation were discovered and controlled by statistical means.

Although the Study utilized a sample of about 1,800 adolescents who varied widely in their calcium intake and in the difference between their chronological and skeletal ages, and appreciably in their total serum calcium, no significant relationship was found between galvanic hyperirritability and any of the three indicators of calcium nutrition. It must be borne in mind, however, that the Study furnished no assessment of calcium nutrition *per se,* and that it was possible to approach it only indirectly through such possibly related factors as calcium intake, serum calcium, and relative skeletal maturity.

That the galvanic test should have diagnostic value for tetany but not for such mild states of calcium deficiency as presumably inhered in the Study sample may mean that the deficiency characteristic of tetany is much more marked than the usual dietary deficiency, or qualitatively different from it. It is known that tetany is induced much more readily by factors which disturb the ionic calcium level in the blood than by simple dietary deficiency of calcium. Whatever the explanation, no evidence is provided that the neuromuscular response to galvanic stimulation provides a satisfactory basis for appraising the adequacy of the calcium nutrition of groups of adolescents.

References

1. Sherman, Henry C.: Food and Health. New York, The Macmillan Company, October, 1934, p. 100.

2. Stiebeling, Hazel K. and Phipard, Esther F.: Diets of Families of Employed Wage Earners and Clerical Workers in Cities. United States Department of Agriculture, Circular No. 507, Washington, D. C., U. S. Government Printing Office, 1939, p. 53.

3. Sherman, Henry C.: Chemistry of Food and Nutrition. New York, The Macmillan Company, 6th edition, May, 1941, p. 264.

4. Erb, W.: Zur Lehre von der Tetanie nebst Bemerkungen über die Prüfung der electrischen Erregbarkeit motorischen Nerven. *Archiv für Psychiatrie und Nervenkrankheiten,* 1874, 4, pp. 271-316.

5. Escherich: Idiopathische Tetanie im Kindersalter. *Wiener klinische Wochenscrift,* Wien, October 2, 1890, iii, No. 40, pp. 769-774.

6. Thiemich, M.: Ueber Tetanie und tetanoide Zustände im ersten Kindesalter. *Jahrbuch für Kinderheilkunde und Physische Erziehung,* Berlin, 1900, 51, pp. 99-120 (vii); pp. 222-226 (x).

7. Mann, L.: Untersuchungen über die elektrische Erregbarkeit im frühen Kindesalter mit besonderer Beziehung auf die Tetanie. *Monatsschrift für Psychiatrie und Neurologie,* Berlin, 1900, 7, pp. 14-38.

8, 9. Loeb, J.: On the Different Effect of Ions Upon Myogenic and Neurogenic Rhythmical Contractions and Upon Embryonic and Muscular Tissue. *American Journal of Physiology,* 1900, 3, p. 383; and 1901, 5, p. 362.

10. Mathews, A. P.: The Nature of Chemical and Electrical Stimulation. I. The Physiological Action of an Ion Depends Upon Its Electrical State and Its Electrical Stability. *American Journal of Physiology,* 1904, 11, p. 455.

11. MacCallum, W. G. and Voegtlin, C.: On the Relation of Tetany to the Parathyroid Glands and to Calcium Metabolism. *Journal of Experimental Medicine,* 1909, 11, p. 118.

12. Howland, J. and Marriott, W. McK.: Observations on the Calcium Content of the Blood in Infantile Tetany and Upon the Effect of Treatment by Calcium. *Quarterly Journal of Medicine,* 1917-1918, 11, p. 289.

13. Hess, A. F., et al.: The Induction of Tetany in Rachitic Rats by Means of a Normal Diet. *Journal of Biological Chemistry,* March, 1931, 90, pp. 737-746.

14. Greenberg, D. M. and others: Factors Concerned in the Development of Tetany by the Rat. *American Journal of Physiology,* September, 1942, 137, pp. 459-467.

15. Hess, Alfred F.: RICKETS INCLUDING OSTEOMALACIA AND TETANY. Philadelphia, Lea & Febiger, 1929, Chapter xiv.

16. Shohl, Alfred T.: MINERAL METABOLISM. New York, Reinhold Publishing Corporation, 1939, Chapter 6.

17. McLean, F. C. and Hastings, A. B.: Concentration of Calcium Ions in Biological Fluids. *Proceedings of the Society for Experimental Biology and Medicine,* June, 1933, 30, pp. 1344-1346.

18. McLean, F. C. and Hastings, A. B.: A Biological Method for the Estimation of Calcium Ion Concentration. *Journal of Biological Chemistry,* October, 1934, 107, pp. 337-350.

19. McLean, F. C. and Hastings, A. B.: The State of Calcium in the Fluids of the Body. I. The Conditions Affecting the Ionization of Calcium. *Journal of Biological Chemistry,* 1935, 108, pp. 285-322.

20. McLean, F. C. and Hastings, A. B.: Clinical Estimation and Significance of Calcium-Ion Concentrations in the Blood. *American Journal of the Medical Sciences,* 1935, 189, pp. 601-603.

21. Todd, T. Wingate: The X-Ray in Assessing the Nutritional State. NUTRITION: THE NEWER DIAGNOSTIC METHODS. Proceedings of the Round Table on Nutrition and Public Health, Sixteenth Annual Conference of the Milbank Memorial Fund, March 29-31, 1938, pp. 9-17.

22. Mack, Pauline Beery: Human Nutrition Research and Improvement in Mass Nutritional Status. *The Pennsylvania State College Bulletin,* April 16, 1942, xxxvi, No. 16.

23. Clark, E. P. and Collip, J. B.: Tisdall Method for Determination of Blood Serum Calcium with a Suggested Modification. *Journal of Biological Chemistry,* 1925, 63, p. 461.

24. Holmes, J. B.: The Reliability of the Electrical Diagnosis of Tetany with Especial Consideration of the Electrical Value Found in Normal Children. *American Journal of Diseases of Children,* July, 1916, 12, No. 1, pp. 1-29.

25. Stinzing, R.: Ueber elektrodiagnostische Grenzwerte. *Deutsches Archiv für klinische Medizin,* 1886, 39, p. 76.

26. Wiehl, Dorothy G.: Medical Evaluation of Nutritional Status. VII. Diets of High School Students of Low-Income Families in New York City. The Milbank Memorial Fund *Quarterly,* January, 1942, xx, No. 1, pp. 61-82.

27. Sjollema, B. and Seekles, L.: Die neuromuskuläre Reizbarkeit in Beziehung zur Biochemie der Minerale; der Einfluss einer Anderung des Ca/P-Verhältnisses in der Nahrung. *Biochemische Zeitschrift,* 1933, 258, pp. 471-479.

28. Schmidt, W. M.: Medical Evaluation of Nutritional Status: Roentgen Appraisal of Development. *American Journal of Public Health,* October, 1941, 31, No. 10, pp. 1068-1072.