Table to Show the Influence of Very Dilute Thyroxin Solutions upon *Bufo halophilus* larvae. Measurements taken after 42 days.

	Total length	Trunk length	Hind leg length
Control in tap water			
	mm.	mm.	mm.
Normal	23.63	10.23	.77
Thyroidectomized	22.96	9.37	.85
Hypophysectomized	27.17	11.43	1.31
Thyroxin solution 1:40,000,000			
Normal	18.30	8.76	3.81
Thyroidectomized	30.47	12.77	5.63
Hypophysectomized	24.32	10.12	4.68
Thyroxin solution 1:20,000,000			
Normal	19.05	8.77	4.04
Thyroidectomized	26.15	10.60	4.65
Hypophysectomized	19.67	8.00	3.63

quantitative difference in response but the writer feels justified in concluding that (1) metamorphosis of thyroidectomized and hypophysectomized tadpoles is readily induced by extremely dilute solutions of thyroxin. (2) The early secretion of these glands does not render the tadpoles either more or less sensitive to thyroxin than the normal ones. (3) The presence of neither the hypophysis nor the thyroid gland is essential or even apparently conducive to the reaction of the tadpole to the thyroxin. Work done several years ago in feeding iodine to thyroidectomized and to hypophysectomized tadpoles demonstrated the fact that elemental iodine would produce metamorphosis when fed to thyroidectomized and hypophysectomized tadpoles. The present work tends to show that there is no difference in the speed of their response to thyroxin.

4593

Effect of Fatigue on Protein Consumption.

FREDERICK P. BROOKS. (Introduced by W. deB. MacNider.)

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Some recent papers on protein consumption and basal metabolism set forth the fact of regular weekly variations in urinary nitrogen excretion. Borgstrom and Bost[1] and Borgstrom, Hafkesbring and Bost[2] showed that in a series of analyses of urine collected over a period of months there was evidence of a weekly cycle of values.

[1] Borgstrom, P., and Bost, R. W., *Am. J. Physiol.*, 1926, lxxix, 229.

[2] Borgstrom, P., Hafkesbring, R., and Bost, R. W., *Am. J. Physiol.*, 1926, lxxix, 237, 245.

On certain days of every week high values were observed for one subject on the basis of fatigue and on the basis of muscular exercise for the other. The writer had observed a recurrent variation in some weekly values determined for a few individuals. In these cases it was difficult to explain the variation on the grounds of exercise but it appeared more likely that fatigue played the more important rôle. It seemed worth while to secure more evidence in support or refutation of this idea. It would be of interest in regard to certain values reported for students,[3, 4, 5] for if certain days of the week give high or low values and the data reported were obtained on these days, these reports would represent levels which might be well above or below the weekly average, and the protein habit of the individuals studied would be incorrectly represented.

If fatigue exerted an appreciable effect upon the protein consumption, this effect would be revealed in the daily urinary nitrogen excretion of medical students since this group lives under a rather strenuous and regular schedule. In this institution the schedule of the first year is rather full, allowing little time for outside activities, and few students take any regular exercise. It would be expected that a gradual increase in fatigue would be seen from Monday to Friday. From Friday night until Monday represents a period of relaxation and, in many instances, an unusual amount of muscular activity. If this condition of fatigue should exert an appreciable influence on the protein intake the weekly curve of urinary nitrogen excretion should show values above average for Saturday, Sunday, Monday and probably Tuesday, with low average values the remainder of the week. The curve would probably run from a maximum on Monday or Tuesday to a minimum on Friday with the intermediate days grading between. The general slope of the curve should be downward from the first to the last of the week. Curve 1 of Charts I and II represents this idea.

It was not to be expected that such a variable as urinary nitrogen on an uncontrolled diet would regularly give any such smooth curve as Curve I even if the effect existed as postulated. It would indeed be remarkable if a large percentage of all the cases studied should show conformity.

Experimental. In 1928 a group of students collected 24-hour urine specimens on one day a week for 7 weeks. A different day of each week was selected so that results were obtained representing

[3] Beard, H. A., *Am. J. Physiol.*, 1927, lxxxii, 577.

[4] Brooks, Frederick P., *Ibid.*, 1929, lxxxix, 403.

[5] Denis, W., and Borgstrom, P., *J. Biol. Chem.*, 1924, lxi, 109.

the daily excretion and at the same time representing the protein habit over a period of 2 months. On the whole the collections were trustworthy. The completeness of the collection was judged from specific gravity—volume relationships and the results were discarded where obvious error appeared. The analyses here reported were all made by the writer using the Gunning modification of the macro Kjeldahl method.

In Chart I are shown graphically certain of the results. These were selected because they represented complete 7-day collections, while those not reported were incomplete due to the closing of the term. The values represent the nitrogen from the urine collected on

CHART I.—*Daily Urinary Nitrogen Excretion in gm.*

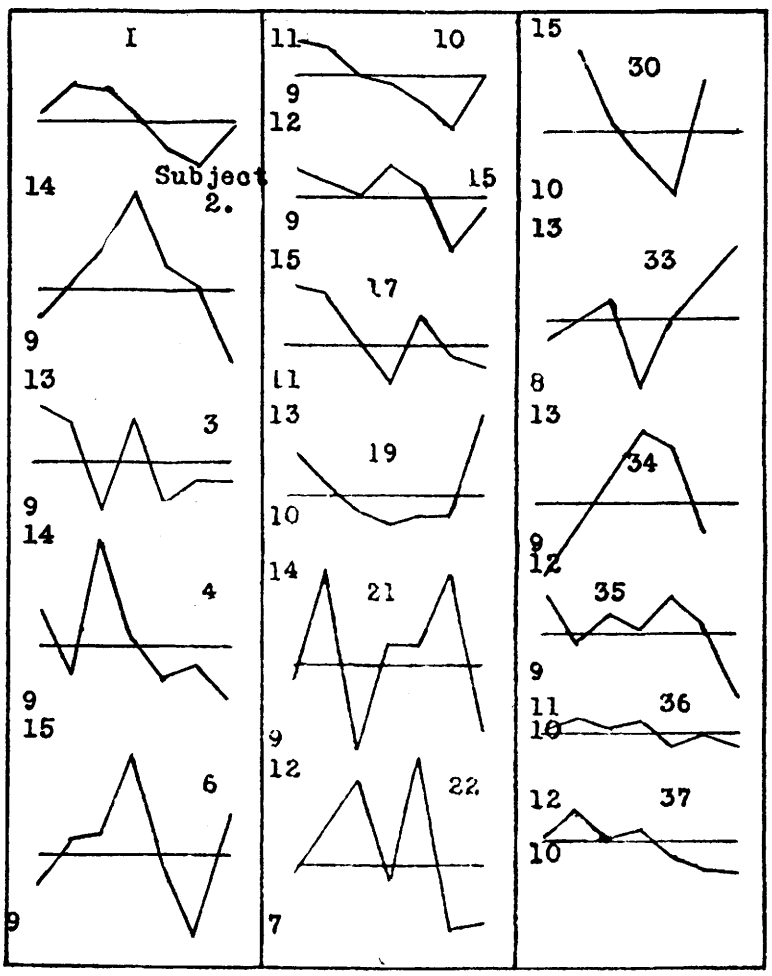


CHART II.—*Daily Urinary Nitrogen Excretion in gm.*

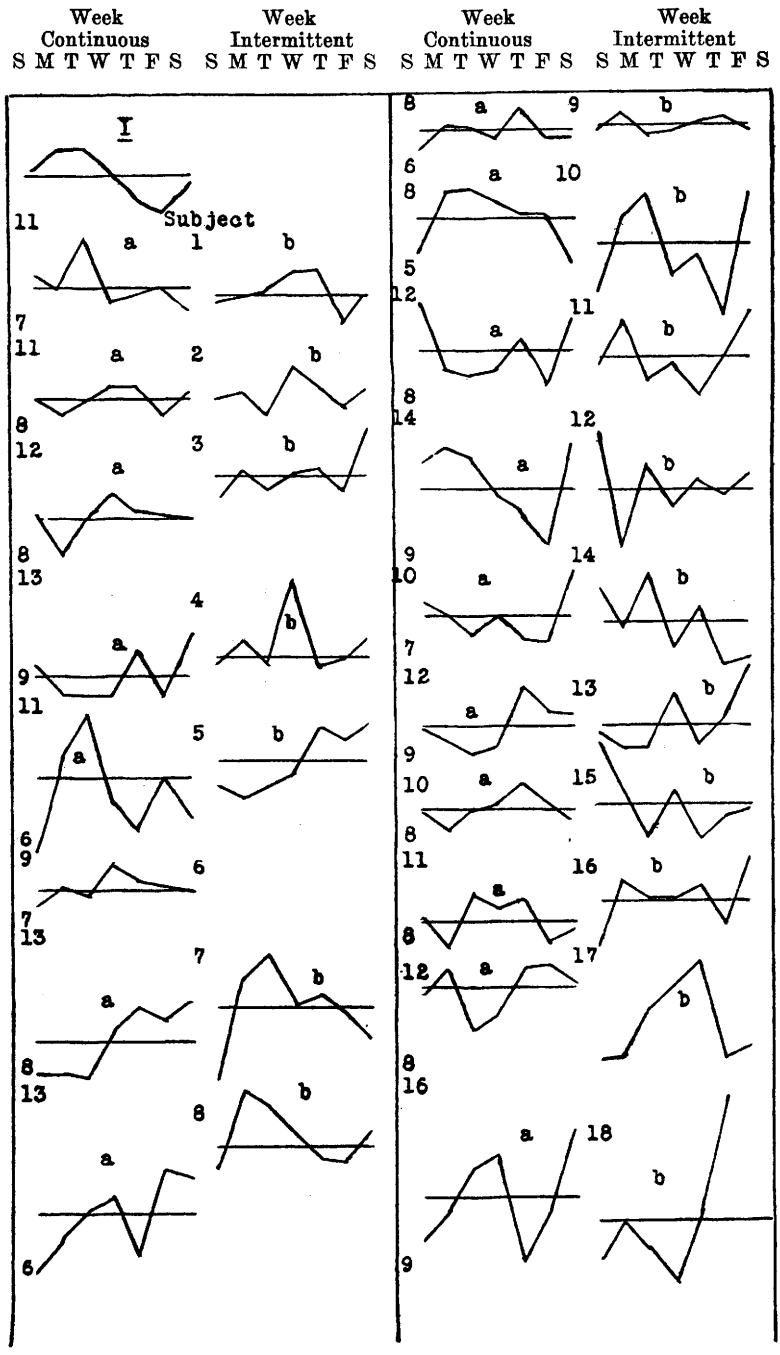
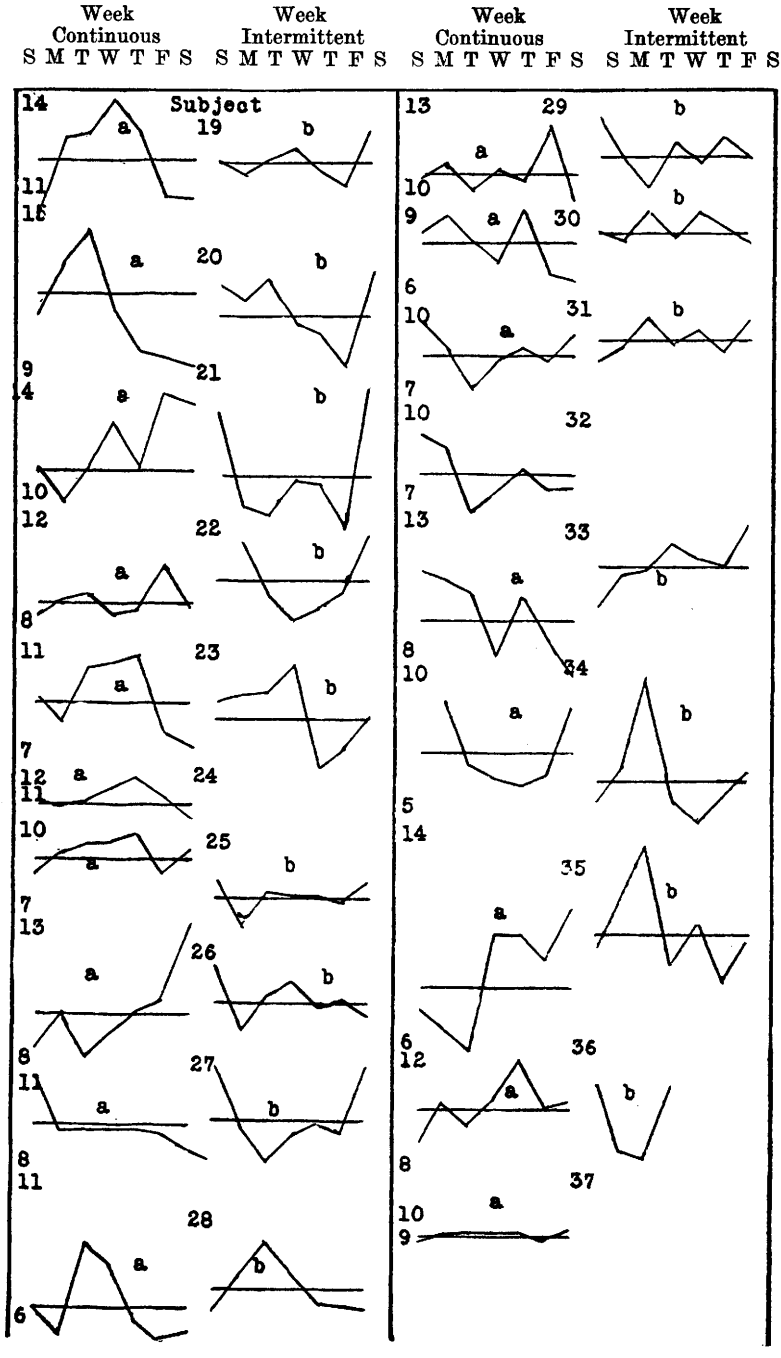


CHART II. *(Continued).*



the day designated. The specimens were completed on the morning of the day following the day labeled, *i. e.*, a Monday specimen is that collection made from 7 a. m. Monday until 7 a. m. Tuesday, etc. These data were collected during February and March of the year noted. The average value for the group, the per 70 kilo equivalent and the temperature of the period have been reported in a previous paper.[4]

In January and February, 1929, a second series of determinations was made from a group of 36 male medical students. The system of collection was the same for 2 months. Each student made a daily collection for one continuous week. The sample was analyzed and the completeness of the collection was checked by the specific gravity volume relationships of the various samples of each individual. The data for this series is shown in the plotted curves of Chart II.

Discussion. A study of Chart I shows the average of the group in fair agreement with Curve 1. Compare Curves 1 and 36 of Chart I. Curve 36 represents the average daily values for the entire group of 35 subjects, while Curve 37 represents the average of the curves shown in Chart I. These are both in fair agreement with Curve I, which is the postulated form of the curve which should show the fatigue effect under the described conditions. An analysis of the curves in Chart I is given below in Table I.

A study of the average values for Group II reveals a remarkably close agreement of all the days of the week and of the week of continuous collections with the week of intermittent collections over a period of 2 months. Curves 37 and 37b, Chart II, show the average daily values for the continuous and intermittent weeks respectively as almost horizontal lines. This would seem to indicate a failure of the protein consumption to respond to fatigue. It certainly indicates that with a sufficiently large group of subjects specimens collected on any day of the week will give the protein habit of the group accurately. From this it appears that the results of Borgstrom and Denis, Beard, and Brooks do represent the habits of the groups studied in so far as any fatigue effect would have vitiated them. Many of the variations observed show maximum values where minimum values were expected. But an analysis of the individual curves of Charts I and II gives more support to the idea of a fatigue effect than do the total averages of the groups.

These curves may be classified in 5 groups as follows: (1) Curves showing maxima toward the first of the week; (2) Curves showing maxima toward the last of the week; (3) Curves showing maxima

TABLE I.
Classification of Curves in Charts I and II.

Data	No. of Curves	Type 1	Type 2	Type 3	Type 4	Type 5
Chart I	16	9	1	2	2	2
Chart IIa	36	13	9	5	3	6
Chart IIb	32	11	3	3	4	11
Total	84	33	13	10	9	19
Percentage of each type		39.9	15.5	12	10.6	22.6

in the middle of the week; (4) Curves showing minima in the middle of the week; (5) Curves showing no tendency toward either maxima or minima. Type *1* is seen in Curve I. Type *2* is seen in Curve 4, Chart II. Type *3* is shown by Curve 2, Chart I. Curve 19, Chart I, represents type *4*. Type *5* is found in Curve 35, Chart I.

From this analysis it is evident that there is a preponderant number of curves of type *1* which certainly shows a strong suggestion of an effect which I have postulated, a fatigue effect. It is quite surprising that types *1* and *5* should make up nearly two-thirds of the total number. It is more surprising that the remainder of the curves which are divided into 3 equal size groups should be able to overcome the tendency shown by type *1* curves and bring the total average to a curve of the neutral type. This is explicable only on the basis of the very wide variations which are observed on some curves on all days of the week.

These weekly variations might be explained on the basis of menu if the group of subjects ate at the same place. However, the group patronized 12 different eating places and not more than 6 ate at the same place. Many ate at cafeterias and cafes where the menu was determined by their choice and financial condition.

The above results seem to indicate a response of protein consumption to fatigue in the case of medical students living on a rather regular schedule. They also show that any day of the week will be satisfactory for the collection of urine specimens for the determination of protein consumption provided a large group of subjects is used for obtaining the average. This apparent inconsistency, *viz.:* a tendency for the daily excretion to diminish in response to fatigue and a representative average for the group on any day of the week is explainable on the grounds of the great irregularity in the nitrogen excretion of certain individuals on each day.

Summary. 1. The results of approximately 105 weeks of urine collections by 70 individuals are given in terms of N excretion. 2. The daily values are plotted and it is found that there is a decided ten-

dency toward a decreasing daily excretion from Sunday to Saturday. 3. These results are interpreted as supporting the claim of a fatigue effect upon protein consumption. 4. It is shown that in spite of this tendency specimens collected on any day of the week may be used to give an accurate idea of the protein consumption of the group if the group is large enough.

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Source of Bioelectricity, Investigated by the Relation Between Stainability and Electric Charges in Tissues and Artificial Models.

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Little is known about the electrical action inside of living tissue. Its nature and cause can be elucidated to a certain degree by comparing stainability and electromotive forces.[1]

Numerous previous experiments have demonstrated the following relation between the stainability of tissues and bioelectric currents. Structures bearing a relatively negative charge are preferably stained by eosin and certain other acid dyes, while electrically positive struc-

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[1] It may be added that electrical potential differences must be present in tissue everywhere, *viz.*, at every phase boundary and also at any place where diffusion occurs. The electromotive property of the skin of plants is analyzed by its extraordinarily large and regular variations following changes in the concentration of the solutions in contact with it. This effect can *not* be reproduced by means of protein as has been maintained by J. Loeb. (Loeb, J., *Proteins and Colloidal Behavior*, 1922; Höber, *Zeitschr. physik chemic.*, 1924, cx, 142.) None of the values given by Loeb, Höber, and their collaborators amounts to more than one-fifth of the maximal effect of concentration obtainable in plant and animal tissue.

The so-called 'protein' effects demonstrated by Höber with various salts are, moreover, just water effects. His assertion that this of itself exclusively should explain bioelectricity is contradicted by numerous facts. With a *few selected* substances only the maximal effect of concentration can be reproduced. Among these substances is dried collodion. The electromotive action of this substance need not necessarily be explained as L. Michaelis (*Biochem. Zeitsch.*, 1925-26, nine papers; also *J. Gen. Physiol.*, 1927-29, eight papers) suggest, as due to pores of molecular dimensions which cause selective ionic permeability. This case does not necessarily require an entirely different theory from other similar cases.