THE INFLUENCE OF THE PROTEIN INTAKE ON THE BASAL METABOLISM.

By GEORGE MACFEAT WISHART.

(*From the Institute of Physiology, University of Glasgow.*)

In view of Rubner's theory of secondary dynamic action of protein, and the frequent observation that the human basal metabolic rate is increased by liberal protein diet, the parallelism (noted in a previous paper (1)) between the daily variations in urinary nitrogen and variations in basal metabolism in one subject are, perhaps, not very surprising. As this subject, Wn., had partaken of somewhat abnormal diets, and the correlation between urinary nitrogen and basal metabolism was a close one (correlation coefficient + 0·70), it was thought worth while to confirm the relationship in another subject, whose diet should be kept as normal as possible, consistent with wide variations in the protein component.

The subject was the author, and two series of observations were made. During the first series, lasting 20 days, the diet was uncontrolled, the object being to discover whether the slight daily fluctuations in nitrogen intake on ordinary diet would show any relation to the daily variations in basal metabolism. A second series was then carried out over a period of 40 days; during this period the protein quota of the diet was varied from approximately 30 gm. per diem to 150 gm. per diem. In addition to the estimation of basal metabolism and the total urinary nitrogen, daily observations of the urea, ammonia, creatinine, uric acid, and phosphate-ratio of the urine were made. These subsidiary determinations were done in the hope that, if, on the whole, the basal metabolism was found to be correlated with the total nitrogenous metabolism, deviations from this general rule would be found to be associated with a change in the excretion of some particular nitrogenous urinary constituent.

Methods. The following methods were used:

Basal metabolism ...	Douglas Bag and Haldane Air Analysis.
Total nitrogen ...	Kjeldahl.
Urea	Urease.

Ammonia ...	...	Folin.
Uric acid ...	...	Hopkins-Folin.
Creatinine ...	...	Folin.
Phosphate-ratio	...	Leathes.

The sample of expired air for the basal metabolism estimation was collected by a skilled operator immediately before the subject rose in the morning at 8 a.m. The urine was collected from 8 a.m. to 8 a.m., and, throughout the following discussion, the basal metabolic data are compared with the urinary data for the collection ending at the time of the basal estimation. A short series of unpublished observations, wherein the estimation of the basal metabolism took place at the mid-point of the urine collection period, seemed less likely to yield fruitful results, and was abandoned.

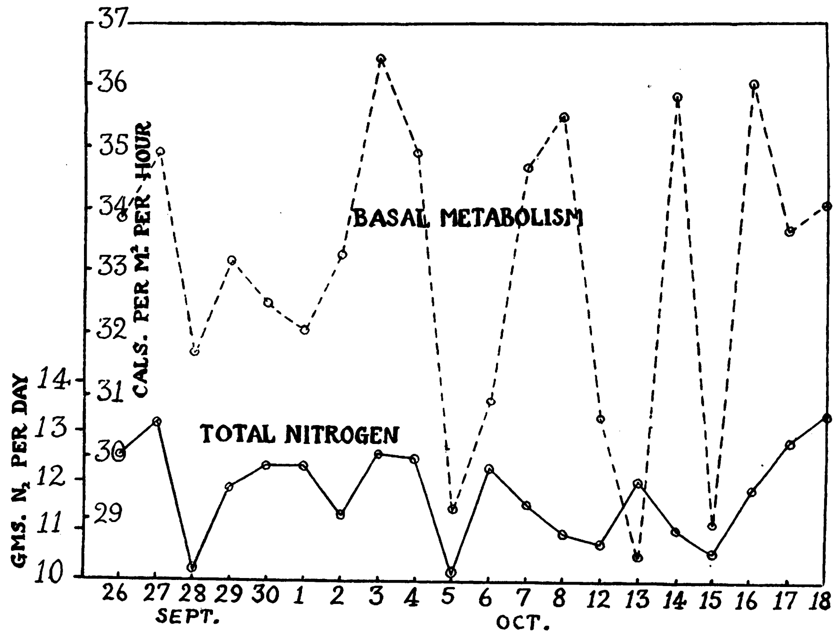
Before commencing the experiment, it was realised that, in an investigation of this kind, the greatest possible accuracy of technique was essential. The most extreme care was therefore taken with all the analyses, each recorded datum being the average of two agreeing analyses; wherever the slightest doubt existed as to the technical accuracy of an analysis, the analysis was repeated. On December 1st, in the second series, owing to a leaky valve, the collection of expired air was faulty and all data for this day had to be omitted. This omission along with a day's interval between the normal diet period and the first period of low protein diet were the only breaks in an otherwise complete set of consecutive daily observations.

The dietary during the first series was the subject's ordinary everyday diet. During the second series the dietary arrangements were as follows: (1) 6 days on normal diet as in first series; (2) 11 days on a fixed diet containing about 30 gm. protein per diem; (3) 12 days on a fixed diet containing about 150 gm. protein per diem; and (4) 11 days' repetition of (2). The low-protein diet was composed of bread, potatoes, butter, tapioca, and syrup, and had a calorie value of approximately 2900. In the high-protein diet the tapioca and syrup were omitted and eggs, ham and meat substituted, the calorie value being approximately 3100. The extra 200 calories were found to be necessary to prevent loss of body-weight. On the whole, the body-weight remained fairly constant throughout the experiment, varying from 64·0 kilos on the first day to 61·6 kilos on the last day of the second series.

Results.

First Series.

The 20 consecutive daily observations on normal diet of the first series showed that (1) the fluctuations of urinary nitrogen on uncontrolled diet were comparatively small (max. 13·44 gm., min. 10·08 gm.) and (2) afforded indications of some parallelism between nitrogenous output and basal metabolic rate. Graphical evidence of this parallelism is given in Graph I.



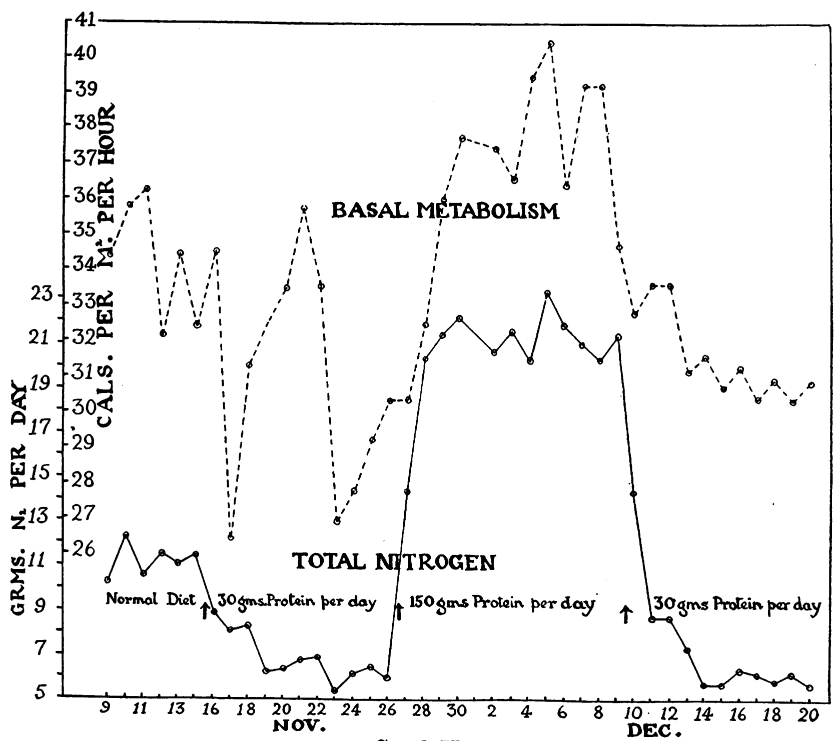
Graph I.

The degree of similarity between the two curves may be given a numerical value by calculating the statistical correlation coefficient r. This was found to be $r_{\mathrm{B.T.N.}} = +\ 0{\cdot}41 \pm 0{\cdot}125$, a significant correlation. The correlation coefficient between basal metabolic rate and the urinary excretion of urea was almost as high—$r_{\mathrm{B.\,Urea}} = +\ 0{\cdot}38 \pm 0{\cdot}129$. Correlation between each of the other nitrogenous constituents determined and the basal metabolism gave coefficients which were insignificant in comparison with their probable errors.

Second Series.

Since, in the first series, variations due to errors of estimation were large in comparison with the nitrogen fluctuations, more striking results were expected from the second series, in which the protein intake was altered as widely as possible without making the diet excessively abnormal.

The fluctuations in urinary nitrogen and basal metabolism are shown in Graph II. The transient fall in basal metabolism reported by Borgstrom, Hafkesbring and Bost (2), as following increased nitrogenous metabolism has not been confirmed, the curves showing a parallel relation to one another, similar to, but more marked than in the case of the first series.



Graph II.

Table I gives the coefficients of correlation between the basal metabolism and (1) the total nitrogen, and (2) each of the other urinary constituents estimated.

TABLE I. (40 observations.)

Correlated variables	Correlation coefficient
B.M. and total nitrogen	$r_{B.T.N.} = +0{\cdot}75 \pm 0{\cdot}047$
B.M. and urea	$r_{B.\ Urea} = +0{\cdot}75 \pm 0{\cdot}047$
B.M. and ammonia	$r_{B.\ Amm.} = +0{\cdot}71 \pm 0{\cdot}053$
B.M. and creatinine	$r_{B.\ Creat.} = +0{\cdot}76 \pm 0{\cdot}045$
B.M. and uric acid	$r_{B.\ Uric\ Ac.} = +0{\cdot}73 \pm 0{\cdot}050$
B.M. and acidity*	$r_{B.\ Acid} = +0{\cdot}44 \pm 0{\cdot}086$

* As expressed by phosphate-ratio.

Since, as may be observed from the graph, there is a distinct tendency for the changes in basal metabolism to lag behind the changes in urinary nitrogen, correlation coefficients were also calculated for the series of 31 observations remaining after the arbitrary exclusion of three consecutive days at each of the three transition periods when dietary changes were made. These values are given in Table II.

TABLE II. (31 observations.)

Correlated variables	Correlation coefficient
B.M. and total nitrogen	$r_{B.T.N.} = +0{\cdot}84 \pm 0{\cdot}036$
B.M. and urea	$r_{B.\ Urea} = +0{\cdot}84 \pm 0{\cdot}036$
B.M. and ammonia	$r_{B.\ Amm.} = +0{\cdot}76 \pm 0{\cdot}051$
B.M. and creatinine	$r_{B.\ Creat.} = +0{\cdot}82 \pm 0{\cdot}040$
B.M. and uric acid	$r_{B.\ Uric\ Ac.} = +0{\cdot}85 \pm 0{\cdot}034$
B.M. and acidity*	$r_{B.\ Acid} = +0{\cdot}59 \pm 0{\cdot}079$

* As expressed by phosphate-ratio.

DISCUSSION OF RESULTS.

A. *Correlation between Basal Metabolism and Nitrogen Excretion.*

There exists a remarkable parallelism between the urinary nitrogen excretion and the basal metabolic rate, as evidenced by the graphs and the high correlation coefficients of + 0·84 for the 31 days and + 0·75 for all 40 days. A much higher degree of correlation would have resulted in the absence of the curiously aberrant values of 18th to 22nd November; no emotional or other cause could be determined to account for these high values. This relation between urinary nitrogen and basal metabolism is evident even in the slight fluctuations occurring on a normal uncontrolled diet.

Comparing the coefficients for all 40 observations with those obtained after exclusion of the transitional periods, the latter are seen to be not only actually higher in every case, but also greater in proportion to their probable errors.

Moreover, in each series, with the exception of the acidity coefficient, the correlation between basal metabolism and each nitrogenous constituent is of approximately the same value; this excluded any possibility of demonstrating, as had originally been hoped, that those basal metabolism values which were aberrant in relation to the total nitrogen excretion would be found to be associated with some marked deviation in the excretion of one particular nitrogenous component. To determine whether such aberrant values were associated with alterations in urinary acidity, as estimated by the phosphate-ratio, the partial correlation coefficient between basal metabolism and acidity, nitrogen being kept constant, was calculated. The coefficient so obtained is an indication of any association of changes in basal metabolism with urinary acidity, when the average variations of each with the nitrogen excretion are eliminated. This partial correlation coefficient was ${}_{N}r_{B.\ Acid} = -0{\cdot}28 \pm 0{\cdot}112$; it was small in comparison with its probable error, and is therefore of doubtful significance.

B. *Prediction in the Individual of the Basal Metabolism from the Urinary Nitrogen Excretion.*

As a test of the value of the statistical correlation between total nitrogen and basal metabolism, one may attempt to predict the latter from the former. Assuming straight-line regression, the regression equation calculated from the series of 31 observations (transitional periods excluded) was

$$x = 28{\cdot}456 + 0{\cdot}449y,$$

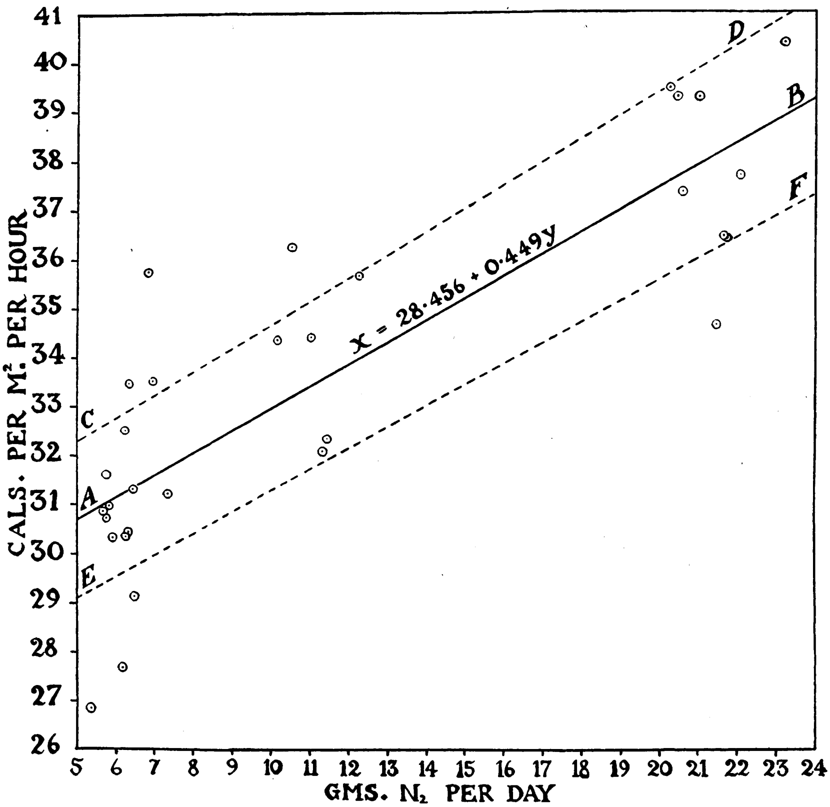
where x = basal metabolism in cals. per sq. meter per hour,

and y = urinary total nitrogen in gm. per day.

If the basal metabolism be expressed, not as per unit area, but in total calories per hour, the equation becomes

$$x = 52{\cdot}645 + 0{\cdot}831y.$$

In this subject, therefore, the basal metabolic rate altered by about eight-tenths of a calorie per hour or by about half a calorie per hour when the metabolism was related to his surface-area, for a change of 1 gm. in the urinary nitrogen excretion. The graphic representation of these equations gives, for this subject, the average value of the basal metabolism associated with any particular value of urinary nitrogen. Graph III is a "scatter" diagram showing the relation of the actually observed values to these average values as represented by the regression line *AB*. Lines *CD* and *EF* are drawn at distances from *AB* equivalent to a deviation of the basal metabolism by 5 p.c. Of the eight observations lying outwith this range, six occurred on consecutive days.



Graph III.

Such results indubitably show that, in assessing the value of the normal basal metabolism of an individual, the level of the protein intake must be taken into account. It is doubtful if this has been sufficiently appreciated, except by Krogh, who demands that the subject shall subsist on a low protein dietary for two days previous to the basal estimation (3).

In the writer's experiments the difference between the metabolism as observed, on the day following the lowest nitrogen excretion (5·292 gm.), and the day after the highest nitrogen output (23·240 gm.), was 13·56 calories per square metre per hour or 51 p.c. of the lower value. Using the predicted values from the regression equation for such nitrogen excretions, the difference would be 26 p.c. The protein intake is therefore a factor of very considerable importance in modifying the basal metabolic rate.

C. *Correlation in Other Subjects.*

Similar correlations have been calculated for three other subjects in whom, in this laboratory, both urinary nitrogen and basal metabolism had been determined daily over fairly long periods. Unfortunately, in only one of these (Wn.—male), who was frequently altering his diet for other experimental purposes, were there any great fluctuations in nitrogen output. The other two (D.—male, and Be.—female), on the contrary, were on fixed diet and endeavouring to maintain their nitrogen metabolism at constant level. The results were as follows:

TABLE III.

Subject	Wn.	D.	Be.
No. of observations	77	41	31
Correlation coefficient $r_{B.T.N.}$	$+0{\cdot}70 \pm 0{\cdot}05$	$+0{\cdot}30 \pm 0{\cdot}096$	$+0{\cdot}15 \pm 0{\cdot}118$

It will be observed that, in Wn., in whom the nitrogenous intake fluctuated widely, a coefficient, approximating to that obtained in the writer as subject, was found, and, even in the other two subjects, D. and Be., in whom, on account of the constant diet, little correlation would be expected, the coefficients were both positive in trend though only one, D., was significant in relation to its probable error. This tendency to parallelism between nitrogenous and basal metabolism is apparently, therefore, a general one, and may be rendered evident, by statistical treatment of a series of observations, even in the comparatively minute fluctuations of nitrogen output that occur on a constant diet.

D. *Prediction of the Basal Metabolism from the Urinary Nitrogen in Different Individuals.*

The existence of a positive correlation in all the subjects examined suggested that one of the factors, and possibly the main factor, in the variations in basal metabolic rates of different individuals lay in differences of nitrogenous metabolism. This idea gained much support when it was found that the regression equation calculated from the observations on Wn. was $x = 28{\cdot}269 + 0{\cdot}448y$, being thus almost identical with the equation $x = 28{\cdot}456 + 0{\cdot}449y$ obtained from the observations on the writer as subject. This similarity in the reactions of Wn. and the writer to alterations in nitrogenous metabolism is shown in another way in Table IV, wherein the data from Wn. are divided up into dietary periods, during each of which the nitrogen output remained fairly constant. For each of these periods, the basal metabolism as observed on Wn. is compared with the metabolism as computed from the nitrogen output by the regression equation developed from the metabolism values of the writer.

TABLE IV.

Urinary nitrogen gm. per day	B.M. observed on Wn.	B.M. calculated from writer's data	Percentage difference
3·80	30·6	30·2	−1·3
3·86	29·7	30·2	+1·7
6·46	30·5	31·4	+2·9
7·25	31·7	31·7	0·0
9·24	32·7	32·6	−0·3
12·46	34·6	34·1	−1·5
13·89	34·2	34·7	+1·4
16·52	36·6	35·9	−1·9

The similarity between these two subjects was so close that it seemed highly improbable that it was due to mere coincidence. It should be noted, however, that both subjects are assistants in this laboratory; their daily work and mode of life were similar, and their calorie intakes almost identical when expressed per unit of surface-area.

The validity of the prediction formula was then tested on the other subjects examined in this laboratory for whom suitable data were available. In applying such regression equations to the computation of the basal metabolism of different individuals, one may use either the formula $28{\cdot}456 + 0{\cdot}449y$, which deals with the metabolism as expressed per unit of surface, or the formula $52{\cdot}645 + 0{\cdot}831y$, which gives results in terms of total metabolism and takes no account of the surface-area at all. Moreover, it was hoped that, by finding which formula had the better prediction value, some indication would be obtained of whether the basal metabolism was a function of the surface-area, or was entirely a matter of the "active protoplasmic mass," as is Benedict's view. In the latter case, since the nitrogen excretion alone is presumably an indication of the amount of "active protoplasmic mass," one would expect better prediction results from the formula $52{\cdot}645 + 0{\cdot}831y$, in which considerations of surface-area are omitted.

In the following table (V) the actual average metabolic rates of four subjects of this laboratory for whom the necessary data were available, are compared with the predicted values obtained by each of the two formulæ. One female subject has been included, though the justification for this is obviously somewhat doubtful.

Table V.

Subject	A Observed metabolism per sq. m. per hour	B Calculated metabolism per sq. m. per hour	Percentage difference A and B	C Observed total metabolism per hour	D Calculated total metabolism per hour	Percentage difference C and D
Wn. (male)*	31·7	31·7	0·0	53·6	58·7	− 8·7
D. (male)	37·9	35·3	+ 7·4	64·8	65·3	− 0·8
M. (male)	36·7	32·8	+11·9	59·4	60·7	− 2·1
Be. (female)	31·2	33·3	− 6·4	51·8	61·8	−16·2
		Average variation	+ 3·2		Average variation	− 6·9

* Period of normal diet only.

It is rare to find in the literature instances of simultaneous observation of urinary nitrogen and basal metabolism where the daily data are given, but the prediction values of both formulæ were also tested on (1) results reported by Borgstrom, Hafkesbring and Bost (4), and (2) data taken from the classical paper by Benedict, Miles, Roth and Smith (5) on under-nutrition. The application of the formulæ to such data as could be obtained from these papers showed divergences between predicted and observed values even greater than those given in the table above. In view of the excellent results obtained by the application of the formulæ to subject Wn., the results on other subjects in this laboratory and on the American subjects were therefore surprisingly disappointing in their irregularity.

Further, while it has been a general experience in this laboratory to obtain results considerably below the Benedict or Du Bois' standards, a difference probably mainly associated with the fact that all our subjects were trained laboratory workers and thoroughly accustomed to metabolic measurements, even when allowance is made for this difference, the predicted values afford no indication as to whether the basal metabolism is primarily determined by the "active protoplasmic mass" or by surface-area.

The unsatisfactory results obtained by the application of the prediction formulæ to such data as were available suggests that differences in nitrogenous metabolism will not alone account for the differences in basal metabolic rates of different individuals. In the two subjects, Wn. and the writer, in whom good agreement was obtained, the calorie intakes per unit of surface-area were almost identical. In the work of Benedict and his co-workers on under-nutrition previously referred to, despite a fall in urinary nitrogen per man per day of only 1·5 gm., restriction of the calorie value of the diet by approximately 30 to 40 p.c. produced an average fall in the basal metabolic rate of about 20 p.c. Habitual differences in the calorie intake of different individuals might therefore quite well act as an additional factor in occasioning differences in their resting metabolisms.

E. *Difference in Behaviour of the Nitrogenous Urinary Constituents at the Dietary Transition Periods.*

A minor point of interest which might prove worthy of further investigation is the different behaviour of the various nitrogenous constituents of the urine when abrupt changes in the protein intake were made. The two extremes of this different behaviour are exhibited, on the one hand, by uric acid, which moves immediately, in the course of the first day after the dietary change is made, either up or down to its new value; and, on the other hand, by the ammonia which shows a gradual progressive change to its new level. The urea and creatinine excretion show changes of a type intermediate between these two extremes.

Summary.

1. A marked parallelism exists between the daily variations in basal metabolism and the variations in the output of nitrogen in the urine. This relation has been demonstrated in several subjects, even in the slight fluctuations that occur on normal diet, but has been much more strikingly shown by a series of daily observations on the writer, during which his protein intake was extensively altered.

2. The correlation between urinary nitrogen and basal metabolism in this series was remarkably close; when expressed statistically, the coefficient of correlation was $+ 0{\cdot}84 \pm 0{\cdot}036$.

3. This statistical relationship was used for the prediction of the basal metabolism in the writer, at any given level of nitrogenous metabolism, with fairly satisfactory results.

4. Basal metabolism values predicted from the same formula showed remarkably good agreement with the observed value in another subject whose protein intake had been extensively varied.

5. General application of the formula to the prediction of the basal metabolism of a number of individuals, both of this and other laboratories, from their average protein metabolism, met with little success.

6. In the writer, the fluctuations of basal metabolism with alterations in protein intake were considerable. The lowest value observed, when the subject was on a diet containing 30 gm. protein per day, was 26·83 cals. per square metre per hour; the maximum value, when the diet contained 150 gm. protein per day, was 40·39 cals. per square metre

per hour. This emphasises the need for something approaching standard dietary conditions previous to basal metabolism estimations.

It is a pleasure to record my indebtedness to Prof. E. P. Cathcart for his helpful advice and criticism during the course of this work.

REFERENCES.

1. Wishart. Quart. Journ. Med. 20. p. 203. 1927.
2. Borgstrom, Hafkesbring and Bost. Amer. Journ. Physiol. 79. p. 245. 1926.
3. Krogh. C.-R. Soc. Biol. 87. p. 458. 1922.
4. Borgstrom, Hafkesbring and Bost. Loc. cit.
5. Benedict, Miles, Roth and Smith. Carnegie Inst. Washington, Pub. No. 280. 1919.