reprecipitated from PI in aqueous solution at pH 5.3, ionic strength of 0.15 at 0°C. The precipitate was removed by centrifugation and was dissolved to the original volume of PI in 0.15 M sodium acetate at pH 7.0 and the solution was clarified by centrifugation. This fraction (PII) contained 920 LD_{50} per ml and 5260 LD_{50} per mg N (average values). This represents an average yield of 81% of PI with a purification factor of 17.5. The overall yield at this step was 51%. The N/P ratio was found to be 43.1.

(e) *Electrophoretic analysis.* Electrophoretic patterns of the water extract, the 0.05 M $CaCl_2$ extract, Fraction PI and Fraction PII from *H. pertussis,* strain No. 29, are shown in Fig. 1. The most active fraction, PII, was found to be heterogeneous.

Discussion. It has been found that the most favorable conditions for the separation of pertussal toxin involve extraction of organisms dried from the frozen state with 0.05 M $CaCl_2$ at pH 6.5 followed by precipitation at pH 4.4 in 15% methanol, ionic strength of 0.13 at −5°C and reprecipitation at pH 5.3, ionic strength of 0.15 at 0°C. The most active fraction, PII, contained 5260 LD_{50} per mg N which represented a 17.5-fold purification over the water extract with an average overall yield of 51%. Fraction PII was found to be heterogeneous by electrophoretic analysis.

The relationship of toxin found in strain No. 29 to that found in true phase I strains has not been definitely established. Strain No. 29 has been shown to satisfy only one of the serological criteria for a phase I organism, namely agglutination by a phase I antiserum(13). This organism contains protective bacterial antigens against the phase I strain used in the mouse protection test(13). The bulk of the available experimental evidence suggests that the toxin found in the variant strain No. 29 is the same as that found in all strains of *H. pertussis.*

Summary. Methods have been described for the separation of pertussal toxin from a 0.05 M $CaCl_2$ extract of *H. pertussis,* strain No. 29, dried from the frozen state. The most active fraction contained 5260 LD_{50} per mg N and was found to be heterogeneous by electrophoretic analysis.

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Influence of Feeding Schedule on Nitrogen Utilization and Excretion. (17815)

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The object of the experiment reported in this paper is to find the answer to a practical question of nutrition: With a given total dietary intake does the feeding schedule have any influence on the efficiency of N utilization, and if so, is it more efficient to have 2, 3 or 4 feedings a day?

According to the current concept of dynamic equilibrium of body constituents, tissue proteins are continually broken down into amino acids and new tissues are continually formed from amino acids. When the rate of protein formation equals the rate of protein breakdown, the body is in protein equilibrium. Besides the reversible reaction between proteins and amino acids, the latter are continually removed irreversibly from the metabolic pool by excretion as such and by conversion into nitrogenous waste products, chiefly urea. The rates of removal of amino acids from the metabolic pool, whether by formation of protein or urea, or by excretion, should vary with the concentration of the assorted amino acids, other things being equal. If the rate of removal of amino acids equals the rate of replenishment from the digestive tract, the concentration of amino acids in the metabolic pool would remain con-

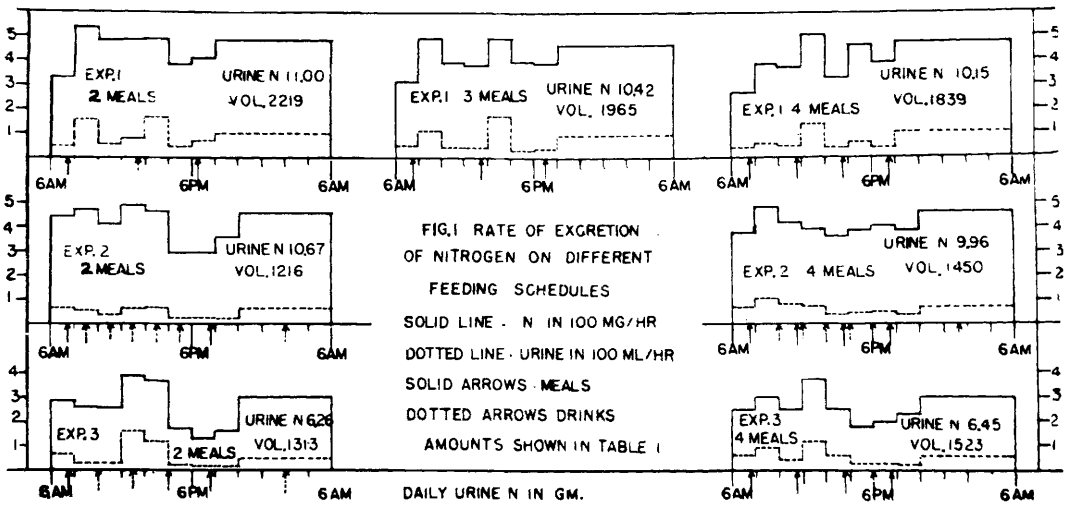

FIG. 1.

stant and the body would be in N balance. Actually, the concentration of amino acids in the metabolic pool must fluctuate with feeding. With this fluctuation, the rates of removal of amino acids by whatever process must fluctuate also. The well known specific dynamic effect of proteins is largely, if not wholly, an expression of the increase in the rate of metabolism of amino acids, as their concentration in the pool is raised by feeding. Following each meal, there is an increase in amino acid concentration in blood, which falls again to the pre-feeding level after several hours. In each day the amino acid concentration in the metabolic pool rises and falls like a wave. With each wave the rates of removal of amino acids rise and fall also.

While the increased catabolism of protein at the trough of the wave may be compensated by the increased anabolism at the crest, the increased loss of amino acids by excretion and by urea formation at the crest, is not compensated by a reverse reaction at the trough. It would seem from these considerations that the amount of amino N irreversibly removed from the pool would depend on the frequency and amplitude of the waves of amino acid concentration which in turn depends on the feeding schedule. Given the same total intake of protein, the loss of N by irreversible removal of amino acids should be smaller and consequently the efficiency of N utilization should be higher with frequent feedings of light meals than with fewer feedings of heavy meals, other things being equal. The experiment to be reported is an attempt to test this hypothesis. Incidentally, it throws some light on the question of the rhythm of N metabolism.

Experimental. The subject was a normal adult who had maintained his body weight (68.5 kg) for many months and was presumably in N equilibrium. The diet of free choice before the experiments contained 78 g protein and supplied 2500 calories. The diet in Exp. 1 was constructed to give the same amounts of protein and calories.

Exp. 1 consisted of periods of 4 days each. The diet was exactly the same throughout the experiment, but it was divided into 2, 3 and 4 meals, approximately equal with respect to protein and calories, in the first, second and third periods respectively.

Exp. 2 was performed 3 months after Exp. 1. The diet of free choice before this experiment also contained 78 g protein but the calorie value (2300) was a little lower. The diet in Exp. 2 was constructed accordingly.

Exp. 3 was performed 2 weeks after Exp. 2. In the interval between these 2 experiments, the protein intake was gradually reduced from 78 to 50 g, the calorie intake re-

TABLE I.
Composition of Diets.

	Exp. 1	Exp. 2	Exp. 3
Orange, E. P.	260	280	240
Lettuce, "	140	140	140
Tomato, "	200	—	—
Grape, "	170	170	—
Apple, "	—	170	200
Rice	100	68	40
Bread	72	120	96
Eggs, E. P.	108	108	108
Ham	200	130	50
Milk	250	400	230
Butter	30	50	40
Sugar	30	30	30
Jam	—	—	215
Grape juice	250	—	—
Mayonnaise	—	—	21
Total protein	78.4	78.3	49.5
" calories	2540	2250	2300
Water intake			
2-meal	2650*	2420†	2000‡
3- "	2690	—	—
4- "	2550	2320†	2250§

* Including 360 ml in drink at 1:30 p.m.

† Including 6 drinks of 100 ml each at times shown in Fig. 1.

‡ Including 4 drinks of 200 ml each at times shown in Fig. 1.

§ Including 2 drinks of 350 ml each at 11:30 a.m. and 3:30 p.m., 200 ml at 6 p.m., and 150 ml at 7:30.

maining the same. With 3 feedings a day, nitrogen balance was attained before Exp. 3 began. In this experiment, the diet contained 50 g proteins and supplied 2300 calories.

Exp. 2 and 3 each consisted of two 4-day periods. The diet was divided into 2 and 4 meals approximately equal with respect to proteins and calories, in the first and second periods respectively.

The composition of the diets is shown in Table I. In Exp. 1 and 2, 70%, and in Exp. 3, 65%, of the protein were of animal origin. The feeding schedules are shown in Fig. 1. All foods were weighed to the gram. The protein and calorie intakes were calculated from standard food tables, but the nitrogen content of the ham used in each experiment was determined by analysis. Throughout the experiments, the same mode of living was maintained. There was no change of body weight and there was no untoward event of any kind. In all the experiments, the first day of each period was considered as preliminary. For the following 3 days, urine was collected at 2-hour intervals during the day and as often as convenient during the night. In Exp. 2 and 3, the 3-day feces marked with charcoal were collected. Nitrogen was determined in the urine and feces in the usual manner. The results are shown in Table II.

Discussion. It will be noted that in Exp. 1 the N excretion diminished with increasing number of feedings. In Exp. 2 the N excretion in the 2-meal period was also higher than that in the 4-meal period. The differences are, however, small in both experiments. If the results of these experiments in which the N intakes were the same, are combined, the mean N excretions in the 2-meal and 4-meal periods were 10.83 $\pm$ 0.19 and 10.06 $\pm$ 0.18 g respectively. The difference between them was 0.77 $\pm$ 0.26 g, which was barely significant statistically. This difference may be taken as the difference in N utilization, if the efficiency of absorption is assumed to be the same with different feeding schedules.

There was a slight difference in fecal N between the 2-meal and 4-meal periods in Exp. 2. This difference is uncertain on account of the well known difficulty of separating the feces of different periods. If this difference is taken into account and assuming the same values for Exp. 1 as for Exp. 2, the difference in N balance in both experiments would be 0.54 g in favor of the 4-meal schedule.

If the loss of N through the skin is taken as 0.36 g per day(1), there would be a slight negative balance in the 2-meal period and a slight positive balance in the 4-meal period in both experiments. And with 3 meals there would presumably be exact balance.

In Exp. 3, there was no significant difference in N balance between the 2-meal and 4-meal periods.

It may be concluded, therefore, that with a liberal protein intake of 1.14 g per kg, there is a definite indication of a higher efficiency of N utilization with the 4-meal schedule as compared with the 2-meal schedule. With a

1. Mitchell, H. H., *Arch. Biochem.*, 1949, v21, 335.

TABLE II.
Influence of Feeding Schedule on Nitrogen Balance.

	Exp. 1			Exp. 2		Exp. 3	
Level of protein intake, g	78			78		50	
" " calorie "	2500			2300		2300	
No. of feedings	2	3	4	2	4	2	4
Food N, g	12.53	12.60	12.54	12.50	12.65	7.92	7.92
Fecal N, g				1.80	2.12	1.68	1.55
N absorbed, g				10.70	10.53	6.24	6.37
Urine N, g	11.00	10.42	10.15	10.67	9.96	6.26	6.45
P. E.*	±.32	±.07	±.17	±.24	±.35	±.19	±.17
Diff. between 2 and 4 feedings		.85 ± .37		.71 ± .43		—.19 ± .25	
N balance, g				+.03	+.57	—.02	—.08
Diff. between 2 and 4 feedings				.54		.10	

* $P.E. = 0.67\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

lower protein intake (0.73 g per kg) no significant difference in N utilization was observed. This is understandable, as with lower protein intake, the height of the amino-acid concentration reached in the metabolic pool after feeding will be lower and consequently the irreversible loss by excretion and by urea formation will be smaller. The pattern of N excretion differed with different feeding schedules. In spite of some variation due to uncontrollable factors, each feeding schedule produced a more or less characteristic pattern, which repeated itself with remarkable uniformity from day to day in each period. There were one or more crests and troughs of N excretion during the day with one crest at night (not shown in the composite diagram in Fig. 1 which shows the average rate of excretion from 8 or 10 P.M. to 6 A.M.)

In Exp. 1 there seemed to be as many waves of N excretion as there were feedings, suggesting a rise of amino acid concentration after each meal. In Exp. 2 and 3, however, there seemed to be, broadly speaking, only one crest in the morning or early afternoon and a trough between 4 and 8 P.M. The difference in the N excretion pattern observed between Exp. 1 on the one hand and Exp. 2 and 3 on the other, even when the periods with the same number of feedings are compared, may be due to the difference in the level of N or water intake, but possibly also to other uncontrolled factors (such as salt intake and perspiration) which render theoretical interpretation of the patterns difficult if not impossible. In any case the variations of N excretion do not necessarily correspond to variations of amino acid concentration in the metabolic pool. It is well known that N excretion lags behind N metabolism. In Exp. 1 the crest of N excretion came too soon to be accounted for by increased N metabolism. Furthermore, it will be noted that the variations of N excretion were almost always accompanied by corresponding variations in urine volume. Since the rate of excretion of urea is known to increase with urine volume, it is impossible to tell whether an increase in N excretion is due to an increase in urea formation or to an increase in excretion of urea already formed.

There has been some discussion in the literature on the rhythm and diurnal cycle of N metabolism. In adults kept in bed and receiving 3 equal meals a day, Forsgren and Schnell(2) observed a rise of N excretion in the morning and forenoon with a maximum after midday and a fall in the afternoon and evening with a minimum after midnight. These authors ascribed this variation of N excretion to a rhythmic function of the liver which was supposed to have its assimilatory phase during the night and its dissimilatory

2. Forsgren E., and Schnell, R., *Acta Med. Scand.*, 1934, v82, 155.

phase during the day, irrespective of meal hours and sleep. This theory was supported by Gerritzen(3) who reported some observations on healthy young adults, some of whom drank hourly and had meals at the usual hours while others drank and ate the same amount of food hourly.

From observations on children (5-10 years of age) receiving 4 meals at 6-hourly intervals, Manchester(4) found the maximum excretion of N to be between 6 A.M. and 12 noon and the minimum between 12 midnight and 6 A.M. Examination of the data reported by these authors showed that the variation in N excretion was always accompanied by a corresponding variation in urine volume. The so-called rhythm of N excretion may be simply a rhythm of water excretion which depends not only on food and water intake but on a host of other factors which impinge on the complex nervous and hormonal control of water metabolism. Unless the rate of water excretion is fairly uniform, it is impossible to locate the real crest and trough of N metabolism or indeed to decide whether there is a cycle or rhythm of N metabolism independent of feeding and other routine of living.

Although the present experiment was originally conceived as a test of the influence of feeding schedule on N utilization through its effect on the amino acid concentration in the metabolic pool, it is realized that N balance experiments can reveal not this effect alone but rather the net effect on a whole series of processes from digestion to excretion. Furthermore, as the present experiment has been performed on a single subject and the number of observations is relatively small, the conclusions must be regarded as tentative. However, the result does indicate that feeding schedule is a factor to be considered in metabolism experiments.

Summary. A normal adult in N equilibrium was given the same diet divided into 2, 3, and 4 approximately equal meals in different periods. With a liberal protein intake (1.14 g per kg), there was a definite indication of a higher efficiency of N utilization with 4-meal than with 2-meal schedule, but the difference was not appreciable to be of practical importance. With a lower protein intake (0.73 g per kg), no significant difference in N utilization was observed.

With the same total intake of food, the pattern of N excretion differed with different feeding schedules and with total N and water intakes. This finding casts doubt on the existence of a diurnal cycle or rhythm of N metabolism irrespective of water intake and feeding schedule.

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3. Gerritzen, F., *Acta Med. Scand.*, 1936, v89, 101.

4. Manchester, R. C., *J. Clin. Invest.*, 1933, v12, 995.

Effect of Adrenocorticotropic Hormone and Cortisone Therapy on Human Plasma Aminopeptidase Activity.* (17816)

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Recent reports have indicated that a considerable increase in serum peptidase activity occurred following the injection of ACTH or adrenal cortical extract in mice(1) and that much less striking increases in various serum peptidases appeared after the administration

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