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GROWTH AND DEVELOPMENT

With Special Reference to Domestic Animals

XXIX. Age Curves of Creatinine and Urinary Nitrogen Coefficients in Dairy Cattle, and Their Relations to Energy Metabolism.

URAL S. ASHWORTH AND SAMUEL BRODY

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FOREWORD

The special investigation on growth and development is a co-operative enterprise in which the departments of Animal Husbandry, Dairy Husbandry, Agricultural Chemistry, and Poultry Husbandry have each contributed a substantial part. The plans for the investigation in the beginning were inaugurated by a committee including F. B. Mumford, A. C. Ragsdale, E. A. Trowbridge, H. L. Kempster, A. G. Hogan. Samuel Brody served as Chairman of this Committee and has been chiefly responsible for the execution of the plans, interpretation of results and the preparation of publications resulting from this enterprise.

The investigation has been made possible through a grant by the Herman Frasch Foundation represented by Dr. R. W. Thatcher, who has given valuable advice from the beginning of the investigation.

F. B. MUMFORD,
Director Agricultural Experiment Station

GROWTH AND DEVELOPMENT

With Special Reference to Domestic Animals

XXIX. AGE CURVES OF CREATININE AND URINARY NITROGEN COEFFICIENTS IN DAIRY CATTLE, AND THEIR RELATIONS TO ENERGY METABOLISM.

URAL S. ASHWORTH AND SAMUEL BRODY

ABSTRACT.—Urine was collected from 6 Jersey and 4 Holstein females between ages 7 and 40 months, and analyzed by standard methods with the following results: The average preformed creatinine nitrogen coefficient (mgs. preformed creatinine nitrogen per kilo live weight) was 9.5, and it remained practically constant during the entire age interval; the average creatine nitrogen coefficient was 7.6, and followed the same course as the preformed creatinine coefficient (that is, the total creatinine-nitrogen coefficient was 17.1). The total urinary-nitrogen coefficient (mgs. urinary nitrogen per kilo live weight) fluctuated from 150 to 350, paralleling largely the nitrogen intake (the animals were fed according to "good dairy practice"). The lowest value of this coefficient (150) was observed shortly before calving. The percentage ratio of preformed creatinine nitrogen to total urinary nitrogen ranged from 2.0 to 6.5 with an average of 3.6. The percentage ratio of total creatinine nitrogen to urinary nitrogen ranged from 6.3 to 6.8. Urea plus ammonia (there was really no ammonia in the urine) nitrogen constituted 79% of the urinary nitrogen. The ratio of Calories (basal metabolism) to milligrams of preformed creatinine nitrogen declined steadily with increasing weight from 3.3 at 6 months to 1.4 at 40 months. The ratio of Calories of "resting" energy metabolism to milligrams of urinary nitrogen excretion fluctuated from 0.06 to 0.12 with an average of 0.09. Several possible practical applications are discussed in the text.

INTRODUCTION

The preceding two papers (Missouri Research Bulletins 189 and 190) were concerned with total creatinine and urinary-N excretion in the rat, and their relations to energy metabolism and food intake. The present paper reports on a preliminary attempt to obtain similar data for dairy cattle. These results are preliminary, because unlike in the case of the rat, the conditions of food supply could not, for economic reasons, be controlled. The animals were regular members of what is practically a commercial herd which had to be fed according to the practices customary in this herd. The data are of interest in showing the results obtained under conditions of good commercial herd management, and as a preliminary step to a more intensive investigation of this problem under conditions of suitably controlled food supplies.

This bulletin contains a part of the dissertation of Ural Stephen Ashworth, presented to the Graduate School of the University of Missouri, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1933.

Paper No. 61 in the Herman Frasch Foundation Series.

METHODS

The urine was collected directly into receptacles at about two hour intervals, after inducing micturition by gentle massage starting below the ventral commissure of the vulva and taking its course upward and laterally terminating beside the labium vulva. This method, developed in this station, was described in detail by Turner and associates in Missouri Research Bulletin 150. This method involves a urinary loss of not over 10%. It sometimes happens that the animals urinate unexpectedly, shortly after collections, due possibly to incomplete preceding urination. This was particularly troublesome in calves preceding age 7 to 8 months; hence, the data here presented begin with this age. So far collections were made up to about $3\frac{1}{2}$ years.

All measurements were made in triplicate by micro methods. Total nitrogen was determined by the method of Koch and McMeekin (J. Am. Chem. Soc., 1924, 46, 2066). Many check analyses were made by the macro Kjeldahl method, however. The urea plus ammonia determinations were made by the Urease Direct-Nesslerization method. The amount of ammonia present in these urines was negligible as was demonstrated by several analyses. This was probably due to the nature of the diet which yielded a very alkaline urine. The range of pH was 7.4-8.4, most of the urines having a pH of 8.0 or over.

Preformed creatinine was determined by Folin's micro method, using samples containing 1 mg. of creatinine, 20cc of saturated picric acid solution, and 1cc of 15% NaOH in a volume of 100cc.

Creatine was converted to creatinine by heating the sample on a water bath for three hours with HCl. After neutralization to litmus the technique was identical with that for preformed creatinine.

To prevent decomposition of the highly alkaline urine, the receiving receptacles were kept distinctly acid by adding dilute H_2SO_4 . A 5% solution of thymol in toluene was added as an additional precaution.

The concentration of the constituents of these urines was quite variable. During the summer, daily excretions of urine of 20 liters or over were not uncommon, while during the winter the same animals would excrete but 3 to 4 liters.

In connection with the preservation of the urine, a study was made of the effect of pH on the stability of creatine and creatinine. A large sample of urine was divided into several portions imme-

diately after excretion and to these were added various amounts of acid. The concentration of preformed creatinine in each portion was then determined at 2-hour intervals. The pH values were secured by colorimetric technique using La Motte standards.

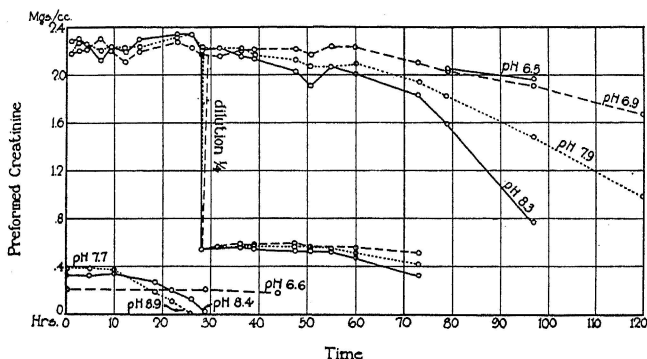


Fig. 1.—The effect of pH and dilution on the decomposition of creatinine at 30-40° C.

The results are shown in Fig 1. At pH 6.5, a very dilute urine (0.2 mg/cc) kept (at temperature of 30-40°C) the original concentration of creatinine for 40 hours; at pH 6.5 a very concentrated urine (2.2 mg/cc) kept the original concentration of creatinine for 80 hours; at pH 8.4, a dilute urine (0.33 mg/cc) kept its original concentration only 10 hours at temperature of 30-40° C., while for a concentrated urine (2.2 mg/cc) the creatinine showed gradual slight decline until about 60 hours, then a progressive loss was noted. Artificial dilution (25/100) of the concentrated urine (2.2 mg/cc) did not seem to influence its rate of decomposition.

The loss of total creatinine is generally a little more rapid than the loss of preformed creatinine, as seen from the following table.

Loss of Preformed and Total Creatinine in Per Cent
(P.C. 2.2, T.C. 3.6 mg. per c.c., Conc. Urine)

After Hours	pH 8.3		pH 7.8		pH 6.9		pH 6.5		pH below 5	
	Pref.	Total	Pref.	Total	Pref.	Total	Pref.	Total	Pref.	Total
51	16.7	20.0	5.0	16.7	0.5	15.9	0.0	13.0	0.0	11.1
97	66.2	52.2	32.6	45.2	12.9	24.2	7.5	26.1	2.8	15.0

The data of this research are presented in graphic form only. With the exception of Fig. 1 the data were plotted in all cases on arithlog paper, the advantage of such plots being, as previously noted, that the percentage changes of the curves are in all cases directly proportional to their slopes and regardless of the absolute units employed.

The creatinine and total nitrogen data are presented in terms of milligrams of preformed creatinine nitrogen, or total creatinine nitrogen, or total urinary nitrogen per kilo of live weight, and these ratios are here termed coefficients.

CREATININE AND URINARY-NITROGEN COEFFICIENTS

The coefficients for preformed and total creatinine nitrogen, and for total urinary nitrogen, are presented in Fig. 2. The breed averages for the 4 Holstein and 6 Jersey heifers (we have no data for males) are shown in the upper rectangles of Fig. 2, while the data for the individuals are shown in the lower and central rectangles.

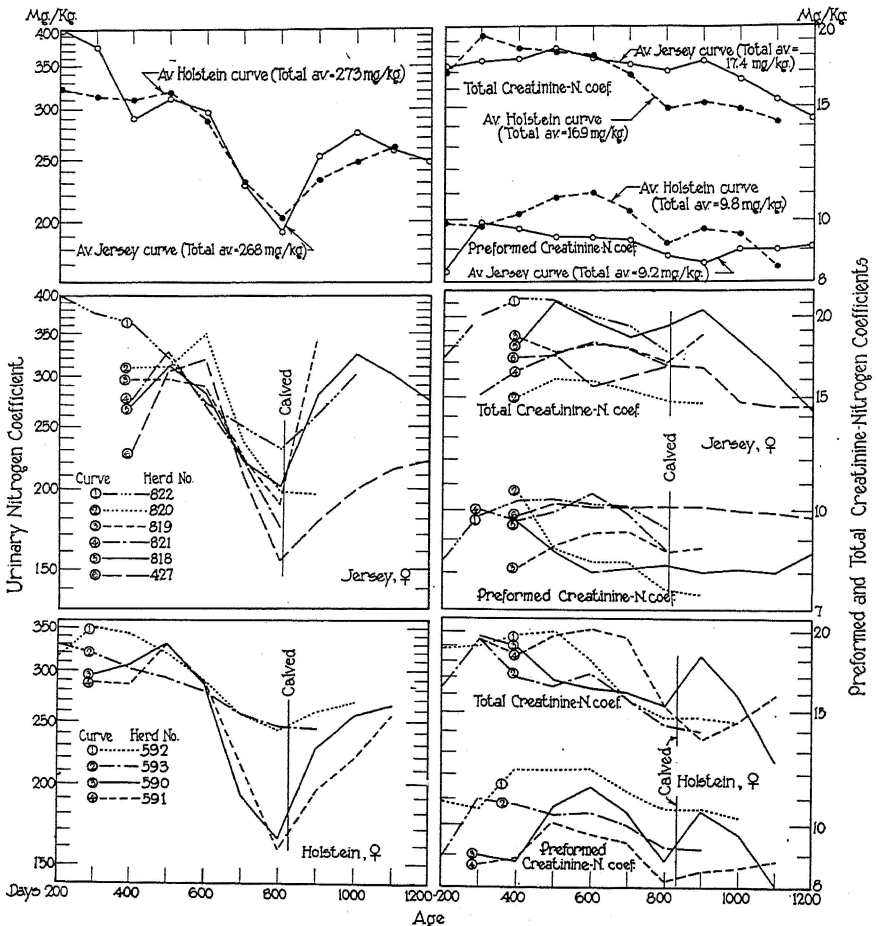


Fig. 2.—Total urinary-nitrogen and creatinine-nitrogen coefficients of our animals plotted as functions of age. The upper rectangles represent the breed averages while the others represent the curves of the individual animals.

angles. The creatinine-nitrogen coefficients are represented on the right side of the chart, while the total urinary nitrogen coefficients are represented on the left side.

The preformed creatinine coefficient of our cattle remains fairly constant, or, in several cases, falls slightly with increasing body weight. This latter result may be indicative of the formation of adipose tissue. Holsteins have a slightly higher preformed creatinine coefficient than Jerseys.

The total creatinine coefficient of these animals seems to parallel that of the preformed creatinine. Unlike in the case of the preformed creatinine coefficient Jerseys have a slightly higher average value for this total creatinine coefficient showing possibly a breed difference in the proportion of creatine dehydrated by the body.

The average *preformed*-creatinine-nitrogen coefficient of our animals (upper right curves in Fig. 2) is seen to be of the order of 9.5. This is quite close to the published values for humans and dogs (8 to 11 for normal adult males; 5.8 to 9.8 for normal adult human females; 10 for adult dogs. (See Lusk's *Science of Nutrition*, 1928, pp. 253-257.) Carpenter's fasting steers (*Am. J. Physiol.*, 1927, lxxxii, 519) gave average coefficients (averages of each individual as computed by us from Carpenter's data) ranging from 8 for the pasture steers C and D; to 9 for the submaintenance steers C and D.

The average *total* creatinine nitrogen coefficients of our animals is 17.1; that is, the average creatine-nitrogen coefficient is of the order of 7.6. This is above the values found by Carpenter on his fasting steers (3.4 for pasture steers C and D; 0.4 for submaintenance steers C and D). It is generally known that normal adult human males do not excrete creatine at all, and normal adult human female excrete it only intermittently. However, fasting, or merely specific carbohydrate fasting, is said to result in creatine excretion (see Lusk, loc. cit.). It may be recalled that in the case of the white rat (*Mo. Res. Bul.* 189), the average total creatinine nitrogen coefficient is 15.1, being somewhat higher for the lighter than for the more fleshy rats. The problem of the amount of creatine in the urine of rats has not yet been solved.

As regards our data for total urinary nitrogen excretion (left side of Fig. 2), the average value of the coefficient is seen to be 268 (268 mg. N per kilo live weight) for the Jerseys, and 273 for the Holsteins. These values are perhaps without absolute significance,

since the total nitrogen excretion (unlike the creatinine excretion) is largely a function of the excess (above the amount utilized for growth and maintenance) of nitrogen intake, and of the biological value of the protein ingested. They may, however, be interesting from comparative points of view.

Thus, we may compare our values with Carpenter's results on fasting steers (*loc. cit.*). His pasture steers C and D had an average coefficient of 115; his submaintenance steers C and D had a coefficient of 62.

We may also compare the results on our cattle with our results on the rats (Mo. Res. Bul. 189). In the case of the rats, we found an average minimum coefficient of 142 for the low protein group of rats, reaching this minimum value after being on a "Nitrogen-free" but otherwise complete diet from 10 to 15 days. The high-protein group of rats reached an average minimum of 140 after being 15 to 20 days on the N-free diet. However, during these periods of specific nitrogen starvation, the results on the rats showed several considerably higher values, some as high as 190. On the normal stock diets, the rats showed coefficients as high as 800 to 1000 for the low protein stock diet, and 1500 to 4000 for the high-protein stock diet (see, for example, Fig. 2, Mo. Res. Bul. 189). It is obvious that other conditions being the same, rats tend to have very much higher N-coefficients than cattle.

Mitchell (Bul. Nat. Res. Council, Number 67, 1929) cites data from Forbes to the effect that the N-coefficient for cows on the 6th and 9th day of fasting is about 100, which agrees with Carpenter's values for his fasting pasture steers C and D, but is higher than Carpenter's submaintenance steers C and D. Mitchell also cites Bull and Grindley who brought two steers on nitrogen equilibrium on a low-protein diet consisting of 1 part of clover hay to 5 parts of corn with a resulting nitrogen coefficient of 46-48; Titus, who found coefficients of 42 to 52 in steers receiving a diet of 40% alfalfa hay and 60% paper pulp; Möllgaard ("The Laboratory of Agricultural Research in Copenhagen"), who brought into nitrogen equilibrium two dry cows receiving only 46 to 47 gm. nitrogen daily with resulting nitrogen coefficients of 29 to 34; Honcamp, Koudela, and Müller, who fed two lactating cows on oat straw, corn, potatoes, molasses, dried beet pulp with a resulting nitrogen coefficient of 35 to 42 on a nitrogen intake of 85 to 89 grams; Hart, Humphrey, and Morrison, who fed two 300 to 400 pound heifers

on wheat straw, corn starch, cane sugar, with a coefficient of 30 to 36; Steenbock, Nelson, and Hart who obtained on a calf a minimum coefficient of 45.

Assuming that a urinary-nitrogen coefficient of 50 is a reasonable minimum of adult maintenance requirements under a "normal" food supply of cattle, then it would seem to appear that our animals having urinary-nitrogen coefficients ranging from 150 to 350 (Fig. 2) have been excreting from 3 to 7 times the assumed adult minimum values of nitrogen, or they have been "wasting" from 100 to 300 mg. nitrogen per kilo per day. Is it necessary to have this waste?

Fig. 2 shows that at 27 months, shortly before calving, when the fetus was gaining in weight most rapidly, the nitrogen excretion was 150 milligrams per kilo; while at 13 to 15 months, it was 350. Let us assume that the minimum nitrogen excretion on the given diet compatible with normal growth is 150 mgs. per kilo rather than 50 as assumed in the preceding paragraph for adult animals. The nitrogen waste would then be 200 mg. per kilo.

The excess of the 200 mg. per kilo of urinary nitrogen excreted by the animal at age of about 13 months above that at 27 months is, of course, the same as 0.2 gm. nitrogen per kilo live weight, or 0.2 pounds nitrogen per 1000 pounds live weight of the animal; or 1.25 pounds (0.2×6.25) of digestible crude protein per 1000 pounds of live weight (or 0.6 pound protein per 500 pounds live weight). As the animals were consuming about 1.3 pounds of digestible crude protein at weight of 500 pounds, when the nitrogen coefficient was 350, they were wasting nearly half ($0.60/1.3$) of their protein intake. It would thus seem that at about one year of age, our heifers received a theoretical daily excess of at least 0.60 pounds of digestible crude protein, or 1.25 pounds of protein per 1000 pounds of live weight per day. The problem as to whether or not it is practically necessary to have this excess of protein for normal growth (or milk secretion), of course, remains to be solved experimentally.

THE RATIOS OF CREATININE-NITROGEN TO TOTAL URINARY NITROGEN

It is now established with considerable certainty that the excretion of preformed creatinine, at least, is constant, and is not influenced by the dietary protein level. Since, according to Folin, the creatinine nitrogen in humans on a N-free diet is about 17%

of the total urinary nitrogen, then if the same ratio holds true for cattle, it is possible to compute the endogenous urinary nitrogen in cattle by multiplying the creatinine-nitrogen excretion by 6. Deuel found a creatinine nitrogen percentage of 33, and Smith found the still higher percentage of 35, in which case the endogenous urinary nitrogen would be computed by multiplying the creatinine nitrogen by 3 (see Lusk, p. 360). Assuming that the ratio of creatinine to endogenous urinary nitrogen is constant (an assumption which we have reasons to doubt), then a knowledge of this ratio would be of considerable practical as well as of theoretical interest.

Thus, assuming 9.5 as an average preformed creatinine-N coefficient of our cattle shown in Fig. 2, then the endogenous urinary nitrogen excretion should be of the order of 57 mgs. per kilo per day according to Folin's value, and 28.5 mg. per kilo per day according to Deuel and Smith's results. Actually, our animals excreted from 150 to 350 mg. nitrogen per kilo per day.

The observed ratios of *preformed* creatinine-N to total urinary-N of our animals are seen in Fig. 3 to range in individual cases from 2 to 6.5 with an average of 3.6% for the Jersey group, and 3.8% for the Holstein group. The highest ratios naturally correspond with the lowest total nitrogen excretion. Our average values of this ratio for cattle are identical with the value found by Folin for humans (3.6) on a high protein, but meat-free, diet. The range of this ratio for humans on ordinary mixed diets is 3 to 7.

The age curves for the ratios of *total* creatinine nitrogen to total urinary nitrogen parallels the course, but on a higher plane, of the ratios of preformed creatinine nitrogen, to urinary nitrogen. The numerical values of the ratios are seen, in Fig. 3, to be 6.3% for the Holstein group, and 6.8% for the Jersey group.

Here, also, it will be instructive to compare our data for the normally-fed dairy females with Carpenter's data for fasting beef steers. The average percentage ratios of *preformed* creatinine nitrogen to total urinary nitrogen in Carpenter's steers range for the several individuals from about 7 for the pasture steers C and D to 16 for sub-maintenance steers C and D. Similar percentage ratios of the total creatinine nitrogen to *total* urinary nitrogen vary from about 10 for the pasture steers C and D to about 16.8 for the sub-maintenance steers C and D. It thus appears that in the case of Carpenter's steers, there was practically no creatine excretion by the animals that were kept on a submaintenance ration, while in

the case of the pasture steers the animals were excreting about 3 mg. of creatine nitrogen per kilo live weight. Is it possible that the high creatine-nitrogen coefficient (7.6) of our animals is due to exogenous protein metabolism (since Carpenter's submaintenance steers practically excreted no creatine while the pasture steers showed a coefficient of only about 3)?

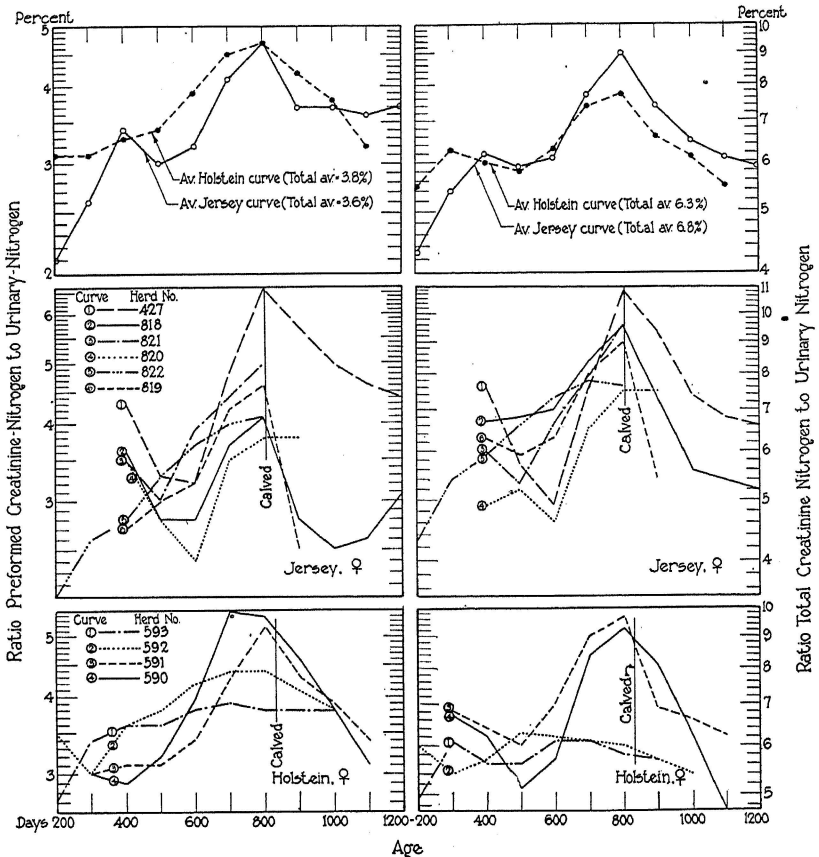


Fig. 3.—Age curves of the percentage ratios of preformed creatinine-nitrogen to total urinary nitrogen (left) and of total creatinine nitrogen to total urinary nitrogen (right). The high values in these curves correspond to the low values in Fig. 2 of the urinary nitrogen coefficient.

THE RATIOS OF UREA PLUS AMMONIA NITROGEN TO TOTAL URINARY NITROGEN

According to Folin, the urea plus ammonia nitrogen excretion of a human on a high (but meat-free) protein diet is 90 per cent of the total urinary nitrogen; while on a nitrogen-free diet (starch

and cream), it is 73% of the urinary nitrogen. Smith reported a minimum of 37% urea plus ammonia on the 24th day of a nitrogen-free diet (see Lusk's book, p. 360). The upper rectangle of Fig. 4 shows that the average of this ratio for our Holstein animals is 78%, and for the Jersey animals 80%. These values are intermediate between the two values reported by Folin for humans, and they are near the average of published data for humans on an average mixed diet.

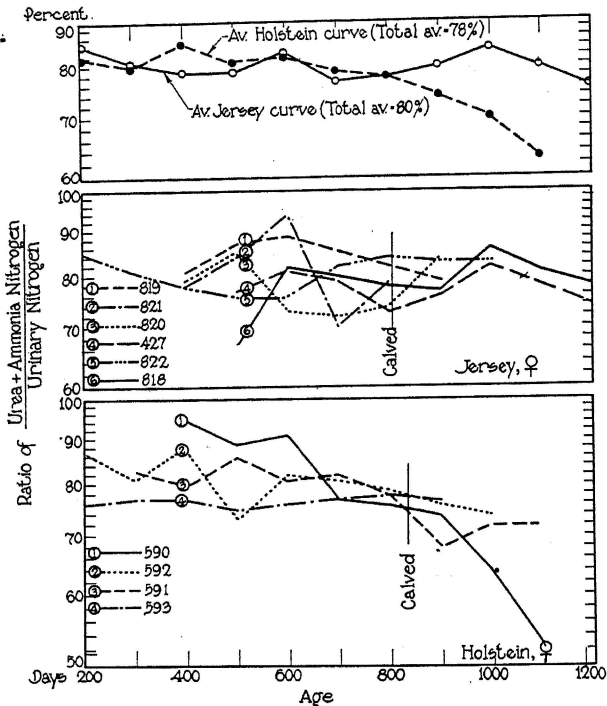


Fig. 4.—Ratios of urea plus ammonia nitrogen to total urinary nitrogen.

Carpenter's pasture steers C and D gave a percentage ratio for urea plus ammonia nitrogen to total urinary nitrogen of about 77; his submaintenance steers C and D gave a ratio of about 63 (as compared to this percentage ratio of about 79 given by our growing dairy cattle). Carpenter's relatively low values for this ratio may perhaps be explained as due to absence of exogenous metabolism, but also in part by the considerable hippuric acid and to a less extent "amino acid" nitrogen elimination in cattle. In some cases, Carpenter's data show as much as 27% of the urinary nitro-

gen in the form of hippuric acid and 17% in the form of amino acid. It is probable that the ratio of urea plus ammonia nitrogen to total urinary nitrogen does not have the same significance as an indication of exogenous metabolism in cattle as it does in humans, on account of the variable excretion of hippuric acid and other substances in cattle.

RATIOS OF BASAL METABOLISM TO CREATININE EXCRETION

Fig. 5 presents the ratios of basal metabolism to performed creatinine nitrogen (upper curves) and of basal metabolism to total creatinine nitrogen (lower curves). The Jerseys are represented on the left side, and the Holsteins on the right side. These ratios decline with increasing age in roughly the same manner as the ratios of basal metabolism to body weight presented in Mo. Res. Bull. 176. This result is in agreement with what might be expected from the fact that while the creatinine coefficient is practically the same for all weights, the caloric coefficient declines with increasing weight.

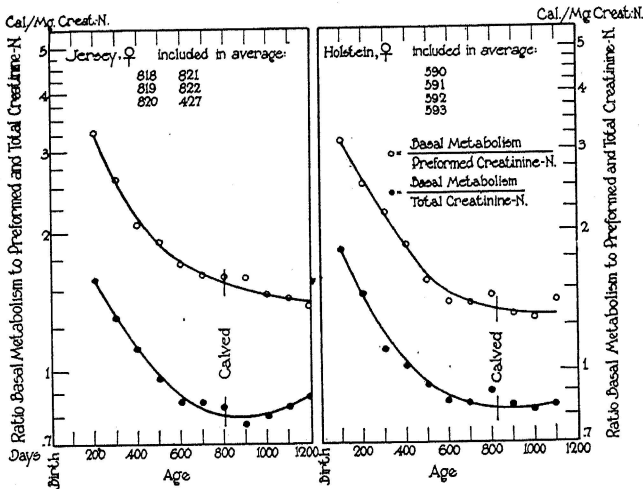


Fig. 5.—Age curves of ratios of basal metabolism to mgms. performed (upper curves) and to total (lower curves) creatinine excretion.

The numerical values of the ratios of Calories to milligrams *performed* creatinine nitrogen range from about 3.3 at six months to about 1.5 at 40 months; the values of the ratios of Calories to milligrams *total* creatinine nitrogen decline during the same time from about 2.0 to about 0.75.

For purposes of comparison, we have computed similar ratios for the fasting steers of Benedict and Ritzman (Public. 377, Carnegie Institution of Washington, 1927). With respect to preformed creatinine, these average ratios (Calories per mg. creatinine nitrogen) are about 2.3 for the pasture steers C and D (ave. weight 715 kilos) and 1.5 for submaintenance steers C and D (ave. weight 628 kilos); with respect to total creatinine, these ratios (Calories per mg. total creatinine nitrogen) are about 1.7 for the pasture steers, and about 1.4 for the submaintenance steers. These ratios for the steers are not strictly comparable to the ratios for our cattle since they differed not only with respect to live weight (which, as pointed out, constitutes a very important determining influence on this ratio), but also as regards the method of computing the energy metabolism. Our animals were completely at rest (lying) while their metabolism was measured, while the steers were standing half of the time. Nevertheless, these ratios for Benedict, Ritzman, and Carpenter's data agree quite closely with our average results.

It may be recalled (see Bul. 190) that in the case of the rats, these ratios likewise declined rapidly with increasing weight, and rats being smaller than cattle, the ratios (Calories/mg. total creatinine N) for the rats were much higher (13 to 6) than for cattle (2 to 0.7).

It may be recalled that Palmer, Means, and Gamble found the ratio of Calories (basal metabolism) to mg. creatinine to be 0.98 for men, and 1.26 for women. These rather low values for humans are undoubtedly due to the fact that the basal energy metabolism of humans is relatively much lower than of cattle.

RATIOS OF "RESTING" METABOLISM TO URINARY NITROGEN EXCRETION

As previously defined (Mo. Res. Buls. 166 and 176), "basal" metabolism differs from "resting" metabolism only in the fact that the latter includes the energy of specific dynamic action—the animals not being in post-absorptive condition. We have plotted in Fig. 6 the ratios of this "resting" metabolism to total urinary nitro-

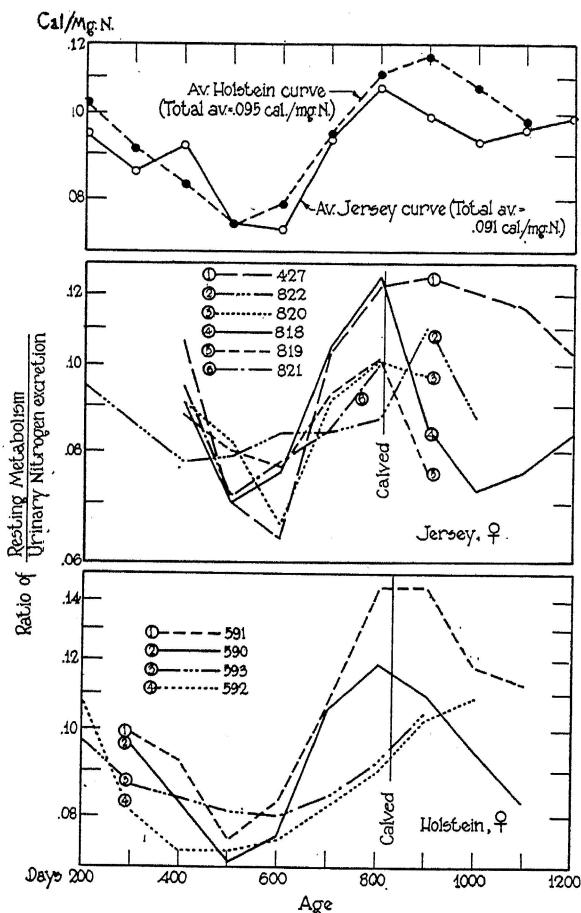


Fig. 6.—Age curves of "resting" energy metabolism to urinary nitrogen excretion.

gen excretion. It is somewhat difficult to interpret these curves, and they have no very definite significance since both of these are variables being undetermined functions of the dietary levels. However, we are presenting these curves as a matter of record for future reference.

SUMMARY AND CONCLUSIONS

The important theoretical contributions of this bulletin are:

(1) The preformed creatinine-nitrogen coefficient (9.5) of growing dairy cattle is the same as in humans. This coefficient was found to be constant during the age interval (7 to 40 months) under observation. The fluctuations in this coefficient may be explained by fluctuations in the relative degrees of fatness of the individual animals; the fatter the animal, the lower the coefficient tends to be.

(2) The dairy females under observation have a high creatinine-nitrogen coefficient (7.6) which is parallel to the preformed creatinine nitrogen coefficient. From a comparison with Carpenter's data for fasting steers, it appears possible that the high creatinine coefficient of our cattle may be of dietary origin.

(3) The ratio of basal (energy) metabolism to creatinine excretion declines with increasing live weight in about the same manner as the decline of the ratio of basal metabolism to live weight.

The following are some practical implications of these theoretical contributions: (1) The *maintenance requirements* for feed energy per unit live weight decline with increasing live weight, while the *maintenance requirements* of feed protein per unit live weight of normally-fed animals *may* remain roughly constant (if the ratio of creatinine nitrogen to total endogenous nitrogen metabolism is constant, an assumption which has not yet been established as a fact); (2) Dairy cattle fed according to "good dairy practice" (as our animals were fed) probably receive far too much protein in their diet; it is probable that the urinary nitrogen excretion need not exceed 150 milligrams per kilo live weight. However, this conclusion is quite tentative and must be confirmed experimentally.