CLINICAL AND BIOCHEMICAL STUDIES ON THE BASIS OF KETONE BODIES IN CATTLE
I. BLOOD KETONE BODIES IN NORMAL DAIRY CATTLE

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INTRODUCTION

The increase of acetone in urine at the time of pregnancy had been observed in man and animals from many years ago. Firstly, in domestic animals, CHRISTALON reported that the value for blood ketone bodies of cow was higher than that of man. Since STINSON drew attention to the fact that post-parturient dyspepsia of bovines was accompanied by the presence of ketone bodies in the milk and urine, there have been a number of clinical and biochemical investigations into the changes in the course of that disease, which is frequently called acetonemia or bovine ketosis. Especially, since the perfection of the micro-diffusion-method by THIN and ROBERTSON,[16] using salicyl-aldehyde quantitative estimation has become so easy and exact that the studies on this disease have made rapid progress in various countries of the world. As to studies on ketosis in Japan, following the fundamental research in ruminants by UMEZU et al.,[20] also a great many studies have been carried out both clinically and biochemically. Up to the present, it has been thought that ketosis was associated with a disturbance of carbohydrate metabolism resulting from reduced availability of carbohydrates with a consequent overproduction of ketone bodies derived from increased fat catabolism.[19,21,23] The excess ketone bodies in the blood were regarded as the cause of the symptoms. Recently, SHOW et al.[13,14] and PUNTRIANO expressed the opinion that ketosis is one of the diseases belonging to the so-called "general adaptation syndrome"[12] caused by the hormonal unbalance of the pituitary-adreno-cortical system resultant from some internal or external stress such as pregnancy, parturition, lactation, failure of feeding-management, starvation or others. At the time of the diagnosis of this disease, great importance has been attached to the estimation of ketone bodies in blood or urine. But as shown in tables 1 and 2,[17,22,24] the distribution of ketone bodies in normal cows as well as in ketosis ranges so widely that the

TABLE 1. *Total Ketones in Normal Cows*

AUTHORS		BLOOD TOTAL KETONE (mg/100 ml)			URINE TOTAL KETONE (mg/100 ml)		
		No. of Cases	Mean	Range	No. of Cases	Mean	Range
SAMPSON et al.	(1933)	30	2.98	2.11~ 4.66	9	7.83	3.45~13.15
BODDIE	(1935)	50	2.18	0.69~ 9.90	16	3.37	2.50~ 6.37
DUNCAN et al.	(1939)	176	2.99	0.66~ 5.54	86	10.00	2.50~70.00
SAMPSON & BOLEY	(1941)	8	1.92	0.87~ 3.97	8	22.01	4.96~34.46
KNODT et al.	(1942)	427	2.66	0.31~ 6.95	427	11.81	0.61~34.26
SAMPSON & BOLEY	(1945)	49	4.25	0~ 7.44	·	·	·
JONSON et al.	(1951)	·	·	0~ 5.00	·	·	·
THIN & ROBERTSON	(1953)	20	6.10	2.87~10.88	7	9.54	4.10~22.55
UMEZU	(1954)	·	2.00	0.60~ 6.00	·	·	0.30~ 3.00
YOSHIDA	(1955)	·	·	·	99	5.60	0~12.00

TABLE 2. *Total Ketones in Bovine Ketosis*

AUTHORS		BLOOD TOTAL KETONE (mg/100ml)			URINE TOTAL KETONE (mg/100ml)		
		No. of Cases	Mean	Range	No. of Cases	Mean	Range
SAMPSON et al.	(1933)	40	37.60	12.47~ 63.83	1	80.00	·
BODDIE	(1935)	1	10.06	·	2	77.22	38.12~ 116.32
FINCHER	(1936)	4	55.20	38.70~ 86.25	4	727.00	570.00~1,025.00
SAMPSON & HAYDEN	(1936)	35	32.00	10.00~ 72.85	28	371.10	11.00~1,309.16
DUNCAN et al.	(1939)	40	34.68	4.06~100.00	49	395.80	18.30~1,309.20
STEYN	(1941)	3	44.23	28.04~ 56.30	·	·	·
SAMPSON & BOLEY	(1941)	8	40.76	21.95~ 70.93	8	562.09	139.40~1,214.90
SYKES et al.	(1941)	4	37.70	2.40~ 89.30	4	694.50	338.00~1,568.00
THIN & ROBERTSON	(1953)	89	48.06	10.00~116.68	24	305.77	32.32~ 757.37
UMEZU	(1954)	·	·	5.00~ 50.00	·	·	Over 3

cause for the fluctuation in the ketone bodies cannot be attributed only to the individual differences. It may be natural that the opinion on physiological range for ketone bodies and also the idea respecting ketosis which had been thought in the past, have become matters of great doubt. In consequence, in the cases of diagnosis and treatment in dairy cattle, these views are very important. Therefore the authors have first of all examined the clinically healthy cows in the field to make certain regarding the natural state of the ketone bodies in blood and urine; also they have examined some cows which suffered from various diseases and have attempted to make sure of the diagnostic significance of ketone bodies. In

the present paper, the results of investigations on the blood ketone bodies of clinically healthy cows are described.

Materials and Methods

As the experimental cases, 368 clinically healthy pure or crossbred Holstein-Friesian cows clear off clinical signs, including lactating and dry, bred in the various dairy herds in the vicinity of Sapporo have been studied during the past 2 years, from April, 1954 to March, 1956. For the experimental cows, common clinical examinations were carried out; after that, samples of blood were collected. As the anticoagulant to blood, sodium fluoride was employed. The samples were examined as soon as possible, respectively.

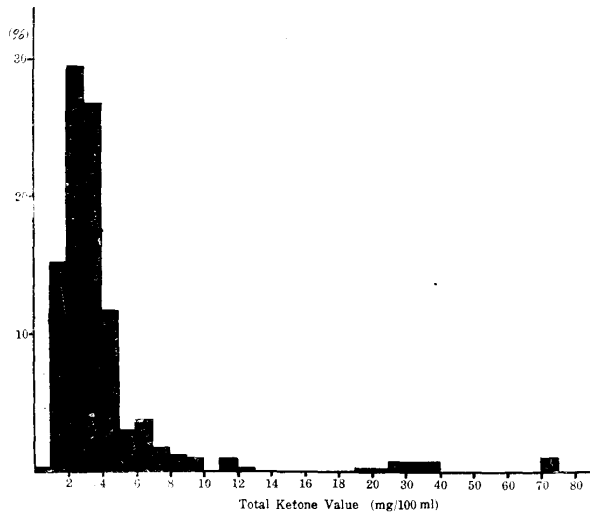
Each of the individual ketone bodies, acetone, acetoacetic acid and total ketone was estimated by the method described by Thin and Robertson and blood sugar determinations were made by ferricyanide-ferric iron method.[9] The results of the experiments were analysed by the statistical methods. In addition, the correlations of internal and external factors with the rise and fall of ketone bodies and also connections of ketone bodies with the levels of blood sugar and with the numbers of eosinophilic leucocytes were examined.

Experimental Results

1. The Values for Total Ketone Bodies in the Blood

The distribution of frequencies of the values for total ketone bodies (abbreviated ketones) in the blood of 270 clinically healthy cows is indicated in chart 1. Namely, this is an exponential distribution in which the range of 2~3 mg/100 ml of blood was highest; the mean value was 3.32 mg/100 ml and its range was 0.87 ~ 73.80 mg/100 ml. After the conversion by logarithms to this distribution, rejection limits under 5% level of significance were adopted. The under and upper limits were 0.99 mg/100 ml and 11.17 mg/100 ml, respectively; the mean value of the 257 cases between the rejection limits was 3.11 mg/100 ml. It may be reasonable to consider this as the mean value for ketones of the normal cows, excepting certain special cases, from the clinically healthy cows in the field. On the other hand, there were 12 cases over the upper limit of the rejection limit in this distribution. Their ratio of appearance was 4.45% and their mean ketone value was 39.10 (12.30~73.80) mg/100 ml. In the present report, these cases above

Chart 1. *The Distribution of the Values for Total Ketones in Blood*

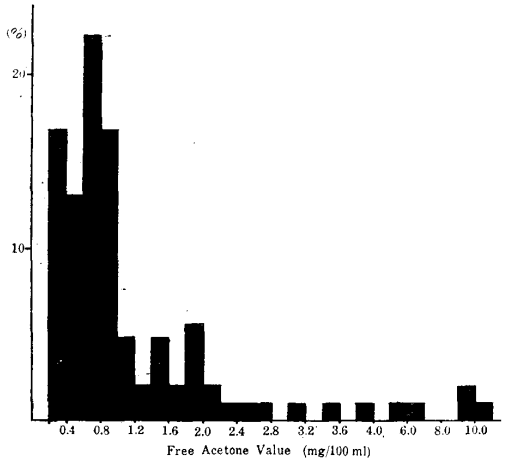


the upper limit are grouped as the "statistically sub-normal group (abbreviated sub-normal group)" and the cases between the rejection limits are called "statistically normal group (abbreviated normal group)", respectively. This is the writers' own provisional classification.

2. The Values for Free Acetone in the Blood

The distribution of frequencies of the values for free acetone (abbreviated acetone) in the blood of 107 clinically healthy cows is shown in chart 2.

CHART 2. *The Distribution of the Values for Free Acetone in Blood*



This distribution is also exponential just as in the case of the ketones. The range of 0.6~0.8 mg/100 ml of blood is highest. The mean value was 0.89 mg/100 ml and the range of this distribution was 0.22~10.50 mg/100 ml. By the statistical methods, as in the ketones, the rejection limits were determined as 4.53 mg/100 ml in upper limit and as 0.17 mg/100 ml in under limit. The mean value of the 102 cases between these two limits was 0.79 mg/100 ml. This value may be considerable as the mean value for acetone of the normal cows, excepting special cases, from the clinically healthy cows in the field. The appearance rate of sub-normal 5 cases to all cases was 4.7%; their mean value was 8.23 (5.20~10.50) mg/100 ml.

TABLE 3. *The Values for Ketone Bodies in Blood*

GROUP	TOTAL KETONE (mg/100 ml)			FREE ACETONE (mg/100 ml)		
	No. of Cases	Mean	Range	No. of Cases	Mean	Range
Clinically healthy cows	270	3.32	0.87~73.80	107	0.89	0.22~10.50
Normal group	257	3.11	0.99~11.17	102	0.79	0.22~ 4.53
Sub-normal group	12	39.10	12.30~73.80	5	8.23	5.20~10.50

3. The Values for Each of the Individual Ketone Bodies in the Blood

The blood samples obtained from 45 cases were employed for fractionations in 3 kinds of ketone bodies: free acetone, acetoacetic acid and β-hydroxybutyric acid. The values of all cases were divided into 9 sub-groups in obedience to each value of total ketone bodies, and the correlation among them was examined.

As shown in table 4, the values for free acetone showed tendency to increase in accordance with the gradual increase in total ketone bodies. However, in the ratio to the values for total ketone bodies, they were extremely low in a sub-group under

TABLE 4. *The Values for Each of the Individual Ketone Bodies in Blood*

LEVEL FOR TOTAL KETONE	NO. OF CASES (45)	FREE ACETONE		ACETOACETIC ACID		β-HYDROXY-BUTYRIC ACID		MEAN VALUE FOR TOTAL KETONE (mg/100ml)
		Mean (mg/100ml)	Ratio to total ketone (%)	Mean (mg/100ml)	Ratio to total ketone (%)	Mean (mg/100ml)	Ratio to total ketone (%)	
Under 2	2	0.19	11.0	1.09	63.5	0.44	25.6	1.72
2~ 3	12	0.70	32.3	1.09	50.3	0.92	42.4	2.17
3~ 4	12	1.00	26.5	1.25	36.8	1.14	33.7	3.39
4~ 5	5	1.38	29.4	1.58	33.7	1.73	36.9	4.69
5~ 6	4	1.17	21.5	1.97	36.4	2.28	42.1	5.42
6~ 8	2	1.21	19.5	1.62	26.1	3.38	54.4	6.21
8~10	4	2.09	22.9	2.89	31.8	4.11	45.3	9.09
10~12	2	2.67	24.2	3.31	29.8	5.09	46.0	11.07
Over 12	2	4.35	16.0	10.41	38.5	12.30	45.5	27.06

2 mg/100 ml, but they were almost highest between 2~3 mg/100 ml. In other sub-group over 3 mg/100 ml, free acetone showed a decreasing tendency antagonistically to the increase of the total ketones. The values for acetoacetic acid increased in accordance with the increase of the values for total ketones, but in the ratio to the values for total ketones, they decreased antagonistically as did also the free acetone. In the values for β-hydroxybutyric acid and in their ratio to the total ketone values, they showed an increasing tendency in parallel with the increase of the values for total ketone bodies.

4. The Relation Among the Values for Ketones and Some External and Internal Factors

1) The relation between age and ketone values: The values for ketones of 269 cases were averaged in each of 13 sub-groups in obedience to age as shown in table 5.

In this table, 5 sub-groups, viz., 7, 2, 6, 14~15 and 8 year olds were 1.9~22.7% higher in mean values than the normal group, respectively. In the other 8 sub-groups, their mean values were all lower than that of the normal group, the variations being 2.56~2.96 mg/100 ml. From the above observations, it may be said that the mean values for ketones generally increased in company with the advance of the age. The appearance rate of cases in sub-normal group to all cases in each of the sub-groups was higher in 4~8 year's sub-group than in the other ages, but with some exception.

2) The relation between the period of pregnancy and ketone values: The influence of pregnancy upon ketone value was examined employing 269 pregnant and non-pregnant cases; the results are shown in table 6.

TABLE 5. *The Relation between Age and Ketone Values*

AGE	NO. OF CASES (269)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (257)	Mean (mg/100ml)	No. of Cases (12)	Appearance Rate (%)
1	2	2	2.81	0	0
2	30	29	3.31	1	3.3
3	42	42	2.67	0	0
4	20	19	2.56	1	5.0
5	23	21	2.86	2	8.7
6	23	22	3.41	1	4.3
7	19	18	3.17	1	5.3
8	26	24	3.82	2	7.7
9	26	26	2.96	0	0
10~11	12	12	2.89	0	0
12~13	19	17	2.65	2	10.5
14~15	21	19	3.67	2	9.5
Over 16	6	6	2.70	0	0

TABLE 6. *The Relation between the Period of Pregnancy and Ketone Values*

MONTH OF PREGNANCY	NO. OF CASES (269)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (257)	Mean (mg/100ml)	No. of Cases (12)	Appearance Rate (%)
Non-Pregnancy	157	151	2.89	6	3.8
1~2	15	13	3.37	2	13.3
3	28	26	4.01	2	7.2
4	13	13	3.85	0	0
5	12	12	3.89	0	0
6	13	13	3.44	0	0
7	10	9	3.59	1	10.0
8	13	12	2.89	1	7.7
9	8	8	2.62	0	0

The mean value of the non-pregant sub-group was 2.89 mg/100 ml which was a decrease by 7.1% compared with that of the normal group. In the pregnant group, the values of all sub-groups among 1~7 months were markedly higher than that of the non-pregnant sub-group. The appearance rate of the sub-normal cases in the non-pregnant sub-group is 3.8%; in the pregnant sub-group, they were 7.2~13.3% in the sub-groups of 3, 8, 7 and 1~2 months, respectively. However, as noted in the table, in the middle stage of pregnancy, no cases were observed. From the above results, the ketone values of the pregnant groups may be stated to be higher than that of the non-pregnant group, especially in the initial stage a marked increase appeared.

3) The relation between the calving frequency and ketone values: One hundred and forty cases were divided into 12 sub-groups according to their calving frequency and the relations among them were examined.

TABLE 7. *The Relation between the Calving Frequency and Ketone Values*

CALVING FREQUENCY	NO. OF CASES (140)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (128)	Mean (mg/100ml)	No. of Cases (12)	Appearance Rate (%)
0	25	23	3.56	2	8.0
1	22	20	2.56	2	9.1
2	14	14	3.28	0	0
3	16	12	3.74	4	25.0
4	10	10	3.58	0	0
5	18	18	3.16	0	0
6	7	6	2.74	1	14.3
7	5	4	2.94	1	20.0
8	9	9	3.78	0	0
9	4	3	1.99	1	25.0
10	4	3	2.86	1	25.0
Over 11	6	6	3.61	0	0

As shown in table 7, the mean values for ketones in each sub-groups ranged from 1.99~3.78 mg/100 ml. In comparison with the mean value of the normal group, those of the sub-groups such as non-calving, 2~5, 8 and over 11 were all higher, that is; they increased by 1.6~22.0%, respectively. But in other 5 sub-groups, the mean values were all under that of the normal group. The sub-normal cases were not observed at all in the five sub-groups such as 2, 4, 5, 8 and over 11, but in the other seven sub-groups they appeared in the rate of 8.0~25.0%, respectively. From the above results, in general, the mean values for ketones showed a tendency to increase in the sub-groups 2~5 calving and also there was a tendency for the appearance rates of the sub-normal cases to become higher in accordance with the increase of the calving frequencies, but there were some exceptions.

4) The relation between the time after calving and ketone values: For this purpose, 118 cases divided into 10 sub-groups according to the time after calving were examined.

The mean values for the 3 sub-groups from 1~6 months were 3.18~3.61 mg/100 ml, that is, they increased by 2.3~16.0% in comparison with the mean value of the normal group, respectively. But the mean values of 2 groups between 7~10 months decreased by 1.57 and 5.2%, respectively. And also the mean values from over 11 months showed the increasing tendency. In the sub-groups, except three consisting of 13~20 and over 25 months, the appearance rate of the sub-normal cases was 5.2~20.0%, especially in the period less than 12 months, the increasing tendency was observed.

TABLE 8. *The Relation between the Time after Calving and Ketone Values*

MONTH AFTER CALVING	NO. OF CASES (118)	NORMAL GROUP No. of Cases (109)	NORMAL GROUP Mean (mg/100ml)	SUB-NORMAL GROUP No. of Cases (9)	SUB-NORMAL GROUP Appearance Rate (%)
1~ 2	19	18	3.34	1	5.2
3~ 4	15	14	3.18	1	6.6
5~ 6	19	18	3.61	1	5.2
7~ 8	6	5	2.62	1	16.6
9~10	19	16	2.95	3	15.7
11~12	18	17	3.43	1	5.5
13~16	5	5	3.19	0	0
17~20	9	9	3.99	0	0
21~24	5	4	3.69	1	20.0
Over 25	3	3	3.39	0	0

5) The relation between milk yield and ketone values: In 269 cases, including lactating and dry, the rise and fall of the ketone values in connection of lactating were examined with the results displayed in table 9.

TABLE 9. *The Relation between Milk Yield and Ketone Values*

VOLUME OF MILK (kg)	NO. OF CASES (269)	NORMAL GROUP No. of Cases (257)	NORMAL GROUP Mean (mg/100ml)	SUB-NORMAL GROUP No. of Cases (12)	SUB-NORMAL GROUP Appearance Rate (%)
0	114	112	3.02	2	1.8
1~ 5	22	21	2.96	1	4.5
5~10	55	53	3.17	2	3.6
10~15	30	29	2.26	1	3.3
15~20	24	19	3.44	5	2.1
Over 20	24	23	4.02	1	4.2

The mean ketone values of 112 cases of dry cows in the normal group was 3.02 mg/100 ml. In comparison with the mean values for the normal group and also with the mean values of lactating cows, ketone values decreased by 2.8 and 3.4%, respectively. In the lactating cases, the values for 2 sub-groups, viz., 1~5 and 10~15 kg, were 2.96 and 2.26 mg/100 ml, respectively, that is, they decreased by 4.8 and 27.0% in comparison with the mean value of the normal group. However in the three sub-groups, viz., 5~10, 15~20 and over 20, the values all increased, especially in over 15 kg. On the other hand, the appearance rate of the sub-normal cases in each division always ranged higher from 2.1~4.5% in lactating sub-groups than in the dry sub-group. From the above data, it may be said that there was a tendency for ketone values to increase in the lactating cows compared with the dry cows. Among only lactating sub-groups, did those values show

increasing tendency in accordance with the increase of milk yield.

6) The relation between the season and ketone values: A year was divided into 5 terms as shown in table 10.

TABLE 10. *The Relation between the Season and Ketone Values*

SEASON (Month)	NO. OF CASES (269)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (257)	Mean (mg/100ml)	No. of Cases (12)	Appearance Rate (%)
I (5 ~ 6)	60	51	3.66	9	15.0
II (7 ~ 8)	31	30	2.09	1	3.2
III (9 ~10)	87	87	3.25	0	0
IV (11~ 1)	49	47	4.36	2	4.1
V (2 ~ 4)	42	42	2.30	0	0

The rise and fall of the ketone values in each term was examined using 269 cases. In the 1st term (May~June) in Hokkaido, the grass grows newly, pasturage begins in the day-time; the term is that of a great deal of milk production, because it continues after the March to May period in which the calving happen most frequently. The atmospheric temperature is most comfortable. The mean ketone value of the 51 cases examined in this term was 3.66 mg/100 ml, which increase by 17.6% compared with the mean value of the normal group. Nine cases were counted as sub-normal cases, but the appearance rate was 15.0%. In the 2nd term (July~August), the grass grows in abundance; it is the most suitable time for pasturage but also the weather is very warm. The mean ketone value of 30 cases in this term was 2.09 mg/100 ml, which is the lowest of all. The appearance rate of sub-normal cases was 3.2%. The 3rd term (September~October) is the harvest time, in which the food is richest and the temperature is favorable. The mean value of 87 cases in the term was 3.25 mg/100 ml, which was an increase by 4.5% over that of the normal group. No cases which belonged to the sub-normal group were observed. In the 4th term (November~January), cows are fed mainly with hay and ensilage in stall on account of the heavy snow; this is the coldest time in a year. The mean ketone value of the 47 cases in this season, was 4.36 mg/100 ml, which shows an increase by 40.2% in comparison with the mean value of the normal group; this is the highest value in the year. Two cases belonged to sub-normal group; the appearance rate was 4.1%. The 5th term (February~April) is the terminal time in stall. In this period, the food for cattle is mainly hay and ensilage and the weather becomes gradually warmer from the cold time. The mean ketone value of the 42 cases studied in this term was 2.30 mg/100 ml, which decreased by 26.1% below the mean value of the normal group; no sub-normal cases at all were observed.

Observing the above results, the mean ketone values were highest in the 4th term (November~January), next in the 1st term (May~June) but the lowest were in the 2nd term (July~August).

7) The relation between feeding-management and ketone values: Studying 269 cases,

relations between feeding-management and ketone values were observed. At this time, the quality of the feeding-management was divided into 3 sub-groups according to judgement based upon the arrangement of the cow-house, condition of the management, presence of pasture, condition of feeding, etc.

TABLE 11. *The Relation between Feeding-Management and Ketone Values*

QUALITY OF FEEDING-MANAGEMENT	NO. OF CASES (269)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (257)	Mean (mg/100ml)	No. of Cases (12)	Appearance Rate (%)
Superior	86	75	4.01	11	12.8
Moderate	150	149	2.88	1	0.7
Inferior	33	33	2.83	0	0

The cows kept in the "model farms" neighbouring Sapporo were grouped into a superior sub-group and the herds fed in the common management and also the herds maintained in careless condition as in newly reclaimed land were grouped into the moderate and inferior sub-groups, respectively. As shown in table 11, the mean values of these 3 sub-groups were 2.83~4.01 mg/100 ml, respectively, viz., in comparison with the mean value of the normal group, the increase rate was 28.9% in the superior sub-group but in the moderate and in the inferior sub-groups, values decreased by 7.3 and 9.0%, respectively. Judging from the above results, in accordance with the better quality of the feeding-management, the tendency of the mean ketone values to increase was recognizable. Also the appearance rate of sub-normal cases showed a tendency to run parallel with the mean ketone values.

5. The Relation between the Ketone Values and the Blood Sugar Levels

The mean level for the blood sugar of the clinically healthy 270 cows was 62.1 (10~154), but in this distribution, the mean levels of the 257 cases between the rejection limits under 5% level of significance was 60.6 (22.3~101.9 mg/100 ml). This value may be considered the mean value for blood sugar levels of the normal cows, excepting special cases, from the

TABLE 12. *The Relation between the Ketone Values and the Blood Sugar Levels*

GROUP	KETONE VALUE (mg/100 ml)	BLOOD SUGAR	
		No. of Cases (251)	Mean (mg/100ml)
Normal	0~ 2	35	62.7
	2~ 4	146	60.1
	4~ 6	37	61.0
	6~ 8	15	61.1
	8~10	4	75.0
	10~12	3	67.5
Sub-normal	Over 12	11	67.1

clinically healthy cows in the field. The relations between the ketone values and the blood sugar levels were examined on the 251 cases estimating both at the same times. On the basis of the ketone values, the experimental cows were divided into 7 sub-groups and the mean values of the blood sugar in each of them were calculated. The results are given in table 12. In the blood sugar levels for 4 sub-groups between 0~8.0 mg/100 ml, no great difference was observed in comparison with the mean value for the normal group, but in the two sub-groups over 8 mg/100 ml in the normal group and also in the sub-normal group, the mean blood sugar levels increased markedly. With regard to the above results, it may be said that the blood sugar levels in the sub-normal group showed tendency of increase.

6. The Relation between the Ketone Values and the Number of Eosinophilic Leucocytes

The mean number of eosinophilic leucocytes calculated for 249 clinically healthy cows was 485 (52~3,400). The mean number of 237 cases between the rejection limits under 5% level of significance was 436 (130~1,670). On the basis of the ketone values, 228 cases were divided into 7 sub-groups, in which the mean numbers of eosinophilic leucocytes were counted, respectively. The relations among them are shown in table 13.

TABLE 13. *The Relation between the Ketone Values and the Number of Eosinophilic Leucocytes*

GROUP	KETONE VALUE (mg/100ml)	NO. OF EOSINOPHILIC LEUCOCYTES No. of Cases (228)	Mean
Normal	0~ 2	40	404
	2~ 4	115	469
	4~ 6	39	361
	6~ 8	14	479
	8~10	6	452
	10~12	3	452
Sub-normal	Over 12	11	318

There was no wide difference found among the mean numbers of the sub-groups except the one sub-group in 4~6 mg/100 ml. But in the sub-normal group, it was 318, that is, the eosinophilic leucocytes decreased markedly. In comparison with the mean number of the normal group, the sub-normal group was much smaller than any of the normal group showing a decrease by 27.0% compared with the mean value for the normal group. From the above data, it may be said that there are scarcely any differences among the numbers of eosinophilic leucocytes of sub-groups in the normal group, but in the sub-normal group, the number of eosinophilic leucocytes decreased markedly.

DISCUSSION

In these experiments, the total ketone and also free acetone contents of blood for the clinically healthy cows, presented such wide differences from individual

to individual that they were divided into 2 groups statistically, viz., "normal and sub-normal groups." In these cases, the values of the "normal group" were supposed to be the values for the greater part of the normal cattle, excepting special cases, from the apparently healthy cows in the field. Now, in comparison with the results quoted by previous workers[17,23~25], the mean value of the total ketones of the normal group is found to be higher than values reported by SAMPSON et al. (1933)[17], BODDIE[17], DUNCAN et al.[17], SAMPSON and BOLEY (1941)[17], KNODT et al.[17] and UMEZU and it is considerably lower than those of SAMPSON and BOLEY (1945)[17] and THIN and ROBERTSON but it almostly agreed with results of SAMPSON et al. (1933) and DUNCAN et al. On the other hand, the mean value of free acetone of the normal group is slightly higher than any of the results reported by UMEZU, YOSHIDA and USHIMI et al. Such differences may be ascribed to the fact that the ketone levels vary considerably from animal to animal and the slight differences noted may be associated with diet, environment, etc. In view of the herein reported results, all of the internal and external factors more or less influenced the ketone levels, especially, parturition, lactation, season and feeding-management seem to be the most important factors exerting influence upon the ketone values. In other words, in our examinations, the mean ketone values became higher in accordance with the increase of milk yield. As CHRISTIAN and SEGARD reported, it may be thought that the large and sudden demand of milk yield after the parturition effects cattle as a great stress. HOLM and FIRCH observed that 17-ketosteroid in urine decreased markedly after the parturition. CRAIGE stated that the decreasing tendency of blood sugar levels was observed after parturition and easily happened in high lactating cows; UMEZU et al.[22] and HENDERSON also expressed the same opinions. Further, MCAULIFF et al. made it clear that in the time of after-parturition the internal secretion systems were stressed so unusually that the metabolic disturbance in carbohydrate was apt to appear. The marked increase of ketone value in November~January may be caused by the coldness and the changes of the feeding-management. Also, the marked increase of the ketone values observed in the sub-group in which the feeding-management was very good seems to be due to the great demand in milk yield as well as over-feeding with concentrated feedstuffs. That is to say, the environment supposed by the observer to be good at a glance, may act to generate some stress upon the cattle themselves. Regarding from the authors' results, it may be thought that the various factors such as age, period of pregnancy, calving frequency and time passage after parturition are not individual factors respectively but rather they act under mutual interference with lactation and parturition etc. as reported by SAMPSON.[10]

By the present authors, it has been shown clearly that the "sub-normal group"

among the clinically healthy cows is a special group showing not only the increase of the blood ketone bodies but also the increase of blood sugar level and marked decrease in number of eosinophilic leucocytes.These findings observed in this group coincided with the findings in cases of the functional increase in the pituitary-adreno-cortical system caused by stress. Sub-normal group may be situated in the stage of resistance in the general adaptation syndrome reported by SELYE. Also, the "sub-normal group" seems to be one analogous to the "physiological ketosis" of SAMPSON,[11] "sub-clinical ketosis" of THIN and ROBERTSON[17] and the "sub-physiological syndrome" of YOSHIDA.

SUMMARY

These clinical and biochemical studies were made on the basis of the blood ketone bodies in 368 clinically healthy dairy cows. The result thus obtained may be summarized as follows.

1. The values for the blood ketone bodies of the clinically healthy cows varied so widely that they were statistically divided into 2 groups, viz., "normal and sub-normal groups."

2. The mean values for the ketone bodies of the normal group were 3.11 (0.99～11.17) mg/100 ml in total ketone, and 0.79 (0.22～4.53) mg/100 ml in free acetone.

3. The mean values for the ketone bodies of the sub-normal group were 39.10 (12.30～73.80) mg/100 ml in total ketone, and 8.23 (5.20～10.50) mg/100 ml in free acetone.

4. The values for free acetone, acetoacetic acid and β-hydroxybutyric acid increased in accordance with the increase of the total ketone.

5. All of the internal and external factors exerted influence upon the rise and fall of the total ketone, especially, it may be supposed that the stress of parturition, milk yield, season and feeding-management were very important.

6. It was clarified that the sub-normal group showed not only the increase of the ketone bodies but also increase of the blood sugar level and marked decrease in number of eosinophilic leucocytes.

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