

## Studies on 'the Utrecht Abnormality of Milk' in the Miyuki Dairy Farm

### V Magnesium depletion of Soft Tissues and Nephrocalcinosis.

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(Tables 1–9)

A great deal of abnormal milk secretions had been observed amongst the lactating dairy herd at the Miyuki Dairy Farm which belongs to the Faculty of Fisheries and Animal Husbandry of Hiroshima University. This abnormality of the milk proved to be identical with that of 'the Utrecht abnormality of milk' which occurred in the Netherlands in nineteen thirty. (1, 12, 13) The abnormal milk in the Netherlands showed alcohol-test positive, heat-test positive and a high content in ionized Ca. The abnormal milk in the Miyuki Dairy Farm however showed not only alcohol-test positive, heat-test positive and a high content in ionized Ca but moreover a high content in total Ca and a low content in total Mg. (14, 16)

The cows showed a low Mg content and a high Ca content in the blood serum and a high Ca level in the whole blood. (15, 17) Further more various metabolic disorders such as ketosis, hypocalcaemia or milk fever, hypomagnesaemia or grass tetany, parturition disturbances such as paresis, abortion, still-birth, retaining of the placenta occurred at the Miyuki Dairy Farm. (19) Abnormal movement of blood serum alkaline phosphatase activities were detected too and so it was found out that the herd were suffering from 'osteoporosis' on the bone surface accompanied by some clinical signs such as articular disease, lameness, diarrhea, broken incisor, kidney disturbance, etc. (19)

From these signs, especially from the low content of Mg in milk and blood serum, from the occurrence of hypomagnesaemia, and from the poor Mg content in roughage, the author has brought to the conclusion that those cows must have been suffering from chronic Mg deficiency accompanying bone disease.

It is said that the Mg content of the soft tissues of dietary Mg deficiency in the experimental animals varie but little and that this Mg deficiency induced nephrocalcinosis. From these finding, the electrolytes of soft tissues of the cows in Miyuki Dairy Farm were analysed.

#### *Experimentals*

Animals; Experimental samples were obtained from the cows of Miyuki Dairy Farm after slaughter. Bone samples, cardiac muscles, skeletal muscles, livers, kidneys, spleens, mammary glands and suryvary glands were collected from 14 cows and 2 heifers.

Cow No.1(13th calving), slaughtered after severe milk fever.

Cow No.2(10th calving)

Cow No.3( 7th calving)

Cow No.4( 6th calving)

Cow No.5( 6th calving)

Cow No.6( 6th calving)

Cow No.7( 8th calving)

Cow No.8( 7th calving)

Cow No.13(4th calving)

Cow No.14(4th calving)

Cow No.19(3rd calving)

Cow No.20(1st calving)

Cow No.28(1st calving)

Cow No.30(1st calving)

Heifer 1

Heifer 2

The details of these cows have been shown in earlier report. (18)

Bone samples were reduced to ash after alcohol, ether, acetone treatments and then were dissolved by a dilute HCl solution. Soft tissues were reduced to ash by 70% HClO<sub>4</sub> in micro-kjel-dahl flasks at 130–160°C in a glycerin bath. The ashes were then diluted by means of deionized water, SrCl<sub>2</sub>, and ethanol. These solutions contained 1000 ppm Sr, 20% ethanol, Ca 1–5 ppm, Mg 0.1–0.5 ppm, Na 5–50 ppm, and K 5–50 ppm. Determinations of Ca and Mg were made by the atomic absorption method and determination of Na and K were made by the flame photometric procedure using Hitachi 208 type atomic absorption spectroscopy.

### *Results and Discussions*

The results of electrolytes of soft tissues and bone ashes of the individual cows of Miyuki Dairy Farm are shown in tables. The most important characteristics were a decrease of Mg contents in the cardiac muscle, the skeletal muscle, the liver, the kidney cortex layer, the kidney medulla layer, and the nephrocalcinosis, especially the calcification of kidney medulla layer.

As for the kidney, it is usually accepted that normal values of ox are as follows, and there is little change between cortex and medulla, and the concentrations of K is higher in cortex whereas Na is higher in medulla.(3)

	normal value of kidney(3)	values of Miyuki Dairy Farm	
	(meq/kg)	kidney cortex (meq/kg)	kidney medulla (meq/kg)
Ca	5.4 – 7.0	3.22	9.98
Mg	16.5 – 19.5	11.87	10.45
Na	63 – 74	70.47	106.11
K	57.6 – 64.1	57.62	51.33

In the case at Miyuki Dairy Farm, the kidney cortex layer showed a progressive fall in Mg, no consistent change in Ca, Na and K, but the kidney medula layer showed a marked increasing in Ca, a progressive fall in Mg, a increasing in Na and a decreasing in K. There were marked differences amongst the individual cows of kidney medula Ca, such as a 7 fold increase for Cow No.5, a 4 fold in Cow, No.4, a 2 fold in Cow No.14, Cow No.19 and Cow No.28. Stones were observed in the kidneys of Cow No.11 and Cow No.14, as shown in an earlier paper. (19) Before wet-ashing the stones were carefully removed from the tissues.

In Mg-deficient young growing rats, MacIntyre (1958) reported a rise of Ca content in the kidney, as much as 400% high. (6) Mainard (1957) too reported a rise in the kidney Ca, about 25 fold. (7) Goulding (1968) pointed out that nephrocalcinosis was much more severe in females than in males. (4).

In Mg-deficient full-grown rats, Martindale (1964) reported an accumulation of Ca in the kidney as much as 500%.(5) Tuft (1937) and Martindale (1964) reported the accumulation of kidney Ca at the early stage of Mg-deficiency. (5, 11)

Bunce (1973) also reported that the Ca accumulation in the kidney began between 24–48 hr after the first low Mg meal and was confined to the nuclear-debris and mitochondrial fractions at its earliest detection time.(2)

The Mg concentrations in the cardiac muscle, the skeletal muscle, the liver, the kidney cortex, the kidney medula and the spleen of the Miyuki Dairy Farm fell down into the respectively corresponding values as shown below.(8).

	normal value(8)	normal value(20)	value of Miyuki Dairy Farm
	(meq/kg)	(meq/kg)	(meq/kg)
cardic muscle	28.78	23.46	17.51
skeletal muscle	23.03	20.57	14.85
liver	18.09	18.94	13.14
kidney cortex	} 15.6	16.31	11.87
kidney medula		13.45	10.45
spleen	— —	20.32	13.80

The K concentration in the cardiac muscle for the sample of Miyuki Dairy Farm was 67.65 meq/kg and thus slightly decreasing from the corresponding normal value of 72.4 meq/kg.(3) The skeletal muscle K was 74.08 meq/kg for the sample of Miyuki Dairy Farm, this also slightly decreased from the normal value of 84 meq/kg.(3)

A marked increase of Ca in the mammary gland and in the suryvary gland were observed. In those glands that showed high values for Ca, the Mg contents were high also. These organs such as the kidney, the mammary gland and the suryvary gland have an important role as excretory organs of body fluids that are in excess of Ca demineralized from the bone.

The Ca and Mg contents of bone ashes were Ca 36.44% and Mg 0.682% for mandible and Ca 36.46% and Mg 0.683% for caudal vertebrae, they had normal values, but the bone showed a characteristic pattern of osteoporosis as shown in earlier papers. (18, 19)

From this experiment, the characteristic sign of the Miyuki Dairy Farm samples are the slight falls of Mg concentrations in various soft tissues, a decreasing tendency of K and an increasing tendency of Na in the kidney, the cardiac muscle and the skeletal muscle, and a marked increase of nephrocalcinosis, especially accumulation of Ca in kidney medula layer. Also a slight fall of blood serum Mg was observed in these cows and abnormal milk secretion, osteoporosis, ketosis, hypocalcaemia, and hypomagnesaemia were observed as reported in previous papers.(18,19)

It is possible that small animals are easy to get Mg-deficiency within the limits of the experiments, by dosing Mg-free feedstuff. It is impossible however to consider farm animals typical cases of Mg-deficiency under spontaneous conditions, because of the reason there fore is that the rations contain various smounts of Mg and that the cows need a big amount of feedstuff. In the case of Miyuki Dairy Farm experiments, the Mg content of the ration should be on the borderline of the limit of maintenance of the body and then should be only a little under the line of sufficiency during the pregnancy conditions and long term milking.

The results obtained from the Miyuki Dairy Farm experiments agreed well with the experimental data for the soft tissues of Mg-deficient rat (4, 5, 6, 7) and also agreed with the results of the bone and teeth examinations.(9)

The characteristics of the Miyuki Dairy Farm cows agreed with the chronic disorder of Mg-metabolism in farm animals, which occurred in Calcareous Alps in 1967, in that case the Ca contents of the lung and the kidney rose, the Mg contents of the blood serum showed a slight depletion, and Mg contents of the bone ash decreased, but in that case no description of bone abnormality and milk abnormality was reported.(10)

#### SUMMARY

'The Utrecht abnormality of milk' and osteoporosis in question occurred in the Miyuki Dairy Farm belonged to the Faculty of Fisheries and Animal Husbandry of Hiroshima University. Milk and blood had showed high Ca levels and low Mg levels during a whole year. So all the cows were taken into close observation in order to follow up systematically this lactational chronic disorder of Mg-metabolism.

The Mg concentrations in the cardiac muscle and the skeletal muscle, the liver, the kidney cortex and the kidney medula layer, the spleen of the cows, they all fell proportionally beneath the corresponding values in normal conditions. Nephrocalcinosis, especially calcification of the kidney medula layer was observed. A gradual increasing tendency in Na and a gradual decreasing tendency in K were detected in the cardiac muscle, in the skeletal muscle and in the kidney medula layer. These signs agreed well with the experiments of Mg-deficient rats and with spontaneous Mg-deficient farm animals.

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## 御幸牧場に発生した低酸度二等乳に関する研究

## V. 各臓器のMg 含量の低下と腎臓の石灰沈着について

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1970年に広島大学水畜産学部附属農場で集団発生した低酸度二等乳は1930年代にオランダ、ユトレヒトを中心に発生した“the Utrecht abnormality of milk”と全く同じ現象であり、これらの乳牛群には骨軟症の一種である骨粗鬆症 osteoporosis が発生している事が判った。牛乳と血清はCaが高くMgが低いこと、牧草中のMg含量がDM中の0.2%未満で必要量を充していないこと及びグラスステニーが発生していることからこれらの乳牛群に慢性Mg欠乏による泌乳障害が発生していることが判った。

実験動物によるMg欠乏症においては筋肉及び各臓器のMg含量の低下、Naの増加とKの減少、臓器によってはCaの沈着などの電解質の変動がみられていて、とくに各臓器のMg含量の低下と腎臓の石灰沈着が特徴であると云われている。

御幸牧場の乳牛から屠殺時に採取した心筋、骨格筋、肝臓、腎臓、ひ臓、乳腺及び唾液腺と、灰化した骨中の電解質を測定したところ、心筋、骨格筋、肝臓、腎臓皮質部及び髓質部、ひ臓においてはMg含量が低下し、更に心筋、骨格筋、腎臓髓質部においてはNaの増加とKの減少がみられた。腎臓の石灰沈着は皮質部においてはみられなかったが髓質部においては16頭の平均で約2倍であり、Cow No.5では7倍、Cow No.4では4倍にものぼった。骨の灰分中のCa及びMg含量は正常値と差はなかったが骨は骨粗鬆症を呈していた。

御幸牧場の各臓器の電解質は実験動物におけるMg欠乏症とよく一致するし、1967年オーストリアで発生した乳牛の慢性Mg代謝障害とも一致しているが、実験動物のMg欠乏症においては腎臓の石灰沈着を皮質部と髓質部に区別して測定していないし、オーストリアの乳牛の場合には骨の異常と低酸度二等乳の発生についてはふれていない。

Table 1. Electrolite in kidney cortex at Miyuki Dairy Farm (meq/1 kg fresh tissue)

Cow No.	Ca (meq/Kg)	Mg (meq/Kg)	Na (meq/Kg)	K (meq/Kg)
1	3.30	13.54	67.27	41.98
2	3.31	12.08	70.04	58.79
3	3.88	8.48	78.66	52.66
4	3.95	12.40	71.02	58.98
5	3.27	11.16	70.89	53.77
6	3.33	10.93	73.89	53.40
7	3.61	9.42	72.18	55.13
8	3.47	10.99	70.86	63.55
13	3.91	11.77	63.99	54.40
14	3.20	12.09	82.49	54.60
19	2.15	13.65	59.32	67.19
20	3.36	11.44	71.35	57.11
28	2.37	12.60	69.97	60.24
30	3.48	12.81	66.33	66.46
H-1	4.13	13.02	55.67	60.76
H-2	2.44	13.54	83.53	62.89
mean	3.32	11.87	70.47	57.62
± sx	0.57	1.45	7.35	6.22

Table 2. Electrolite in kidney medula at Miyuki Dairy Farm (meq/1 kg fresh tissue)

Cow No.	(meq/kg)	(meq/kg)	(meq/kg)	(meq/kg)
1	7.77	12.13	67.61	39.77
2	6.64	11.37	126.14	51.01
3	4.44	12.82	95.49	47.65
4	19.63	11.15	121.47	51.80
5	34.67	10.28	123.53	50.38
6	5.65	9.41	130.91	46.48
7	6.81	8.59	119.88	53.15
8	7.38	9.83	118.65	56.23
13	8.54	9.74	99.37	48.77
14	12.67	9.54	100.03	50.68
19	10.81	10.61	85.37	57.32
20	5.94	9.47	93.33	51.85
28	12.39	11.25	95.51	52.69
30	5.95	10.31	115.40	55.78
H-1	6.24	10.24	108.74	53.36
H-2	4.18	10.39	96.38	54.41
mean	9.98	10.45	106.11	51.33
± sx	7.66	1.09	17.27	4.30

Table 3. Electrolyte in cardiac muscle at Miyuki Dairy Farm (meq/ 1 kg fresh weight)

Cow No.	Ca (meq/kg)	Mg (meq/kg)	Na (meq/kg)	K (meq/kg)
1	2.85	17.80	42.89	61.08
2	2.31	18.85	37.37	74.98
3	2.54	15.90	37.67	56.89
4	2.12	18.19	41.67	67.50
5	3.05	16.36	34.36	61.94
6	2.65	17.18	35.46	62.90
7	1.85	16.55	33.73	75.16
8	2.02	16.35	35.80	72.64
13	2.85	17.83	31.61	68.18
14	2.35	17.47	33.17	64.33
19	1.91	16.14	40.94	57.80
20	2.00	16.79	31.13	62.03
28	1.40	18.26	38.58	74.22
30	1.57	18.05	29.06	78.17
H-1	2.35	17.68	36.42	67.37
H-2	1.40	20.80	33.03	77.24
mean	2.20	17.51	35.84	67.65
± sx	0.51	1.23	3.92	6.99

Table 4. Electrolyte in skeletal muscle at Miyuki Dairy Farm (meq/1 kg fresh tissue)

Cow No.	Ca (meq/kg)	Mg (meq/kg)	Na (meq/kg)	K (meq/kg)
1	1.92	15.93	40.13	66.40
2	2.22	16.09	31.89	85.33
3	4.59	14.67	21.49	70.76
4	4.38	13.45	36.06	61.03
5	2.68	14.62	22.21	70.96
6	3.61	12.34	28.86	56.41
7	1.67	12.84	34.94	79.23
8	1.66	15.29	48.54	91.92
13	4.84	15.34	26.84	69.58
14	3.25	13.19	33.39	59.16
19	1.19	16.52	21.78	83.54
20	2.91	13.99	30.75	67.49
28	1.52	15.03	30.21	83.58
30	1.46	15.61	21.86	89.30
H-1	8.04	14.15	25.68	60.89
H-2	1.47	18.56	22.41	89.65
mean	2.96	14.85	29.82	74.08
± sx	1.81	1.56	7.65	11.97

Table 5. Electrolite in liver at Miyuki Dairy Farm (meq/1 kg fresh tissue)

Cow No.	Ca (meq/kg)	Mg (meq/kg)	Na (meq/kg)	K (meq/kg)
1	1.75	16.39	36.54	74.68
2	2.06	14.28	31.17	82.03
3	2.26	11.67	33.43	63.71
4	1.93	13.50	31.66	70.63
5	2.44	12.41	33.49	60.02
6	2.26	12.05	33.70	59.45
7	2.23	12.53	38.83	72.79
8	3.52	12.17	34.59	70.40
13	2.77	13.34	29.23	67.39
14	2.17	12.33	30.87	63.76
19	1.43	13.03	31.76	70.70
20	2.53	12.81	32.34	62.17
28	1.50	14.15	30.53	72.80
30	1.90	12.39	23.60	81.11
H-1	2.52	14.01	28.48	65.14
H-2	1.23	13.12	29.81	72.47
mean	2.16	13.14	31.88	69.33
± sx	0.57	1.16	3.47	6.77

Table 6. Electrolite in spleen at Miyuki Dairy Farm (meq/1 kg fresh tissue)

Cow No.	Ca (meq/kg)	Mg (meq/kg)	Na (meq/kg)	K (meq/kg)
1	2.79	11.68	52.32	53.92
2	1.98	14.30	38.54	92.10
3	2.32	14.00	29.95	81.17
4	2.32	14.79	34.81	93.13
5	2.09	13.40	34.46	75.61
6	2.48	14.73	32.28	84.91
7	2.23	12.98	32.84	91.78
8	2.65	13.85	23.52	92.47
13	2.65	14.77	32.72	88.24
14	2.29	14.45	33.22	81.78
19	1.67	15.40	34.57	88.64
20	2.21	13.79	30.98	76.75
28	1.74	14.61	33.64	95.48
30	2.29	13.31	33.81	89.92
H-1	2.64	15.26	33.86	93.07
H-2	0.95	9.51	23.09	56.63
mean	2.21	13.80	33.41	83.48
± sx	0.46	1.48	6.39	12.53



Table 7. Electrolyte in mammary gland Miyuki Dairy Farm (meq/l kg fresh tissue)

Cow No.	Ca (meq/kg)	Mg (meq/kg)	Na (meq/kg)	K (meq/kg)
1	31.64	11.28	39.41	48.96
2	887.1	61.01	58.69	38.52
3	92.9	11.01	57.88	37.84
4	300.2	31.80	52.65	38.33
5	306.2	30.85	47.59	39.76
6	384.2	35.87	58.43	40.46
7	—	—	—	—
8	—	—	—	—
13	92.9	12.68	31.07	38.78
14	252.0	25.05	51.89	43.24
19	—	—	—	—
20	129.0	13.52	43.45	36.79
28	46.9	9.84	26.94	41.20
30	—	—	—	—
H-1	—	—	—	—
H2	—	—	—	—
mean	252.3	27.29	46.80	40.39

Table 8. Electrolyte in suryvary gland at Miyuki Dairy Farm (meq/ 1 kg fresh tissue)

Cow No.	Ca (meq/kg)	Mg (meq/kg)	Na (meq/kg)	K (meq/kg)
1	33.52	16.96	50.17	58.50
2	31.28	14.98	43.48	58.26
3	21.87	10.97	39.15	52.87
4	25.51	12.68	39.58	51.52
5	—	12.12	34.58	63.24
6	21.98	12.46	42.31	57.78
7	33.27	13.57	33.27	64.14
8	21.33	11.51	41.58	65.68
13	21.87	11.85	42.66	51.72
14	30.47	12.45	37.82	54.86
19	—	—	—	—
20	22.05	9.99	37.00	53.59
28	25.15	13.27	48.45	69.73
30	30.03	12.96	46.82	62.43
H-1	36.32	13.55	29.79	47.65
H-2	—	—	—	—
mean	27.28	12.81	40.48	58.00

Table 9. Ca and Mg content in bone ashes at Miyuki Dairy Farm (% in ash)

Cow No	Mandible		Caudal vertebrae	
	Ca (%)	Mg (%)	Ca (%)	Mg (%)
1	37.16	0.760	34.79	0.677
2	35.92	0.613	—	—
3	36.76	0.650	37.04	0.615
4	36.29	0.779	35.92	0.719
5	37.00	0.710	37.25	0.670
6	36.89	0.600	37.57	0.639
7	—	—	—	—
8	—	—	—	—
13	36.76	0.623	37.04	0.654
14	36.48	0.789	35.70	0.725
19	35.30	0.617	34.82	0.612
20	35.96	0.720	36.33	0.633
28	37.18	—	35.51	0.678
30	—	—	—	—
H-1	—	—	37.36	0.826
H-2	35.59	0.645	36.23	0.749
mean	36.44	0.682	36.46	0.683