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ON THE THYROID GLAND ACTIVITY OF RUMINANT

I. THE SEASONAL CHANGES OF SERUM PROTEIN-BOUND IODINE IN THE DAIRY CATTLE

By

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There are many studies on the thyroid gland function of livestock and it has been demonstrated that the function can be influenced by various factors (1-7). This, together with other findings, indicated clearly that the thyroid gland is of importance in the survival and production of the animal. In addition, as a result of the progress in techniques for experimental modification of nearly any degree of thyroidal activity, some productivities of livestock has been considerably improved (1).

As indicative of the thyroid activity, serum protein-bound iodine (PBI) seemed to be one of the most useful measures (7-11), because of its rather simplicity in measurement.

Little evidence on PBI of the livestock in Japan, so far the authors know, has hitherto been presented, except for the values of horse grazed outdoors in all seasons (12). Therefore, it was desired, as an initial investigation, to determine the normal range and the average PBI value throughout a year in dairy cattle fed under the usual practical conditions.

Materials and Methods

The animals used in this investigation consisted of ten dairy cattle of the University Farm: five Holsteins and Jerseys, respectively. Their conditions at the time of the beginning of determination are shown in Table 1, and subsequent changes in the condition and management are illustrated in Table 1 and Fig. 1. The animals were divided into two groups as regards their management during summer, depending on the differences in lactation. That is, the lactating ones (HHA-5, HRO-4; J-2, J-4, in 1959: HRO-4, H-7, H-8; J-4, J-6) were kept in stables put in the field part of the day for grazing. The rest were put on a native mountain pasture, 500 m above the sea level, during the term from the beginning of June (1959) or the middle of May (1960) to the end of October. The cattle on the mountain pasture was brought back down for drawing blood samples.

Table 1. Condition of the dairy cattle employed (July, 1959)

Breed and animal no.	Age in month	History in reproduction Latest birth (later changes)	Milk yield kg
HHA-5#	66	Dec., 1958 pregnant, Sept., '59	11.9
HRO-4	59	Feb., 1959	14.2
H-6*#	20	— pregnant, Feb., '60	—
H-7*	20	— calved, Feb., '60	—
H-8*#	18	— infertile	—
J-2#	36	Aug., 1958 infertile	2.3
J-3	35	Dec., 1958 pregnant, Aug., '59	3.6
J-4*	20	— calved, Jan., '60	—
J-5*#	20	— infertile	—
J-6*	20	— calved, Mar., '60	—

* and # indicate the cows which were put on the native mountain pasture from June to October in 1959 and from the middle of May to October in 1960, respectively.

The ration for the animals consisted of silage (maize and legume), hay, beat, and commercial concentrate for dairy cattle. The feeds were given making allowance of body weight and milk yield. As salt, iodine derivate was not particularly supplemented.

Blood samples were taken monthly at about the middle of every month and left to stand in the refrigerator overnight before transportation. The estimation of PBI was carried on by the method of Barker, Humphrey and Soley (7) and was run in duplicate.

The average monthly temperature with each maximum and minimum of the month was calculated from daily records, and used to examine the annual change of PBI.

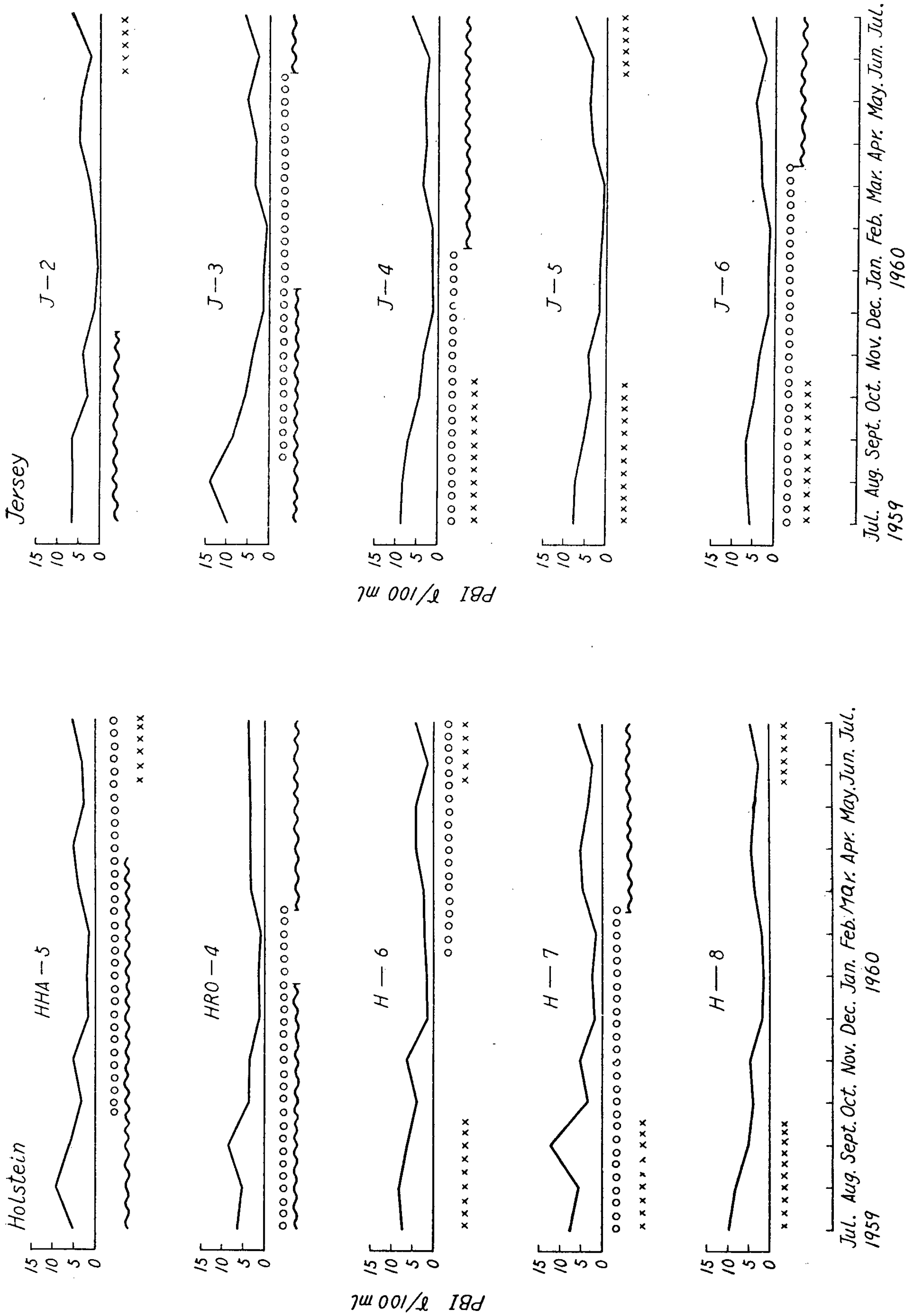
Results

Individual PBI values and curves according to monthly changes are given in Table 2 and Fig. 1, respectively. In general, a seasonal change in PBI was observed, in spite of some fluctuations in individuals.

Examinations were made of factors which would affect the PBI concentration, although the number and condition of the animals seemed insufficient to draw a final conclusion.

i) *Breed and Age*

The PBI contents of three Holsteins and five Jerseys, not so different in age (between 18 to 36 months) were compared (Table 3-1, Fig. 2). Likewise



○○○○○ : lactating × × × × × × : pregnant ~~~~ : grazing on the mountain pasture (non-lactating)

Fig. 1. Monthly changes of individual value of PBI (γ/100 *ml*)

Table 2. Monthly changes of the serum PBI content of dairy cattle (1959-60)

	month / anim. no.	1959						1960						Av.	Jul.
		Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.		
Holstein	HHA-5	5.17	9.34	6.22	3.32	5.60	1.90	2.25	1.98	4.01	5.61	2.94	3.34	4.32	5.61
	HRO-4	6.74	5.61	8.66	3.77	3.77	1.34	1.65	1.06	3.09	3.77	3.33	3.77	3.88	8.44
	H-6	7.28	8.22	6.44	4.00	6.56	1.49	1.74	2.34	2.71	4.19	4.14	1.65	4.23	5.32
	H-7	7.63	5.33	12.31	3.11	5.03	1.34	2.08	1.34	4.77	5.32	3.32	2.16	4.48	5.32
	H-8	9.80	8.03	5.17	4.00	4.94	1.99	1.54	1.59	3.54	4.65	3.77	2.34	4.28	5.61
	Av.±s.d.	7.32	7.31	7.76	3.64	5.19	1.74	1.86	1.67	3.66	4.71	3.77	2.68	4.24	6.06
		1.66	1.57	2.54	0.36	0.91	0.30	0.27	0.45	0.72	0.68	0.58	0.80	2.49	1.50
Jersey	J-2	6.75	6.56	6.92	3.22	4.48	1.65	0.83	1.54	2.91	5.04	4.77	2.53	3.43	6.56
	J-3	9.80	14.05	8.90	5.92	4.00	1.57	1.34	0.90	3.44	3.21	5.32	2.53	5.08	5.90
	J-4	8.87	8.44	7.09	4.34	3.67	1.28	1.50	1.65	3.89	2.80	3.21	2.16	4.08	6.22
	J-5	7.63	7.27	5.03	3.77	4.24	1.49	1.50	1.26	0.90	3.66	4.26	3.33	3.70	7.28
	J-6	5.61	6.56	6.92	4.34	3.77	1.49	1.66	1.13	3.09	3.44	4.52	2.16	3.72	5.32
	Av.±s.d.	7.75	8.58	7.98	4.32	4.03	1.49	1.38	1.29	2.84	3.63	3.91	2.54	4.10	6.21
		1.49	2.70	1.58	0.90	0.28	0.31	0.90	0.26	0.24	0.76	0.86	0.40	2.65	1.24
Over-all average ±s.d.		7.53	7.94	7.99	3.98	4.61	1.55	1.62	1.48	3.25	4.17	3.91	2.61	4.17	6.16
		1.50	2.37	2.31	0.77	0.86	0.23	0.37	0.41	0.97	0.90	0.73	0.64	2.56	1.05

a similar comparison was also attempted between the three Holsteins and three among the five Jerseys, all of these six having received the same management except in June 1960 (Table 3-2). The results, in general, indicated tendencies of no breed difference, but of seasonal changes. However, the Jerseys were likely to show a lower PBI only during the cold season than the Holsteins, $(0.05<P<0.1)$, though they were not different in PBI from Holstein in the warm season. At the same time, it was interesting to note that PBI differences among these two breeds were significant $(0.02<P<0.05)$, in November and April, the beginning and the end of the cold season.

Table 3-1. Comparison of PBI in regard to Breed(γ/100 *ml* serum)

Breed (anim.no.)	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Av
Holstein (3)	8.24	7.19	7.96	3.70	5.51	1.64	1.80	1.76	3.67	4.72	3.74	2.05	
Jersey (5)	7.75	8.58	7.98	4.32	4.03	1.49	1.38	1.29	2.84	3.63	4.42	2.54	

Table 3-2

Holstein (3)	8.24	7.19	7.96	3.70	5.51^{*1}	1.64	1.80	1.76	3.67	4.72^{*2}	3.74	2.05	4.33
Jersey (3)	7.37	7.42	6.53	4.15	3.89	1.45	1.55	1.35	2.63	3.30	4.00	2.55	3.85

* indicates a significant difference of $0.02>P>0.05$, and $t=2.90$ and 3.35 for 1 and 2, respectively

Difference between Holstein and Jersey was significant at the level of $0.05<P<0.1$ during cold season which corresponds to the stage shed feeding

Furthermore, PBI of the two older Holstein (of 59 and 66 months) seemed not to exhibit any marked difference from that of the younger cattle mentioned above (Fig. 2).

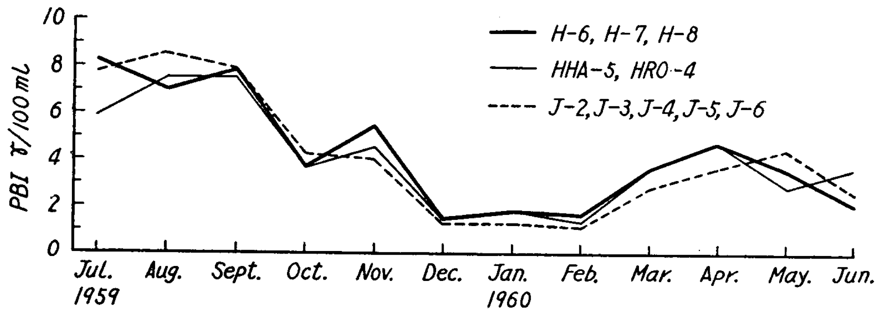


Fig. 2. Breed and age difference in PBI (γ/100 *ml*)

ii) *Pregnanry*

It was difficult to compare, in strict sense, the PBI changes during pregnancy, due to both the shortage in animal number and the difference of the stage in pregnancy at a given month. But, if the authors divide the animals into pregnant and non-pregnant groups, both showed a change with the same tendency of seasonal variations (Table 4 and Fig. 3). Whereas a significant high PBI ($P<0.05$) was detected in September (at the 4th and 5th month of gestation) when the average of the pregnant (HRO-4, H-7, J-4, J-6) was

Table 4. Comparison of PBI with regard pregnancy (γ/100 *ml* serum)

	1959 Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	1960 Feb.
Pregnant	7.22 ±1.2 (4)	6.32 ±1.40 (4)	8.79 ±1.94 (5)	4.13 ±0.90 (6)	4.30 ±0.94 (6)	1.49 ±0.32 (6)	1.74 ±0.32 (6)	1.46 ±0.57 (6)
non-pregnant	7.74 ±1.59 (6)	8.71 ±2.69 (6)	7.19 ±1.25 (5)	3.75 ±0.31 (4)	5.05 ±0.90 (4)	1.65 ±0.20 (4)	1.44 ±0.34 (4)	1.51 ±0.17 (4)

Figure in parenthesis indicates number of animals.

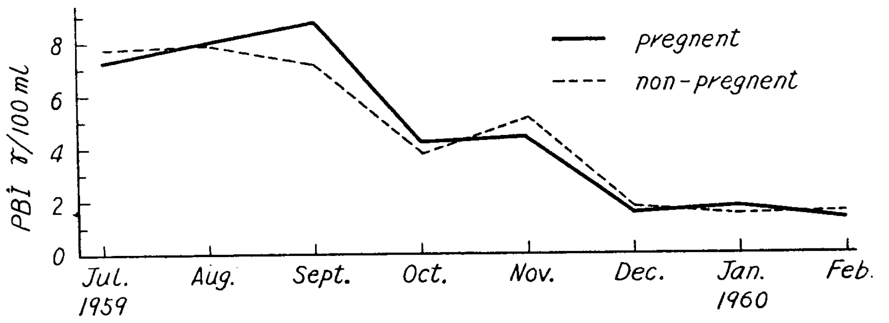


Fig. 3. PBI comparison with regard pregnancy

compared with the rest (non-pregnant) : the average values were 8.75±2.50 and 5.95±0.82 γ/100 *ml*, respectively. It was not clarified in this investigation which factor, the pregnancy stage or the season, was the cause of this difference. No essential differences were noticed either before or after the stage.

iii) *Grazing on mountain pasture*

Although this factor could be complicated with lactating or drying, as was described in "Materials and Methods", it could also be said that no detectable differences were found between the groups of shed and mountain pasture feeding (Table 5).

Table 5. Effect of the mountain pasture feeding on PBI concentration (γ/100 *ml*)

Group (anim. no.)	July	August	September	October/1959	June/1960	Average
Feeding near shed (4)	7.21	8.89	7.68	4.06	6.63	5.06
Feeding on pasture (6)	7.80	7.31	7.16	3.93	5.85	4.86

iv) *Reproductive efficiency and lactation*

Among the animals there were some that did not become pregnant for a long time. Their PBI, however, did not indicate particularly low values and stayed within the range of variation (Table 2).

Nor did the lactating appear to give a high PBI (Table 2 and 5).

v) *Season*

It might be too early to conclude that all of the factors examined above did not influence the measure of PBI at all, while, as far as the present investigation was concerned, none of them would probably provide any clear

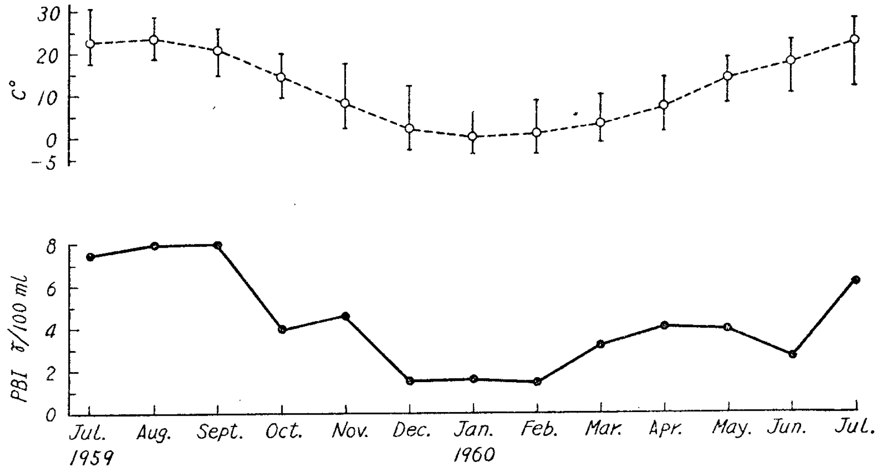


Fig. 4. Monthly changes of PBI and ambient temperature

differences. Consequently, all cows and heifers were treated together to compare with the seasonal changes. PBI plotted against ambient temperature and months (seasons) is illustrated in Fig. 4. The temperature was expressed as the monthly average with the maximum and minimum of each month.

The curve of PBI variation demonstrated a one phase cycle with a good coincidence with that of the temperature, *i.e.* higher in summer and lower in winter. This relation was further ascertained by the calculation of seasonal average of PBI (Table 6), and then the statistical analyses led the author to note a distinct evidence that apparent differences existed between the seasons ($P<0.01$), except between summer and autumn.

Table 6. Seasonal change of PBI γ/100 *ml* serum.

	a	b	c	d
	Jun.-Aug.	Sept.-Nov.	Dec.-Feb.	Mar.-May
HHA-5	5.98	5.05	2.04	4.19
HRO-4	5.37	5.40	1.35	3.40
H-6	5.72	5.67	1.86	2.30
H-7	5.04	6.82	1.59	4.49
H-8	6.72	3.70	1.72	3.99
J-2	5.28	4.87	1.27	4.24
J-3	8.79	6.27	1.27	3.99
J-4	6.49	5.03	1.48	3.30
J-5	6.08	4.35	1.33	2.94
J-6	4.78	5.01	1.42	3.68

a-b, no significant: a-c*, ($t=3.60$): a-d*, ($t=2.29$): b-c*, ($t=3.88$): b-d*,($t=2.32$): c-d*, ($t=2.98$)   * means significance at 1% level.

Discussion

It has been well known that various genetical and physiological factors would accompany the changes of PBI in livestock: Breed (13–16), growth or age (13, 16–19); pregnancy, lactation and reproductive efficiency (8, 18–25) and others. But the present investigation showed no evident relationship between PBI and those factors, although the animals were insufficient both in number and condition to deduce a conclusion. The only exception was the finding of a possible significance; the PBI of the Jerseys in the cold season decreased to a more extent than that of the Holsteins.

The most striking change obtained was a plain cycle of PBI with regard to season being higher in summer and lower in winter. This was closely associated with the change of environmental temperature. The results demonstrated by this investigation showed a complete agreement with those of Matumoto and Tonoue (12) with horses in Hokkaido (northern island of Japan). Lewis and Ralston, Michigan, U.S.A. presented data of the same tendency as ours, but the seasonal changes were less marked (18).

On the other hand, most of the papers so far published have indicated a reverse relation in the seasonal curve of PBI, *i.e.* generally, higher in the cold and lower in the warm season. From this relation, it is principally accepted that the thyroid gland activity is stimulated as the ambient temperature is decreased, although the seasons when the highest or the lowest PBI obtained varied a little among the papers probably depending on the locality where the investigation was undertaken.

Describing in detail the result of some previous researches, Søresen of Denmark proved PBI to be the heighest in autumn and postulated that this might be due to the warmer temperature of cowsheds in winter than in autumn (23). Lee, III, King, and Conley (26) Louisiana, U.S.A., showed increased PBI in the warm season and decreased PBI in cold seasons with dairy cattle of four breeds. But as their cows were given 1 per cent trace mineralized salt, and then PBI was extremely high; 28.17 and 14.79 $\gamma/100\,ml$ for Holsteins and Jerseys in July, respectivey.

There are some papers in which the measures of thyroxine secretion rate are employed. The evidence of the minimum in July–August and of the maximum PBI in March–April (three times that of summer) was presented by Premachandra, Pipes and Turner of Missouri, U.S.A. (27). They pointed out an interesting observation that, in general, PBI changes in approaching summer were more rapid than in approaching winter. A similar result was obtained by Lodge and his collaborators (28), Michigan, U.S.A., as well as Premachandra *et al.* Furthermore, Mixner and Lennon, Jr., in New Jersey, U.S.A., failed to show any evident differences of the seasonal change in PBI value of the lactating cows, but found the trend of the secretion rate to be upward in the winter and spring, and downward in summer and autumn (11).

As has been widely known that the variation of thyroid function could be considerably influenced by other factors, besides the ambient temperature. Among these factors are the geographical location and the property of soils where the animals were kept. Accordingly, the sort or quality of foodstuffs must be taken into consideration, because, in the end, all of these factors would greatly influence the iodine intake of animals, directly or indirectly (5, 29–31). For instance, an unusual magnitude and variation of PBI in dairy cattle happened when grass silage was given, and in this case the increase of PBI indicated an extremely high level of circulating thyroid hormone (33). It is impossible at the moment to account for the causes of descrepancy in the results between the present work and the others cited above. But considering the good agreement between our result and that of Matumoto *et al.* (12), it may be finally contributed to something specific in Japan, as well as to the ambient temperature, or the combination of the two.

The only possible explanation for the specificity of PBI change in Japan at this stage are, i) feeding animals with fresh grass which is probably rich in iodine content would produce a high value in summer, and ii) the elevated demand for thyroid hormone due to exposure to cold circumstance would result in the lower value of PBI in winter. As to the first point, soils and agricultural products of Japan have characteristically proven to have a very high content of iodine (34–37). A conceivable role may have been played in the maintenance of the thyroid gland activity by the feeding on pasture (1).

Next, the authors here consider concerning the physiological significance of PBI, again. PBI *per se* represents originally a balance between hormone secretion rate from the thyroid gland and disappearance rate at the peripheral tissue. At the same time a high coefficient of correlation between PBI and thyroxine-secretion rate shown clearly ($\gamma=0.70$) by Mixner and Lennon, Jr. (11). However, some interesting evidences have been brought to notice. Briefly stated, i) the metabolic response to cold is not directly related to the amount of circulating hormone, showing a reduction in the blood level of hormone at the time of sustained thyroid hyperactivity resulted from chronic exposure to cold (38–40), ii) heat production is regulated by a different hormone from thyroid (thermothyrin) other than thyroxine (41). iii) the significance of high PBI (serum thyroxine) both in pregnancy and hyperthyroidism should be distinguished from the basal metabolic rate. In pregnancy, the elevated PBI is regarded as a result of some compensatory mechanism, probably caused by a drop in the free or diffusible thyroxine (42–44).

Since a simple "feed-back" hypothesis can not adequately account for the present results, as well as the preceding observed changes in thyroid hormone activity, further knowledge is required, especially about the metabolism and mode of action of the hormone at the peripheral tissue. As a future test, the changes of PBI caused by the kind of feeds under controlled temperature will also propose available evidence to explain the present findings.

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Summary

Holsteins and Jerseys, ten in total, being pastured some of them on the mountain during summer, were used for estimation of PBI in the serum

throughout the year. The monthly measurement of PBI hardly showed any significant differences in regard to breed, age, reproductive and lactational condition, and putting on the mountain pasture. However, a clear seasonal change was in truth detected, being high in summer and low in winter and this was in good accordance with Matumoto *et al.* with horse in our country, but was entirely different from other works. The present results led the authors to the conclusion that the thyroid of dairy cattle in Japan showed a one phase cyclic change. The results obtained were discussed in comparison with those from different areas in the world. The authors suggest something specific for Japan in the raising PBI during the summer.

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