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Studies in Mineral Metabolism XXX.

Variations in the Iodine Content of Grasses at different stages of Growth and a note on the Iodine Content of Milk.

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MANY anomalous results regarding the iodine content of plants are encountered in the literature. These cannot be attributed solely to experimental errors, and it seems quite feasible that, like some of the other more common elements, the iodine present in plants and vegetables may vary at different stages of growth.

Von Fellenberg (1926) reported on the seasonal variations in the iodine present in plants. He analysed the leaves obtained from *Helianthus tuberosus* at different times of the year. The leaves were collected on the 13th September, 4th December and 3rd March the following year, and the iodine contents expressed in γs per 100 grams dry weight were 12·5, 71·5 and 44·5 respectively. Similar variations were observed in the case of leaves from Beech trees. Leaves collected in May contained 1·3 γ, those collected in August, 13·8, while the former year's leaves contained 10·7 γ iodine per 100 grams calculated on the dry weight.

Prior to the commencement of the present study, experimental grass plots were established at Onderstepoort for another investigation. At the end of January, 1932, the grasses were firmly established and all the plots were cut and then allowed to grow freely. A portion of each plot was cut at monthly intervals so that samples were obtained representing the grass at stages of growth of 1, 2, 3, etc., up to 12 months. These samples were dried in the shade, finely ground and the iodine determined.

The rainfall in inches recorded at Onderstepoort for the twelve months was as follows:—

February	4·8	inches.
March	2·1	,,
April	0·8	,,
May	0·2	,,
June	nil	,,
July	nil	,,
August	nil	,,
September	0·9	,,
October	1·8	,,
November	2·6	,,
December	3·5	,,
January	1·5	,,

In the following tables descriptions of the samples as well as their iodine contents at different stages of growth are given.

TABLE I.

Species: *Panicum maximum.*

Stage of Growth in Months.	Description of Sample.	Iodine Content Expressed as γ % on the Dry Weight.
1	Green, with flower heads	26·5
2	Green, seeds falling out	26
3	Mixed, mainly green, seeds falling out	14·5
4	Mixed, seeds fallen out	15
5	Mixed, mainly brown, seeds fallen out	16
6	Brown, seeds fallen out	16·6
7	Brown, seeds fallen out	21
8	Mixed, mainly brown, seeds fallen out	31
9	Mixed, new flower heads present	20
10	Mixed, mainly green, new flower heads present	22·4
11	Mixed, mainly green, with flower heads	12
12	Mixed, mainly green, seeds falling out	16

TABLE II.

Species: *Themedra triandra.*

Stage of Growth in Months.	Description of Sample.	Iodine Content Expressed as γ % on the Dry Weight.
1	Green, with flower heads	14
2	Green, with flower heads	14
3	Mixed, mainly green, seeds falling out	10·5
4	Mixed, seeds falling out	18·5
5	Mixed, mainly brown, seeds falling out	25
6	Brown, seeds fallen out	23
7	Brown, seeds fallen out	26
8	Mixed, mainly brown, seeds fallen out	51
9	Mixed, mainly brown, new flower heads present	35
10	Mixed, new flower heads present	37
11	Mixed, mainly green, with flower heads	20·5
12	Mixed, with flower heads	16

TABLE III.

Species: *Rhynchelythrum roseum.*

Stage of Growth in Months.	Description of Sample.	Iodine as γ % calculated on the Dry Weight.
1	Green, with flower heads	20
2	Green, seeds falling out	16·5
3	Mixed, mainly green, seeds falling out	9
4	Mixed, seeds falling out	16
5	Brown, seeds fallen out	19
6	Brown, seeds fallen out	25
7	Brown, seeds fallen out	32
8	Mixed, mainly brown, seeds fallen out	35
9	Mixed, mainly brown, new flower heads present	39
10	Mixed, mainly brown, new flower heads present	22
11	Mixed, mainly brown, with flower heads	33
12	Mixed, mainly brown, seeds falling out	28·5

TABLE IV.

Species: *Cymbopogon plurinodis.*

Stage of Growth in Months.	Description of Sample.	Iodine as γ % calculated on the Dry Weight.
1	Green, with flower heads	15
2	Green, seeds falling out	13
3	Mixed, mainly green, seeds falling out	12
4	Mixed, mainly brown, seeds falling out	22
5	Mixed, mainly brown, seeds falling out	30·5
6	Brown, seeds falling out	36
7	Brown, seeds falling out	22·5
8	Mixed, mainly brown, seeds fallen out	31
9	Mixed, mainly brown, seeds fallen out	16
10	Mixed, new flower heads present	24·5
11	Mixed, with flower heads	28·5
12	Mixed, mainly green, with flower heads	8

TABLE V.

Species: *Urochloa pullulans.*

Stage of Growth in Months.	Description of Sample.	Iodine as γ % calculated on the Dry Weight.
1	Green, with flower heads { Stalks	8·2
	Green, with flower heads { Leaves	11
2	Green, seeds falling out	8
3	Mixed, mainly green, seeds falling out	6
4	Brown, seeds fallen out	14
5	Brown, seeds fallen out	16
6	Brown, seeds fallen out	17
7	Brown, seeds fallen out	20·5
8	Mixed, mainly brown, seeds fallen out	24·5
9	Mixed, seeds fallen out	33
10	Mixed, new flower heads present	30
11	Mixed, with flower heads	21
12	Mixed, mainly green, seeds falling out	21·5

The monthly samples obtained from three other plots were divided into stalks, leaves, and tops. The tops represented the flower heads at different stages of development and as these usually formed only a small fraction of the total weight of the samples, iodine determinations were carried out on only a few of these flower heads.

TABLE VI.

Species: *Hyparrhenia hirta.*

Stage of Growth in Months.	Description of Sample.	Fraction.	Air-dried Weight in Gm.	Iodine in Fraction as γ % on Dry Weight.
1	Green, with flower heads............	Stalks...	90	4·2
		Leaves..	200	11
		Flowers..	17	24
2	Green, with flower heads............	Stalks...	740	3·8
		Leaves..	540	14
		Flowers..	202	29
3	Green, mainly green, seeds falling out	Stalks...	720	Nil.
		Leaves..	620	17
4	Mixed, seeds falling out.............	Stalks...	1,015	Nil.
		Leaves..	512	20·5
5	Mixed, mainly brown, seeds falling out	Stalks...	905	6·2
		Leaves..	543	26·5
6	Brown, seeds fallen out.............	Stalks...	788	6
		Leaves..	490	28
7	Brown, seeds fallen out.............	Stalks...	1,010	4
		Leaves..	590	28
8	Mixed, mainly brown, seeds fallen out	Stalks...	620	10
		Leaves..	415	28
9	Mixed, mainly brown, new flower heads present	Stalks...	420	11·5
		Leaves..	240	34·6
10	Mixed, new flower heads present....	Stalks...	745	10·6
		Leaves..	415	41
11	Mixed, with flower heads............	Stalks...	925	12
		Leaves..	875	26
		Flowers..	300	34·6
12	Mixed, seeds falling out.............	Stalks...	1,567	12
		Leaves..	1,155	28
		Flowers..	577	16

TABLE VII.

Species: *Amphilophis insculpta.*

Stage of Growth in Months.	Description of Sample.	Fraction.	Air-dried Weight in Gm.	Iodine in Fraction as γ % on Dry Weight.
1	Green, with flower heads............	Leaves..	330	10·8
2	Green, seeds falling out.............	Stalks...	290	Nil.
		Leaves..	380	Trace.
		Flowers..	90	15·2
3	Mixed, mainly green, seeds falling out	Stalks...	420	Nil.
		Leaves..	570	Trace.
4	Mixed, mainly brown, seeds fallen out	Stalks...	565	6·5
		Leaves..	572	9
5	Brown, seeds fallen out.............	Stalks...	516	6·5
		Leaves..	439	11
6	Brown, seeds fallen out.............	Stalks...	290	8·6
		Leaves..	305	25·6
7	Brown, seeds fallen out.............	Stalks...	500	19·6
		Leaves..	486	32·6
8	Mixed, mainly brown, seed fallen out	Stalks...	270	11·0
		Leaves..	185	40
9	Mixed, mainly brown, new flower heads present....................	Stalks...	—	7·0
		Leaves..	—	40
10	Mixed, new flower heads present.....	Stalks...	400	16
		Leaves..	425	36
11	Mixed, mainly green, with flower heads	Stalks...	600	22
		Leaves..	1,290	33
12	Mixed, mainly green, seeds falling out	Stalks...	1,260	6·5
		Leaves..	1,674	26

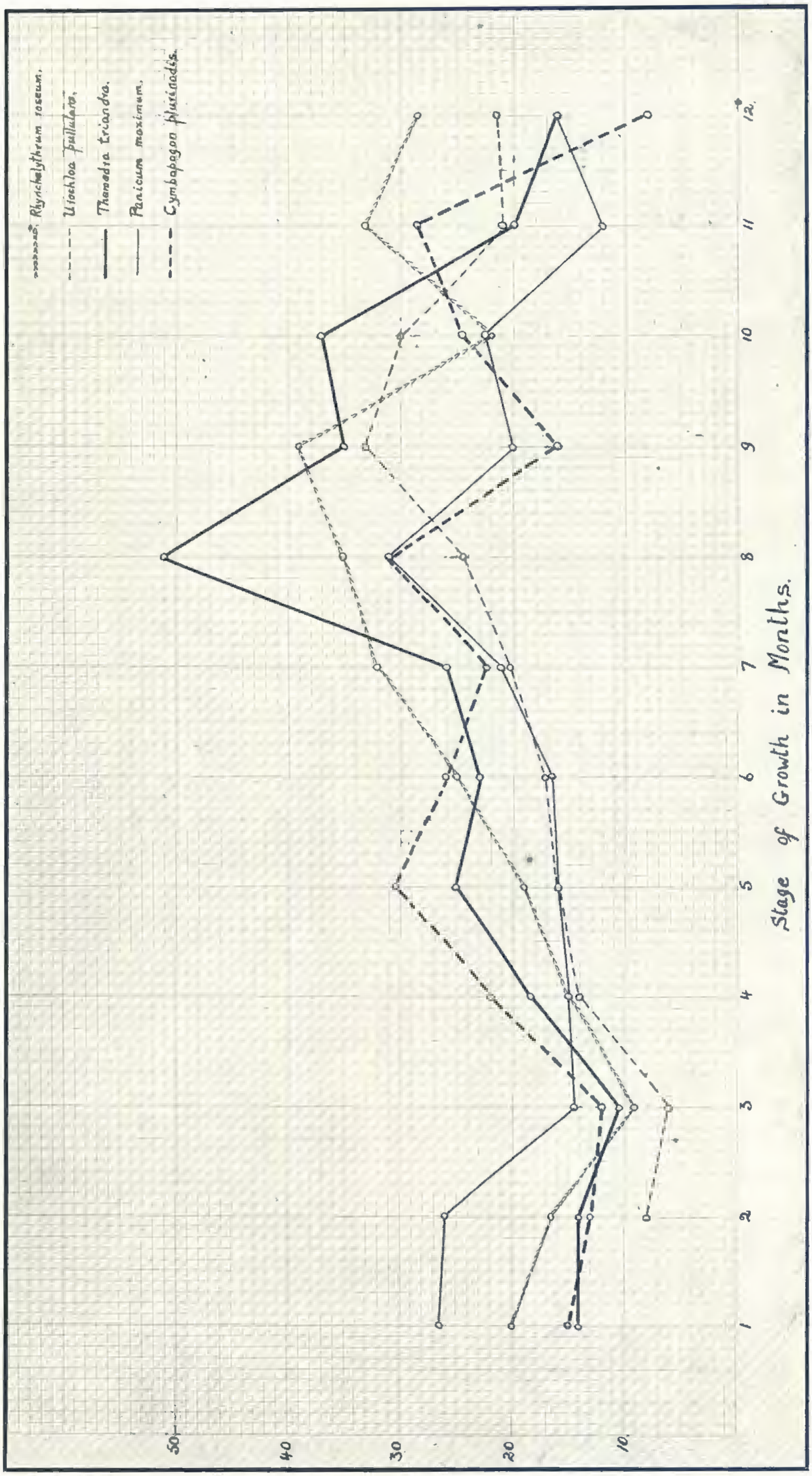
TABLE VIII.

Species: *Eragrostis superba.*

Stage of Growth in Months.	Description of Sample.	Fraction.	Air-dried Weight in Gm.	Iodine in Fraction as γ % on Dry Weight.
1	Green, with flower heads............	Leaves..	—	11·3
2	Green, with flower heads............	Total.... Sample	—	8
3	Mixed, mainly green, seeds falling out	Stalks... Leaves.. Flowers..	200 180 90	Trace. 12·6 8
4	Mixed, seeds falling out.............	Stalks... Leaves..	559 340	4 10
5	Mixed, mainly brown, seeds falling out	Stalks... Leaves..	365 320	6 9
6	Mixed, mainly brown, seeds fallen out	Stalks... Leaves..	370 230	6 18
7	Brown, seeds fallen out.............	Stalks... Leaves..	450 171	6 16
8	Mixed, mainly brown, seeds fallen out	Stalks... Leaves..	335 275	15 31
9	Mixed, mainly brown, new flower heads present	Stalks... Leaves..	319 355	15 27
10	Mixed, new flower heads present.....	Stalks... Leaves..	117 395	20 29
11	Mixed, mainly green, with flower heads	Stalks... Leaves..	957 1,056	11·5 18
12	Mixed, seeds falling out.............	Stalks... Leaves..	2,280 2,760	11·5 13·5

Graphical representations of the results (figures I, II and III) show conclusively that there is an appreciable difference in the iodine content of the same species of grass cut from the same plot at different stages of growth. It is interesting to note that at the end of the third month, i.e. when the grass is turning brown, the iodine content is at a minimum. From then onwards there is a progressive increase, the amount of which varies for the different grasses analysed. In some cases this increase is of such an order that the iodine in the samples cut from four months onwards is higher than that of the first month. This fact appears to be very important for, as stated further on, several investigators find that the iodine content of thyroids shows seasonal variations which may bear some relation to the seasonable iodine content of the pasture consumed.

FIG. I.


Rhynchelythrum roseum.
Urochloa pullulans.
Themeda triandra.
Panicum maximum.
Cymbopogon plurinodis.
Stage of Growth in Months.
1
2
3
4
5
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7
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9
10
11
12
50
40
30
20
10

FIG. II.

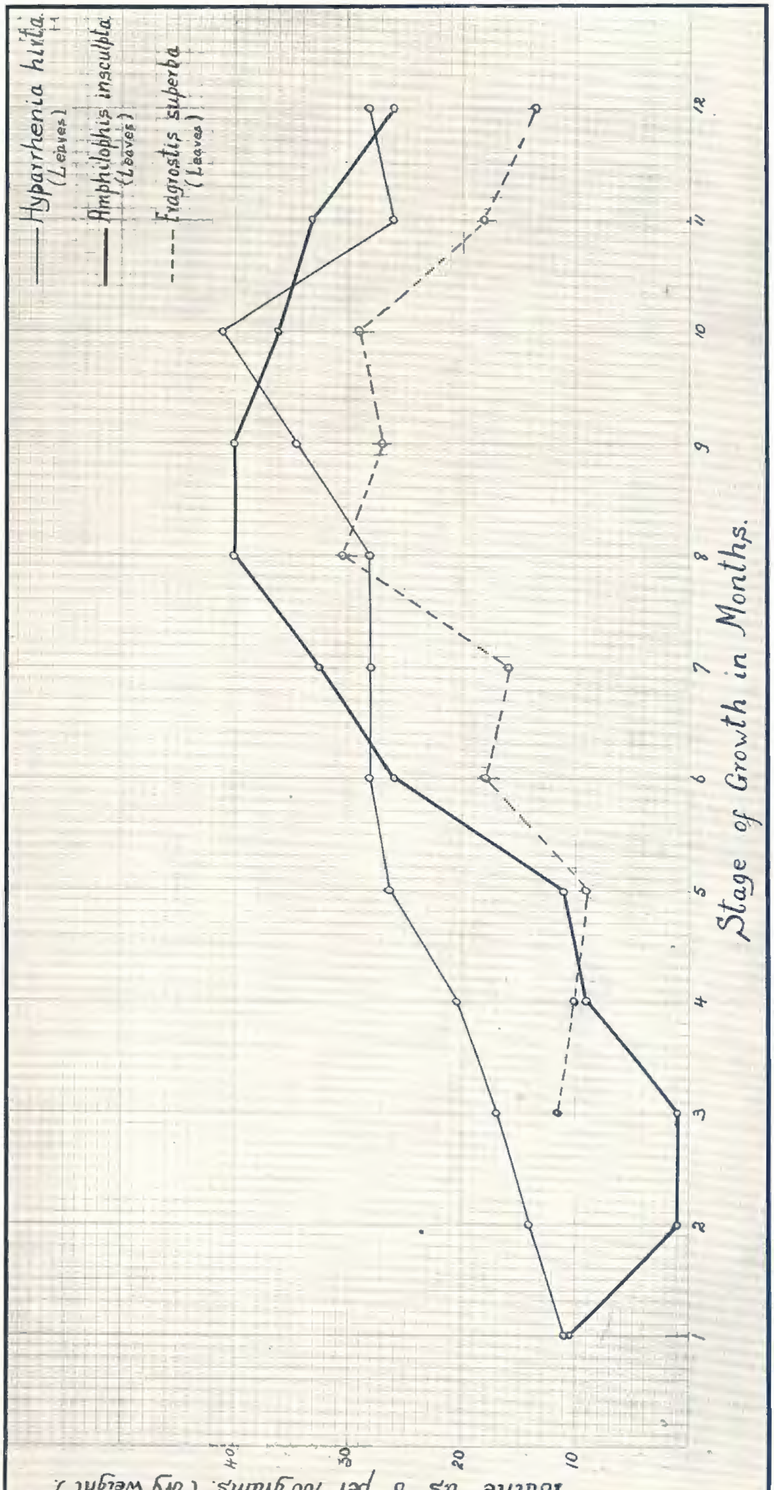
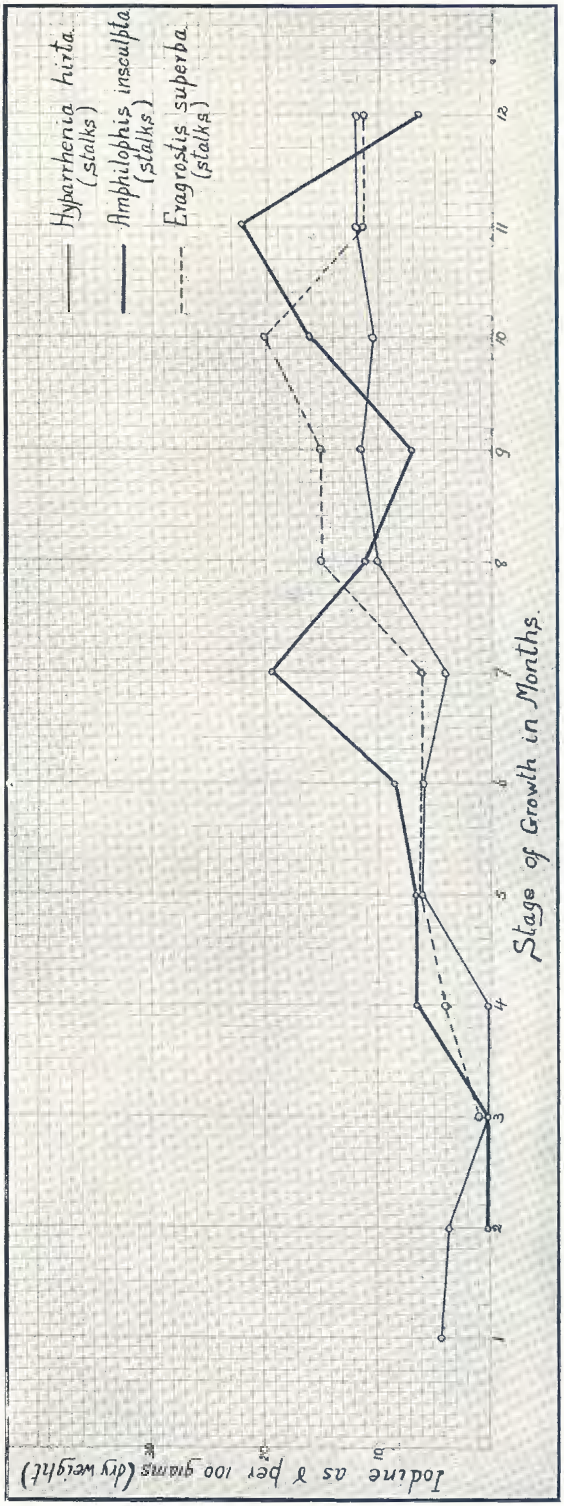

Hyparrhenia hirta (Leaves)
Amphilophis insculpta (Leaves)
Eragrostis superba (Leaves)
Stage of Growth in Months.
1 2 3 4 5 6 7 8 9 10 11 12
Iodine as γ per 100 grams (dry weight)
10 20 30 40

FIG. III.


Hyparrhenia hirta (stalks)
Amphilophis insculpta (stalks)
Eragrostis superba (stalks)
Stage of Growth in Months.
Iodine as γ per 100 grams (dry weight)

After the first rains in the spring there is a stage of active growth and the iodine content reaches a maximum about the eighth or ninth month. Beyond this stage the iodine content fluctuates but, in general, the trend is downwards so that at the end of the test, i.e. after twelve months the iodine is practically at the same level as at the end of the first month. The wide variations in the iodine content of grasses at different stages of growth are well worth recording. The iodine present in the composite sample is made up of that present in the various constituents, i.e. the stalks, leaves, and flowers or seeds or whatever remains after the seeds have fallen out. The weights as well as the iodine content of the different fractions vary from month to month and consequently the iodine present in the sample will vary accordingly. The results of the three grasses divided into fractions show that the leaves and flowers contain more iodine than the stalks. Hence the increase of iodine content during the winter would depend in some measure on the relative quantity of leaves as compared with stalks in the stand of grass.

The results are in close agreement with those of von Fellenberg on beech leaves. Hercus and Roberts (1927) in studying the seasonal variations in the iodine content of vegetable matter reported that the iodine concentration was at a maximum in autumn and winter and a similar increase in the autumn has been observed by Orr and Leitch (1929).

It has also been established by Orr and others that the iodine content of the thyroid can be raised by the administration of small doses of iodine. Furthermore, it was shown by Martin (1912, 1913) and confirmed by Seidell and Fenger (1912, 1913) that the iodine of sheep thyriods showed a regular variation with a maximum in autumn and a minimum in spring. Orr naturally assumes that this is due to the variations in the amount of iodine present in the pasture.

Dawbarn and Farr (1932) in a survey of the thyroids of about 700 sheep in Australia found results in close agreement with those of Martin and others. Further evidence for the assumption of Orr was obtained by these authors who found that after a period of severe drought the iodine concentration in the thyroid was much higher than during or after a good season.

The wide variations in the iodine content of grass collected at different stages of growth and the consequent seasonable variations in the iodine concentration in the thyroid suggest that similar variations might be encountered in the case of foodstuffs. This has been established by Hercus and Roberts (1927) who obtained figures for beef ranging from 1·2 to 80 γ per 100 gm. during a period of 9 months and the iodine content of eggs was 6·4, 16·0 and 29 γ per 100 gm. respectively for three consecutive months. In order to obtain comparative values for the iodine content of foodstuffs, samples for analysis should be collected at the same time and at the same stage of growth or development.

The Iodine Content of Milk.

During the course of the present investigation iodine analysis were carried out from time to time on milk from cows in a nutrition experiment (du Toit, Malan and Groenewald). All the cows were fed on the same diet with the addition of certain mineral salts, all with either a trace or only a minute quantity of iodine in the form of an impurity. In addition to this bovine 3,677 was given 0·1 gm. potassium iodine daily for a period of three years. The results are recorded in Table IX. The effect of the administration of iodine to bovine 3,677 is reflected in the high iodine content of its milk.

TABLE IX.

Bovine No.	Date of Sampling.	Time of Sampling.	Iodine Expressed as γ per 100 c.c. Milk.
3640......	24.11.32................	Afternoon................	4·6
3640......	25.11.32................	Morning.................	5·1
3643......	25.11.32................	Morning.................	5·9
3645......	24.11.32................	Afternoon................	4·6
3645......	25.11.32................	Morning.................	8·4
3649......	24.11.32................	Afternoon................	5·5
3649......	25.11.32................	Morning.................	1·7
3655......	24·11·32................	Afternoon................	5·5
3655......	25.11.32................	Morning.................	2·5
3642......	21.11.23................	Afternoon................	4·5
3642......	22.11.32................	Morning.................	3·1
3642......	22.11.32................	Afternoon................	2·6
3653......	21.11.32................	Afternoon................	6·7
3653......	22.11.32................	Morning.................	6·7
3653......	22·11·32................	Afternoon................	1·7
3677......	24.11.32................	Afternoon................	107·0
3677......	25·11·32................	Morning.................	51·3

Another case worth recording is that of bovine 3643. This cow was treated for metritis and the uterus was douched with Lygol's iodine on the 10th, 17th and 20th October, 1932. The iodine content of the milk sampled after the treatment is given in Table X.

TABLE X.

Iodine Content of Milk from bovine 3643.

Date of Sampling.	Time of Sampling.	Iodine as γ per 100 c.c. Milk.
24.10.32......................	Afternoon......................	121·0
25.10.32......................	Morning.......................	120·0
31.10.32......................	Morning.......................	70·4
31.10.32......................	Afternoon......................	49·0
4.11.32......................	Afternoon......................	9·6
5.11.32......................	Morning.......................	17·4
18.11.32......................	Afternoon......................	6·7
21.11.32......................	Morning.......................	4·5

The non-protein portion of milk was next considered. The proteins were precipitated by the addition of trichloracetic acid to the milk and iodine was determined in the filtrate. These results, together with the total iodine present in the milk, are given in Table XI.

TABLE XI.

Bovine No.	Date of Sampling.	Time of Sampling.	Iodine Expressed as γ per 100 c.c. Milk.	
			Iodine Present in Filtrate.	Total Iodine.
3677......	19.10.32..............	Morning...............	29·6	99
3677......	19.10.32..............	Afternoon..............	15·2	62
3677......	21.10.32..............	Morning...............	17·0	64
3677......	21.10.32..............	Afternoon..............	18·8	83
3677......	22.10.32..............	Morning...............	28·6	86
3677......	24.10.32..............	Afternoon..............	31·0	39
3677......	25.10.32..............	Morning...............	31·0	45
3677......	27.10.32..............	Afternoon..............	39·2	59
3677......	28.10.32..............	Morning...............	24·5	59
3642......	24.10.32..............	Afternoon..............	121·0	121·0
3642......	25.10.32..............	Morning...............	119·0	120·0
3640......	24.10.32..............	Afternoon..............	2·7	5·6
3649......	24.10.32..............	Afternoon..............	3·7	9·2
3655......	24.10.32..............	Afternoon..............	4·3	8·6

It appears that in some cases all the iodine in milk is present in the non-protein portion but in others only a fraction of the total iodine is present in the filtrate and some of the iodine must be associated either mechanically or chemically with the proteins which are precipitated.

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