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STUDIES ON IN VITRO ERYTHROCYTE UPTAKE OF I^{131}-LABELLED l-TRIIODOTHYRONINE AS A TEST OF THYROID FUNCTION IN DAIRY CATTLE*)

By

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Studies of thyroid function in cattle, particularly during the last decade, have mainly been concerned with the relation with milk production, milk fat content, and growth (*Premachandra et al.* 1958; *Sorensen* 1958) as well as with the differences in levels of thyroid activity between different breeds, ages, and seasons (*Mixner et al.* 1962; *Pipes et al.* 1963). Most of these studies have been based upon radioiodine methods and determinations of thyroxine secretion rate seem to have been most valuable (*Post & Mixner* 1961). The disadvantages of this method are that it is time-consuming, requires the administration of radioiodine to the animals, and that it usually demands that the animals be kept under artificial experimental conditions. These disadvantages often rule out the use of the method for diagnostic purposes.

The uptake of I^{131}-l-triiodothyronine (I^{131}-T3) by erythrocytes *in vitro* for the assessment of thyroid function was suggested by *Hamolsky et al.* in 1957. Neither the mechanism by which the erythrocytes take up I^{131}-T3 nor the way the test reflects the thyroid activity are fully understood. The test, however, has been

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shown to be a useful clinical tool in human medicine (e. g. *Robbins* 1959; *Parrow & Werner* 1962; *Yen* 1963) and its possible use in veterinary medicine has also been discussed (*Wilson et al.* 1961; *Hansard* 1962). The aim of the present investigation was further evaluation of the method as a means of estimating thyroid funçtion in dairy cattle with special reference to normal age differences.

MATERIAL AND METHODS

The erythrocyte uptake of I^{131}-T3 was measured according to *Hamolsky et al.* (1957) with the modifications suggested by *Yen* (1963). A two ml sample of heparinized venous blood was mixed well in a test tube with 0.2 ml 1^{131}-T3 containing about 0.02 μc I^{131} and between 0.001 — and 0.008 μg T3*) after dilution of the stock solution with 0.9 per cent NaCl solution. The mixture was incubated at + 37°C for one hour in a water bath. The radioactivity in the sample was determined using a well-type scintillation counter and a single channel gamma spectrometer. The blood cells were then washed five times in ten ml physiological saline, made up to the original volume with saline solution, and counted. The erythrocyte uptake (EU) of I^{131}-T3 was calculated using the formula:

$$EU = \frac{\text{CPM of the erythrocytes}}{\text{CPM of whole blood}} \times \frac{100}{\text{haematocrit}}$$

in which the haematocrit is expressed as a decimal fraction. The test was always performed in duplicate and the mean of the determination was used. The error of the method based on 100 double determinations has been calculated to be 0.41 EU. A comprehensive study on the effects of variations in the performance of the test on blood from cattle has been made and will be published elsewhere (*Thorell* 1965). The test was performed within 48 hours of taking the blood samples.

Haematocrit determinations were carried out with a "Kemila" haematocrit centrifuge (type 543) by centrifugation of heparinized whole blood for five minutes at 12,000 rpm. Duplicate tests were always made and the mean of the determinations was used. The difference between duplicate tests was less than one per cent of the mean.

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*) Supplied by the Radiochemical Centre, Amersham, England.

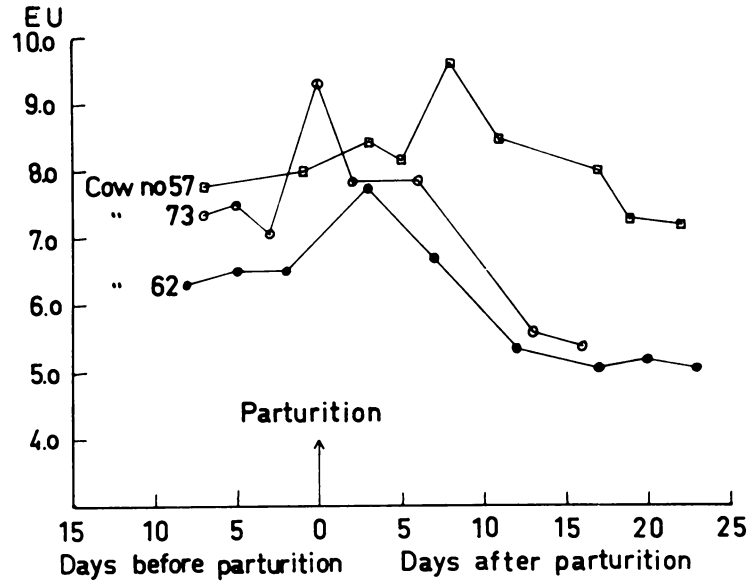

Figure 1. Changes of EU in conjunction with parturition.

The EU determinations were made on blood samples obtained during October, November, and December from 366 Swedish Red-and-white heifers and cows up to more than eight years of age and kept on farms in the counties of Södermanland, Östergötland, and Västergötland in southern Sweden. The farms were of the usual type in this part of the country with the animals tied up in stanchions. The mean outside temperature during the sampling period was +3.3°C and the mean stable temperature was +12°C.

All the animals used for the survey were clinically healthy.

RESULTS

The first point dealt with was to see whether there was an age difference in EU values among cows with one or more parturitions. Since variations in thyroid activity could be expected just prior to and after parturition, animals from which blood samples had been taken during the last month of gestation or the first month of lactation were not included in this part of the study. Table I shows that the EU values for two-year-old cows are significantly lower than the values for cows four years of age or older. The normal EU value for 141 cows more than three years

Table I. EU values for milking cows of different ages with a statistical comparison between the groups.

Age, years	2	3	4	5—6	7—8	>8
Mean, EU.	6.23	6.56	7.02	6.86	7.18	7.02
Standard deviation, EU.	0.76	1.04	0.99	0.99	0.84	0.92
Range, EU.	4.86—8.53	4.64—9.18	5.16—10.23	4.85—9.75	5.78—9.38	5.09—8.82
Number of cows	35	38	36	56	30	19
Statistical comparison between the groups. Student's t-test.						
>8 years	$0.01 > \overset{**}{P} > 0.001$	$P > \bar{0}.05$	$P > \bar{0}.05$	$P > \bar{0}.05$	$P > \bar{0}.05$	
7—8 „	$P < \overset{***}{0}.001$	$0.05 > \overset{*}{P} > 0.01$	$P > \bar{0}.05$	$P > \bar{0}.05$		
5—6 „	$0.01 > \overset{**}{P} > 0.001$	$P > \bar{0}.05$	$P > \bar{0}.05$			
4 „	$P < \overset{***}{0}.001$	$P > \bar{0}.05$				
3 „	$P > \bar{0}.05$					

old, with the exception of the period just before and after parturition, is 6.99 ± 0.95 (mean ± standard deviation of the mean).

The changes in EU values associated with parturition are illustrated in Fig. 1 for three cows. Table II shows EU values for 54 cows of different ages within one month after parturition. There is a significant difference ($0.01>P>0.001^{**}$) between the EU values for two-year-old cows within a month after parturition and the values for the same age group during the rest of the lactation period. The corresponding difference for the three-year-old cows is nearly significant ($0.05>P>0.01^{*}$) and the difference for cows more than three years old is statistically significant ($0.01>P>0.001^{**}$). On the other hand, there were no statistically significant differences between the EU values obtained during the month preceding parturition and the normal value given above.

Table II. EU values for cows within one month after calving.

Age, years	2	3	>3
Mean, EU	6.90	7.46	7.61
Standard deviation, EU	0.93	1.45	0.78
Range, EU	5.72—8.96	5.77—9.50	5.76—8.77
Number of cows	21	16	17

The higher EU values of heifer calves decline with age to reach the same level as in cows by 12 to 18 months of age (Table III). The EU values for 12 to 18-month-old heifers, however, were statistically significantly higher than those for two-year-old cows.

DISCUSSION

According to current concepts (*Christensen* 1960a,b; *Ingbar* 1960) the uptake of I^{131}-T3 by erythrocytes expresses the degree of saturation of the plasma proteins which bind thyroid hormones. This binding is reversible. There is a certain amount of free thyroid hormone in the blood plasma and the constant exchange between the free and the bound hormone is in equilibrium. It is generally considered that only the free form is biolagically active (*Ingbar & Freinkel* 1960). Erythrocytes accumulate T3 in proportion to the amount of free thyroid hormone in the plasma. Changes in the EU value, then, reflect changes in the amount of free hormone. These changes can in turn be re-

Table III. EU values for calves of different ages with a statistical comparison between the groups.

Age, months	<1	1—2	2—4	4—6	6—9	9—12	12—18
Mean, EU.	13.46	12.77	10.55	9.57	9.20	7.95	6.91
Standard deviation, EU	4.00	3.10	1.70	1.35	0.78	0.85	0.51
Range, EU.	8.97—24.13	10.08—17.02	7.85—13.92	7.97—12.66	8.53—10.30	5.90—9.68	6.17—7.91
Number of animals	11	6	17	13	5	24	19
Statistical comparison between the groups Student's t-test.							
12—18 months	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	
9—12 „	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$0.01>P>0.001^{**}$		
6— 9 „	$0.05>P>0.01^{*}$	$0.05>P>0.01^{*}$	$P>0.05^{-}$	$P>0.05^{-}$			
4— 6 „	$0.01>P>0.001^{**}$	$0.01>P>0.001^{**}$	$P>0.05^{-}$				
2— 4 „	$0.05>P>0.01^{*}$	$0.05>P>0.01^{*}$					
1— 2 „	$P>0.05^{-}$						

ferred either to changes in the protein available for binding, their amount and their binding capacity (*Parrow* 1962).

In this context erythrocytes serve as non-specific accumulators of T3. The criss-cross experiments of *Hamolsky et al.* (1959) demonstrated this; T3 uptake was the same for a particular plasma sample and erythrocytes from different donors with different levels of thyroid function. It has subsequently been shown that resin can substitute for the erythrocytes (*Mitchell et al.* 1960).

The EU values obtained here are lower than those reported by *Hansard* (1962) probably because of differences in methods. Hansard incubated his samples for four hours instead of one hour. *Wilson et al.* (1961) obtained a mean of 7.06 for 36 cows at an incubation time of two hours. Incubation for two hours gives EU values some 45 per cent higher than those obtained after incubation for one hour (*Thorell* 1965). The increase after incubation for four hours was 95 per cent.

The EU values for the cattle in this series were obtained by a method very similar to that used by *Yen* (1963) for human beings. For euthyroid subjects, the normal range was 10.0 to 18.5, somewhat higher than the values for cattle. Species differences in the *in-vitro* uptake of labelled T3 have recently been dealt with by *Slebodzinski* (1963) and can probably be ascribed to differences in the properties of the thyroxine-binding plasma proteins. Animals are known to possess thyroxine-binding globulin (TBG) but little is known of the TBG capacity of the different species. Most studies have been carried out on human serum (*Robbins & Rall* 1960). Nor is much known about thyroxine-binding prealbumin in the different species.

The calves in this survey had significantly higher EU values than the cows, i. e. their level of thyroid activity was higher. Studies on other species and with different methods have given a similar result. *Schultze & Turner* (1945), for example, demonstrated that thyroxine secretion was 25 per cent greater in chicks two to five weeks old than in six-month-old chickens. In rats, thyroxine secretion decreases with increasing age (*Monroe & Turner* 1946). Higher serum PBI values in calves than in cows have also been reported by *Long et al.* (1951), *Lewis & Ralston* (1953) and *Sorensen* (1958).

There is no readily apparent reason for the two-year-old cows after parturition having lower EU values than older cows. One

possible link is the lower milk production during the first lactation.

After parturition the EU values increased rapidly. *Sorensen* (1958) has reported higher PBI values early in pregnancy than later. This gives a clear association between the magnitude of thyroxine secretion and milk production but does not necessarily imply a causal and reciprocal relation between them. Exogenous thyroid hormone, however, increases milk production (*Moustgaard & Thorbeck* 1949; *Owen* 1948) to make it appear that thyroid function is a primary factor.

This study of thyroid function based on the *in vitro* uptake of labelled T3 by erythrocytes has demonstrated the suitability of this method for screening thyroid function of large numbers of animals in a geographical region. The method is sensitive enough to detect the changes in thyroid function which accompany physiological events. It remains to establish whether the method is suitable for the diagnosis of hypo- or hyperfunction of the thyroid in cattle. Preliminary trials, however, suggest that this is the case. The method will undoubtedly contribute to greater knowledge of thyroid function in cattle and particularly its relation to different production qualities.

REFERENCES

Christensen, L. K.: Triiodothyronine uptake by erythrocytes. Endocrinology 1960a, *66*, 138—140.

Christensen, L. K.: Triiodothyronine uptake by erythrocytes. Acta med. scand. 1960b, *166*, 141—146.

Hamolsky, M. W., Golodetz, A. & Freedberg, A. S.: The plasma protein-thyroid hormone complex in man. III. Further studies on the use of the *in vitro* red blood cell uptake of I^{131}-l-triiodothyronine as a diagnostic test of thyroid function. J. clin. Endocr. 1959, *19*, 103—116.

Hamolsky, M. W., Stein, M. & Freedberg, A. S.: The thyroid hormone-plasma protein complex in man. II. A new *in vitro* method for study of uptake of labelled hormonal components by human erythrocytes. J. clin. Endocr. 1957, *17*, 33—43.

Hansard, S. L.: Evaluation of labelled triiodothyronine *in vitro* for measuring thyroid status of animals. J. Animal Sci. 1962, *21*, 382 (Abstr.).

Ingbar, S. H.: The interaction of the thyroid hormones with the proteins of human plasma. Ann. N. Y. Acad. Sci. 1960, *86*, 440—453.

Ingbar, S. H. & Freinkel, N.: Regulation of the peripheral metabolism of the thyroid hormones. Recent Progr. Hormone Res. 1960, *16*, 353—403.

Lewis, R. C. & Ralston, N. P.: Protein-bound iodine levels in dairy cattle plasma. J. Dairy Sci. 1953, *36*, 33—38.

Long, J. F., Gilmore, L. O., Curtis, G. M. & Rife, D. C.: Protein-bound iodine as related to age, sex and breed. J. Animal Sci. 1951, *10*, 1027—1028.

Mitchell, M. L., Harden, A. B. & O'Rourke, M. E.: The *in vitro* resin sponge uptake of triiodothyronine-I^{131} from serum in thyroid disease and in pregnancy. J. clin. Endocr. 1960, *20*, 1474—1483.

Mixner, J. P., Kramer, D. H. & Szabo, K. T.: Effects of breed, stage of lactation, and season of year on thyroid secretion rate of dairy cows as determined by the chemical thyroxine turnover method. J. Dairy Sci. 1962, *45*, 999—1002.

Monroe, R. A. & Turner, C. W.: Thyroid secretion rate of albino rats during growth, pregnancy and lactation. Res. Bull. Mo. agric. Exp. Sta. 1946, No 403.

Moustgaard, J. & Thorbeck, G.: Undersøgelser over jodkaseinets virkning på mælksekretionen og stofskiftet hos køer. 240. Beretn. Forsøgslab., Copenhagen 1949.

Owen, E. C.: The effects of thyroxine on the metabolism of lactating cows. I. General results and nitrogen metabolism. Biochem. J. 1948, *43*, 235—243.

Parrow, A.: The plasma protein binding of triiodothyronine and its relation to the uptake of I^{131}-labelled triiodothyronine by human erythrocytes *in vitro*. Scand. J. Clin. Lab. Invest. 1962, *14*, 192—202.

Parrow, A. & Werner, I.: Determination of the triodothyronine uptake by erythrocytes — a practical routine test of the thyroid function. Acta med. scand. 1962, *171*, 621—631.

Pipes, G. W., Bauman, T. R., Brooks, J. R., Comfort, J. E. & Turner, C. W.: Effect of season, sex and breed on the thyroxine secretion rate of beef cattle and a comparison with dairy cattle. J. Animal Sci. 1963, *22*, 476—480.

Post, T. B. & Mixner, J. P.: Thyroxine turnover methods for determining thyroid secretion rates in dairy cattle. J. Dairy Sci. 1961, *44*, 2265—2277.

Premachandra, B. N., Pipes, G. W. & Turner, C. W.: Variation in the thyroxine secretion rate of cattle. J. Dairy Sci. 1958, *41*, 1609—1615.

Robbins, L. R.: Experience with the *in vitro* erythrocyte uptake of I^{131}-labelled l-triiodothyronine in a routine clinical laboratory. J. clin. Endocr. 1959, *19*, 1292—1301.

Robbins, J. & Rall, J. E.: Proteins associated with the thyroid hormones. Physiol. Rev. 1960, *40*, 415—489.

Schultze, A. B. & Turner, C. W.: The determination of the rate of thyroxine secretion by certain domestic animals. Res. Bull. Mo. agric. Exp. Sta. 1945, No. 392.

Ślebodziński, A.: Uptake of iodothyronines by erythrocytes *in vitro* in various species. Nature (Lond.) 1963, *199*, 75—76.

Sorensen, P. H.: Jodstoftskifte og thyreoideafunktionen hos kvaeg og svine (Iodine metabolism and thyroid function in cattle and pigs). 302. Beretn. Forsøgslab. Copenhagen 1958.

Thorell, C. B.: To be publ. 1965.

Wilson, R. B., Dickson, W. M. & Frost, F. N.: A procedure for assay of thyroid status in animals. Vet. Med. 1961, *56*, 285—289.

Yen, W.: In vitro studies of thyroid function with erythrocyte uptake of ^{131}-I-labelled triiodothyronine. Acta endocr. (Kbh.) 1963, *42*, 1—44, Suppl. 76.

SUMMARY

Uptake of I^{131}-labelled triiodothyronine by erythrocytes (EU test) as a test of thyroid function has been studied in cattle. The normal EU value for 141 Swedish Red-and-white cows was 6.99 ± 0.95 (range 4.45 — 10.23) at an incubation time of one hour. Statistically significantly higher values were obtained for newly-calved cows. The EU values for calves are significantly higher but by 12 to 18 months of age decline to the same level as for older cows.

The principle of the test is described and the results obtained compared with those for human beings. The EU test is suitable for screening thyroid function for large groups of animals and for studying associations between thyroid activity and different production qualities.

ZUSAMMENFASSUNG

Untersuchungen über die Aufnahme von J-131-gemerkten l-Trijodthyronins in Erythrocyten in vitro als Test der Thyreoideafunktion bei Rindern.

Eine Untersuchung über die Aufnahme gemerkten Trijodthyronins (genannt EU-Test, *E*rythrocyte *U*ptake) als Test der Thyreoideafunktion bei Rindern wurde vorgenommen. Basiert auf eine Untersuchung von 141 SRB-Kühen erhielt man einen EU-Normalwert von 6,99 ± 0,95 (Grenzwerte 4,45 — 10,23), wobei doch die Zeit nahe der Kalbung ausgeschlossen wurde, nachdem man statistisch signifikant höhere EU-Werte bei neugekalbten Kühen erhielt. Jungtiere zeigen höhere EU-Werte, die doch mit steigendem Alter sinken, um nach 12—18 Monaten die gleichen, wie die bei älteren Tieren zu sein.

Das Prinzip, worauf der Test baut, wird mit Leitung der Literaturangaben diskutiert. Die erhaltenen Resultate werden mit denen, besonders beim Menschen erhaltenen, verglichen. Die Verfasser schliessen daraus, dass der EU-Test für eine grobe Aussiebung des Thyreoideastatus bei grösserem Tiermaterial, sowie für Untersuchungen des Zusammenhanges zwischen Thyreoideastatus und verschiedenen Produktionseigenschaften sehr anwendbar ist.

SAMMANFATTNING

Undersökningar över erythrocyternas upptag i n v i t r o av J^{131}-märkt l-trijodthyronin som en test på thyreoideafunktionen hos nötkreatur.

En undersökning har företagits över erythrocyternas upptag av märkt trijodthyronin (EU-testen) såsom en test på thyreoideafunktionen hos nötkreatur. Baserat på en undersökning av 141 SRB-kor erhölls ett normalvärde på EU av 6,99 ± 0,95 (gränsvärden: 4,45 — 10,23) varvid dock tiden närmast efter kalvningen uteslutits, eftersom statistiskt signifikant högre EU-värden erhålles hos nykalvade kor. Ungdjur visar högre EU-värden,, vilka sjunker med stigande ålder för att vid 12—18 månaders ålder vara desamma som hos äldre kor.

Den princip varpå testen bygger diskuteras med ledning av litteraturuppgifter. De erhållna resultaten jämföres med motsvarande resultat hos speciellt människa. EU-testen är väl lämpad för screening av thyreoideastatus på ett större djurmaterial liksom vid undersökning av sambandet mellan thyreoideastatus och olika produktionsegenskaper.

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