

## STUDY ON THE THYROID FUNCTION OF THOROUGHBRED HORSES BY MEANS OF "IN VITRO" $^{125}\text{I-T}_3$ MODIFIED AND $^{125}\text{I-T}_4$ TESTS

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**SUMMARY:** Sera of 71 animals, divided in groups of males and females, in repose and after activity were studied.

The method to establish the percentage of the  $^{125}\text{I}$ -lyothyronine retention in resin (Test  $^{125}\text{I-T}_3$  or  $\text{T}_3$ ) was modified by the use of 0.2 ml of serum on the resin column, after addition of the marked hormone.

This modification served to prove that thoroughbred equines show binding of the I-lyothyronine to the serum four times reduced, indicating, therefore, that these animals have four times more ligation sites of triiodothyronin saturation in the serum, when compared with the results obtained from human beings.

The variance analysis applied to the  $\text{T}_3$  Test showed no significant results at the 95% level as regards to activity. For the 71 animals, the author has found an average of 50.30% of the  $^{125}\text{I}$ -lyothyronine in resin retention, being the confidence interval for this group between 48.75% and 51.85% to a 95% confidence coefficient.

Evaluating the results of the  $\text{T}_4$  Test by means of the variance analysis, we noticed that the male and female groups in repose differed statistically from the groups after activity to a 95% confidence coefficient.

The author has grouped the results of the  $\text{T}_4$  Test of 32 equines, 18 males and 14 females, in repose, obtaining an average of 0.61 mcg and 0.51 mcg and 0.71 mcg  $\text{T}_4/100$  ml as confidence interval to a 95% confidence coefficient.

We have listed 39 results of  $\text{T}_4$  Test, being 23 males and 61 females, after activity, obtaining an average of 2.01 mcg of thyroxin by 100 ml of serum and 1.72 mcg and 2.30  $\text{T}_4/100$  ml as confidence interval to a 95% confidence coefficient.

**UNITERMS:** Thoroughbred horse\*; Thyroid function\*; Test "in vitro".

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## INTRODUCTION

When considering particularly big sized animals, this semiology becomes highly important in view of the difficulty to explore them with human resources only.

Several author who specialized themselves in the use of radioisotopes, devoting themselves not only to basic research but to applied research as well<sup>7,20,16,5</sup>, demonstrate that this branch of radiology is a method of capital importance, since it offers a precision that is an excellent means of investigation.

Since it is kept in feeding and handling conditions similar, associated to the constitution, size and use selected by man, the thoroughbred equine appears as an ideal specimen for the application of the radioisotopic methods.

Thus, the author decided to study the thyroid function of thoroughbred equines by "in vitro" methods, using radioisotopes and modifying the technique that evaluates the percentage of the  $^{125}\text{I}$ -lyothyronine retention in Sephadex G-25 resin and, besides, to verify the relationship between this test and the one that furnishes the amount of thyroxin, measured in micrograms per 100 ml of serum. For this study, the author collected sera of male and female animals chosen at random or presenting a normal electrophoretic picture.

## LITERATURE

The binding of the hormones secreted by the thyroid glands into the blood proteins<sup>9,10,11,40</sup> and the places liable to being saturated with these hormones<sup>30,31,29,36,2,39</sup> confirm the "in vitro" methods as capable of evaluating its functional conditions<sup>38,6,4,8</sup>.

The substitution of the erythrocytes by the ion changing resin<sup>22,23,24,26,27,28,29,1,3</sup> excludes countless difficulties such as storage, anti-clotting, hemolysis, handling, washing and hematocrit, combined with the modification in the technique, where

the marked hormones were fixed in the resin, furnished patterns of precision and sensibility to the method<sup>34,35,19,5</sup>.

In the equines, the PBI used to verify the functional thyroid conditions<sup>37,14,15,18,21,32,33</sup> was the proof that furnished the best information; however, being a difficult technique, it was not accepted by part of the practitioners.

The functional thyroid proof in these animals with radioactive isotopes<sup>19,15,16</sup>, particularly those "in vitro"<sup>40,12,13,17,25,5</sup>, have contributed to increase the diagnosis of hypo and hyperthyroidism possibilities in equines.

## MATERIAL AND METHODS

The author has employed serum taken from 71 animals, 41 males (23 after activity and 18 in repose) and 30 females (16 after activity and 14 in repose), the males in the bracket age between three and seven years and the females between three and ten years; 13 males and 10 females showed a normal electrophoretic picture and the rest was considered clinically normal.

Before feeding or performing any other activity, an average of 30 ml of blood was removed from the jugular vein. After clot retraction, the centrifugation was done and, then, the serum was kept in a test tube in a freezer at  $-10^\circ\text{C}$ .

To measure the  $^{125}\text{I}$  bound to the thyroid hormones, the author has used a model 8725 gamma spectrometric system, of Nuclear Chicago, coupled to a well detector with a two inch diameter NaI (T1) crystal, the inner diameter of the well being 16 mm, gauged for the  $^{125}\text{I}$  power, with a 15 kev window.

*Method to establish the percentage of the  $^{125}\text{I}$ -lyothyronine retention in resin (Test $^{125}\text{I-T}_3$  of  $\text{T}_3$  Test)\*, modified*

\* Trilute, Ames Company. Israel.

For this work we have used the SCHOLLER<sup>34</sup> (1962) method, introducing modifications and have varied the sera amount of 0.05, 0.1 and 0.2 ml deposited directly on the resin column after addition of the  $^{125}\text{I}$ -lyothyronine, with the aim to measure the places not bound to the triiodothyronine.

*Method to establish the total thyroxin amount (Test  $^{125}\text{I-T}_4$  of  $\text{T}_4$  Test)\**

The author has used the method suggested by MURPHY & PATTEE<sup>26</sup> (1964).

We have established the standard averages and deviations as well as Pearson's variability coefficients of the values included in the tables that show the results.

Later on, we have equalled the values by allotment and before the treatment these were converted into angles (angle = arc. sen.  $\sqrt{\text{percentage}}$ ).

To verify the statistical significance with a 95% confidence coefficient, we have applied the variance and regression analysis.

The author has made Tukey's test in order to elucidate differences between the groups that showed significance in the variance analysis.

We have also tried to determine the confidence limit at the 95% level for the tables where the values obtained through the use of Tests  $\text{T}_3$  and  $\text{T}_4$  were grouped.

## R E S U L T S

The results from this study are shown in six tables.

In Table I we listed the results found in the sera of five equines chosen at random, two males and three females, which were tested by varying the amount of serum or the number of drops of marked hormone, in order to determine the percentage of  $^{125}\text{I}$ -lyothyronine retention in resin. We have also grouped the total amount of thyroxin of these sera, measured in micrograms by 100 ml. We have

thus shown that in the first test, corresponding to the percentage of the  $^{125}\text{I}$ -lyothyronine retention, in which we have used five drops of marked hormone and 0.05 ml of serum, three results above 100% and a 99.04% average were found. In the second test, with seven drops of marked hormone and 0.05 ml of serum, we have found four results above 100% and a 102.23% average. In the third one, in which we also used seven drops of  $^{125}\text{I}$ -lyothyronine and 0.1 ml of serum, the results were under 100% and the average was 88.63%. In the fourth test, again with the same amount of marked hormone and 0.2 ml of serum, the average observed was 56.72%. The average value corresponding to the total amount of thyroxin for this group was of 1.57 mcg/100 ml of serum.

In Table II we have studied 10 females, aged from three through six years, with the serum electrophoretic picture considered normal.  $\text{T}_3$  Test performed with seven and five drops of  $^{125}\text{I}$ -lyothyronine and 0.05 ml of serum, showed the averages of 97.62% and 93.72% of retention in the resin, respectively, and, performed with seven drops of the marked hormone and 0.2 ml of serum, showed an average retention of 52.38%. Now, the average obtained in this case for the  $\text{T}_4$  Test was of 1.16 mcg of thyroxin per 100 ml of serum.

In Table III we have examined 13 males, aged between three and six years, with electrophoretic picture of the serum considered normal, showing average retentions of the marked hormone obtained by means of  $\text{T}_3$  Test, with five and seven drops of  $^{125}\text{I}$ -lyothyronine and 0.05 ml, of 100.11% and 98.07%, while with seven drops and 0.2 of serum the average was 52.69%. For  $\text{T}_4$  Test the average for this group was of 0.73 mcg of thyroxin per 100 ml of serum.

Before the results obtained with these initial studies, we have always used in the other analysis shown in the tables presented next seven drops of  $^{125}\text{I}$ -lyothyronine and 0.2 ml of equine serum.

We have used the variance analysis to the results obtained with the help of  $\text{T}_3$

\* Tetralute, Ames Company. Israel.

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Test for males and females, in repose as well as after activity, verifying statistical non-significance for 95% in both sexes and with respect to activity.

We have also evaluated the results of  $\text{T}_4$  Test and noticed that the groups of males and females in repose differed statistically from the groups after activity to a 95% confidence coefficient.

In Table IV we have listed the results of  $\text{T}_3$  Test of the 71 animals under study, finding an average retention of the  $^{125}\text{I}$ -lyothyronine in resin of 50.30%, with a confidence interval for this group between 48.75% and 51.85% to a 95% confidence coefficient.

In Table V we have grouped the results of  $\text{T}_4$  Test, made with 32 equines, 18 males and 14 females, in repose, obtaining an average of 0.61 mcg and a confidence in-

terval of 0.51 mcg and 0.71 mcg  $\text{T}_4/100$  ml to a 95% confidence coefficient.

In Table VI we have listed 39 results of  $\text{T}_4$  Test, made with 23 males and 16 females after activity, obtaining an average of 2.01 mcg of thyroxin per 100 ml of serum and a confidence interval of 1.72 mcg and 2.30 mcg  $\text{T}_4/100$  ml to a 95% confidence coefficient.

## DISCUSSION

The special conditions imposed by man to the thoroughbred equine on the one hand offer special material for the basic or applied research and on the other determine restrictions capable of limiting certain experiences.

The observation of these animals behavior during clinical examination in the

TABLE I - Averages, standard deviations and Pearson's variability constant. Results obtained by establishment of  $\text{T}_3$  Test, altering the amount of marked hormone and of the serum of the thoroughbred equine as well as those referring to  $\text{T}_4$  Test. São Paulo, 1973.

ANIMAL		SEX	T <sub>3</sub> TEST (% RETENTION)				T <sub>4</sub> TEST mcg T <sub>4</sub> /100 ml
NO	NAME'S INITIAL		5 DROPS	7 DROPS			
			SERUM	S E R U M			
			0,05 ml	0,05 ml	0,1 ml	0,2 ml	
1	B.V.	M	101,40	100,80	82,11	52,23	1,19
2	J.	F	101,00	103,09	95,16	56,95	2,25
3	E.	F	89,74	96,27	76,56	48,75	0,46
4	F	F	99,03	105,19	95,70	58,43	2,25
5	T.	M	104,07	105,01	93,61	67,25	1,68
x̄ (Mean)			99,04	102,23	88,63	56,72	1,57
s̄ (Standard deviation)			5,50	3,87	8,74	7,03	0,76
Pearson's variability coefficient			5,6	3,8	9,9	12,4	48,6

TABLE II - Averages, standard deviations and Pearson's variability coefficients of the results obtained to establish  $\text{T}_3$  Test, altering the amount of the marked hormone and of the serum of the female thoroughbred equine, with electrophoretic data considered as normal as well as the results referring to  $\text{T}_4$  Test. Sao Paulo, 1973

ANIMAL		AGE (YEARS)	ELECTROPHORETIC PATTERNS								T <sub>3</sub> TEST (% RETENTION)			T <sub>4</sub> TEST mcg T <sub>4</sub> /100 ml
NO	NAME'S INITIAL		ALBUMIN (%)	GLOBULINS (%)					7 DROPS	SERUM				
				α 1	α 2	β 1	β 2	γ		0,05 ml	0,05 ml	0,2 ml		
1	F.	3	45,0	3,0	9,0	13,0	-	22,0	89,37	82,37	44,90	0,58		
2	C.	3	52,0	3,0	10,0	14,0	-	17,0	93,28	96,96	59,46	0,55		
3	U.B.	4	51,0	4,0	8,0	14,0	-	17,0	94,71	88,19	44,43	0,60		
4	E.	4	54,0	5,0	10,0	14,0	-	17,0	96,27	89,74	48,75	0,46		
5	F.	4	50,4	3,4	9,3	12,6	6,7	17,7	105,19	99,03	58,43	2,25		
6	U.B.	4	45,2	4,5	7,9	14,7	9,0	18,1	101,77	96,47	58,32	2,37		
7	M.	5	50,6	4,6	10,4	12,6	8,1	13,8	97,59	98,62	49,56	0,84		
8	Q.	5	47,9	4,1	9,9	12,4	8,3	17,4	99,29	96,19	54,49	1,15		
9	J.	5	49,0	4,1	8,2	13,3	8,2	17,3	103,09	101,00	56,95	2,25		
10	K.	6	51,7	3,7	8,6	12,3	7,4	16,0	92,68	88,68	48,49	0,57		
x̄ (Mean)		4,3	49,7	3,9	9,1	13,3	7,9	17,3	97,62	93,72	52,38	1,16		
s (Standard deviation)		0,95	2,92	0,67	0,96	0,79	0,80	2,03	5,40	6,0	5,8	0,80		
Pearson's variability coefficient		22,1	5,9	17,0	10,5	6,0	10,0	11,7	5,5	6,5	11,1	69,1		

TABLE III - Averages, standard deviations and Pearson's variability coefficient of the results obtained to establish  $\text{T}_3$  Test, altering the amount of the marked hormone and of the serum of the male thoroughbred equine, with electrophoretic data considered as normal as well as the results referring to  $\text{T}_4$  Test. Sao Paulo, 1973

ANIMAL		AGE (YEARS)	ELECTROPHORETIC PATTERNS								T <sub>3</sub> TEST (% RETENTION)			T <sub>4</sub> TEST mcg T <sub>4</sub> /100 ml
NO	NAME'S INITIAL		ALBUMIN (%)	GLOBULINS (%)					γ	SERUM				
				α <sub>1</sub>	α <sub>2</sub>	β <sub>1</sub>	β <sub>2</sub>	7 DROPS		5 DROPS	7 DROPS			
												0,05 ml	0,05 ml	
1	H.G.	3	56,0	6,0	10,0	12,0	-	16,0	98,86	80,62	49,99	0,39		
2	N.	3	54,0	4,0	11,0	14,0	-	16,0	97,19	97,82	43,21	0,46		
3	D.	3	53,0	4,5	8,0	10,2	9,1	15,5	101,02	100,87	57,70	1,15		
4	A.	3	50,8	4,3	9,7	11,9	6,5	16,2	105,16	99,92	42,17	0,84		
5	T.	4	49,7	3,2	9,7	9,7	8,6	18,4	105,81	104,07	67,25	1,68		
6	K.	4	53,0	3,9	7,8	14,3	7,8	13,0	102,12	98,23	50,59	0,40		
7	P.	4	45,5	4,5	11,1	10,0	10,0	18,9	80,96	99,54	58,18	0,38		
8	S.	4	49,0	4,0	11,0	9,0	5,0	18,0	107,30	95,84	51,04	1,30		
9	C.	4	40,0	4,0	11,0	14,0	-	19,0	93,93	96,89	41,78	0,20		
10	B.V.	5	53,0	5,0	11,0	11,0	-	20,0	100,80	101,40	52,23	1,19		
11	Q.P.	5	47,6	3,9	9,7	11,7	8,7	18,5	95,73	99,53	52,70	0,46		
12	C.F.	6	54,0	4,3	8,1	12,6	5,7	18,0	107,30	95,84	62,13	0,84		
13	U.	6	47,0	3,0	13,0	14,0	-	24,0	105,26	104,37	56,06	0,23		
x̄ (Mean)		4,1	50,2	4,2	10,0	11,9	7,7	17,8	100,11	98,07	52,69	0,73		
s (Standard deviation)		1,07	4,39	0,75	1,49	1,83	1,76	2,65	7,23	5,90	7,64	0,47		
Pearson's variability coefficient		25,7	8,7	17,9	14,8	15,4	23,0	14,9	7,2	6,0	14,5	64,3		

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TABLE IV

Averages, standard deviations, Pearson's variability coefficient and confidence interval to 95% of results obtained with the help of T Test in sera of male and female thoroughbred equines in repose and after activity. Hippodrome Cidade Jardim São Paulo, 1973

ANIMAL		Sex	Age (years)	T Test (% de retention) serum 0,2 ml
N.º	Name's initial			
1	Q. P.	M	5	63,30
2	L. T.	F	5	63,15
3	N.	F	4	62,58
4	C.	M	6	62,13
5	G.	M	4	61,62
6	T.	M	3	60,11
7	C.	F	3	59,46
8	D.	M	5	58,88
9	F.	F	4	58,43
10	U. B.	M	4	58,32
11	P.	F	4	58,18
12	D.	M	3	57,70
13	L.	M	5	57,12
14	I.	F	4	57,06
15	J.	M	5	56,95
16	V.	F	6	56,06
17	D.	F	3	55,66
18	H.	F	3	55,44
19	E.	F	3	55,19
20	Q.	M	5	54,49
21	A.	F	7	54,40
22	P. V.	F	4	54,18
23	Q.	F	8	52,87
24	A.	M	3	52,81
25	G.	M	5	52,75
26	Q. P.	M	3	52,70
27	B. V.	M	5	52,23
28	E. B.	M	5	51,77
29	L.	M	4	51,15
30	G.	M	3	50,85
31	K.	F	4	50,59
32	H.	M	5	50,51
33	A.	M	3	50,13
34	H. G.	M	3	49,99
35	P. B.	M	3	49,99
36	I.	F	5	49,69
37	M.	M	5	49,56
38	C. O.	M	5	49,20
39	R.	M	5	48,97

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ANIMAL		Sex	Age (years)	T Test (% de retention) serum 0,2 ml
N.º	Name's initial			
40	B.	F	5	48,94
41	E.	F	4	48,75
42	Z.	M	5	48,69
43	X.	F	5	48,69
44	K.	F	6	48,49
45	R.	F	5	48,47
46	G.	F	10	48,37
47	M.	M	4	48,26
48	E.	F	6	48,22
49	G.	M	3	48,10
50	F.	F	6	47,95
51	N.	F	3	47,16
52	B.	M	4	46,28
53	N.	M	4	45,71
54	H.N.	M	3	45,54
55	O.	F	5	45,27
56	F.	M	3	44,90
57	U.B.	F	4	44,43
58	H.	F	4	44,33
59	M.B.	F	6	43,31
60	N.	M	3	43,21
61	X.	M	5	42,38
62	A.	M	3	42,17
63	Z.	M	6	42,02
64	C.	M	4	41,78
65	H.B.	M	4	40,57
66	U.	M	5	40,06
67	L.	F	9	39,95
68	E.	F	3	39,67
69	G.	M	3	38,70
70	T.	M	4	38,27
71	Y.T.	M	3	36,84
x (Mean)			4,5	50,30
s (Standard deviation)			1,42	6,62
Pearson's variability coefficient			32,0	13,2
Confidence interval (95%)			—	48,75 — 51,85



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TABLE V

Averages, standard deviations, Pearson's variability coefficient and confidence interval to 95% of results obtained with the help of T Test in sera of male and female thoroughbred equines in repose and after activity. Hippodrome Cidade Jardim São Paulo, 1973

ANIMAL		Sex	Age (years)	T Test mcg T /100 ml
N.º	Name's initial			
1	D.	M	3	1,15
2	A.	M	7	0,99
3	G.	F	10	0,91
4	L.	F	5	0,91
5	H.	F	5	0,89
6	C.	M	6	0,84
7	M.	F	5	0,84
8	N.	F	4	0,84
9	N.	F	3	0,84
10	R.	M	5	0,84
11	A.	F	3	0,76
12	P.B.	M	5	0,76
13	L.T.	F	5	0,69
14	L.	F	9	0,67
15	F.	M	6	0,61
16	X.	M	5	0,61
17	U.B.	F	4	0,60
18	K.	F	6	0,57
19	N.	F	4	0,48
20	N.	M	3	0,46
21	Q.P.	M	3	0,46
22	C.O.	M	5	0,45
23	Q.	F	8	0,45
24	B.	M	5	0,41
25	I.	M	4	0,40
26	K.	M	4	0,40
27	H.G.	M	3	0,39
28	P.	M	4	0,38
29	C.	F	3	0,35
30	H.N.	M	3	0,23
31	V.	M	6	0,23
32	C.	M	4	0,20
x (Mean)			4,8	0,61
s (Standard deviation)			1,76	0,25
Pearson's variability coefficient			36,7	40,2
Confidence interval (95%)			—	0,51 — 0,71

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TABLE VI

Averages, standard deviations, Pearson's variability coefficient and confidence interval to 95% of results obtained with the help of T Test in sera of male and female thoroughbred equines after activity. Hippodrome Cidade Jardim São Paulo, 1973.

ANIMAL		Sex	Age (years)	T Test mcg T /100 ml
N.º	Name's initial			
1	T.	M	4	3,67
2	E.	M	6	3,36
3	E.	F	3	3,36
4	G.	M	5	3,36
5	A.	M	3	3,21
6	E.	F	3	3,06
7	G.	M	4	3,06
8	H.B.	M	4	3,06
9	Y.T.	M	3	2,91
10	M.B.	M	6	2,75
11	G.	F	3	2,67
12	O.	M	5	2,44
13	X.	M	5	2,44
14	Z.	F	5	2,44
15	U.B.	F	4	2,37
16	F.	F	4	2,25
17	J.	F	5	2,25
18	B.	F	4	2,21
19	D.	F	3	2,21
20	H.	F	3	2,14
21	P.V.	F	4	2,06
22	U.	M	5	1,98
23	Z.	M	6	1,98
24	H.	F	4	1,91
25	R.	F	5	1,75
26	G.	M	3	1,68
27	I.	M	5	1,68
28	L.	M	4	1,37
29	B.V.	M	5	1,19
30	Q.	F	5	1,15
31	D.	M	5	1,07
32	N.	M	4	1,07
33	G.	M	3	0,91
34	A.	M	3	0,84
35	Q.P.	M	5	0,60
36	F.	F	3	0,58
37	T.	M	3	0,42
38	E.B.	M	5	0,41
39	E.	F	4	0,40
x (Mean)			4,3	2,01
s (Standard deviation)			0,97	0,94
Pearson's variability coefficient			23,20	46,95
Confidence interval (95%)			—	1,72 — 2,30

course of preparations preliminary to the race and during the race show that they are undoubtedly highly influenced by the thyroid glands.

The functional thyroid test in which radioisotopes are administered to the patient gives results of high precision; however, the immobilization, the adaptation of devices and the authorization to administer the radioactive compound are strong restrictive reasons for its use in animals, particularly in the thoroughbred. Therefore, with the advent of the "in vitro" experiences performed with radioisotopes these difficulties were eliminated, with the further advantage that they offer the same possibilities of precision along with great practicability.

In order to establish the influence of the thyroid glands on equines, we have used the chemical PBI and the thyroxin secretion rate (TSR), methods which were not accepted in the laboratory routine as can be observed by the scarce bibliography on the subject.

It is believed that hipo and hyperthyroidism are rare in equines due to the difficulty presented by the auxiliary means of diagnosis. The red blood cells test, however, showed that its use in equines and other species, as well as the TBI that offers the advantage of not being altered by the administration or application of iodine, a largely difused therapy among groomers and trainers, associated to clinical information made it possible to differentiate hipo and hyperthyroidean equines.

The retention levels, between 43% and 59%, for Tests  $\text{T}_3$  found by the author are extremely low when compared to those found in this study, where the technique commended for human beings, that is, seven drops of  $^{125}\text{I-lyothyronine}$  and 0.05 ml

of serum, showed and average of 99.98% of marked hormone retention in resin (see Tables I, II and III).

By altering the amount of serum from 0.05-ml to 0.2 ml we offer a greater number of binding places for the  $^{125}\text{I-lyothyronine}$  and, therefore, we are in the position to state that the thoroughbred shows such bindings four times reduced, suggesting, thus, that these animals have four times more places saturated with triiodothyronine in the serum when comparing the results with those obtained from human beings.

The results obtained in this study and presented in Table IV show that for Test  $\text{T}_3$  there were no statistically significant differences between sexes and activities, with an average retention of 50.30% ( $S = 6.62$ ) of  $^{125}\text{I-lyothyronine}$  in resin. Estimating the confidence interval to a 95% confidence coefficient, we found the limits of 48.75 and 51.85%.

The values found by different authors for the Test  $\text{T}_4$ , indicating 0.76 to 2.60 mcg of  $\text{T}_4/100$  ml, showed the non-existence of statistically significant differences as to sex, the revers occurring as to activity. We have reached the same conclusion in this study, finding an average of 0.61 mcg ( $S = 0.25$  - Table V) for males and females in repose, and an average of 2.01 mcg ( $S = 0.94$ ) of thyroxin per 100 ml of serum (Table VI) for the group after activity.

#### A G R A D E C I M E N T O

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DE MARTIN, B. W. — Study on the thyroid function of throughbred horses by means of "in vitro"  $^{125}\text{I-T}_3$  modified and  $^{125}\text{I-T}_4$  tests. *Rev. Fac. Med. vet. Zootec. Univ. S. Paulo*, 12:107-20, 1975.

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RFMV-A/10

DE MARTIN, B. W. — *Estudo da função tireóidea em eqüinos Puro Sangue Inglês, machos e fêmeas, mediante utilização dos testes "in vitro"  $^{125}\text{I-T}_3$  modificado e  $^{125}\text{I-T}_4$* . *Rev. Fac. Med. vet. Zootec. Univ. S. Paulo*, 12:107-120, 1975.

**RESUMO:** Foram estudados soros de 71 animais, divididos em lotes de machos e fêmeas, em descanso e em trabalho.

O método de determinação da porcentagem de retenção da  $^{125}\text{I}$ -liotironina em resina (Teste  $^{125}\text{I-T}_3$  ou  $\text{T}_3$ ) modificado pela utilização de 0,2 ml de soro sobre a coluna de resina, após a colocação do hormônio marcado.

Esta modificação veio demonstrar que os eqüinos Puro Sangue Inglês apresentam ligação de  $^{125}\text{I}$ -liotironina ao soro diminuído de quatro vezes, sugerindo, portanto, que estes animais têm quatro vezes mais lugares saturados por triiodotironina no soro, se compararmos os resultados com os obtidos para o homem.

A análise de variância aplicada aos resultados do Teste  $\text{T}_3$  revelou não serem significantes ao nível de 95% para sexo e quanto à atividade. Para os 71 animais, encontramos como média, 50,30% de retenção da  $^{125}\text{I}$ -liotironina em resina, estando o intervalo de confiança para este grupo, entre 48,75% e 51,85% para 95%.

Avaliando os resultados dos Testes  $\text{T}_4$ , através da análise de variância, verificamos que os lotes de machos e fêmeas em descanso diferiam estatisticamente dos lotes em trabalho, para 95% de confiança.

Agrupamos os resultados do Teste  $\text{T}_4$ , de 32 eqüinos, 18 machos e 14 fêmeas, em descanso, obtendo-se como média 0,61 mcg e como intervalo de confiança para 95%, 0,51 mcg e 0,71 mcg  $\text{T}_4/100$  ml.

Relacionamos 39 resultados do Teste  $\text{T}_4$ , sendo 23 machos e 16 fêmeas em trabalho, obtendo como média de tiroxina por 100 ml de soro, 2,01 mcg e como intervalo de confiança para 95%, 1,72 mcg e 2,30 mcg  $\text{T}_4/100$  ml.

**UNITERMOS:** Eqüinos P.S.I.\*; Função tireóidea\*; Teste "in vitro\*."

## BIBLIOGRAPHY

1. BAUER, R. et al. — The use of Sephadex G-25 in the estimation of total serum thyroxine. *Clin. Chem.*, 16:526, 1970.
2. BEIERWALTES, W. H. et al. — Plasma levels of thyroid hormone In WAGNER Jr., H. N. — *Principles of nuclear medicine*. Philadelphia, W. B. Saunders Co., 1968, p. 320-2.
3. BRAVERMAN, I. E. et al. — Evaluation of a simplified technique for the specific measurement of serum thyroxine concentration. *J. clin. Endocr.*, 32:497-502, 1971.
4. CLARK, F. & HORN, D. B. — Assessment of thyroid function by the combined use of the serum protein bound iodine and resin uptake of  $^{131}\text{I}$  — triiodothyronine. *J. clin. Endocr.*, 25:39-45, 1965.
5. DE MARTIN, B. W. — Estudo da função tireóidea em eqüinos Puro Sangue Inglês, através do  $^{131}\text{I}$  — T. B. I. *Rev. Fac. Med. vet. Zootec. Univ. S. Paulo*, 10:35-44, 1973.
6. FOCKLER, F. H. et al. — Comparative thyroid function studies with the resin T-3 test. *J. nucl. Med.*, 4:239-43, 1963.

DE MARTIN, B. W. — Study on the thyroid function of thoroughbred horses by means of "in vitro"  $^{125}\text{I-T}_3$  modified and  $^{125}\text{I-T}_4$  tests. *Rev. Fac. Med. vet. Zootec. Univ. S. Paulo*, 12:107-20, 1975.

7. GILLETTE, E. L. — Nuclear medicine. In CARLSON, W. D. — *Veterinary radiology*. Philadelphia, Lea & Febiger, 1971, p. 637-49.
8. GORMAN, C. A. & MCCONAHEY, W. M. — Diagnosis of hyperthyroidism and hypothyroidism by laboratory methods. *Med. Clin. N. Amer.*, 54:1037-47, 1970.
9. HAMOLSKY, M. W. — The plasma protein thyroid hormone complex in thyrotoxicoses vs euthyroidism in man. *J. clin. Invest.*, 34:914, 1955.
10. HAMOLSKY, M. W. et al. — 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.*, 17:33-44, 1957.
11. HAMOLSKY, M. W. et al. — The plasma protein — thyroid hormone complex in man. III: Further studies on the use of the in vitro red blood cell uptake of  $^{131}\text{I}$ -l-triiodothyronine as a diagnostic test of thyroid function. *J. clin. Endocr.*, 19:103, 1959.
12. HIGHTOWER, D. & MILLER, L. F. — Thyroid function test in veterinary medicine. I. A review. *S. West Vet.*, 22:200-5, 1969.
13. HIGHTOWER, D. et al. — Thyroid function tests in veterinary medicine. *S. West Vet.*, 23:15-21, 1969.
14. IRVINE, C. H. G. — Protein bound iodine in the horse. *Amer. J. vet. Res.*, 28: 1687-92, 1967.
15. IRVINE, C. H. G. — Thyroid function in the horse. In: Ann. Conv. Amer. Assoc. Equine Practitioners, 12th., Los Angeles, 1966. *Proceedings*, p. 197-206.
16. IRVINE, C. H. G. — Thyroxine secretion rate in the horse in various physiological states. *J. Endocr.*, 39: 313-20, 1967.
17. KALLFELZ, F. A. & LOWE, J. E. — Some normal values of thyroid function in horses. *J. Amer. vet. med. Ass.*, 156:1888-91, 1970.
18. KANEKO, J. J. — Thyroid function. In: KANEKO, J. J. & CORNELIUS, C. E. — *Clinical biochemistry of domestic animals*. New York, Academic Press, 1970, v. 1, p. 293-311.
19. KANEKO, J. J. — Thyroid function studies in the horse. *J. Amer. vet. med. Ass.*, 146:262, 1965.
20. LUICK, J. R. — Use of radioactive isotopes in veterinary clinical biochemistry. In: KANEKO, J. J. & CORNELIUS, C. E. — *Clinical biochemistry of domestic animals*. New York, Academic Press, 1971, v. 2, p. 271-312.
21. McDONALD, L. E. — *Veterinary endocrinology and reproduction*. Philadelphia, Lea & Febiger, 1970, p. 38-52.
22. MITCHELL, M. L. — Resin uptake of radiothyroxine in sera from non-pregnant women. *J. clin. Endocr.*, 18:1437-40, 1958.
23. MITCHELL, M. L. et al. — The in vitro resin sponge uptake of triiodothyronine- $\text{I}^{131}$  from serum in thyroid disease and in pregnancy. *J. clin. Endocr.*, 20:1474-83, 1960.
24. MITCHELL, M. L. et al. — Resin uptake of radiothyroxine from serum in thyroid disease and in pregnancy. *J. clin. Endocr.*, 21:1448-54, 1961.
25. MOTLEY, J. S. — Use of radioactive triiodothyronine in the study of thyroid function in normal horses. *Vet. Med. Small Anim. Clin.*, 67:1225-8, 1972.
26. MURPHY, B. P. & PATTEE, C. J. — Determination of thyroxine utilizing the property of protein-binding. *J. clin. Endocr.*, 24:187-96, 1964.
27. MURPHY, B. P. & JACHAN, C. — The determination of thyroxine by competitive protein-binding analysis employing an anionexchange resin and radiothyroxine. *J. Lab. clin. Med.*, 66:161-7, 1965.
28. MURPHY, B. P. et al. — Clinical evaluation of a new method for the determination of serum thyroxine. *J. clin. Endocr.*, 26:247-56, 1966.
29. MYANT, N. B. & OSORIO, C. — Competition for triiodothyronine between serum proteins and red cells. *J. Physiol. London*, 157:529-41, 1961.
30. ROBBINS, J. & RALL, J. E. — Zone electrophoresis in filter paper of serum  $\text{I}^{131}$  after radioiodide administration. *Proc. Soc. exp. Biol. New York*, 81: 530-6, 1952.

DE MARTIN, B. W. — Study on the thyroid function of throughbred horses by means of "in vitro"  $^{125}\text{I-T}_3$  modified and  $^{125}\text{I-T}_4$  tests. *Rev. Fac. Med. vet. Zootec. Univ. S. Paulo*, 12:107-20, 1975.

31. ROBBINS, J. & RALL, J. E. — The interaction of thyroid hormones and protein in biological fluids. *Recent. prog. Hormone Res.*, 13:161-202, 1957.
32. ROONEY, J. R. — The musculokeletal system. In: CATCOTT, E. J. & SMITHCORS, J. F. — *Equine medicine & surgery*. 2.<sup>a</sup> ed. Wheaton, American Veterinary Publications, 1972, p. 489-501.
33. SALUTINI, E. et al. — L'esplorazione funzionale della tiroide nel cavallo stallone. *Ann. Fac. Med. vet. Pisa*, 23: 30-7, 1971.
34. SCHOLER, J. F. — A simple measure of thyro-binding by plasma: A test of thyroid function. *J. nucl. Med.*, 3:41-6, 1962.
35. STERLING, K. & TABACHNICK, M. — Resin uptake of  $\text{I}^{131}$  triiodothyronine as a test of thyroid function. *J. clin. Endocr.*, 21:456-64, 1961.
36. TATA, J. R. — Distribution and metabolism of thyroid hormones. In: PITT-RIVERS, R. & TROTTER, W. R. *The thyroid gland*. London, Butterworths, 1964, v. 1, p. 163-86.
37. TRUM, R. F. & WASSERMAN, R. H. — Studies on the depression of radioiodine uptake by the thyroid after phenothiazine administration. II. Effect of phenothiazine on the horse thyroid. *Amer. J. vet. Res.*, 17:271-5, 1956.
38. URELES, A. L. et al. — The erythrocyte uptake of  $\text{I}^{131}$  labeled triiodothyronine as a measure of thyroid function. *J. Lab. clin. Med.*, 54:178-85, 1959.
39. VISSCHER, R. D. — T  $\text{I}^{131}$  binding capacity of serum proteins. A test for evaluation of complications of pregnancy. *Amer. J. Obstetr. Gynec.*, 86: 829-34, 1963.
40. WILSON, R. B. et al. — A procedure for assay of thyroid status in animals. *Vet. Med.*, 56:285-9, 1961.

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