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Responses Upon Multiple Administration of L-Thyroxine in Hens*

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Summary. Studies on the influence of repeated injections of L-thyroxine on enzyme activity and total protein level in the blood plasma of White Rock and Sussex hens have shown that:

1. The total protein level in both races decreased significantly.
2. Activity of aldolase increased in White Rock hens while in Sussex hens it increased considerably only after the last injection.
3. Activity of alanine aminotransferase did not change in White Rock hens and increased in the blood plasma of Sussex hens.
4. In both races, the activity of aspartate aminotransferase increased initially and changed after L-thyroxine injection.
5. Activity of alkaline phosphatase increased in White Rock hens, while in Sussex hens it decreased.
6. Statistically significant differences between activities of examined enzymes in both races after L-thyroxine administration were found.

Key words: Hens – L-thyroxine effect – Blood plasma enzymes – Blood plasma protein

Introduction

It is quite clear that the administration of L-thyroxine produces effects on overall metabolic activity through action on metabolism of the organism (Dratman 1974; May and Packer 1976; Bray and Goodman 1965; Freedland 1965; Freedland et al. 1968; Murad and Freedland 1967). Many authors, however, have concluded that thyroid hormones are much more closely concerned with growth and differentiation than with metabolism and that these hormones are more important at the time of development than in adult life (Heinonen 1975; Weichsel 1977; Clark and Weichsel 1977; Snedecor and Camyre 1966; Hendrich and Turner 1966).

It seemed, therefore, very interesting to examine the response of adult organisms to hyperthyroidism by observing changes in the activity of certain enzymes. Another question was whether the various races of animals response in a similar way to the same change of physiological homeostasis.

To resolve this problem the activity of the following enzymes in the blood plasma of adult hens of White Rock and Sussex breeds during multiple treatments with L-thyroxine were determined: aldolase (fructose-1, 6-diphosphate D-glyceraldehyde-3-phosphate lyase, E.C. 4.1.2.13), alanine aminotransferase (GPT), (L-alanine: 2-oxoglutarate aminotransferase, E.C.2.6.1.2), aspartate aminotransferase (GOT), (L-aspartate: 2-oxoglutarate aminotransferase, E.C.2.6.1.1) and alkaline phosphatase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1). In addition, the total protein level in the blood plasma was evaluated.

Material and Methods

The experiments were carried out on 12 White Rock hens, 12 months old, of mean body weight 3.1 kg and on 15 Sussex hens of the same age and having a mean body weight of 2.1 kg. The birds were maintained under the same conditions and came from the farm of the Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, Poland.

The hens were divided into two groups. Birds of the first group (7 White Rock and 8 Sussex hens) received L-thyroxine at dosages of 600 μg per kg of body weigt. L-thyroxine (T_4) was injected intramuscularly every 48 hours for 22 days. A corresponding control group (5 White Rock and 7 Sussex hens) was injected at the same time with saline.

During the experiment blood samples were taken from hens in

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Table 1. The occurence of statistically significant differences between the examined breeds of hens during multiple injections of L-thyroxine

Compared groups	Protein	Aldolase	GOT	GPT	Alkaline phosphatase
WR 0 – WR 1b	xx	xx	xx		
WR 0 – WR 2b	xx	xx	xx		x
WR 0 – WR 3b	xx	xx			x
WR 0 – WR 4b	xx				
WR 0 – WR 5b	xx				xx
WR 1a – WR 1b	xx	xx	xx		
WR 2a – WR 2b	xx	xx	xx		xx
WR 3a – WR 3b	xx	xx	x		x
WR 4a – WR 4b	xx		x		x
WR 5a – WR 5b	xx				xx
WR 1b – SX 1b		xx	xx		xx
WR 2b – SX 2b		xx	xx		xx
WR 3b – SX 3b			x		xx
WR 4b – SX 4b			xx		
WR 5b – SX 5b					xx
SX 0 – SX 1b			x		
SX o – SX 2b	xx				x
SX o – SX 3b	xx		x		xx
SX 0 – SX 4b			xx		
SX 0 – SX 5b			xx		xx
SX 1a – SX 1b	xx		x	x	xx
SX 2a – SX 2b	xx				xx
SX 3a – SX 3b	xx				xx
SX 4a – SX 4b	xx				x
SX 5a – SX 5b	xx		x		
WR 0 – SX 0					xx

x = $P < 0.05$, xx- $P < 0.01$, WR-White Rock, SX-Sussex
a = control, b- L-thyroxine treatment
0 = prior to the injection, 1 – after 1st injection, 2 – after 4th injection,
3 = after 8th injection, 4 – after 11th injection, 5 – after 12th injection

both groups. This occurred prior to the injection, 48 hours after the first injection and 24 hours after the 4th, 8th, 11th and 12th injections of the T_4 or saline.

L-thyroxine was dissolved in a small amount of 0.1 N NaOH and diluted with saline to a concentration of 600 μg per ml. The pH of the T_4 solution as well as that of the control saline was adjusted to 9.

The activity of aldolase, GPT, GOT, alkaline phosphatase and the total protein level in the blood plasma were determined. The activity of aldolase was determined according to the method of Bruns (1954), the activity of alanine and aspartate aminotransferase by Reitman's and Frankel's method (1957), the activity of alkaline phosphatase according to the method of Hansen (1966) and the total protein level by the biuret method (Krawczyński and Osiński 1967). The activity measurements were conducted with spectrocolorimeter Specol (Carl Zeiss, Jena, DDR).

The activity of aldolase was expressed as μmoles of substrate utilized per 1 minute per 1 liter of the blood plasma, the activity of aminotransferase in Reitman-Frankel's units, of alkaline phosphatase in King-Armstrong's units and the total protein level in mg per ml. of the blood plasma. Moreover, the activity of the enzymes and the protein content were expressed as a percentage of the activity or concentration prior to the injection.

48 hours after the last injection of the T_4 or saline both groups of birds received an injection of ^{131}J at a dose of 1 μCi into the wing vein. After 6 hours, the hens were decapitated, their thyroid

glands were isolated and incorporation of iodine into the glands was detected by scintillation counting. The results obtained were presented as c.p.m. per 1 mg of fresh thyroid glands.

The results were subjected to a statistical analysis with the application of Duncan's test (Ruszczyc 1970).

Results

The occurrence of significant differences in the level of protein and activity of enzymes between examined breeds are shown in Table 1.

The influence of T_4 on the total protein level in the blood plasma of White Rock and Sussex hens is shown in Table 2.

In White Rock hens, the total protein level had already decreased considerably 48 hours after the first injection of T_4, reaching 35.0 mg/ml. This represents 79.1% of the level observed before the injection ($P < 0.01$), (Tables 1, 2). Subsequent injections caused a further decrease in the total protein level, which reached its minimum 24 hours after the 8th injection (30.5 mg per ml, 68.7% of the initial value). After the 11th and 12th injections the total protein level reached 73.2 and 80% of the value observed to the injection, respectively.

In the Sussex hens the total protein level in the blood plasma fell from the initial value of 44.3 mg/ml to a minimum of 34.4 mg per ml (77.4% of the initial value) ($P < 0.01$) after the 8th injection (Tables 1, 2). The protein concentration showed the tendency to increase slightly after further injections (87.4 and 88% of pre-injection levels, respectively) although it never exceeded the initial values prior to the first injection, as in the White Rock hens. In Sussex hens, contrary to the White Rock hens, statistically significant changes in the total protein level, when compared with the value obtained prior to the hormone administration, occurred after the 4th and 8th injections ($P < 0.01$) (Table 1).

The statistical analysis did not demonstrate significant differences in the protein levels between the T_4-treated groups of both breeds but within the White Rock breed there occurred significant differences between the control (injected with saline) and experimental group (Table 1). Similar relations were observed in Sussex hens (Table 1).

The changes in the activity of aldolase in the blood plasma of both hormone-treated hens breeds are presented in Table 3.

In White Rock hens the activity of aldolase in the blood plasma increased rapidly, reaching 216.4% of the initial activity after the first injection and its maximum after the 4th injection (239.3%). The lowest increase of this enzyme activity occurred after the 11th injection of hormone. In this breed, significant differences between the control and experimental groups after the 1st, 4th, 8th injections of L-thyroxine were observed (Tables1, 3).

Changes in the activity of aldolase in the blood plasma of Sussex hens were slight and not significant in relation to the activity observed prior to the hormone administra-

Table 2. The changes of the total protein level in the blood plasma of White Rock and Sussex hens during multiple injections of L-thyroxine

Breed	Total protein level (mg per ml)					
	Prior to the injection	48 hours after 1st injection	24 hours after 4th injection	24 hours after 8th injection	24 hours after 11th injection	24 hours after 12th injection
White Rock Control	44.4 ± 4.4	47.0 ± 4.3	47.4 ± 3.6	41.3 ± 4.8	43.7 ± 2.2	44.1 ± 5.0
T_4		35.1 ± 5.9	34.8 ± 7.2	30.5 ± 4.9	32.5 ± 3.8	35.5 ± 3.1
% of level prior to the injection	100	79.1	78.4	68.7	73.2	80.0
Sussex Control	44.3 ± 7.2	53.3 ± 3.9	53.7 ± 5.7	45.6 ± 3.3	49.3 ± 1.1	47.4 ± 9.6
T_4		38.1 ± 6.3	34.5 ± 5.6	34.3 ± 6.5	38.7 ± 6.1	39.0 ± 5.6
% of level prior to the injection	100	86.0	77.9	77.4	87.4	88.0

Values are mean ± SE
For statistically significant differences see Table 1

tion and in comparison with the control birds (Tables 1, 3). Statistically significant differences between T_4-treated groups of both breeds occurred after the first and 4th injections (Tables 1, 3).

The changes in the activity of alanine aminotransferase (GPT) in the blood plasma of White Rock and Sussex hens after the administration of L-thyroxine are shown in Table 4.

As can be observed, the injection of T_4 did not cause changes in the activity of GPT in the blood plasma of

Table 3. The changes of the activity of aldolase in the blood plasma of White Rock and Sussex hens during multiple injections of L-thyroxine

Breed	Activity of aldolase (μmoles of substrate utilized per minute per 1 l of the blood plasma)					
	Prior to the injection	48 hours after 1st injection	24 hours after 4th injection	24 hours after 8th injection	24 hours after 11th injection	24 hours after 12th injection
White Rock Control	6.1 ± 0.54	7.8 ± 1.2	6.8 ± 1.8	7.6 ± 1.1	6.1 ± 1.1	11.4 ± 2.9
T_4		13.2 ± 3.5	14.6 ± 4.9	11.7 ± 3.1	9.0 ± 2.5	9.3 ± 2.3
% of level prior to the injection	100	216.4	239.3	191.8	147.5	152.5
Sussex Control	8.2 ± 1.9	7.2 ± 2.7	6.6 ± 2.3	7.5 ± 0.9	8.5 ± 4.7	14.0 ± 3.8
T_4		8.2 ± 2.0	9.3 ± 1.9	9.5 ± 0.8	7.3 ± 1.4	11.1 ± 3.4
% of level prior to the injection	100	100	113.4	115.9	89.0	135.4

Values are mean ± SE
For statistically significant differences see Table 1

Table 4. The changes of the activity of GPT in the blood plasma of White Rock and Sussex hens during multiple injections of L-thyroxine

Breed	Activity of GPT (in Reitman-Frankel's units)					
	Prior to the injection	48 hours after 1st injection	24 hours after 4th injection	24 hours after 8th injection	24 hours after 11th injection	24 hours after 12th injection
White Rock Control	7.5 ± 4.4	6.4 ± 3.5	7.4 ± 3.4	6.8 ± 3.7	7.8 ± 5.1	5.6 ± 2.5
T_4		9.0 ± 3.7	7.7 ± 3.9	7.3 ± 2.8	7.7 ± 3.4	7.6 ± 2.9
% of level prior to the injection	100	120.0	102.7	97.3	102.7	101.3
Sussex Control	4.3 ± 3.3	5.1 ± 3.5	5.1 ± 3.6	4.9 ± 3.4	6.9 ± 3.4	4.9 ± 3.9
T_4		6.1 ± 3.4	6.1 ± 3.4	6.4 ± 3.4	5.8 ± 4.6	5.4 ± 2.2
% of level prior to the injection	100	141.9	141.9	148.8	134.9	125.6

Values are mean ± SE
For statistically significant differences see Table 1

White Rock hens. Only after the first injection did an increase of the activity (120% of the activity prior to the injection) occur, however that proved not to be statistically significant (Table 1).

Quite different responses from the T_4 administration were observed in Sussex hens (Table 4). After the first injection the activity of GPT increased to a value of 141,9% of that seen prior to the hormone administration. Similar increases were retained after the 4th and 8th injections (141.9 and 148.8% of the initial activity, respectively),

Table 5. The changes of the activity of GOT in the blood plasma of White Rock and Sussex hens during multiple injections of L-thyroxine

Breed	Activity of GOT (in Reitman-Frankel's units)					
	Prior to the injection	48 hours after 1st injection	24 hours after 4th injection	24 hours after 8th injection	24 hours after 11 th injection	24 hours after 12th injection
White Rock Control	231.2 ± 22.6	251.6 ± 15.7	228.2 ± 25.0	207.6 ± 11.7	202.6 ± 13.7	205.4 ± 19.0
T_4		295.9 ± 15.9	287.7 ± 22.2	233.9 ± 19.6	232.3 ± 38.1	214.8 ± 23.3
% of level prior to the injection	100	127.9	124.4	101.2	100.5	92.9
Sussex Control	231.3 ± 24.5	227.4 ± 15.1	206.0 ± 16.6	202.0 ± 35.9	203.4 ± 31.7	225.0 ± 19.3
T_4		260.3 ± 10.7	214.0 ± 19.5	204.0 ± 36.1	196.5 ± 30.2	192.3 ± 26.3
% of level prior to the injection	100	112.5	92.5	88.2	85.0	83.1

Values are mean ± SE
For statistically significant differences see Table 1

Table 6. The changes of the activity of alkaline phosphatase in the blood plasma of White Rock and Sussex hens during multiple injections of L-thyroxine

Breed	Activity of alkaline phosphatase (in King-Armstrong's units)					
	Prior to the injection	48 hours after 1st injection	24 hours after 4th injection	24 hours after 8th injection	24 hours after 11th injection	24 hours after 12th injection
White Rock Control	11.6 ± 5.0	18.0 ± 9.7	7.4 ± 2.8	9.4 ± 3.5	6.9 ± 2.5	9.0 ± 4.5
T_4		19.6 ± 10.8	26.1 ± 12.7	23.2 ± 9.8	19.2 ± 7.8	51.8 ± 10.1
% of level prior to the injection	100	168.9	225.0	200.0	165.5	446.6
Sussex Control	34.4 ± 13.4	15.4 ± 7.4	16.2 ± 10.8	23.5 ± 7.7	11.7 ± 3.6	15.8 ± 7.0
T_4		44.5 ± 14.3	48.8 ± 19.2	58.8 ± 19.2	23.4 ± 9.2	11.5 ± 3.4
% of level prior to the injection	100	129.4	141.9	170.6	68.0	33.4

Values are mean ± SE
For statistically significant differences see Table 1

however it decreased after the 11th and 12th injections (Table 4).

Despite different responses upon injections of L-thyroxine, changes in the activity of GPT proved to be not significant statistically when compared with the relative activity of this enzyme in the T_4-treated White Rock hens as well as with the GPT activity in the Sussex birds, prior to the injection (Tables 1, 4).

The changes in the activity of aspartate aminotransferase (GOT) in the blood plasma of hens of both breeds induced by L-thyroxine injections are presented in Table 5.

The activity of GOT in White hens increased after the first and 4th injections to 127.9 and 124.4% of the initial value, respectively ($P < 0.01$) (Tables 1, 5). Further injections returned it to initial values (101.2%, 100.5%, 92.9%, respectively).

A statistically significant increase in the activity of GOT occurred in Sussex hens only after the first injection of hormone (112.5% of the activity prior to the T_4 injection). Subsequent injections of L-thyroxine initiated a decrease of the activity of GOT below the value prior to the injection. Statistically significant differences occurred after the 8th, 11th and 12th injections ($P < 0.01$) (Tables 1, 5).

The statistical analysis demonstrated the occurrence of significant differences in the activity of GOT between both breeds after the 1st, 4th, 8th and 11th injections of hormone (Table 1).

The influence of repeated L-thyroxine injections on the activity of alkaline phosphatase in the blood plasma of White Rock and Sussex hens is presented in Table 6.

Both breeds of hens demonstrated a statistically significant difference in the initial activity of alkaline phosphatase (Tables 1, 6). In White Rock hens an increase of the activity of the enzyme to 168.9% was observed after the first injection, but a similar difference also occurred after the injection of saline (Table 6). Further injections of L-thyroxine caused a considerable increase in the activity of phosphatase. After the last injection the activity of the enzyme was at its highest and reached 446.6% of the level observed prior to the injection. Only after the 11th injection with the increase in the activity of phosphatase going to 165% of that prior to the first injection was it statistically not significant (Tables 1, 6).

The activity of alkaline phosphatase in the blood plasma of Sussex hens demonstrated different changes after the T_4 injections. After the 1st, 4th and 8th injections a gradual, but smaller increase than seen in the previous breed was observed in the activity of this enzyme. It reached a maximum activity after the 8th injection (170.6%) and then fell below the value seen prior to the T_4 administration. After the 11th and 12th injections the activity of alkaline phosphatase amounted to 68 and 33% of the initial value, respectively. The changes in the activity of alkaline phosphatase between the examined breeds of hens proved to be statistically significant (Table 1).

Table 7. The incorporation of ^{131}J into the thyroid glands of White Rock and Sussex hens

Breed	n	c.p.m. per 1 mg of fresh thyroid gland
White Rock		
Control	5	$2365.2 \pm 900.3^{b,d}$
T_4	5	$69.8 \pm 35.0^{b,a}$
% of control		3.0
Sussex		
Control	6	$4533.5 \pm 1117.1^{c,d}$
T_4	8	$236.0 \pm 133.7^{c,a}$
% of control		5.2

Statstically significant differences were determined by Student's test t
Values having superscript letter in common are significantly different:
a,d $P < 0.05$, b,c $P < 0.01$
Values are mean ± SE

The treatment with L-thyroxine caused an inhibition of the physiological functions of the thyroid glands in hens of both breeds. The incorporation of ^{131}J into the thyroid proteins was inhibited to a considerable degree (Table 7).

In White Rock hens treated with L-thyroxine, the incorporation of iodine into the thyroid glands occurred only in 3% in comparison with the control birds. Similarly, in Sussex hens it only was 5.2% of the control value (Table 7). Significant differences in the incorporation of iodine were observed between both control and experimental groups of birds.

Discussion

It was shown that hens produce about 1.2 μg of thyroxine per 100 g of body weight per day (Tanabe et al. 1967). In our experiments hens were treated every 48 hours with a hormone at a dose of 600 μg per kg of body weight (300 μg per kg per day), thus, a treatment about 25 times larger than physiological doses. The application of this concentration was justified by the results of the earlier experiments by Konecka et al. (1978) which indicated that a repeated treatment with such a dose of L-thyroxine causes considerable changes in the activity of certain enzymes in the blood plasma of adult Leghorn hens.

The repeated injections of L-thyroxine caused a decrease of the total protein level in the blood plasma of the investigated birds, although no significant differences were

observed between the examined groups (Tables 1, 2).

There is no report in the available literature about the influence of thyroxine on the protein in the blood plasma of birds. Results of investigations conducted on other animal species are known, although the results of those experiments differ from those presented here. Thapliyal et al. (1975) demonstrated that during a prolonged treatment of Spotted Munia (*Lonchura punctulate*) with various doses of L-thyroxine, the level of the blood plasma protein increased under the influence of low hormone concentration and decreased under higher hormone dose. However, as in present investigations, Konecka et al. (1978) stated a decrease of the protein level in the blood plasma of Leghorn hens under influence of high doses of L-thyroxine.

The changes in the activity of aldolase in the examined breeds of hens are interesting. The activity of enzyme in the blood plasma of White Rock hens increased to a greater degree, while in the Sussex hens the observed changes were small and not statistically significant (Tables 1, 3). The differences in the activity of this enzyme between the breeds occurring after the 1st and 4th injections of hormone were statistically significant (Table 1). The results presented can be compared with those obtained in rats as there is no data in the literature referring to this problem in birds. Contrary to our investigation, Freedland (1965) did not observe any changes in the activity of aldolase in the rat liver. Similar relations were observed by Saxena et al. (1961) and Freedland et al. (1968) in the rat liver and by Hamburg and Flexner (1957) in the rat brain.

Many experiments, conducted both in vivo and in vitro, indicated that L-thyroxine considerably affects by the amino acids metabolism. Campbell et al. (1964) demonstrated that this hormone stimulated the incorporation of L-leucine into proteins. This was confirmed by Grandhi et al. (1975, 1975a) who observed an influence of the thyroid hormones on the incorporation of methionine, alanine and lysine into the proteins of the blood plasma, liver, kidneys and egg albumines in White Leghorn chicks. The synthesis of alanine and glutamine in the skeletal muscle (Karl et al. 1976) and methionine and other amino acids into the brain of rats (Heinonen 1975) was also stimulated by L-thyroxine. Many authors reported an increase of the activity of the enzymes of amino acids metabolism, i.e., glutamate dehydrogenase (Freedland 1965; Freedland et al. 1968) and serine dehydrase (Freedland et al. 1968). It was demonstrated in our investigations that L-thyroxine had no effect on the activity of GPT in White Rock hens. The results obtained were in agreement with the earlier data of Freedland (1965) and Freedland et al. (1968), referring to the activity of this enzyme in the liver of rat. However, the increase of the activity of GPT in the blood plasma of the Sussex hens was not confirmed in the literature (Table 4). It seems, that the differences between the changes of the activity of GPT in the blood plasma of T_4-treated White Rock and Sussex hens, although not confirmed statistically, may proceed from the genetically conditioned different utilitarian traits of both those breeds.

The increase of GOT activity under the influence of L-thyroxine in the rat liver was observed by Freedland (1965) and Freedland et al. (1968). Snedecor et al. (1972), examining the effect of various doses of L-thyroxine on the activity of aspartate aminotransferase in the livers of 6-week old White Leghorn chickens, stated an increase in the activity of this enzyme. Similarly, in our experiments an increase in the activity of GOT in the blood plasma of White Rock was demonstrated, but only after four injections of this hormone. After further injections, the enzyme activity returned to a level similar to the initial one. In Sussex hens, the increase of the activity of this enzyme occurred only after the first injection of L-thyroxine (Table 5). Those breeds of hens differed considerably in the reaction of this enzyme to repeated hormone injections. In the White Rock birds the activity of GOT returned to the level seen prior to the injection while in Sussex hens, after an initial increase, the activity of this enzyme dropped below the initial value (Table 5). The differences in the activity of GOT between the examined breeds of hens proved to be statistically significant (Table 1).

The activity of alkaline phosphatase increased in the blood plasma of White Rock hens, reaching a maximum after the 12th injection (Table 6). In the Sussex breed an increase of the activity was initially observed, and after the last injections, a significant decrease took place (Tables 1, 6). A statistical analysis demonstrated significant differences in the changes of the activity of alkaline phosphatase under the influence of hormone injections (Table 1). Our results are in agreement with the data of Barker (1951), who observed an increase of the activity of alkaline phosphatase in the blood serum of rat, while in the kidney this enzyme was not sensitive to L-thyroxine.

The presented results indicate that both breeds of hens, which differ in utilitarian traits, are characterised by a different reaction of the organism to homeostatic disturbances by artificial hyperthyroidism.

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