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# Stress by Immobilization, with Food and Water Deprivation, Causes Changes in Plasma Concentration of Triiodothyronine, Thyroxine and Corticosterone in Poultry

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*Abstract*

Measurements were sought for assessment of stress during investigations of welfare in intensively housed poultry. Immobilization was used as a stressor in experiments involving 64 cockerels of various ages. Two parameters were found to be related to acute stress: plasma triiodothyronine ($T_3$) concentration, which fell markedly, and plasma corticosterone concentration, which rose considerably, in all birds during severe stress. Concurrently, plasma thyroxine ($T_4$) concentration also fell in most, but not all, birds.

Thyrotrophin releasing hormone (TRH) injected intravenously after immobilization caused increases in $T_3$ and $T_4$ levels, suggesting that the fall in $T_3$ and $T_4$ during acute stress was not caused by exhaustion of hormone production by the thyroid gland but rather by inhibition of release of TRH at the hypothalamic level.

## Introduction

In evaluating welfare of poultry kept under intensive conditions, we have attempted to evaluate stress by measurements of various behavioural and blood parameters. The results of our behavioural observations are reported elsewhere (Wodzicka-Tomaszewska *et al.* 1980).

Circulating L-3,5,3-triiodothyronine ($T_3$) values were measured because our unpublished data suggested the involvement of this hormone in acute stress. Thyroxine ($T_4$) was measured because data of Klandorf *et al.* (1978*b*) and Bobek *et al.* (1980) for quail suggested that $T_4$ fell during the stress of handling. In addition, both hormones are under the control of thyrotrophin releasing hormone (TRH) which stimulates thyroid stimulating hormone (TSH), causing a release of both $T_3$ and $T_4$ into the circulation.

It is well known in mammals that elevated glucocorticoid levels are associated with thyroidal inhibition, through a depression of pituitary TSH secretion (cf. Brown-Grant 1966; Reichlin 1966). However, these data are lacking for birds.

A rise in plasma corticosterone is regarded as a measure of severe stress in poultry by many authors. For example, Nir *et al.* (1975) found that immersion of 10-day-old chicks in cold water (at 17°C) caused a three- to fourfold increase in plasma corticosterone levels 5 and 10 min after the chicks were immersed. Similar increases were noted in chicks of this age after 1 and 3 days of total starvation. However, Etches (1976) found that the less severe stress of handling and venipuncture did not affect plasma corticosterone concentrations in hens.

Immobilization by tying animals' legs together so that they cannot stand has been found to be stressful in many species, including the domestic fowl (cf. Beuving and Vonder 1978). Confinement to a restricted space with subsequent limitation of movement occurs when birds are moved from deep litter pens to multi-bird laying cages. Intermittent food and water deprivation form part of the rearing regime in modern poultry husbandry.

This paper presents results of the effects of stress by immobilization and subsequent food and water deprivation on some endocrine responses of young cockerels.

## Materials and Methods

White Leghorn × Black Australorp laying-strain cockerels 6–14 weeks old were used. The cockerels had been reared previously in the same type of cages, under the same photoperiod (14 h light : 10 h darkness, with the light period beginning at 0600 h) and with food and water freely available. The 'control' or 'free' birds were kept in these cages with free access to food and water and were only caught when sampled. The immobilized cockerels had their legs tied together and were kept on the floor beside the cages. Each experiment was begun at 0900 h. The pilot experiments, as well as experiments 2 and 3, were carried out in autumn; experiment 1 took place in late July after 2 months of cool weather. The cockerels were held on a table in the same room as the cages when blood samples were obtained, 2 ml being taken at each sampling except for experiment 2 when 5 ml was taken.

In the pilot experiments and experiment 1 blood was taken by venipuncture from the main wing vein and put into heparinized tubes for determination of hormone concentrations in plasma. In experiments 2 and 3, the main wing vein was cannulated (Abbocath, 18 G) to reduce disturbance due to repeated venipuncture. Cannulae were readily inserted within 2 min of catching a bird. The presence of the cannulae did not appear to disturb the birds, nor interfere with their movements. On each occasion that a bird was bled, the sample was obtained within 1 min of catching the bird. Cannulae were kept patent with heparinized saline (20 i.u. per millilitre). There were no differences in plasma $T_3$ and $T_4$ in samples taken immediately after catching a bird and after the insertion of the cannula.

Total $T_3$ was measured with a quantitative radioimmunoassay kit (TRI-TAB-RIA, manufactured by Nuclear-Medical Laboratories Inc.), and total $T_4$ was measured using a TETRA-TAB-RIA kit from the same manufacturer. The minimal detectable dose for $T_3$ and $T_4$ was 0·2 ng/ml. The within- and between-assay coefficients of variation were 6·9 and 10·2% for $T_3$ and 5·5 and 7·6% for $T_4$, respectively. The dose rate of 100 $\mu$g TRH (Beckman) per kilogram liveweight intravenously (i.v.) was used, because preliminary experiments showed that this gave an increase in plasma concentration of about 100% in $T_3$ and 33% in $T_4$. Corticosterone, expressed as total corticoids, was measured by the protein-binding technique of Murphy (1967). The minimal detectable level was 1·0 ng/ml. The within- and between-assay coefficients of variation were 10·1 and 13·1% respectively.

## Results

In the first pilot experiment, blood was taken from 10 6-week-old cockerels immediately after catching each bird and after immobilization for 3 h. Plasma $T_3$ concentrations fell significantly ($P < 0 \cdot 001$) in all birds from $2 \cdot 71 \pm 0 \cdot 38$ (s.d.) to $0 \cdot 64 \pm 0 \cdot 28$ ng/ml.

In a second preliminary experiment the time sequence of the changes in plasma $T_3$ levels was investigated in five 9-week-old and five 10-week-old cockerels, immobilized for 6 and 5 h respectively. At 30 min and at 1 h after immobilization $T_3$ concentrations rose in 7 out of 10 birds. They then fell in all birds, the fall being evident in some birds at 1 h and in others at 2 h. At 2, 3, 5 and 6 h after immobilization $T_3$ concentrations were significantly different ($P < 0 \cdot 01$) from that in the blood sample taken initially, falling from 3·1 to 0·4 ng/ml in the 9-week-old birds and from 1·8 to 0·7 ng/ml in the 10-week-old birds at 3 h after immobilization (Fig. 1).

*Experiment 1*

The results of the pilot experiment showed a marked decrease in $T_3$ levels following immobilization. Hence an experiment was designed to compare plasma levels of $T_3$ and corticosterone in immobilized cockerels with those of similar birds subjected only to handling and blood sampling.

Twenty 14-week-old cockerels were sampled at intervals, 10 of them being tied for 5 h (immobilized group) and the other 10 constituting the 'free' group. Plasma $T_3$ and corticosterone concentrations were measured in all birds before immobilization and 0·5, 1, 3 and 5 h later.

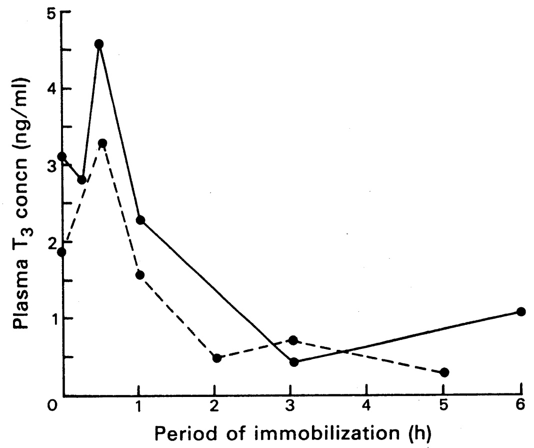


**Fig. 1.** Effects of immobilization and serial sampling on mean plasma $T_3$ concentrations in five 9-week-old (——) and five 10-week-old (---) cockerels.

The results are presented in Fig. 2. $T_3$ concentrations rose briefly in 5 out of 10 birds at 0·5 or 1 h after immobilization. Then, $T_3$ values fell markedly in all birds. The 'free' birds showed a less steep decline in $T_3$. The $T_3$ values of the two groups did not differ at the beginning of the experiment, but differed significantly ($P < 0{\cdot}001$) at 3 and 5 h, the means being 4·5 and 4·2 ng/ml for the 'free' and immobilized groups respectively at the beginning, falling to 3·8 and 2·4 ng/ml at 3 h and 3·6 and 1·9 ng/ml at 5 h. The regression equation on time ($x$) for the $T_3$ values ($y$) of the immobilized birds was:

$$y = -4{\cdot}584x + 4{\cdot}0310,$$

and that of the 'free' birds was:

$$y = -2{\cdot}066x + 4{\cdot}5246,$$

the slopes of the two lines being significantly different ($P < 0{\cdot}05$).

Conversely, corticosterone values rose in the immobilized group (from 4·0 to 11·8 ng/ml at 5 h), being significantly different from the initial value at 0·5 h and all subsequent samplings ($P < 0{\cdot}001$), but did not change significantly during the experiment in the 'free' group. The regression equation on time ($x$) for the corticosterone values ($y$) for the immobilized birds was

$$y = 1{\cdot}175x + 5{\cdot}488,$$

and that for the 'free' group was

$$y = -0 \cdot 108x + 3 \cdot 904.$$

The slopes of the two lines were significantly different ($P < 0 \cdot 05$).

*Experiment 2*

The second experiment was designed to follow the changes during the stress of immobilization more closely by taking samples at shorter intervals. In addition, $T_4$ (also released by TRH) was measured.

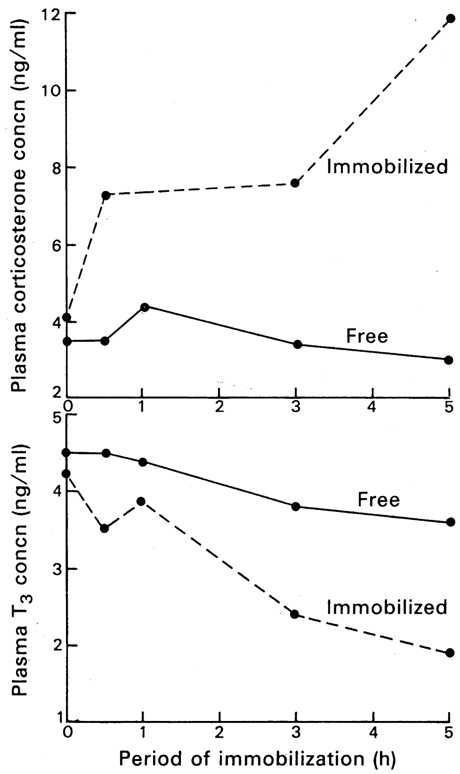


**Fig. 2.** Effects of immobilization and serial sampling on mean plasma concentrations of $T_3$ and corticosterone in 20 14-week-old cockerels immobilized during the whole experiment and in 20 'free' cockerels.

Eleven 8-week-old cockerels were cannulated and immediately sampled before being immobilized for 4 h. After immobilization they were sampled at 20-min intervals for 200 min and plasma $T_3$, $T_4$ and corticosterone concentrations were measured. Fig. 3 shows the mean concentrations of the three hormones. At 40 and 60 min, $T_3$ concentration rose in 6 out of 11 birds and then fell between 40 and 120 min. $T_4$ rose in 3 out of 10 birds and then fell in 8 out of 10 birds ($P < 0 \cdot 05$). The initial fall in the levels of $T_3$ and $T_4$ occurred at varying times (between 40 and 120 min) after immobilization in different birds. Least-squares determinations on the model $y = \alpha + \beta x$ together with the respective standard deviation of coefficients are given in the following equations:

For $T_3$,

$$y = 2 \cdot 2731\ (\pm 0 \cdot 0795) - 0 \cdot 7318\ (\pm 0 \cdot 000672)x.$$

For $T_4$,

$$y = 11 \cdot 833\ (\pm 0 \cdot 595) - 0 \cdot 2513\ (\pm 0 \cdot 00502)x.$$

The homogeneity of slopes for $T_3$ and $T_4$ at 60 min after immobilization and later was tested. There was a suggestion of a difference between slopes ($P < 0 \cdot 10$).

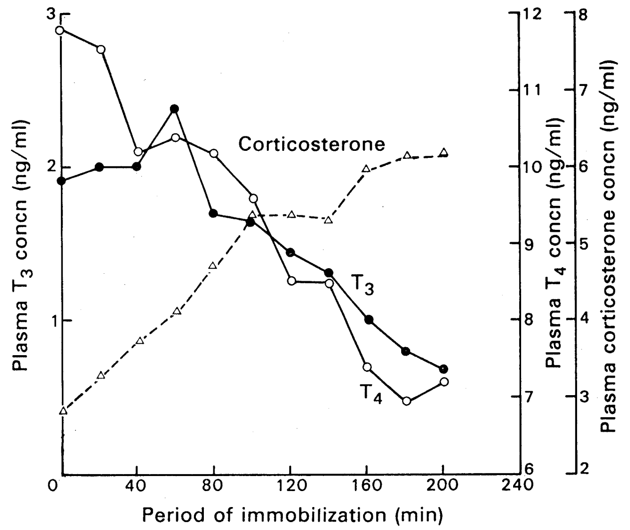


**Fig. 3.** Effects of immobilization for 4 h and serial sampling on mean plasma concentration of $T_3$, $T_4$, and corticosterone in 11 8-week-old cockerels.

Corticosterone rose following immobilization in all birds, the rise being detected in birds at the 20-min sampling and later. Least-square determinations of the above model together with the respective standard deviations of the coefficients are given by the following equation:

$$y = 3 \cdot 128\ (\pm 0 \cdot 334) + 0 \cdot 169\ (\pm 0 \cdot 00282)x.$$

The determinations showed that corticosterone values on time fitted a straight line, rather than a quadratic locus, as did the $T_3$ and $T_4$ values.

*Experiment 3*

The fall in plasma levels of $T_3$ and $T_4$ observed in experiments 1 and 2 could have originated at the hypothalamo-pituitary or the thyroid site. To test this hypothesis 13 14-week-old cockerels were cannulated and immediately sampled. They were then immobilized for 5 h. A second sample was taken 3 h after the initial sample. Seven of the birds (group A) were then injected with TRH; the other birds (group B) were immobilized only. All birds were then sampled at 15, 37, 60, 85 and 120 min after the injection of TRH into the birds of group A.

Fig. 4 shows mean changes in $T_3$ and $T_4$ levels in cockerels of both groups. As expected, circulating $T_3$ levels in all 13 birds were lower 3 h after immobilization ($P < 0 \cdot 001$) (falling from a mean of $3 \cdot 0$ to $1 \cdot 9$ ng/ml in group A and from a mean of $2 \cdot 7$ to $1 \cdot 7$ ng/ml in group B) and remained low throughout the experiment in the six birds of group B. However, after the injection of TRH in birds of group A, $T_3$ levels rose considerably (to a mean maximum 3·7 ng/ml) in all seven birds. The rise occurred at different times for different birds: at 15 min for one bird, at 37 min for five birds and at 60 min for the last bird. The peak values were reached in six of seven birds, between 60 and 90 min after the injection of TRH, but in one bird the peak was reached at 15 min. The $T_3$ values were not significantly different between groups A and B 3 h after immobilization but were significantly higher in the blood samples from group A (TRH-injected) birds ($P < 0·01$) taken 37, 60, 85 and 120 min after the injection of TRH.

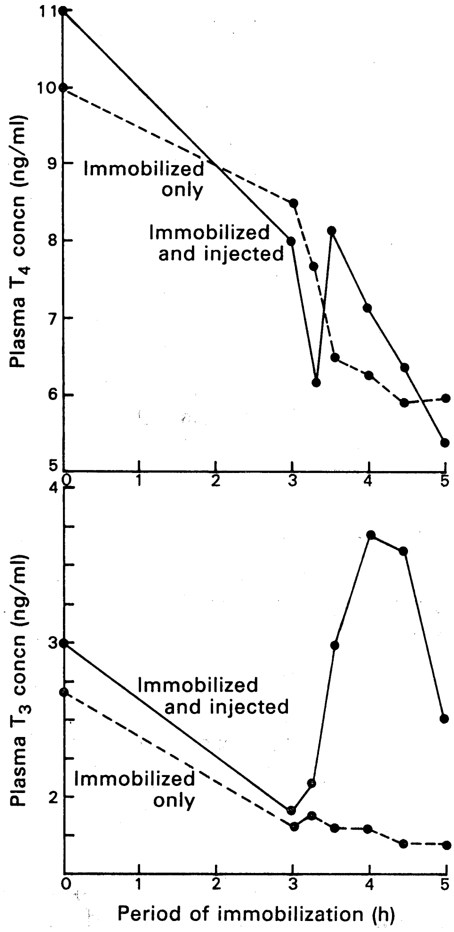


**Fig. 4.** Effects of immobilization of 13 14-week-old cockerels for 5 h and injection of seven of these birds with TRH i.v. (100 $\mu$g/kg liveweight) 3 h after the initial sample was taken, on plasma concentration of $T_3$ and $T_4$.

$T_4$ levels fell in five out of the six birds (from a mean of 9·8 to 8·5 ng/ml, $n = 6$) in group B after immobilization, and six out of seven birds (from a mean of 11·0 to 8·0 ng/ml, $n = 7$) in group A. This difference between first and second samplings was significant ($P < 0·05$). $T_4$ levels rose in six out of the seven birds ($P < 0·05$) 37 min after the TRH injection. In group B, $T_3$ levels continued to fall 3–4 h after immobilization (from 8·5 to 6·3 ng/ml) and stayed at this low level until 5 h after immobilization.

## Discussion

The levels of circulating $T_3$ and $T_4$ before immobilization found in the present experiments were within the range found in 12-week-old pullets by Klandorf *et al.* (1978*a*), and the corticosterone values were within the range in hens found by Etches (1976) and Beuving and Vonder (1977).

A consistent finding was that $T_3$ fell considerably in every bird after immobilization and to a lesser extent after handling and venipuncture. Newcomer (1974) found an increase from approximately 1·5 to 2·0 ng/ml in $T_3$ from 0900 h (on a 16 h light: 10 h dark photoperiod) and practically no change during that time in $T_4$ in 6-week-old cockerels. Klandorf *et al.* (1980) working with 12-week-old pullets on a 14 h light: 10 h dark photoperiod found small increases in both hormones from 0800 to 1230 h. Kühn and Nouven (1978) found a trough in $T_4$ concentrations at about 1400 h, but no diurnal rhythm in $T_4$. The fall in $T_3$ and $T_4$ levels found in our experiments could not therefore be due to a diurnal rhythm in these hormones.

May (1978), working with 7-week-old pullets at 32·2°C, found that $T_3$ levels were lower and $T_4$ levels higher in chickens deprived of food for 16 h. However, when the food-deprived birds were given access to food for 1 h, $T_3$ and $T_4$ values were no different 3·5 h later from *ad libitum* controls. Hence, 2 and 3 h after immobilization, the fall in $T_3$ and $T_4$ levels was unlikely to be due to food deprivation.

The fact that $T_3$ did not decrease in the first 20 min of stress is useful because it indicates that initial concentrations will be similar to undisturbed concentrations. Our results (expt 1), like those of Etches (1976), suggest that corticosterone increases only following more severe stresses (cf. Nir *et al.* 1975) which may not occur often in efficient modern poultry systems.

We do not know why a few birds (3 out of 23) did not exhibit the fall in $T_4$ following immobilization unless in those birds it occurred more than 5 h after the stress was applied. The timing of the initial decline in both $T_3$ and $T_4$ varied in our experiments between birds (40–160 min) after the birds were immobilized.

In some, but not all birds, there was an initial rise in $T_3$ and $T_4$ levels preceding the decline. Since this rise, like the decline, varied in timing after immobilization between birds, it is possible that it did in fact occur in all birds but was missed because it occurred briefly during the 20-min interval between two blood samplings.

Klandorf *et al.* (1978*b*) found a decrease in $T_4$, but not in $T_3$ levels, in 13-month-old hens which were serially sampled every 30 min over a period of 3 h, and attributed this decline to handling. We do not know why their results and ours differ because we found some decrease in $T_3$ in every bird handled and sampled, unless Klandorf's birds had been handled so much that they were not at all disturbed by the samplins procedure. But if that were the case, one would not expect a fall in $T_4$. The birdg in our experiment had not been used to handling and were much younger.

The fact that in the present experiment, TRH caused $T_3$ and $T_4$ concentrations to rise during immobilization, while $T_3$ and $T_4$ concentrations in the immobilized birds, which received no TRH, remained low, suggests that the decline in $T_3$ and $T_4$ during stress was not due to exhaustion of hormone production by the thyroid gland but rather to inhibition of release of TRH from the hypothalamus, possibly due to the suppressive effect of high levels of endogenous corticosterone similar to that found in mammals (cf. Brown-Grant 1966).

Klandorf *et al.* (1978*b*) used the same dose of TRH (Sigma Chemical Co.) as we did but injected it subcutaneously. The rise in $T_3$ in their experiments was somewhat later (60 min with a peak at 80–100 min), but similar to ours for $T_4$ (peak 40–60 min). In the experiments of Kühn and Nouven (1978) 10 $\mu$g TRH (U.C.B. Bioproducts, Brussels, Belgium) (i.v.) gave somewhat smaller increases in $T_3$ in younger, 6-week-old chickens than in the experiments described here. The synthetic TRH preparation used in our experiments was different from that used in the experiments of Klandorf and of Kühn and Nouven.

Recent medical reports also indicate the involvement of the thyroid gland in stress. For example, Adami *et al.* (1978) and Chan *et al.* (1978) found that $T_3$ and $T_4$ levels decreased in humans following surgery. Chan *et al.* sampled more frequently (every 30 min) during the operative period and found an initial transient rise in free $T_3$ and $T_4$. The values returned to normal 4–7 days later.

Our results suggest that $T_3$ may be a more sensitive indicator of acute stress than corticosterone. It remains to be determined how long the decline in circulating $T_3$ and $T_4$ persists during longer-term stress and following the removal of the stress factor. It is likely that longer-term or chronic stresses result in a more complex picture in birds as has been found in mammals (cf. Ducommun *et al.* 1967; Dewhurst *et al.* 1968) with an increase in thyroid hormones following the decrease and, perhaps, a further decline in thyroidal activity as the thyroid gland becomes depleted. We plan to measure changes in the thyroid hormones and corticosterone in situations similar to those occurring under commercial conditions in the Australian poultry industry when stress is alleged to exist, e.g. food deprivation, travel or moving birds from groups kept in floor-pens to small multi-bird laying cages.

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