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# THE EFFECT OF 2,4-DINITROPHENOL ON THE TISSUE CONCENTRATION OF IODINE-CONTAINING COMPOUNDS IN ISOTOPICALLY EQUILIBRATED INTACT RATS

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It has been repeatedly shown (Wolff, Rubin & Chaikoff, 1950; Goldberg & Chaikoff, 1951; Goldberg, Wolff & Greep, 1955, 1957; Castor & Beierwaltes, 1956) that the administration of 2,4-dinitrophenol to mammals is accompanied by a sharp decrease of the plasma PBI without an increase of TSH secretion by the pituitary. (The following abbreviations are used: 2,4-dinitrophenol (DNP); thyroid-stimulating hormone (TSH); protein-bound iodine (PBI); trichloracetic acid precipitable (TCAp); thyroxine-binding globulin (TBG).) These findings are usually considered to be due to 'disruption' of the classical thyroid–pituitary feed-back mechanism. Goldberg *et al.* (1957) showed that the pituitaries of DNP-treated animals had not lost the ability of reacting to other stimuli, and concluded from their results that the data 'were not consistent with the simple classical feed-back theory of thyroid–pituitary interrelationships. It is likely that the pituitary cells secreting TSH are influenced not only by the actual circulating level of thyroid hormone(s) but by one or more of its peripheral actions'.

Data obtained while studying the influence of DNP on the peripheral metabolism of L-thyroxine in thyroidectomized, L-thyroxine-maintained rats (Escobar del Rey & Morreale de Escobar, 1958*a*, *b*) led us to suggest that the sharply lowered plasma PBI of treated animals might not necessarily mean that a similar decrease had occurred in the concentration of thyroid hormone in the peripheral tissues: we had observed and have later confirmed in more extensive experiments *in vivo* and *in vitro* (Morreale de Escobar & Escobar del Rey, 1961) that DNP alters the normal partition of the thyroid hormone between plasma or protein-free incubation media and cells in favour of the latter.

It has been the working hypothesis for this work that, in non-thyroidectomized rats receiving DNP, the tissue concentration of thyroid hormone(s)

might remain within normal limits, despite the very low plasma level. If this were confirmed, the lack of a TSH-secreting response to the sharp lowering of the plasma PBI might be interpreted as an indication that the mechanisms controlling TSH secretion are more sensitive to factors related to the intracellular concentration of thyroid hormone than to its circulating level. Whenever the plasma concentration of thyroid hormone no longer reflected intracellular events, PBI data alone might lead us to conclude wrongly that there was 'disruption' of the thyroid–pituitary servo mechanism.

The concentration of iodinated compounds in tissues was taken as an index of their thyroid hormone content. Owing to difficulties inherent in present techniques for the chemical determination of iodinated compounds in most tissues, an isotope equilibrium dilution method was adopted, similar to that proposed by van Middlesworth (1956).

## METHODS

Unless otherwise stated the general procedure was as follows: either male or female young adult Wistar rats were fed on a low-iodine, medium-residue, Remington diet for at least a month before and during equilibration with radio-iodide. Since its exact iodine content was not known, the same batch of feed was used for a given group throughout the experiment.

Isotopic equilibration of the iodine-containing compounds in the animals was achieved by the daily administration (in the feed or by intraperitoneal injection) of radio-iodide, following in general the procedure of van Middlesworth (1956). The dose range for different experimental groups was $I^-$ 2–4 $\mu$g/rat/day, labelled with less than 1 $\mu$c of $^{131}I$, as determined on the first day of administration. Attainment of isotopic equilibrium was assessed for each experimental group by one or more of the following criteria: attaining a plateau of neck $^{131}I$ count and of the individual urinary and faecal excretions; recovery of at least 95 % of the daily administered dose in the excreta; constancy of plasma, tissue and thyroid $^{131}I$; constancy of the specific activity of the plasma PBI. By these criteria and in these experimental conditions, isotopic equilibration of the major iodine compartments was reached in 16–20 days.

Half the equilibrated group then received 2–4 mg DNP/rat/8 hr in the feed or intraperitoneally. These animals and their untreated controls were killed at different times after the last DNP injection. Blood was withdrawn under ether narcosis after I.V. heparin, and the animals were then exsanguinated from the inferior vena cava. It later became necessary to alter the procedure by perfusing the animals at necropsy with 150 ml. of saline, after extraction of about 5 ml. of blood. The technique was checked in a group of radio-iodide-equilibrated rats, injected with Evans Blue and killed with perfusion half an hour later; it was then found that the ratio of colour to TCAp-$^{131}I$ was the same in both whole plasma and perfusate. The $^{131}I$ distribution throughout the whole body was determined as previously described (Escobar del Rey & Morreale de Escobar, 1958*a*); the procedure for the determination of the TCA-precipitable fraction of iodinated compounds was, however, modified by the addition before fractionation of thiourea and of non-radioactive plasma (the latter to tissue samples) in order to avoid spurious results due to the presence of haemoglobin (Rosenberg, 1959) and to ensure a sufficient thyroxine-binding protein content.

Radioactivity was determined with a thallium-activated sodium iodide well-type scintillation crystal and the results are expressed as percentage of the dose of radio-iodide administered daily. Since the animals were maintained in isotopic equilibrium until necropsy,

it is valid to assume that the concentration of iodinated compounds in a given sample was proportional to its radioactivity. The minimum iodine content in a given sample may be calculated from the latter and from the specific activity of the exogenous iodide. External sources of iodine other than the administered labelled iodide were kept as low and as constant as possible, and should influence figures of both DNP-treated and untreated animals in the same way. Since for technical reasons the total iodine intake was not known, the results reported here are expressed as percentages of the known dose administered daily and not in mass units.

Stable iodine determinations in plasma were carried out by the wet-ashing procedure of Connor, Swenson, Park, Gangloff, Lieberman & Curtis (1949).

Carrier-free $^{131}$I-iodide was obtained from the Radiochemical Centre, Amersham (England). L-Thyroxine, labelled in the 3′- and 5′-positions was supplied by Abbott Laboratories (U.S.A.).

DNP was purified by two crystallizations from acetone; it was dissolved in NaOH solution and neutralized with HCl so that the final solution was approximately 0·9 g/100 ml. NaCl solution. When DNP was injected intraperitoneally, the dose was contained in 0·5 ml.; equal volumes of isotonic saline were injected into the controls.

## RESULTS

Experiments with several different groups of animals as described above repeatedly showed both with males and females (Table 1) that the ratio between the TCA-precipitable iodinated compounds in the carcass to that in the plasma was always higher in the DNP-treated animals, the increase being 33–200% of the control value. (By 'carcass' is understood the mechanical homogenate consisting mostly of skeletal muscle and bones obtained after removal of blood, organs, gastro-intestinal tract, abdominal fat and skin.) Though not shown in this table, the same occurred for the ratio between heart, brain, kidney, spleen, etc., and plasma. This 'shift' in

TABLE 1. The effect of DNP administration on the tissue-plasma partition of iodine-containing compounds in rats isotopically equilibrated with radio-iodide

| | | $\frac{\text{TCAp } ^{131}\text{I in 1 g carcass}}{\text{TCAp } ^{131}\text{I in 1 ml. plasma}}$ | | | |
|---|---|---|---|---|---|
| Group | Time on DNP | Control | DNP-treated | $P$ | Increase over control value (%) |
| I *f* | 24 hr | 0·23 | 0·39 | < 0·001 | 70 |
| | 5 days | 0·23 | 0·42 | < 0·001 | 83 |
| II *f* | 22 hr | 0·21 | 0·28 | < 0·01 | 33 |
| | 5 days | 0·25 | 0·35 | < 0·02 | 40 |
| III *m* | 5 days | 0·10 | 0·16 | < 0·001 | 58 |
| IV *f* | 4 days | 0·20 | 0·60 | < 0·001 | 200 |
| *V *m* | 5 days | 0·10 | 0·25 | < 0·001 | 150 |
| VI *m* | 4 days | 0·15 | 0·40 | < 0·001 | 167 |

Animals of all these groups were in isotopic equilibrium with exogenous labelled iodide as ascertained by several criteria (see Methods); other experimental conditions, such as radio-iodide dose and mode of administration, DNP dose level, route and frequency of administration, moment of observation of $^{131}$I distribution, were different.

* Underlined groups correspond to animals thoroughly perfused at necropsy.

the distribution of iodine-containing compounds between plasma and tissues in favour of the latter resulted from the sharp decrease during DNP administration of the plasma concentration (down to 50–25 % of the control value), whereas the concentration of such compounds in the carcass remained unaltered in most experimental groups or decreased somewhat in others, but always to a lesser extent than did the plasma concentration.

TABLE 2. Influence of DNP on the distribution of iodine-containing compounds in rats isotopically equilibrated with radio-iodide

| | % of daily dose: mean ± S.D. | | |
|---|---|---|---|
| | DNP-treated (4) | Control (4) | *P* |
| Thyroid | 424 ± 59 | 411 ± 63 | — |
| Plasma (1 ml.) | 0·285 ± 0·026 | 0·942 ± 0·084 | < 0·001 |
| Plasma (1 ml.) TCAp | 0·202 ± 0·025 | 0·840 ± 0·100 | < 0·001 |
| Carcass (1 g) | 0·184 ± 0·028 | 0·187 ± 0·013 | — |
| Carcass (total) | 19·5 ± 2·7 | 16·9 ± 2·3 | — |
| Liver (total) | 4·03 ± 1·08 | 6·48 ± 1·73 | — |
| Intestines (total) | 11·61 ± 3·40 | 8·48 ± 2·55 | — |
| Carcass: plasma ratio | 0·66 ± 0·18 | 0·18 ± 0·5 | 0·01–0·001 |

Female rats, weighing 180–200 g, isotopically equilibrated with radio-iodide 4 μg/rat/day for 25 days, treated for 5 days with 3 mg DNP/8 hr; killed 4 hr after the last injection of the drug.

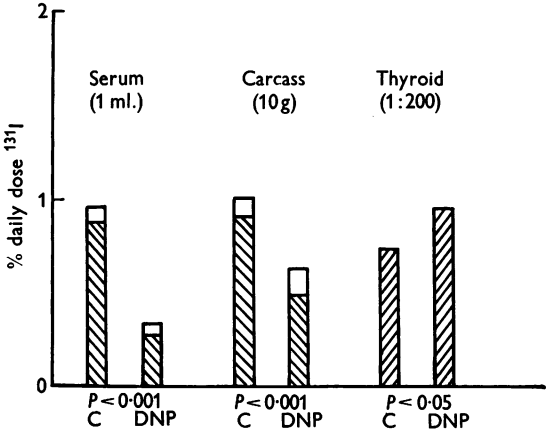


Fig. 1. Influence of DNP on the content of iodine-containing compounds in plasma, carcass and thyroid. Male rats, weighing 240–260 g, isotopically equilibrated with radio-iodide 3 μg/rat/day for 5 days and killed 7 hr after the last injection. The means of data from ten animals in each group are indicated. ▨ TCAp $^{131}I$; □ TCA soluble $^{131}I$; ▨ total $^{131}I$.

In those experimental groups where the carcass radioactivity remained the same in both DNP-treated and control animals, no change was observed in the mean thyroid $^{131}I$ content. On the contrary, in some animals where a decrease of the carcass concentration of iodine-containing compounds had occurred, an increase of the mean total thyroid $^{131}I$ was found.

Table 2 and Fig. 1 show typical examples of both types of response. This difference in response to the administration of DNP from one experimental group to another was most unsatisfactory; similar variability in the response to DNP of other parameters had also been observed by others (Goldberg & Chaikoff, 1951; Goldberg *et al.* 1957). It did not appear to depend on the sex of the animals used. Closer examination of the experimental conditions employed and the type of response obtained showed that whenever a decrease in the concentration of iodine-containing compounds had been observed in the carcass, the animals had been killed 6–8 hr or more after the last dose of DNP. Indeed, the lowest tissue concentrations we observed were found in an experimental group which had been killed 12 hr after the administration of the daily feed containing 12 mg of the drug. The highest tissue concentrations of iodine-containing compounds was found in animals killed about 4 hr after the last DNP injection. On the other hand, *in vitro* experiments which were being carried out simultaneously (Morreale de Escobar & Escobar del Rey, 1961) showed that the increase induced by DNP in 'uptake' of $^{131}$I-L-thyroxine by rat R.B.C. and diaphragms depended on the concentration of the latter and needed time to reach a maximum. It was therefore possible that, as time elapsed after the administration of DNP to the animals, the circulating concentration of the drug decreased, and with it the extent of the 'shift' of iodine-containing compounds from the plasma to tissues.

The effect of DNP on the tissue concentration of iodine-containing compounds was therefore re-investigated at different times after DNP administration; some of the results obtained for a group of female rats weighing 180–200 g at necropsy, isotopically equilibrated on 4 $\mu$g $I^-$/rat/day for 25 days, which had received 3 mg DNP every 8 hr during the last 4 days, are shown in Fig. 2. As may be seen, the concentration of iodinated TCA-precipitable compounds in the plasma of the DNP-treated animals was about 25 % that of the controls during the whole period of observation. On the other hand, the concentration of these compounds in the carcass (and, though not shown in the figure, in brain, heart, etc.) became the same as that of the control group 1 hr after the last intraperitoneal injection of DNP and was still so at 4 hr. By 8 hr it had decreased to about two-thirds of the control value. The carcass:plasma ratio of iodine-containing compounds clearly reflected these findings, showing a rapid increase soon after the injection of DNP and reverting almost to control values by 8 hr, at which time the concentration of DNP in the plasma had fallen to low levels. The thyroid iodine remained unaltered in both groups during the whole period. Since the animals had been receiving the drug periodically (every 8 hr) it seems valid to conclude that a similar pattern of events as that found during the 8 hr observation period (Fig. 2) had been

repeating itself, the plasma concentration of iodinated compounds being continuously low, that in the tissues varying within limits much nearer to the control range.

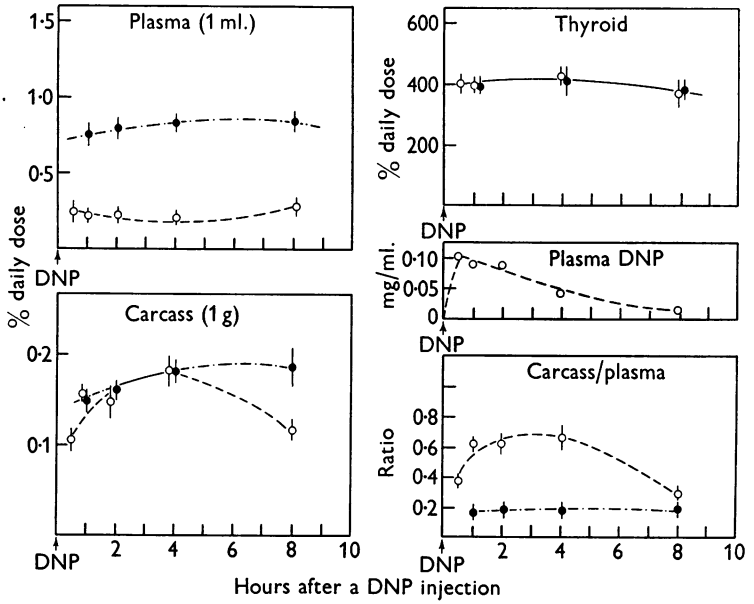


Fig. 2. Influence of DNP on the distribution of iodine-containing compounds in the plasma, carcass and thyroid at different times after the last injection of the drug. Female rats, weighing 180–200 g, isotopically equilibrated with 4 $\mu$g radio-iodide/rat/day over a 25-day period, treated with 3 mg DNP/8 hr for 4 days. Data are expressed as percentage of the administered radio-iodide; the mean $\pm$ S.D. being given. Changes in the plasma concentration of DNP with time are also shown. ○ DNP-treated; ● controls.

From other experiments another factor which might have been influencing these results emerged. In order to determine the ratio of carcass to plasma concentrations of iodine-containing compounds for very low and very high levels of circulating thyroid hormone, thyroidectomized rats were maintained and isotopically equilibrated at different dose levels of L-thryoxine (0·4–100 $\mu$g L-$T_4$/rat/day). It was found that over a very wide concentration range there was roughly ten times more TCA-precipitable iodine in 1 ml. plasma than in 1 g peripheral muscle, heart, brain, etc. This would mean that blood retained in the tissues would give artificially high values of the tissue concentrations of iodinated compounds. It was therefore possible that at least part of the decrease in iodinated compounds that had been observed in the peripheral muscle of some DNP-treated groups was actually due to the great decrease in the concentration of these

compounds in the plasma retained in tissues at necropsy. Assuming the true (no retained plasma) concentration of iodine-containing compounds of the peripheral muscle of DNP-treated and control animals to be the same, the plasma content for DNP-treated rats to be one-third that of controls, and that about 3 ml. of plasma could easily remain in 100 g of peripheral muscle at necropsy, a 20 % decrease of the apparent carcass concentration in DNP-treated animals would be accounted for. A greater decrease in the plasma concentration would result in an even larger artifactual difference between the carcass data for DNP-treated and control animals.

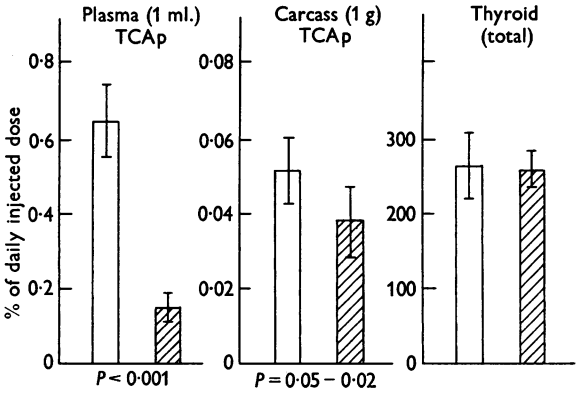


Fig. 3. Plasma, peripheral muscle and thyroid content of iodinated compounds in male rats, weighing 260–280 g, isotopically equilibrated on radio-iodide 4 μg/rat/day over a 26-day period, treated with 10 mg DNP in the feed for 4 days and killed with thorough perfusion. The mean ± S.D. is indicated. ▨ DNP-treated; □ control.

For this reason the experimental procedure was altered to include thorough perfusion of the rats at necropsy. Figure 3 shows the results obtained in such an experiment with a group of male rats, weighing 260–280 g, which had been isotopically equilibrated with 4 μg $I^-$/rat/day and had been treated for 4 days with 10 mg DNP in the feed and 3 mg by intraperitoneal injection 20 and 3 hr before being killed. As may be seen, the plasma concentration of TCA-precipitable iodine-containing compounds was very low in the DNP-treated animals, being about one-fourth that of the controls; the concentration in the perfused carcass decreased about 20 %, the difference between both mean values being near the borderline of statistical significance ($P$ = 0·05–0·02). The carcass:plasma ratio was therefore much higher for the DNP-treated animals. Total thyroid iodine content remained unchanged in both groups.

When the changes with time in the distribution of iodine-containing compounds were determined in groups of male rats, weighing 210–250 g, isotopically equilibrated on 4 μg $I^-$/rat/day, treated during 5 days before

necropsy with 3 mg DNP/8 hr, and killed with thorough perfusion at intervals up to 11 hr after the last DNP injection, it was found (Fig. 4) that the plasma concentration of iodine-containing compounds in DNP-treated animals was about one-third the control value, whereas the carcass concentration was the same as that in control animals in some groups, higher or lower in others, the over-all mean being the same. As has already

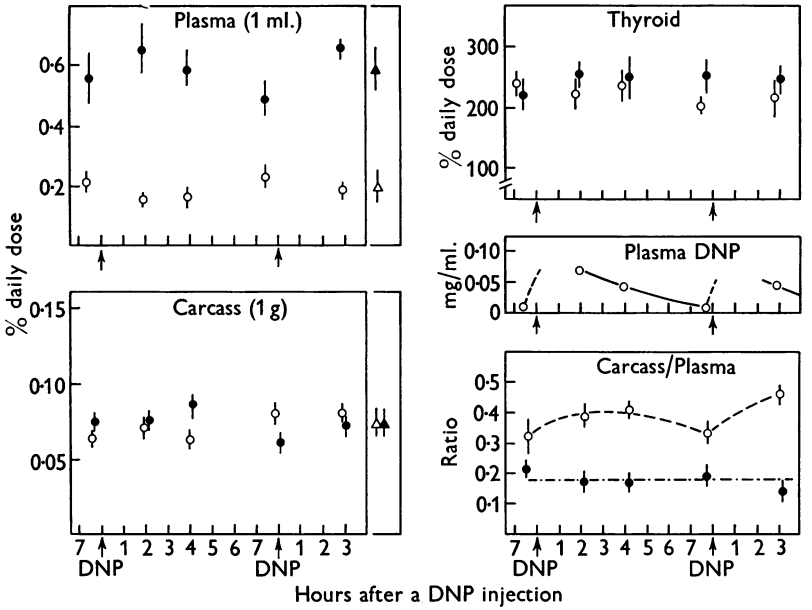


Fig. 4. Variations with time of the effect of DNP on the concentration of iodine-containing compounds in the plasma, peripheral muscle and thyroid of male rats, weighing 220–250 g, isotopically equilibrated on radio-iodide 4 μg/rat/day, treated for 4 days with 3 mg DNP/8 hr and killed with thorough perfusion: mean ± S.D. of groups of four animals are given. The variations with time of the plasma DNP concentration are also shown. ○ DNP-treated; ● controls; △, ▲ over-all means ± S.D. over whole period of observation.

been seen in other experiments (Table 1 and Fig. 2), a 'shift' of iodine-containing compounds from the plasma to tissues occurred in DNP-treated animals and this 'shift' was least when the plasma DNP concentration was lowest. The mean values for the thyroid iodine content were the same for both treated and untreated rats. However, when the individual thyroidal iodine contents were plotted against the concentration of iodine-containing compounds in the carcass for animals of both groups together, a negative correlation was found ($P < 0{\cdot}05$); for DNP-treated rats alone, the thyroid–carcass correlation was significant at a $P = 0{\cdot}01$–$0{\cdot}001$ level. No correlation was found when the thyroid values were plotted against corresponding plasma values.

As has been stated above and shown in Table 2 and Fig. 2, the apparent carcass concentrations of iodine-containing compounds in many DNP-treated groups was the same as that in the corresponding controls. From the results obtained with perfused animals (Fig. 4) it would not seem too speculative to assume that, in some at least of the unperfused groups, the true tissue concentration of iodine-containing compounds in DNP-treated animals had actually been higher than in the controls and that some of the differences between the carcass data for both groups had been smaller.

The present results underline the necessity of determining the pattern of distribution of iodine-containing compounds at different times during the experimental procedure, since data obtained at one time only might be misleading as to the general sequence of events (see Figs. 3 and 4). Moreover, we think that changes in dose level, mode and frequency of administration of DNP might lead to quantitatively different patterns of distribution of iodinated compounds in plasma and tissues; this is a possible explanation for many of the differences in the response to DNP described by others (Goldberg & Chaikoff, 1951; Goldberg *et al.* 1957). Total perfusion of the animals to eliminate plasma from the tissues is also a necessity if data on the iodine content of the latter are sought.

Though the data are not shown here in detail, the concentration of iodinated compounds in the heart was usually lower in the DNP-treated animals than in their controls, even in those perfused groups where no difference was demonstrable in peripheral muscle. The concentrations in the brain, on the contrary, behaved similarly to that in the corresponding peripheral muscle in all groups.

We thought it would be interesting to determine the concentration of iodine-containing compounds in the pituitaries of DNP-treated and control, radio-iodide-equilibrated, intact rats; this was not feasible under our experimental conditions, because of the extremely low radioactivities encountered in the glands. For this reason measurements were attempted in thyroidectomized male rats weighing 200–250 g, isotopically equilibrated on $^{131}$I-L-thyroxine 4 $\mu$g/rat/day. In such experimental conditions isotopic equilibrium is achieved in a much shorter period (5–6 days) and without concentration of radioactivity in a small gland, thus permitting the use of higher initial radioactivities (about 3·5 $\mu$c/4 $\mu$g L-$T_4$ as determined on the first day of administration). Preliminary results with perfused animals show that the iodine content of total pituitaries of DNP-treated animals was the same as that of the controls (0·0048 $\pm$ 0·0007 % dose for the former and 0·0050 $\pm$ 0·0008 for the latter), despite the sharp decrease of the concentration of iodine-containing compounds in the plasma (1·37 $\pm$ 0·17% dose for DNP-treated versus 3·06 $\pm$ 0·21 for the untreated control). The corresponding data for the brain were 0·37 $\pm$ 0·04 % and 0·38 $\pm$ 0·03 %.

Moreover, the variations with time of the iodine content of the pituitaries parallelled the changes occurring in peripheral muscle and brain more closely than those taking place in the plasma.

Because of the low radioactivities of the samples obtained from radioiodide-equilibrated intact rats a reliable chromatographic identification of the nature of the iodinated compounds in the plasma and tissues was not feasible, without using dangerously high initial radioactivities.

## DISCUSSION

From the experiments reported here we have concluded that in DNP-treated rats the lowering of the concentration of TCA-precipitable iodine-containing compounds in the plasma does not reflect changes in the concentration of these compounds in peripheral tissues, such as skeletal muscle, brain, etc. Indeed, the over-all concentration of such compounds in these tissues remains within a range comparable to that in untreated rats, despite the continuously low circulating level. Preliminary data suggest that variations in the iodine concentration in pituitaries of DNP-treated rats reflect alterations occurring in brain and skeletal muscle more closely than those taking place in the plasma.

Such a pattern of distribution of iodine-containing compounds appears to be brought about chiefly by two factors: (*a*) The very rapid decrease in the plasma level of iodinated compounds was shown (Escobar del Rey & Morreale de Escobar, 1958*a*, *b*) to be accounted for by the simultaneous intense increase of their biliary secretion and, ultimately, a faecal excretion. (*b*) The maintenance of a normal or nearly normal concentration of iodine-containing compounds in most peripheral tissues probably results from the alteration induced by DNP in the normal partition of thyroid hormone between plasma and tissues in favour of the latter. This effect is reflected in the variations of the carcass-to-plasma ratios of TCA-precipitable iodine-containing compounds reported here. It is also suggested by the increased red-blood-cell radioactivity in animals injected with labelled thyroxine and DNP (Escobar del Rey & Morreale de Escobar, 1958*b*). Moreover, *in vitro* determinations of the influence of DNP on the 'uptake' of thyroxine by human and rat red blood cells and by diaphragms of perfused rats consistently showed (Morreale de Escobar & Escobar del Rey, 1961) that the drug greatly increases the proportion of available hormone which is found with the red-blood-cell fraction even under experimental conditions which exclude a mechanism involving solely and/or principally an action on the plasma-protein–hormone complex invoked by others (Christensen, 1959, 1960). It was concluded that this DNP effect is chiefly exerted directly on the cells, the exact site remaining undetermined.

It is not known whether the increased biliary secretion of thyroid hor-

mone, which is responsible for the low plasma PBI (Escobar del Rey & Morreale de Escobar, 1958*b*) is also the result of an increased transfer of thyroxine to the liver. If this were so, the entire distribution pattern of iodine-containing compounds in these DNP-treated animals could be accounted for by the 'shift' of the hormone towards the cells.

The situation during DNP administration in the rat may be summarized as follows:

(*a*) There is no increase in the TSH-secreting activity of the pituitary (Goldberg & Chaikoff, 1951; Goldberg *et al.* 1955, 1957).

(*b*) As is shown here, the concentration of iodine-containing compounds in peripheral tissues, pituitary included, remains within normal limits; the over-all peripheral deiodination of L-thyroxine is also unaltered or somewhat increased (Escobar del Rey & Morreale de Escobar, 1958*a*, *b*).

(*c*) It has been repeatedly shown (Wolff *et al.* 1950; Goldberg & Chaikoff, 1951; Goldberg *et al.* 1955, 1957; Escobar del Rey & Morreale de Escobar, 1958*a*, *b*), and is confirmed here, that there is a very sharp and rapid decrease of the plasma PBI.

(*d*) DNP alters both *in vivo* and *in vitro* the distribution of thyroid hormone between plasma and tissues in favour of the latter; data reported elsewhere (Morreale de Escobar & Escobar del Rey, 1961) support the conclusion that this effect of DNP is mainly exerted by a direct action on the cells.

(*e*) A negative correlation between the content of iodine-containing compounds of the thyroid and that of the peripheral muscle was sometimes observed, but none between the thyroid content and the plasma PBI.

These points have led us to the following tentative conclusions: The level of circulating thyroid hormone is not always a good index of its concentration and metabolism in the peripheral tissues. The same might be said of the level of 'free' circulating thyroxine. The behaviour of the TSH-secreting cells of DNP-treated rats would seem to correlate better with parameters such as the concentration and disposal of thyroid hormone in the peripheral tissues than with its level, whether total or 'free', in the circulation.

Though evidence is still fragmentary, there is general agreement that thyroid-hormone–plasma-protein interactions may control the action of the thyroid hormone by determining the level of its 'free' diffusible form in the circulation and hence its transfer to the peripheral tissues (Robbins & Rall, 1957, 1960; Pitt-Rivers & Tata, 1959; Dowling, Freinkel & Ingbar, 1960). The concentration of free diffusible thyroxine in the blood would therefore play a major role in the thyroid–pituitary homoeostatic

mechanism, a system which is believed to be regulated so as to maintain a normal intracellular concentration and metabolism of the thyroid hormone. We would point out that in such a scheme the level of free thyroid hormone in the circulation would be assigned a primary role in the regulation of TSH secretion only in so far as it determined the rate of transfer and of disposal of the hormone in the tissues. However, it appears from the present data and those reported elsewhere (Morreale de Escobar & Escobar del Rey, 1961) that these parameters might be influenced directly via mechanisms not operating at the level of the plasma proteins, but of the cells. We venture to suggest as a working hypothesis that TSH-secreting cells are sensitive to parameters related to the metabolic situation of the thyroid hormone in the peripheral tissues. That this might occur at a hypophysial level would not seem excluded by the present results nor by those reported by others (von Euler & Holmgren, 1956*a*, *b*; Yamada, 1959*a*, *b*). Whenever the concentration of circulating thyroid hormone no longer reflected these tissue parameters, as appears to occur in DNP-treated rats, if the former alone were considered, the situation would be one of an apparent 'disruption' of the thyroid–pituitary servo mechanisms.

These remarks are not meant to convey that the regulating role in thyroid–pituitary interrelationships should be assigned to the tissue thyroid hormone concentration *per se*, but to factor(s) somehow related to it. Too little is known at present about possible interrelationships, if any, between intracellular concentration, degradation, metabolic action, etc., of the hormone to venture a more precise hypothesis.

It seems, therefore, desirable whenever possible to obtain quantitative data on the concentration and metabolism of iodinated compounds in the peripheral tissues, specially at a hypophysial and hypothalamic level. Such information might prove quite valuable in giving us a deeper insight into the regulators more directly involved in thyroid–pituitary interplay and the factors which might modify them.

## SUMMARY

1. The effect of the administration of 2,4-dinitrophenol for several days on the concentration of iodine-containing compounds in plasma and tissues of rats was determined, using animals isotopically equilibrated with radio-iodide.

2. It was found that the concentration of TCA-precipitable iodine-containing compounds in the plasma of DNP-treated animals was one-third or less that of their controls.

3. The interval between the last administration of DNP and the observations influences the actual concentration of iodine-containing compounds of the peripheral tissues found; moreover, because the con-

centration of iodinated compounds in the plasma of DNP-treated animals is much lower than that of controls, the blood retained in the peripheral tissues at necropsy may also give rise to artifactual differences in the apparent tissue concentration of such compounds.

4. When the concentration of iodine-containing compounds in the tissues of perfused rats, killed at different intervals over a 12 hr period, was determined, it was observed that the over-all concentration of these compounds in peripheral muscle and brain remains the same in both DNP-treated rats and their controls, despite the much lower concentration in the plasma of the former.

5. Preliminary figures for the iodine content of total rat pituitaries showed it was the same in DNP-treated and control animals and that its variations parallelled those observed in brain and skeletal muscle.

6. The results obtained indicate that the plasma concentration of TCA-precipitable-iodine-containing compounds is not always a good index of alterations of the concentration of such compounds in the peripheral tissues.

7. The possible bearing of these findings are discussed in terms of the thyroid–pituitary feed-back system. The results reported in the present paper are tentatively interpreted as suggesting that the regulator(s) of thyroid–pituitary interrelationships are closely related to some intracellular parameter, alterations of which might be better reflected by changes in the concentration of iodine-containing compounds in the peripheral tissues, brain and pituitary included, than in the level of circulating total or 'free' thyroid hormone.

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