Table

Oxidative activities of liver homogenates from THNA-treated rats and controls

The experimental conditions are the same as those given in the Figure. The final concentration of the added substrates was 0·02 M. The rates of O_2 uptake are expressed as µl/h/100 mg of wet weight. All values are corrected for endogenous respiration and represent the average of the number of the experiments presented. The significance of the difference between means is indicated by the value of 'P'.

No. of rats	Experimental condition	Substrate added	O_2 uptake mean	Value of 'P'	% Increase in O_2 uptake
6	Controls	Na-citrate	85	$< 0{\cdot}02$	
6	THNA-treated	Na-citrate	112		32
12	Controls	Na-succinate	263	$= 0{\cdot}01$	
12	THNA-treated	Na-succinate	358		36
4	Controls	Na-fumarate	104	$< 0{\cdot}02$	
4	THNA-treated	Na-fumarate	132		27
6	Controls	Na-malate	91	$< 0{\cdot}02$	
6	THNA-treated	Na-malate	116		28
8	Controls	Na-glutamate	188	$= 0{\cdot}05$	
8	THNA-treated	Na-glutamate	215		14

the presence of pyruvate (with fumarate added to furnish a supply of oxalacetate for the condensation reaction which causes citrate formation) is considerably higher when liver homogenates from treated rats are employed.

Endogenous oxygen uptake by liver homogenates from THNA-treated rats shows a slight and somewhat erratic enhancement in comparison with controls. Only in 6 experiments out of 15, a marked increase in endogenous respiration was observed, but its extent never reached that found when pyruvate and fumarate were added.

(2) *Oxidation of some Krebs Cycle intermediates by liver homogenates from THNA-treated rats.* In the sets of experiments shown in the Table, liver homogenates from normal and THNA-treated rats were incubated with some Citric Acid Cycle intermediates.

The added substrates were oxidized at very different rates, however, it can be seen that THNA-administration resulted in all cases in higher oxygen consumption by liver homogenates. An interesting feature of the above experiments is that, regardless of vast differences in oxygen consumption using different Tricarboxylic Acid Cycle intermediates, the percentage increase in O_2 uptake by liver homogenates from treated rats is essentially the same. The oxidation of glutamate proved to be less affected by THNA treatment than that of the above substrates employed.

Discussion.—If conclusions regarding the *in vivo* effects of THNA can be drawn from experiments carried out under *in vitro* conditions, our findings would suggest that THNA stimulates the hepatic Cyclophorase activity of the rat. Judging from the experiments in which pyruvate and fumarate were employed, the preparations from treated rats appear to produce (and to oxidize) 'active acetate' more readily than controls.

Our experiments do not offer any explanation of the mechanism by which THNA stimulates Krebs Cycle activity; however, it seems of interest to note that the *in vivo* effects of THNA resemble, in some respects, those of 'uncoupling' agents such as 2,4-dinitrophenol, thyroxine and triiodo-thyronine. One could therefore put forward the hypothesis that THNA causes an acceleration of the oxidative processes at the expense of chemical efficiency in generating high-energy phosphate bonds required in the anabolism (synthesis) of carbohydrates. Work to study the *in vivo* and *in vitro* effects of THNA upon oxidative phosphorylation, and to control whether the above hypothesis is justified, is now under progress and will form the subject matter of subsequent communications.

In conclusion we admit that our results lend strong support to assume that THNA administration to rats brings about enhanced carbohydrate breakdown by stimulating Krebs Cycle activity.

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Riassunto

Gli omogenati di fegato dei ratti sacrificati dopo 4 h dalla somministrazione di β-tetraidronaftilammina (30 mg pro kg per via intraperitoneale) presentano una aumentata capacità di ossidare alcuni composti del Ciclo di Krebs, in rapporto ai controlli. Viene avanzata l'ipotesi che l'intensa glicogenolisi epatica e muscolare e l'aumentata acetilazione *in vivo* dell'acido para-aminobenzoico provocate nel ratto dalla somministrazione di β-tetraidronaftilammina, siano causate da un esaltato metabolismo intermedio ossidativo.

In vitro Effects of 1,2,3,4-Tetrahydro-β-naphtylamine (THNA) on Succinate Oxidation by Rat Liver Homogenates

Previous experiments[1,2] showed that THNA, a powerful pyrogenic agent, when added to rat liver homogenates, exerts a slight inhibition on oxidation of malate and significantly increases total O_2 uptake, when succinate is employed as substrate. These *in vitro* effects on succinate and malate oxidation tended to prove that THNA acts through a mechanism similar to that of thyroxine, which has recently been described by WOLFF, CLARKE and BALL[3,4], ESTABROOK[5] and BARKER[6]. These investigators admitted that the increased oxidation of succinate by fresh rat heart homogenates in the presence of thyroxine is due to its capacity of inhibiting the malate dehydrogenase

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Table I
Effect of THNA on succinate oxidation by 'aged' rat liver homogenates

Time min	mm^3 O_2 uptake		Value of 'P'	% Variation in the presence of THNA
	Controls	withTHNA		
20	31 ± 5·0	41 ± 4·6	< 0·02	+32
40	48 ± 3·7	65 ± 4·2	< 0·02	+35
60	63 ± 5·5	84 ± 5·8	< 0·02	+33

Each Warburg flask contained: 0·5 ml of 'aged' liver homogenate (5% W/V); 0·3 ml of 0·1 M phosphate buffer (pH 7·6); 0·2 ml of 0·01 M Na-ATP; 0·4 ml of 0·003 M $MgCl_2$; 0·3 ml of 0·05 M Na-succinate; 1·0 ml of 0·12 M KCl-0·02 $NaHCO_3$ solution; 0·3 ml of 0·01 M THNA or water (controls). 0·2 ml of 15% NaOH in the center well. Duplicate estimations were made in all cases. Gas phase: air. Temperature: 38°C. The flasks were allowed to equilibrate for 7 min and readings were taken at 20-min intervals for 60 min. The rates of O_2 uptake are expressed per 25 mg of wet weight of tissue. Mean values of 14 experiments ± standard deviation after endogenous O_2 uptake subtraction are presented. The significance of the difference between means is indicated by the value of '*P*'.

Table II
Effect of THNA on the reduction of methylene blue by rat liver succinic dehydrogenase complex (S. D. C.)

Time min	% reduction of Methylene Blue		% Variation in the presence of THNA
	Controls	with THNA	
3	26	33	+27
6	46	58	+26
9	64	80	+25
12	79	90	+14
15	90	90	–

Thunberg tubes expecially built to be located into the EEL photoelectric colorimeter were used. The systems consisted of: 1 ml of Na-succinate 0·008 M; 1 ml of Methylene Blue (10 mg%); 1·6 ml of 0·1 M phosphate buffer (pH 7·6) and 0·4 ml of 0·02 M THNA or water (controls). 0·5 ml of liver homogenate was placed into the side arm. The tubes were attached to a manifold and evacuated for 3 min with a high-vacuum pump. The contents of the tube and side arm were mixed after 5 min incubation in a 38°C water bath. At 3 min intervals the tubes were removed, wiped and read in the colorimeter with a 660 mμ filter. The activity was followed by observing the time for 90% reduction of Methylene Blue anaerobically. The values recorded in the Table represent the mean of 20 experiments.

activity and the accumulation of oxalacetate, a strong inhibitor of succinic dehydrogenase.

Some preliminary experiments indicated that the enhancing effect of THNA on succinate oxidation by rat liver homogenates is somewhat different from that of thyroxine and could perhaps be ascribed to a 'direct' stimulation of succinic oxidase system activity. In view of this possibility, the *in vitro* effects of THNA on succinate oxidation by rat liver homogenates were further investigated.

Animals and Methods. Male albino rats from a local strain were used throughout. Liver homogenates were prepared by grinding a portion of the liver in a Potter-Elvehjem all-glass apparatus with cold 0·12 M KCl–0·02 M $NaHCO_3$ solution to yield a 5% W/V suspension. The homogenates were employed within a 2 h period of 'aging' in order to allow the maximal loss of DPN (Diphosphopyridine Nucleotide) and the consequent inhibition both of malate dehydrogenase activity and oxalacetate formation.

Succinate oxidation was determined manometrically according to the method of AISENBERG and POTTER[7]. Cytochrome oxidase activity was measured by the manometric technique of SCHNEIDER and POTTER[7]. Succinic dehydrogenase activity was followed by observing the time for 90% reduction of Methylene Blue anaerobically[7]. The experimental conditions are given in connection with Tables I to II.

Results and Discussion. The effect of THNA addition on oxygen uptake by rat liver 'aged' homogenates in the presence of succinate is shown in Table I.

The increased rate of O_2 uptake in the presence of 0·001 M THNA was regularly observed from the earlier time intervals and was maintained over prolonged periods of incubation. Since it is well known that succinate oxidation by rat liver fresh homogenates falls off rapidly with time, unless oxalacetate (which inhibits succinic dehydrogenase activity) is removed, the possibility of THNA effects being similar to those already reported for thyroxine was considered: i.e. that a THNA-inhibited formation of oxalacetate might have been responsible for the enhanced O_2 uptake. Succinate oxidation was then investigated in the presence of glutamate which removes oxalacetate by transamination to form aspartate and α-ketoglutarate, neither of which inhibits the succinic dehydrogenase activity. On a percentage basis, the results obtained were quite similar to those reported in Table I.

These observations focussed our attention on the possibility that the increased oxygen uptake might imply a more specific action of THNA on the succinic oxidase system. According to this assumption, experiments were undertaken to study the enzyme systems bringing about the oxidation of succinate, i.e. succinic dehydrogenase and Cytochrome oxidase. It was observed that THNA addition does not alter significantly the rat liver Cytochrome oxidase activity. In contrast to this, the activity of the succinic dehydrogenase complex (SDC), as judged by the reduction of Methylene blue, is significantly stimulated in the presence of THNA in a final concentration of 0·002 M (Table II).

Thus the results illustrated in Table II might suggest that the increased succinate oxidation in the presence of THNA may be due to a 'direct' stimulation of succinic dehydrogenase activity.

Although the above findings seem to be in agreement with the increased succinate oxidation by liver homogenates from rats previously treated with THNA[8], they can be considered only as indicative, since the greatest care must be taken in drawing conclusions regarding *in vivo* effects from experiments carried out under *in vitro* conditions.

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Riassunto

Il consumo di ossigeno e la decolorazione del Bleu di Metilene dovuti all'ossidazione del succinato da parte di omogenati di fegato di ratto, appaiono nettamente aumentati in presenza di β-tetraidronaftilammina. Viene prospettata la eventualità che tale sostanza sia in grado di esercitare un'azione attivante «diretta» sul sistema succino-deidrogenasico.

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