Effect of Thyroid Function on Prothrombin Time Response to Warfarin in Rats.

Treatment	Number of Animals	Before Anticoagulant, Prothrombin time, s ± S.E.	First Day on Anticoagulant, Prothrombin time, s ± S.E.	Second Day on Anticoagulant, Prothrombin time, s ± S.E.	Third Day on Anticoagulant, Prothrombin time, s ± S.E.
Normal	10	28·8 ± 1·0	100·0 ± 13·7	190·0 ± 14·5	
Hyperthyroid . . .	10	34·5 ± 1·6*	163·1 ± 17·4*	251·0 ± 19·5**	
Normal	10	31·6 ± 1·3	107·9 ± 7·2	241·1 ± 4·8	424·0 ± 50·0
Hypothyroid . . .	10	34·6 ± 0·7	79·1 ± 6·8*	225·0 ± 7·3	245·0 ± 24·6*

* Difference between means of normal and experimental groups are significant at a probability level $< 0{\cdot}01$.

** Difference between means of normal and experimental groups are significant at a probability level $< 0{\cdot}05$.

The decreased response to the anticoagulant in the hypothyroid state may likewise be due to an increased rate of synthesis or more probably a decreased rate of degradation of prothrombin and other coagulation factors at the cellular level, while the increased metabolic activity in the hyperthyroid state would tend to increase the effect of the anticoagulant by accelerating protein degradation.

Recently, MARTIUS *et al.*[7] have postulated that indirect anticoagulants like dicoumarol may act in a manner similar to thyroxine. Both dicoumarol and thyroxine decrease, while Vitamin K increases, oxidative phosphorylation of mitochondria *in vitro*. The inhibitory activity of coumarin derivatives was found to parallel anticoagulant activity. Dicoumarol has also been shown to inhibit DPNH oxidase activity[8].

These observations are difficult to correlate with the anticoagulant activity of these drugs in the intact animal, where their action is highly specific. Doses which cause a significant decrease of prothrombin levels fail to alter liver functions or change the levels of liver enzymes[9], or cause any other widespread physiological changes one would associate with an uncoupling of oxidative phosphorylation. Hence, while the results demonstrate a synergism between thyroid hormone and indirect anticoagulants, their site or mode of action at the cellular level may not necessarily be identical.

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Zusammenfassung

Die Wirkung von Warfarin, einem Anticoagulans der Dicumarolgruppe, ist erhöht bei hyperthyroiden und vermindert bei hypothyroiden Ratten.

[7] C. MARTIUS, *Proceedings of the 3rd International Congress of Biochemistry*, Brussels 1956 (Academic Press Inc., New York 1956).
[8] J. LOWENTHAL, Bull. Soc. chim. Belg. *65*, 124 (1956).
[9] J. P. GREEN, E. SØNDERGAARD, and H. DAM, Acta pharmacol. et toxicol. *11*, 79 (1955).

The Influence of Inorganic Thiophosphate on the Conversion of Parathion to Paraoxon in Rat Liver Slices

As was shown by various authors[1] parathion is converted in the animal body to its oxygen analogue, paraoxon, which is a powerful anticholinesterase; therefore, this metabolic conversion is of primary importance in the toxic action of parathion.

The identity of the enzymic system involved is not clear; the corresponding dehydrogenase seems to be DPN-linked[2]. GAGE[3] suggested that the system may be identical with that of BINKLEY[4] which catalyses the oxidation of inorganic thiophosphate to orthophosphate and perhaps thiosulphate. If this were true, inorganic thiophosphate when present in a sufficiently high concentration should exhibit an inhibitory influence on the conversion of parathion to paraoxon with this system. Examples of such competition of substrates are known even from *in vivo* experiments, e.g. the oxidation of methyl alcohol was found to be retarded by doses of ethyl alcohol[5] etc.

Therefore, the rate of paraoxon formation was followed in female rat liver slices incubated with various concentrations of parathion in the presence of various concentrations of sodium thiophosphate ($Na_2HPSO_3 \cdot 12 H_2O$). The concentrations of the later substance were held within the range that would be innocuous to the experimental animal when injected (the intravenous LD_{50} of the preparation used was 432 mg/kg for female mice and approximately 800 mg/kg for female rats). The incubation was continued until a steady state was reached in which paraoxon was formed as rapidly as it was split by the A-esterase present[6], so that its concentration in the medium did not change. This steady state concentration, which was reached after 60–90 min in the conditions of the experiment, is a measure of the velocity of paraoxon formation, if the rate of this decomposition is supposed to be constant[7]. To be sure that the con-

[1] D. K. MYERS, B. MENDEL, H. R. GERSHMANN, and J. A. A. KETELAAR, Nature *170*, 815 (1952). – R. L. METCALF and R. B. MARCH, Ann. entomol. Soc. Amer. *46*, 63 (1953). – J. C. GAGE, Biochem J. *54*, 426 (1953).
[2] A. N. DAVISON, Biochem. J. *61*, 203 (1955).
[3] J. C. GAGE, Biochem. J. *54*, 426 (1953).
[4] F. BINKLEY, J. biol. Chem. *181*, 317 (1949).
[5] L. P. KENDAL and A. N. RAMANATHAN, Biochem. J. *54*, 425 (1953).
[6] W. N. ALDRIDGE, Biochem. J. *53*, 117 (1953).
[7] J. KUBIŠTOVÁ, Exper. *12*, 233 (1956).

centration of thiophosphate inside the tissue was constant from the beginning of the experiment, the slices were pre-incubated with thiophosphate before parathion was added to them. Controls with the slices of the same animal without thiophosphate were run simultaneously and the results corrected according to them.

In these conditions, inorganic thiophosphate was found to have a distinct inhibitory influence on the enzymic formation of paraoxon from parathion. The inhibition, however, did not decrease with rising concentration of the substrate, i.e. parathion, if the concentration of the inhibitor, i.e. thiophosphate, was kept constant, as would be expected in the case of the competitive inhibition. On the contrary, the inhibition increased with increasing substrate concentration, as may be seen from the following table:

Parathion concentration	Thiophosphate concentration	Paraoxon steady state concentration	Inhibition in %
$5 \cdot 9 \times 10^{-5}\ M$	0	$6{\cdot}1 \times 10^{-7}\ M$	0
$5 \cdot 9 \times 10^{-5}\ M$	$9{\cdot}44 \times 10^{-3}\ M$	$3{\cdot}0 \times 10^{-7}\ M$	49
$1{\cdot}18 \times 10^{-4}\ M$	0	$8{\cdot}2 \times 10^{-7}\ M$	0
$1{\cdot}18 \times 10^{-4}\ M$	$9{\cdot}44 \times 10^{-3}\ M$	$2{\cdot}2 \times 10^{-7}\ M$	73
$3{\cdot}95 \times 10^{-4}\ M$	0	$2{\cdot}6 \times 10^{-6}\ M$	0
$3{\cdot}95 \times 10^{-4}\ M$	$9{\cdot}44 \times 10^{-3}\ M$	$3{\cdot}1 \times 10^{-7}\ M$	88

If the values found are plotted into a diagram, it appears that the relation of the reciprocal of the reaction velocity, substrate (parathion) concentration and inhibitor (thiophosphate) concentration fit reasonably well with the law of anticompetitive inhibition (Fig. 1 and 2)[8]. It seems that this relation is not the result of some non-specific action of thiophosphate, e.g. the suppression of the general metabolism of the cells; it was found in accordance with BINKLEY[4] that thiophosphate is oxidized by liver slices. In the conditions of the experiments, 20–30% of the original thiophosphate present was found to be oxidized at the end of the experiment.

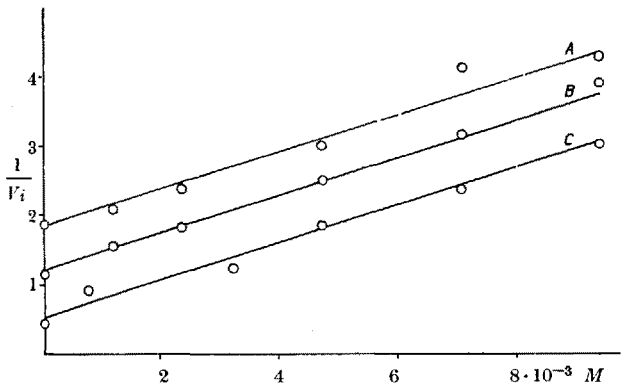

Fig. 1.–Relation between $1/V_i$ and [I] using 3 different concentrations of parathion: *A* $5{\cdot}9 \times 10^{-5}\ M$, *B* $1{\cdot}18 \times 10^{-4}\ M$, *C* $3{\cdot}95 \times 10^{-4}\ M$.

The enhancement of oxygen consumption corresponded well with the oxidation of the sulphur moiety of the molecule to the thiosulphate level, as supposed by BINKLEY[4].

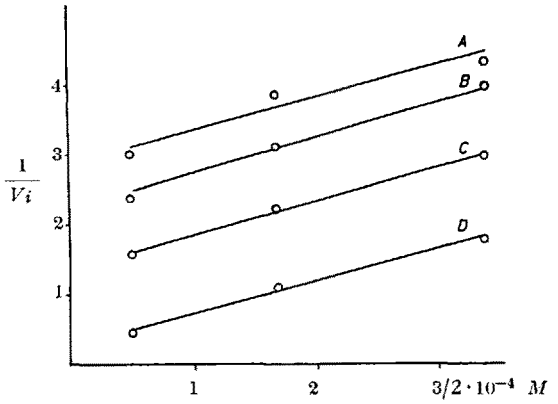

Fig. 2.–Relation between $1/V_i$ and 1/[S] using four different concentrations of thiophosphate: *A* $9{\cdot}44 \times 10^{-3}\ M$, *B* $7{\cdot}08 \times 10^{-3}\ M$, *C* $4{\cdot}72 \times 10^{-3}\ M$ and *D* 0.

Therefore, it seems possible that the above relation reflects the interaction of the substrate and inhibitor with the enzyme responsible for the parathion to paraoxon conversion. The anticompetitive type of inhibition, which was found in this case and which suggests the reaction of the inhibitor with the enzyme-substrate complex does not, however, justify the conclusion that both inorganic thiophosphate and parathion are metabolised by the same enzyme system.

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Zusammenfassung

An Leberschnitten von Rattenweibchen wurde festgestellt, dass anorganisches Thiophosphat die Oxydation von Parathion zu Paraoxon hemmt. Dies hängt von den variierenden Konzentrationen des Substrates und des Inhibitors gemäss der antikompetitiven Hemmung ab.

Ein diuretisch wirksamer Stoff aus Wacholder (*Juniperus communis* L.)

Im Rahmen des Studiums von bewährten Heilpflanzen, welches die Isolierung von neuen biologisch aktiven Substanzen zum Ziele hat, befassten wir uns mit dem diuretischen Prinzip des Wacholders (*Juniperus communis* L.)[1]. Einzelne Fraktionen des Wacholderbeeröles wurden auf ihre diuretische Wirkung geprüft.

Die Wirkung jeder Substanz wurde bei subkutaner Verabreichung an mindestens 12 weissen Ratten, denen vorher 2,5 ml physiologischer Kochsalzlösung verabfolgt worden war, festgestellt und mit der Diurese einer gleich starken Gruppe von Kontrolltieren verglichen. Zur Aus-

[8] D. BURK, quoted by E. R. EBERSOLE, C. GUTTENTAG, and P. W. WILSON, Arch. Biochem. *3*, 399 (1944). – J. B. SUMNER and K. MÜRBÄCK, *The Enzymes* (New York 1950).

[1] V. HEROUT, O. MOTL und F. ŠORM, Chem. listy *43*, 589 (1954); Collection *19*, 990 (1954). – O. MOTL, I. JANKŮ und H. RAŠKOVÁ, Čs. farm. *4*, 240 (1955). – O. MOTL, V. HEROUT und F. ŠORM, Chem. listy *50*, 128 (1956).