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.

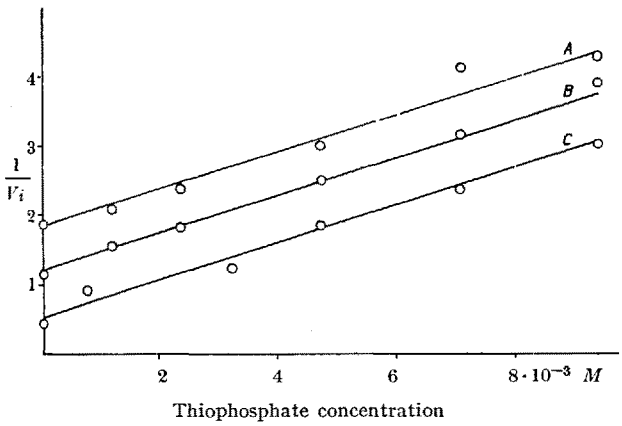

Fig. 1.–Relation between $1/V_i$ and [I] using 3 different concentrations of parathion: *A* $5{\cdot}9 \times 10^{-5}\ M$, *B* $1{\cdot}18 \times 10^{-4}\ M$, *C* $3{\cdot}95 \times 10^{-4}\ M$.

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].

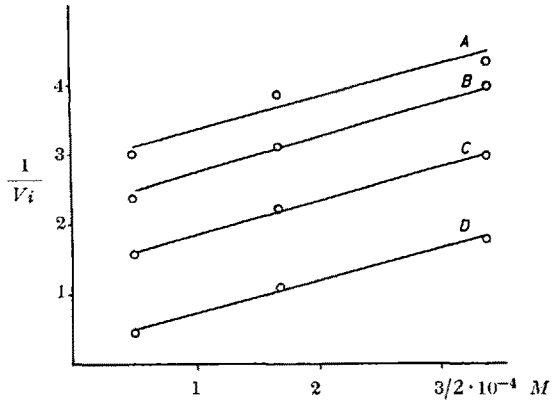

Fig. 2.–Relation between $1/V_i$ and 1/[S] using four different concentrations of thiophosphate: *A* $9{\cdot}44 \times 10^{-3}\ M$, *B* $7{\cdot}08 \times 10^{-3}\ M$, *C* $4{\cdot}72 \times 10^{-3}\ M$ and *D* 0.

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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Institute of Industrial Hygiene and Occupational Diseases, Prague, February 11, 1957.

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).

	Urinmenge als Prozentsatz verabfolgter Flüssigkeit			
	nach 4 h		nach 24 h	
	Durchschnitt	95%-Sicherheitsgrenzen	Durchschnitt	95%-Sicherheitsgrenzen
Kontrolle	14,3 ± 4,1	3,8–24,8	43,8 ± 4,6	32,0– 55,6
Wacholderbeeröl 1,0 ml/kg subkutan	44,0 ± 6,9	26,7–61,0	85,3 ± 7,6	65,8–104,8
Terpinenol-4 0,1 ml/kg subkutan	78,4 ± 7,1	60,2–96,6	157,6 ± 7,1	113,7–201,5

wertung der Wirkung wurde die Menge ausgeschiedenen Urins, ausgedrückt als Prozentsatz verabfolgter Flüssigkeit, nach 4 und nach 24 h bestimmt.

Nach dem Vorversuche mit der Kohlenwasserstofffraktion keine besondere Wirkung zeigten, konzentrierten wir uns ausschliesslich auf die Prüfung der diuretischen Wirksamkeit der sauerstoffhaltigen Fraktion. Das darin enthaltene Terpinenol-4 (1-*p*-menthen-4-ol), Sdp. 89° bei 10 mm Hg, d_4^{20} 0,9259, n_D^{20} 1,4762, $[\alpha_D^{20}] + 28{,}1°$ [2] zeigte im Vergleich mit Wacholderbeeröl, eine gut ausgeprägte diuretische Wirkung (Tabelle).

Dem Terpinenol-4 muss ein grosser Teil der diuretischen Wirkung der Wacholderbeeren zugeschrieben werden.

Die Ergebnisse pharmakologischer Prüfungen und histologischer Untersuchungen nach langdauernder Verabreichung von Terpinenol-4 waren so befriedigend, dass mit der klinischen Prüfung der Substanz begonnen worden ist.

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Summary

It has been demonstrated that terpinenol-4, contained in juniper-berry oil, has a marked diuretic effect. On the basis of this finding, it is assumed that this substance is the proper diuretic factor of juniper-berries.

[2] Ohne Lösungsmittel gemessen.

The Control of Ovary Development in Worker Honeybees (*Apis mellifera*)

Introduction.—It is generally agreed that the ovaries of adequately nourished worker honeybees develop to some extent if the queens are removed from their colonies. For example, HESS[1] found that in nine out of eleven colonies the ovaries of 10% or more of the worker bees developed within a week of the removal of their mated queens.

It has also been shown that ovary development will occur in well-nourished queenless workers kept in small groups in cages at about 30°C, and that the presence of either a living or dead mated queen is sufficient to prevent such development under these conditions[2].

As a result of her experiments HESS[1] suggested that some material substance produced by the queen is circulated in food amongst her workers and is capable of inhibiting the development of their ovaries. The existence of such an inhibitory substance (queen substance) has since been verified[3], and biologically active extracts of it have been obtained from the bodies of queens in a number of organic solvents[4].

In 1954 BUTLER[5] found that bees who had just finished licking the body of their queen offered regurgitated food to other members of their colony more often than other bees of the same colony who had only just examined but not licked their queen. Furthermore, he was able to show that less ovary development occurred amongst a group of well-nourished, queenless worker honeybees to whose food had been added the honeystomach contents of bees who had just licked their queen than amongst a similar group of bees to whose food had been added the honeystomach contents of bees who had had no opportunity to lick a queen for several hours[6]. It thus appeared that queen substance was capable of inhibiting ovary development even when received by worker bees in their food rather than directly from their queen. VOOGD[7], on the other hand, concluded from her own experiments that queen substance was effective *only* when the bees were able to lick it from some object, such as a dead worker bee.

Further experiments have now been made to compare directly the effectiveness of queen substance in inhibiting ovary development in worker bees when supplied to them by these two methods.

Experimental Method.—In July 1956, 216 newly emerged worker honeybees were taken from a normal colony which had made no attempts to swarm nor to supersede its mated, laying queen. The ovaries of 36 of these bees were immediately examined and found to be undeveloped. The remaining bees were divided at random into 5 groups of equal size. Each group of 36 bees was placed in a separate, well-ventilated, perspex cage, 60 mm × 5 mm × 95 mm high. A piece of empty worker brood comb, 40 mm × 40 mm, was fixed in the centre of one of the sides of each cage.

The bees in each cage were supplied continuously with distilled water and also with pollen/candy in separate feeders. The pollen/candy was prepared by mixing icing sugar with 20% by weight of fresh bee-collected pollen and sufficient distilled water to make a stiff paste. The cages of bees were kept in the dark in an incubator at approximately 32°C for 20 days.

An extract of queen substance was prepared as follows: 2 mated laying queens were killed by chilling them in a

[1] G. HESS, Beih. Schweiz. Bienenztg. *1*, 33 (1942).

[2] J. PAIN, *XI. International Beekeeping Congress, Copenhagen 1954*. – A. P. DE GROOT and S. VOOGD, Exper. *10*, 384 (1954).

[3] A. P. DE GROOT and S. VOOGD, Exper. *10*, 384 (1954). – S. VOOGD, Exper. *11*, 181 (1955); *12*, 199 (1956). – C. G. BUTLER, Proc. Roy. Ent. Soc. Lond. (A) *31*, 12 (1956).

[4] A. P. DE GROOT and S. VOOGD, Exper. *10*, 384 (1954). – S. VOOGD, Exper. *11*, 181 (1955); *12*, 199 (1956).

[5] C. G. BUTLER, Trans. Roy. Ent. Soc. Lond. *105*, 11 (1954).

[6] C. G. BUTLER, Proc. Roy. Ent. Soc. London *31*, 12 (1956).

[7] S. VOOGD, Exper. *11*, 181 (1955); *12*, 199 (1956).