	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).

Table I.—Effects of treatments on ovary development in worker honeybees

Cage No.	Treatment	No. bees with developed ovaries	No. bees with undeveloped ovaries	% bees with developed ovaries
1	None	28	2	93
2	Extracted queen . . .	25	9	74
3	Reimpregnated queen.	14	22	39
4	Acetone in food . . .	31	3	91
5	Extract in food . . .	22	10	69

refrigerator. Their bodies were then extracted for 3 h in 10 ml of acetone in a micro-Soxhlet apparatus. This extract of queen substance was subsequently reduced in volume to 2 ml by evaporation and divided into 2 equal parts.

One of the mated laying queens which had been extracted in this way for 3 h was attached to the centre of the comb in one cage. The second mated laying queen which had been similarly extracted was reimpregnated with 1 ml of the concentrated acetone extract obtained from the 2 queens and attached to the centre of the comb in a second cage. (Reimpregnation of this queen's body was achieved by repeatedly bathing it in the extract and allowing the solvent to evaporate at room temperature.) The second 1 ml of the concentrated extract was thoroughly mixed with the pollen/candy (volume 8 ml) in a third cage in which no queen was present. 1 ml of acetone was added to the pollen/candy (8 ml) in a fourth cage, in which there was also no queen. Nothing was added to the food of the bees in the remaining cage nor was a queen present.

After 20 days all the bees remaining alive were killed and the degree of development of their ovaries determined. Five degrees of ovary development similar to those used by HESS[1] were recognised: 'no development', 'possible very slight development', 'some development', 'strong development', 'very strong development'. Since in summer in an average, apparently normal colony headed by a mated laying queen worker bees with ovaries showing 'no development' and 'possible slight development' are regularly found, these two degrees of development were classified together as 'undeveloped' and the remaining three degrees of development were considered together as 'developed'. This classification was also employed by DE GROOT and VOOGD[8].

Results.—The results obtained are shown in Tables I and II.

Discussion.—VOOGD[9] showed that the ovaries of those queenless worker honeybees who licked an object which had been impregnated with an acetone extract of a queen honeybee developed significantly less ($P < 0{\cdot}01$) than those of bees who did not lick the impregnated object; but, when an extract of a queen was added to their food the number of bees with developed ovaries was less obviously reduced although, if one uses a 'single-tail test' the difference which she observed is just on the 5% level (i.e. if one ignores the possibility of a true result in the 'wrong' direction). The results given in Table I in this paper show that an acetone extract of a mated queen, whether given in food or on the body of a dead, previously extracted, queen, inhibited the development

Table II.—Analysis of results in Table I[2]

Comparison of difference in number of bees with and without developed ovaries in cages	Value of P
1 and 2	–
1 and 3	$< 0{\cdot}001$
1 and 4	–
1 and 5	$< 0{\cdot}04$
2 and 3	$< 0{\cdot}01$
2 and 4	–
2 and 5	–
3 and 4	$< 0{\cdot}001$
3 and 5	$< 0{\cdot}03$
4 and 5	$< 0{\cdot}05$

of the ovaries of worker honeybees (cages 1 and 3, 4 and 5, 2 and 3, 3 and 4). Therefore, if the data of VOOGD[9] and of BUTLER[6], as well as those in the present paper, are considered together it seems that an extract of queen substance (obtained in acetone from a queen) taken orally by queenless well-nourished worker honeybees, either in food or from the body of a dead bee, does have a definite inhibitory influence on the development of their ovaries.

It appears, however, from comparison of the degree of ovary development of the bees in cages 3 and 5, that presentation to the bees of the extract on the body of a previously extracted queen (which they were seen to examine and lick frequently) was more effective in inhibiting ovary development by these worker bees than was the addition of an equal quantity of the same queen extract to the food of a similar group of bees. It is possible that this difference may have been due to the bees receiving more queen substance when it was offered on the body of a dead, extracted, queen than when it was offered in food, or that it may conceivably have been due to the bees receiving a psychological stimulus when they licked an object impregnated with queen substance. This latter possibility seems rather unlikely, however.

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Zusammenfassung

Während Übereinstimmung darüber erreicht worden ist, dass die Arbeitsbienen eine ovarienhemmende Substanz von ihren Königinnen erhalten, ist es weder klargestellt worden, wie diese Substanz unter die Arbeitsbienen verteilt wird, noch ob die Königin eine psychische Wirkung auf sie ausübt.

Wenn die Versuchsresultate von VOOGD[9] und BUTLER[6] sowie die Resultate dieser Arbeit zusammen betrachtet werden, scheint es klar, dass ein Extrakt dieser Substanz, wenn er an weisellose Arbeiterinnen *verfüttert* wird, eine deutliche Hemmwirkung auf die Ovarienentwicklung ausübt. Die Versuchsresultate deuten jedoch darauf hin, dass diese Substanz wirkungsvoller ist, wenn sie den Arbeitsbienen auf dem Körper einer toten Biene, oder dem Modell einer Biene, dargeboten wird, als wenn man sie dem Futter beimischt. Es scheint aber unwahrscheinlich, dass dies auf eine psychische Reizwirkung zurückzuführen ist[10].

[8] A. P. DE GROOT and S. VOOGD, Exper. *10*, 384 (1954).
[9] S. VOOGD, Exper. *12*, 199 (1956).
[10] D. B. CARLISLE and C. G. BUTLER, Nature *177*, 276 (1956).