counter for fluids (Twentieth-Century-Electronics, Type M. 6) in order to dosage the radiations emitted by the ^{35}S contained in the various tissue extracts.

Values obtained in the various estimations have been worked out so as to express the 'concentration index', that is the relationship between the relative specific activity (R. S. A.) of repair tissue and that of normal skin and subcutaneous tissue.

Results. Values obtained through the present experiments are reported in the Table and in the Figure.

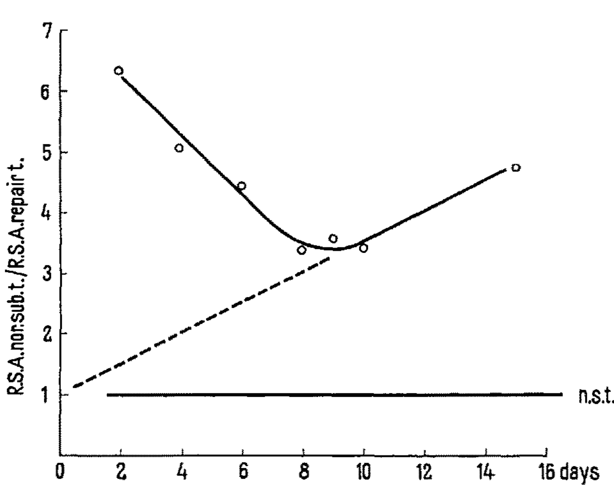
Discussion. The ability of repair tissue to take up a larger amount of ^{35}S that is done by normal skin and subcutaneous tissue (these data being in perfect agreement with those of LAYTON[13]) points a considerably increased metabolism of ^{35}S in the repair tissue.

The very high initial values might find a logical explanation by admitting that ^{35}S in repair tissue is not at first incorporated in the SO_4-group of chondroitinsulphuric acid, since the mechanism of its incorporation surely requires a comparatively long time, but bound to some substance or group of substances present in the tissue, which would take it up in considerable quantities and in a relatively labile way. It is probably only with time, probably in relation to the changes observed by several authors both in the fibrocellular part (DUMPHY *et al.*[14], SYLVÉN[15] etc.) and in the ground substance (SYLVÉN[16], CAMPANI *et al.*[17] etc.) of repair tissue, that ^{35}S pass from 'uptake' to 'incorporation'.

Relative specific activity (R. S. A.) of normal cutaneous and subcutaneous, and of repair tissue of skin wounds at various stages of the healing process.

Guinea pig No.	Tissue type	R. S. A. Normal cutaneous tissue	R. S. A. Repair tissue	Concentration index
1	2-days rep. t.	0·0432825	0·2732230	6·31
2	4-days rep. t.	0·0171934	0·0874926	5·08
3	6-days rep. t.	0·0202942	0·0903792	4·45
4	8-days rep. t.	0·0783679	0·2672030	3·40
5	9-days rep. t.	0·0163303	0·0582180	3·56
6	10-days rep. t.	0·0221658	0·0758071	3·42
7	15-days rep. t.	0·0164203	0·0778325	4·74

$$\text{R.S.A.} = \frac{\mu c \text{ observed}}{\text{sample weight}} \cdot \frac{\text{body weight}}{\mu c \text{ injected}}.$$



Behaviour of the ^{35}S content in the repair tissue of cutaneous wounds

The lowest values of ^{35}S found between the sixth and tenth day are perhaps the expression of the metabolism cycle of chondroitinsulphuric acid of repair tissue.

The amount of ^{35}S incorporated into the chondroitinsulphuric acid of wound repair tissue might probably be expressed as a straight line which, starting from very low values, joins the curve of the Figure by the eighthtenth day and, from that point on, follows it. The large amount of ^{35}S found in the early days might in fact be the result of the ^{35}S uptake plus incorporation, since the method used in these experiments does not allow such differentiation.

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Riassunto

1) Il «tessuto di riparazione» delle ferite cutanee ha la proprietà di «fissare» una quantità di S^{35} notevolmente più grande di quella captata dal connettivo sottocutaneo.

2) La quantità di S^{35} fissata varia durante le diverse fasi del processo di guarigione delle ferite: è molto alta nei primi giorni; scende quindi sino al sesto-decimo giorno, epoca nella quale raggiunge i valori più bassi, e risale poi gradualmente sino al quindicesimo giorno (ultimo giorno preso in considerazione nelle presenti ricerche).

[13] L. L. LAYTON, Proc. Soc. exp. Biol. Med., N. Y. *76*, 596 (1951).
[14] J. E. DUMPHY and K. N. UDUPA, New England J. Med. *253*, 487 (1955).
[15] B. SYLVÉN, Acta chir. scand. *86*, Suppl. 66 (1941).
[16] B. SYLVÉN, Klin. Wschr. *17*, 1545 (1938).
[17] M. CAMPANI and O. REGGIANINI, J. Path. Bact. *62*, 563 (1950).

Queen Recognition by Worker Honeybees (*Apis mellifera* L.)

Introduction. The sight, sound and scent of a queen honeybee do not inhibit either queen rearing by worker bees[1–3] or the development of their ovaries[4–10]. Inhibition occurs only when the bees can touch their queen and obtain 'queen substance' from her[3,10].

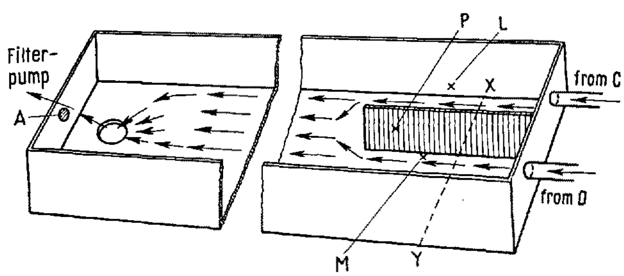
Many beekeepers believe that a queen has an odour attractive to workers. If this true it would be important because the odour would help workers to find the queen and get queen substance, but the only experimental evidence even suggestive of a queen odour seems to be that provided by LECOMTE[11], VOOGD[12], and PAIN[13].

[1] F. HUBER, *Nouvelles observations sur les abeilles* (1814), 2nd ed. Translation (1926), published by Dadant, Hamilton, Ill.
[2] A. MÜSSBICHLER, Z. vergl. Physiol. *34*, 207 (1952).
[3] C. G. BUTLER, Trans, R. ent. Soc., London *105*, 11 (1954).
[4] A. P. DE GROOT and S. VOOGD, Exper. *10*, 384 (1954).
[5] J. PAIN, Insectes sociaux *1*, 59 (1954).
[6] J. PAIN, Insectes sociaux *2*, 35 (1955).
[7] S. VOOGD, Exper. *11*, 181 (1955).
[8] C. G. BUTLER, Proc. R. ent. Soc., Lond. [A] *31*, 12 (1956).
[9] C. G. BUTLER, Exper. *13*, 256 (1957).
[10] C. VERHEIJEN-VOOGD (née S. VOOGD), Z. vergl. Physiol. *41*, 527 (1959).
[11] J. LECOMTE, 14th Internat. beekeep. Congr. Paper 13 (1951).
[12] S. VOOGD, Exper. *12*, 199 (1956).
[13] J. PAIN, C. R. Acad. Sci., Paris *240*, 670 (1955).

The following experiments attempted to find whether a mated queen honeybee has an odour that is attractive to workers and, if so, whether it is produced by her queen substance or something else.

Method. Experiments were made in red light to which workers do not respond, using an olfactometer (Fig. 1) designed by FREE and BUTLER[14,15]. Air was drawn through it as indicated. 'C' and 'D' each contained a different test material, and the air-flow through them was kept as constant and equal as possible.

One worker was tested at a time. It was introduced through 'A' and moved forward against the air stream until it reached the partition 'P' when it had a choice of chambers 'L' and 'M'. When it passed the line 'XY' the chamber concerned was recorded. The bee was then removed, the containers 'C' and 'D' interchanged and another bee put in. Twenty bees at least, taken at random from normal queenright colonies, were used in each experiment. The whole apparatus was washed between experiments and whenever a bee excreted in it. No bee was used more than once.



Olfactometer arrows indicate direction of air flow from the containers C and D whole apparatus made od 'Perspex'.

Materials tested. All the queens and workers used in containers 'C' and 'D' were dead. All the queens, except Nos. 141 and 2, had been dried for at least 4 months. The sample of pure crystalline queen substance was extracted and purified by Dr. R. K. CALLOW and Miss N. C. JOHNSTON of the National Institute for Medical Research, Mill Hill, London, using the method described by BUTLER, CALLOW, and JOHNSTON[16].

'Extracted queens' were queens from which queen substance was removed in ethanol in a micro-Soxhlet apparatus for 48 h.

Solutions in ethanol of pure queen substance, of the materials extracted from whole mated queens, and pure ethanol, were placed on filter-paper in the containers 'C' and 'D'.

Results and conclusions. The results, summarised in the Table, support the idea that queens, alive or dead, attract worker bees at a distance.

FREE and BUTLER[14] showed that even a large number of newly killed worker bees has no odour attractive to other workers; Experiment 1 supports this conclusion.

Of 6 mated queens tested individually (Experiments 2, 3, 4, 5, 9, 10) only one (No. 3) attracted individual workers towards it in preference to the odours of either dead workers, queens that had been extracted in ethanol, or nothing. Even a freshly killed queen (No. 141) was not attractive, but when two or more of these 6 queens were used together their combined odour was atractive (Experiments 6, 7, 8, 11). Therefore, queen honeybees not only produce an odour attractive to worker bees, but this

Results of olfactometer experiments

Exp. No.	No. bees tested	Material in 'C'	Material in 'D'	No. bees that moved towards 'C'	Significance
1	80	12 workers	nil	38	—
2	40	mated queen 1	nil	16	—
3	40	mated queen 141	nil	19	—
4	20	mated queen 2	1 worker	13	—
5	20	mated queen 3	1 worker	15	$P < 0{\cdot}05$
6	80	mated queens 1, 2	4 workers	52	$P < 0{\cdot}01$
7	40	mated queens 141, 1, 2	6 workers	34	$P < 0{\cdot}01$
8	80	mated queens 1–6	nil	63	$P < 0{\cdot}001$
9	20	mated queen 4	extracted mated queen 7	12	—
10	20	mated queen 5	extracted mated queen 8	13	—
11	20	mated queens 1–6	extracted mated queens 9–14	19	$P < 0{\cdot}001$
12	40	2 ml ethanol extract (A) mated queens (wet)	2 ml ethanol (wet)	27	$P < 0{\cdot}05$
13	60	2 ml ethanol extract(A) [a]mated queens (dry)	2 ml ethanol (dry)	35	—
14	40	2 ml ethanol extract(A) [b]mated queens (dry)	2 ml ethanol (dry)	21	—
15	20	2 ml ethanol extract(B)mated queens (wet)	2 ml ethanol (wet)	20	$P < 0{\cdot}001$
16	20	2 ml ethanol extract(B)[c] mated queens (dry)	2 ml ethanol (dry)	15	$P < 0{\cdot}05$
17	40	1·0 mg crystalline queen substance in 2 ml ethanol (wet)	2 ml ethanol (wet)	21	—
18	40	1·3 mg crystalline queen substance	nil	20	—

Extracts were placed on filter-paper and either used immediately before the solvent had evaporated (= wet) or after it had evaporated (= dry).
[a] = 2 h after Experiment 12; [b] = 17 h after Experiment 12; [c] = 1/2 h after Experiment 15.

[14] J. B. FREE and C. G. BUTLER, Behaviour *7*, 304 (1955).
[15] C. G. BUTLER, Proc. R. ent. Soc., Lond. [A] *35*, in press (1960).
[16] C. G. BUTLER, R. K. CALLOW, and N. C. JOHNSTON, Nature *184*, 1871 (1959).
[17] C. G. BUTLER and D. A. GIBBONS, J. Insect. Physiol. *2*, 61 (1958).
[18] C. G. BUTLER, unpublished work.

persists in dead queens dried for more than 4 months. The responsible substance is removed by extraction in ethanol (Experiment 11) and ethanol extracts also attract workers (Experiments 12, 15, 16).

Although the attractive odour persists for months on dried queens, it soon disappears when ethanol extracts are evaporated on filter-paper (Experiments 13, 14, 16). Presumably, therefore, the attractive odour is only gradually released from a queen's body, where it is perhaps being retained by her body waxes.

Queen substance can also be extracted from queens' bodies in ethanol[17] but pure queen substance does not have an odour that attracts workers even when it is offered in relatively large amounts – i.e. certainly several times the quantity present on the body surfaces of ten mated queens together[18] – (Experiments 17, 18) and on a filter-paper surface several times larger than the combined surface areas of 10 queens (Experiment 17). It is clear, therefore, that queen substance is not responsible for the attractive odour. The nature and origin of the responsible substance is unknown; its odour probably serves to attract workers to a queen, and so helps them to find queen substance, but the substance does not itself inhibit queen rearing[1–3].

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Zusammenfassung

Begattete und sogar getrocknete Bienenköniginnen haben einen für Arbeiterinnen anziehenden Geruch, der jedoch, mit einer Ausnahme, nur bei Darbietung von mehr als einer Königin demonstriert werden konnte. Der anziehende Geruch ist wie «Queen Substances» mit Alkohol extrahierbar, stammt aber nicht von der «Queen Substance». Die Herkunft der riechenden Substanz ist nicht bekannt. Der Königinnengeruch dient wahrscheinlich zur Anlockung der Arbeiterinnen und damit zur Erleichterung des Auffindens der «Queen Substance», die allein die Königinnenaufzucht zu verhindern vermag, wenn sie in genügender Menge vorhanden ist.

Pressure Perception in *Ostariophysi*

Concerning the site of pressure perception in the *Ostariophysi*, there is much controversy in the literature. According to some authors, the Weberian ossicles and the labyrinth play a major role in the perception of changes of hydrostatic pressure[1–3]; according to others, pressure perception is mainly localized in the swimbladder wall[4–8], where abundant nerve endings have been described histologically[9–13], or in the surrounding tissues. It seems likely that both principles are realized, each of them under its own circumstances[14]. The following observations add new evidence to this problem.

Among a number of blinded minnows (*Phoxinus laevis*) from which both mallei were removed, some were precisely equilibrated hydrostatically. These fishes stayed almost motionless in the middle of the water, thus offering good opportunity to observe even slight reactions to pressure change. For some fishes, pressure increase was used as a stimulus, while pressure decrease was applied to others. In both cases, spontaneous reactions occurred on pressure changes of only a few (4–7) cm of water pressure (rate of pressure change: about 70 cm/min). At each pressure increase, the fish showed a sudden, quiet ascend (downward beating of pectoral fins) over several cm. This response was always preceded by an interval of 5–8 sec without any visible signs of pressure perception or passive displacement. Frequently, the active ascent occurred without any observable rotation of the animal around a transverse axis; the fish body remained quite horizontal. At each pressure decrease, a similar reaction in the opposite direction was observed. These observations show that a blind minnow without functioning Weberian apparatus is still sensitive to pressure changes of a few cm of water pressure, and that the Weberian ossicles play no main role in the maintenance of hydrostatic equilibrium.

It has been reported that blinded minnows can easily be trained to react to slight pressure changes, both pressure increase and pressure decrease (rate of pressure change about 120 cm water/min[15]). Determination of a threshold has not been tried, but a pressure change of $^1/_2$–1 cm of water pressure evoked still a clear response (feeding reaction). After removal of both mallei, these reactions were abolished, even when the pressure increase used as a training stimulus was put up to 40 cm. When repeating these training experiments, we found in 7 blind fishes without mallei, after a long series of trials (at least 40 stimulus-punishment or stimulus-food combinations) positive conditioning (escape or feeding reactions, respectively) to a pressure increase or decrease of only 7–8 cm water pressure (total number of combinations: 1100). With a pressure change of 5–6 cm, the percentage of positive reactions went down to 50%, and this value is evidently close to the threshold, especially in the case of pressure decrease. The results of our training experiments, therefore, agree well with the conclusion drawn from our earlier equilibrium experiments.

Summarizing, we may conclude that in *Phoxinus laevis* the Weberian apparatus and the labyrinth play no main part in pressure perception underlying the maintenance of hydrostatic equilibrium.

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Zusammenfassung

Blinde, malleuslose Elritzen beantworten unter günstigen Umständen schon Schwankungen von 4–7 cm Wasserdruck mit spontanen Reflexbewegungen. Druckzunahme löst – nach bestimmter Latenzzeit – Aufwärtsbewegung aus, Druckabnahme Abwärtsbewegung. Auch in langfristigen Dressurversuchen wurde entsprechende Reaktion auf Schwankungen bis zu 5–6 cm Wasserdruck erzielt.

[1] E. GUYÉNOT, Bull. sci. France Belg. *42*, 203 (1909).
[2] E. v. KOKAS, Zool. Jb. Physiol. *52*, 179 (1932).
[3] F. P. MOEHRES, Z. vergl. Physiol. *28*, 1 (1940).
[4] S. BAGLIONI, Z. allg. Physiol. *8*, 1 (1908).
[5] K. KUIPER, Proc. K. Akad. Wet., Amsterdam *18*, 572 (1916).
[6] G. FRANZ, Z. vergl. Physiol. *25*, 193 (1937).
[7] C. S. KOSHTOYANTZ and P. D. VASSILENKO, J. exp. Biol. Med. *14*, 16 (1937).
[8] P. D. VASSILENKO and M. N. LIVANOV, Bull. biol. med. Exp. URSS. *2*, 264 (1936).
[9] D. DEINEKA, Z. wiss. Zool. *78*, 149 (1904).
[10] D. SCEVOLA, Monit. zool. ital. *48*, 283 (1938).
[11] A. STEFANELLI, Monit. zool. ital. *55*, 1 (1946).
[12] B. TERIO, R. C. Accad. Napoli *14*, 17 (1948).
[13] A. ABRAHAM and A. STAMMER, Ann. biol. Univ. hungar. *2*, 345 (1954).
[14] S. DIJKGRAAF, Exper. *6*, 189 (1950).
[15] S. DIJKGRAAF, Z. vergl. Physiol. *28*, 389 (1941).