The Iceni Microscopy Study Group
Honey bee dissection - 3rd May 2014

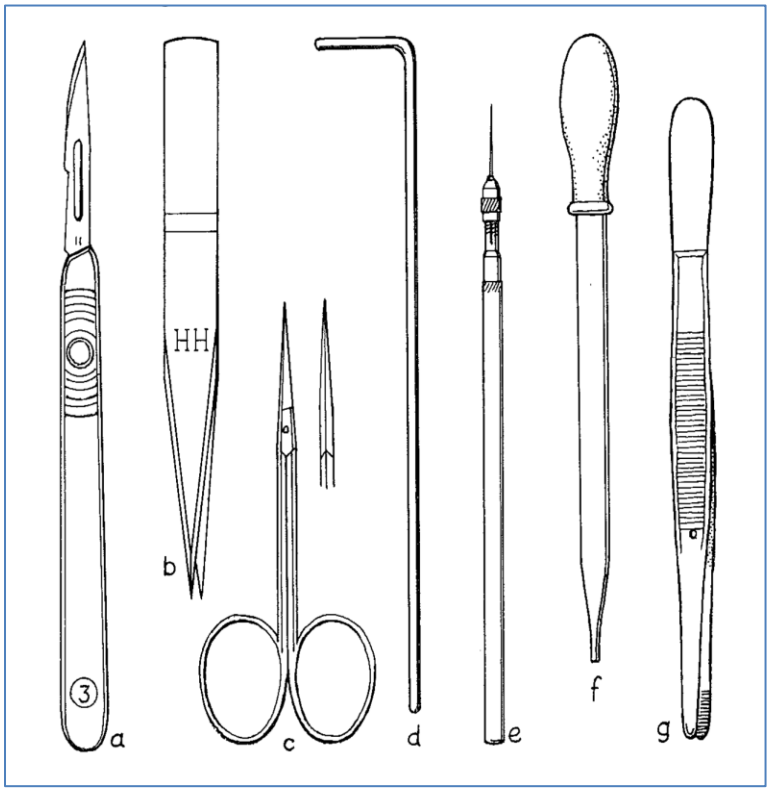
Although the Iceni committee's original brief was *Worker bee dissection and mounting*, it has now agreed that there is enough in the dissection alone to fill time we have. If anyone wants to go on into the mounting of the body parts, I should be delighted to join them in that.
"Why dissect bees?" asks Eric Marson and then tells us: "Preserved insects show only a limited amount of detail when examined under a microscope because little light passes through them or is reflected from them. If insects are softened, cleaned, dehydrated and cleared, whole mounts can be prepared which will allow light to pass through them; but many whole mounts can only show the detail of a limited number of parts in a satisfactory way. This is because, even with careful arrangement, the parts of the whole insect may cover each other." From a study of the dissection and of the parts, we can learn how the bee works and so understand what it does and how it does it.

From personal experience and disappointment, I know that it takes commitment fully to research and prepare for an Iceni session before the event. And I mean that all the members of the class need to research and prepare, not just the presenter! Those who do prepare will understand the tuition given very much better and may actually finish the work. I say this because, to my shame, I have a box of 'work in progress' that, frankly, I am unlikely ever to complete. *To get the most out of this day, please read the following and prepare the necessary equipment.*
For those who have already dissected bees, we have some queen wasps; it would be interesting to compare the insects.

Those who don't have a copy of Dade's *Anatomy and Dissection of the Honeybee* can find the COLOSS BEEBOOK paper by clicking on: http://www.ibra.org.uk/articles/Standard-methods-for-Apis-mellifera-anatomy-and-dissection and then on **Open Access** to download the paper but you shouldn't need to do that as I have copied much of the relevant sections here.
[Enlarged and laminated versions of the plates to accompany this for use at the laboratory bench are available from the IBRA Bookshop by clicking **here**. The paper reproduces much of Dade, explains how to do the work and says what is needed to be able to do it; it is slightly confusing that in the original the 'how' starts on page 3 but continues on page 25.]

Equipment needed (in addition to a dissecting (stereo) microscope):
Only a few instruments are needed (*see below*), but it is important that three of them should be of exactly the right kind.
1. The scissors should be 'cuticle' scissors, with blades not less than 90 mm in length, and not much longer, with very fine points which cut cleanly right up to their tips. Looked at sideways, they should be very slim. Check them using your dissecting microscope. For sharpening advice, see: http://www.wikihow.com/Sharpen-Scissors.

2. Forceps need to have very fine points and grip very firmly at their extreme tips. *Use your dissecting microscope to check the tips of your own forceps and, if necessary, to adjust them.*



3. A very sharp and finely-pointed knife is the third important tool. The Swann-Morton scalpel No. 3 with replaceable Swann-Morton No. 11 blades of the correct shape is widely used.
4. A pair of needles, mounted in metal handles.
5. Pasteur pipettes *(the usual disposable sort)*.
6. Coarse forceps.
7. A stout wire, bent into an L-shape, its long limb being about 150 mm long and the short one 20 mm long. The best material is brass rod, 5 mm thick. This brass rod has to be heated. **Alternatively, (and better) use an electric soldering iron.**
8. Two or three dissecting dishes need to be made from flat round metal tins about 75 mm in diameter. *(Honey jar lids serve but are a little small. Better, perhaps, a* ***metal*** *polish tin.)* These are to be filled with melted beeswax to within 6 mm of the top of the rim; the wax must then be allowed to solidify.
9. The surface of the wax has to be re-melted frequently. While this may be done most conveniently by turning a Bunsen burner flame downwards over the dish, *we generally use a spirit lamp.*

The dissection should be carried out in liquid to support the internal organs and prevent drying but water cannot be used by itself as will not wet the hairy bodies of bees. A mixture of 30% methylated spirit and 70% water is recommended; Meyer suggests a 50% alcohol solution (presumably IPA).

With the exception of queens and drones, it is easier to dissect preserved bees than freshly killed ones.

Insects are usually dissected dorsally (with their backs uppermost).

Go to page 6.

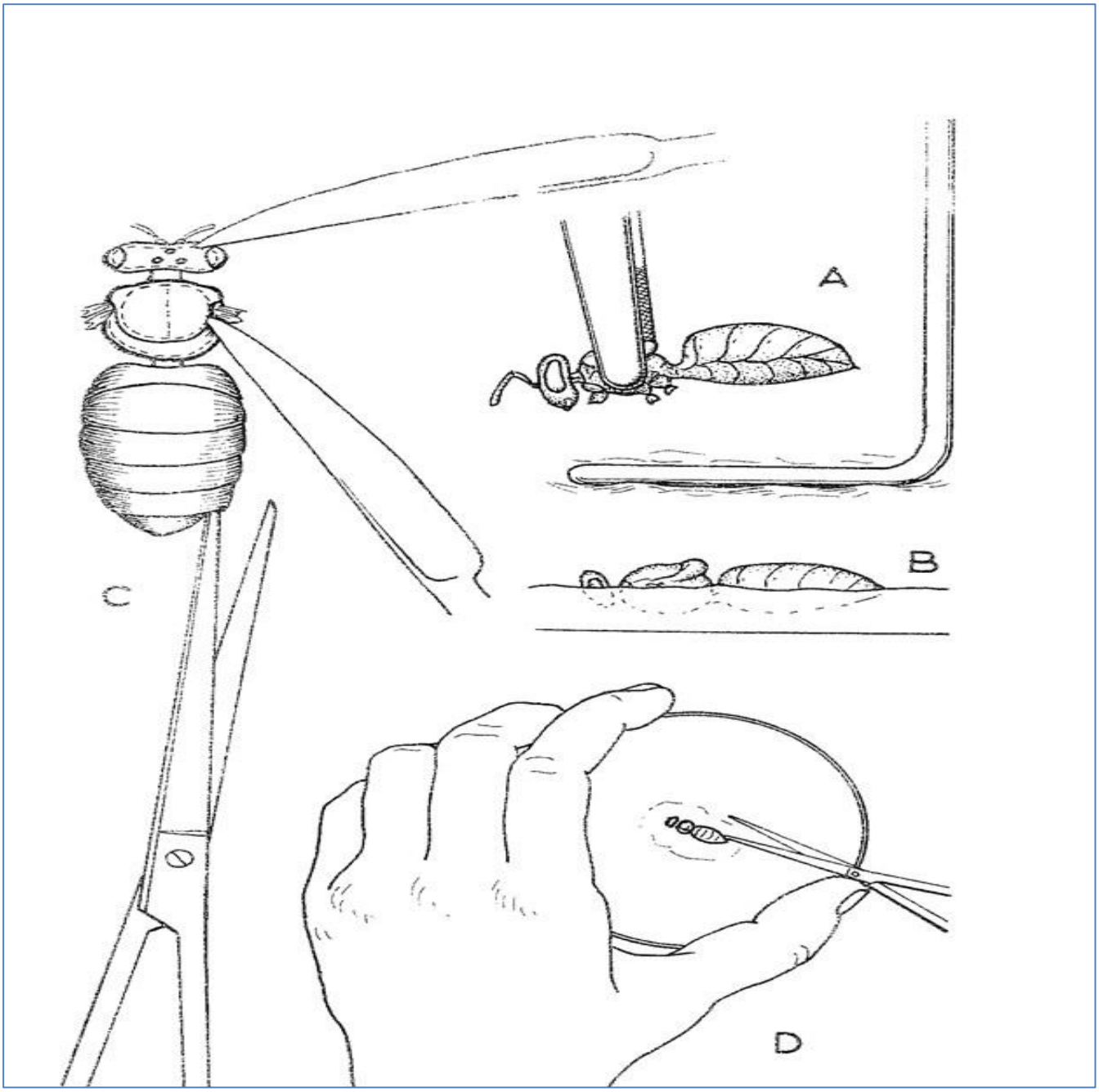


Plate 1
Preparation for dissection and use of instruments.
A, Anchoring bee in pool of melted wax.
B, Bee fixed in wax.
C, Methods of using scissors and scalpel.
D, Holding dissecting dish and steadying scissors against thumb.

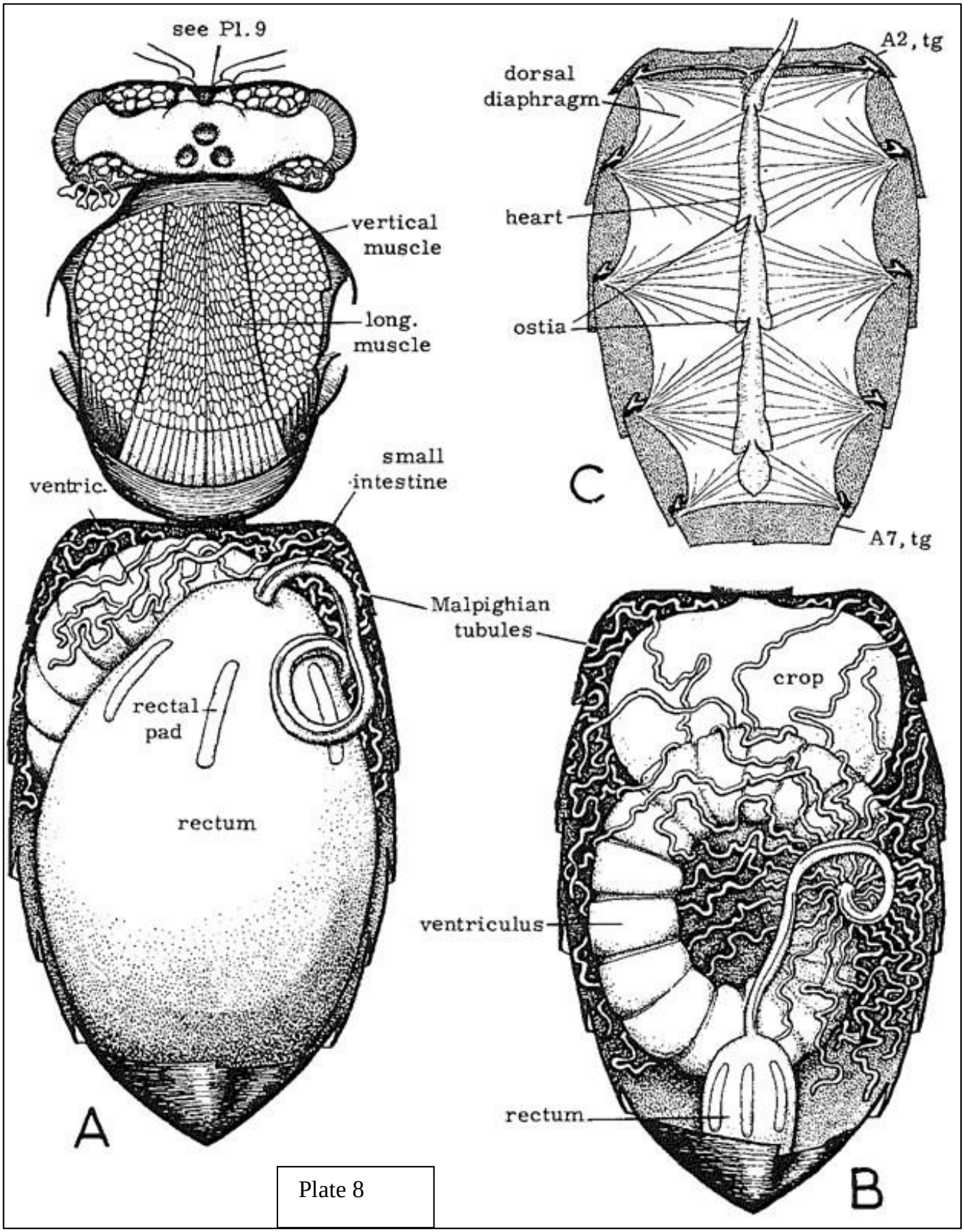

see Pl. 9
vertical muscle
long. muscle
ventric.
small intestine
Malpighian tubules
rectal pad
rectum
A
A2, tg
dorsal diaphragm
heart
ostia
C
A7, tg
crop
ventriculus
rectum
B

Plate 8

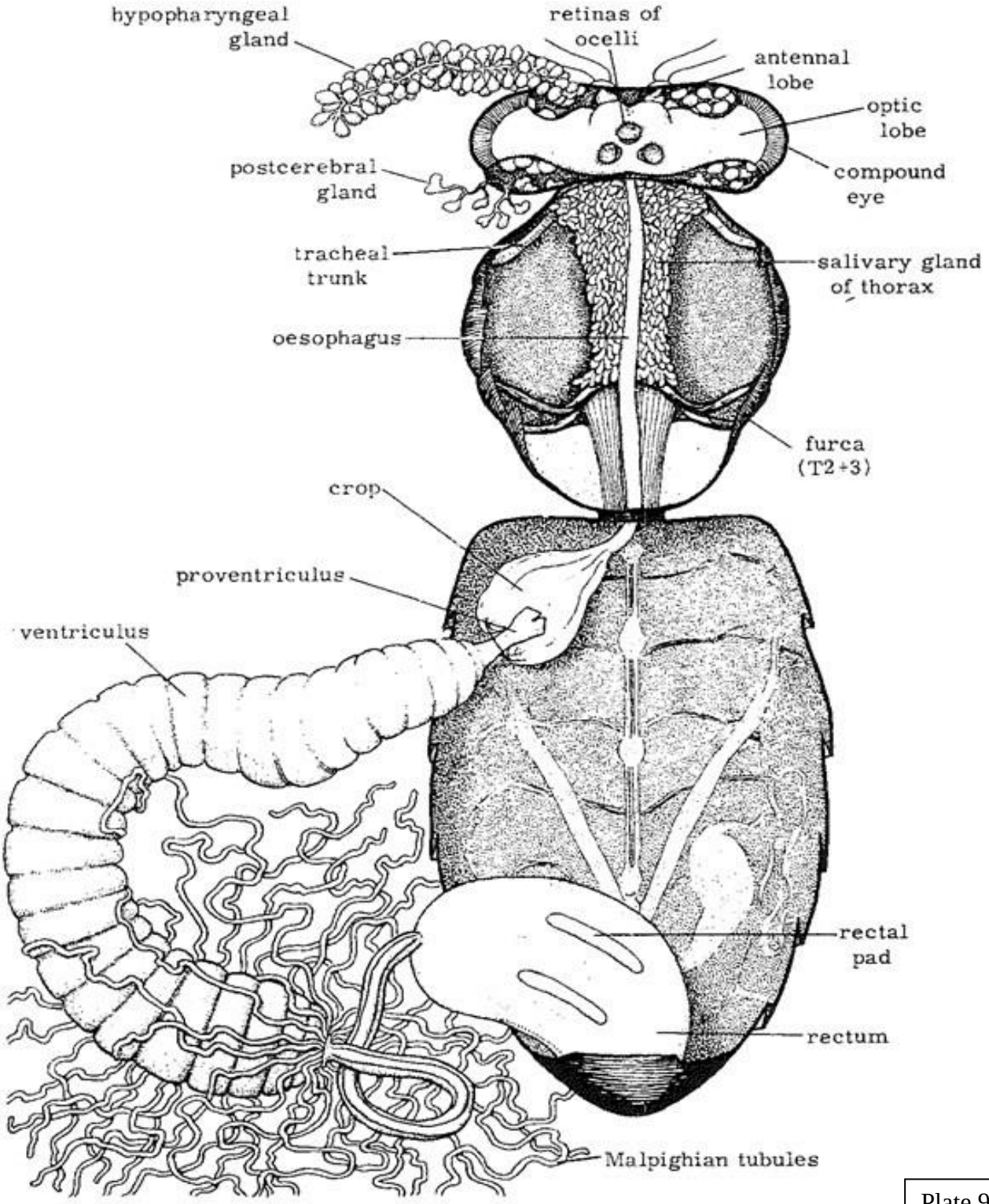

hypopharyngeal gland
retinas of ocelli
antennal lobe
optic lobe
compound eye
postcerebral gland
tracheal trunk
salivary gland of thorax
oesophagus
furca (T2+3)
crop
proventriculus
ventriculus
rectal pad
rectum
Malpighian tubules

Plate 9

1. Take a freshly killed or preserved worker, and cut off its wings, legs and proboscis with scissors; put these (and any other parts detached later) into iso propyl alcohol until there is time to process them further.
2. If the bee has been preserved, dry it as thoroughly as possible by rolling it gently on blotting paper.
3. Consult Plate 1 above.
4. Seize the bee by the thorax, back uppermost with coarse forceps.

5. Take the bent wire with the other hand.
6. Heat its short limb in a flame.
7. Apply the hot wire to the wax in the middle of the dissecting dish, thus forming a small pool of melted wax somewhat bigger than the bee.

Or use your soldering iron to make a pool.

8. Place the bee quickly in the pool, hold it there with the cool end of the wire, and withdraw the forceps.
9. Reheat the wire and melt a little of the wax near the sides and ends of the bee
10. Push this melted wax against the body, so that it piles up slightly and makes good contact. This will ensure that the specimen is firmly anchored and will not come adrift during dissection. The bee should be sunk nearly halfway in the wax. The diagram above shows this operation, and the embedded bee. All this must be done quickly and without overheating the insect. A better posture is obtained if, when lowering the bee into the pool of wax, the tip of the abdomen touches the wax first; it will adhere, and then the body can be drawn forward slightly, thus stretching the abdomen a little. Whole insects, or parts like the head, can be prepared in any posture that may be desired.
11. Pour insect saline or dissecting fluid on at once, enough to cover the bee.
12. Place the dish under the microscope; it then is ready for work.
13. Focus the microscope and adjust the spot lamp.
14. Instruments should be laid out ready for use.

DISSECTION OF THE WORKER BEE

The general dissection

Plates 8 & 9 above illustrate the dissection of the whole body, from the dorsal aspect, in three stages. In practice it will be found convenient to begin with the abdomen, and to complete its examination before starting on the thorax or head, and the directions which follow are arranged in that order.

For the study of the alimentary canal, the heart, the tracheal sacs, and the ovaries in both normal and laying workers, bees should be dissected immediately after killing. These organs become brittle in preserved specimens. For all other purposes, preserved bees are much more satisfactory. Bees with fully distended abdomina will show the heart and nervous system to the best advantage. The specimen is prepared, fixed in the dissecting dish, back uppermost, and covered with dissecting fluid, as described in Section 3.1. and illustrated in Plate 1.

3.2.2. The abdomen

For a right-handed person, turn the dish so that the head of the insect points to 10 o'clock. During operations, remember to move the dish to suit the convenience of the right hand and scissors (see section 3.4.).

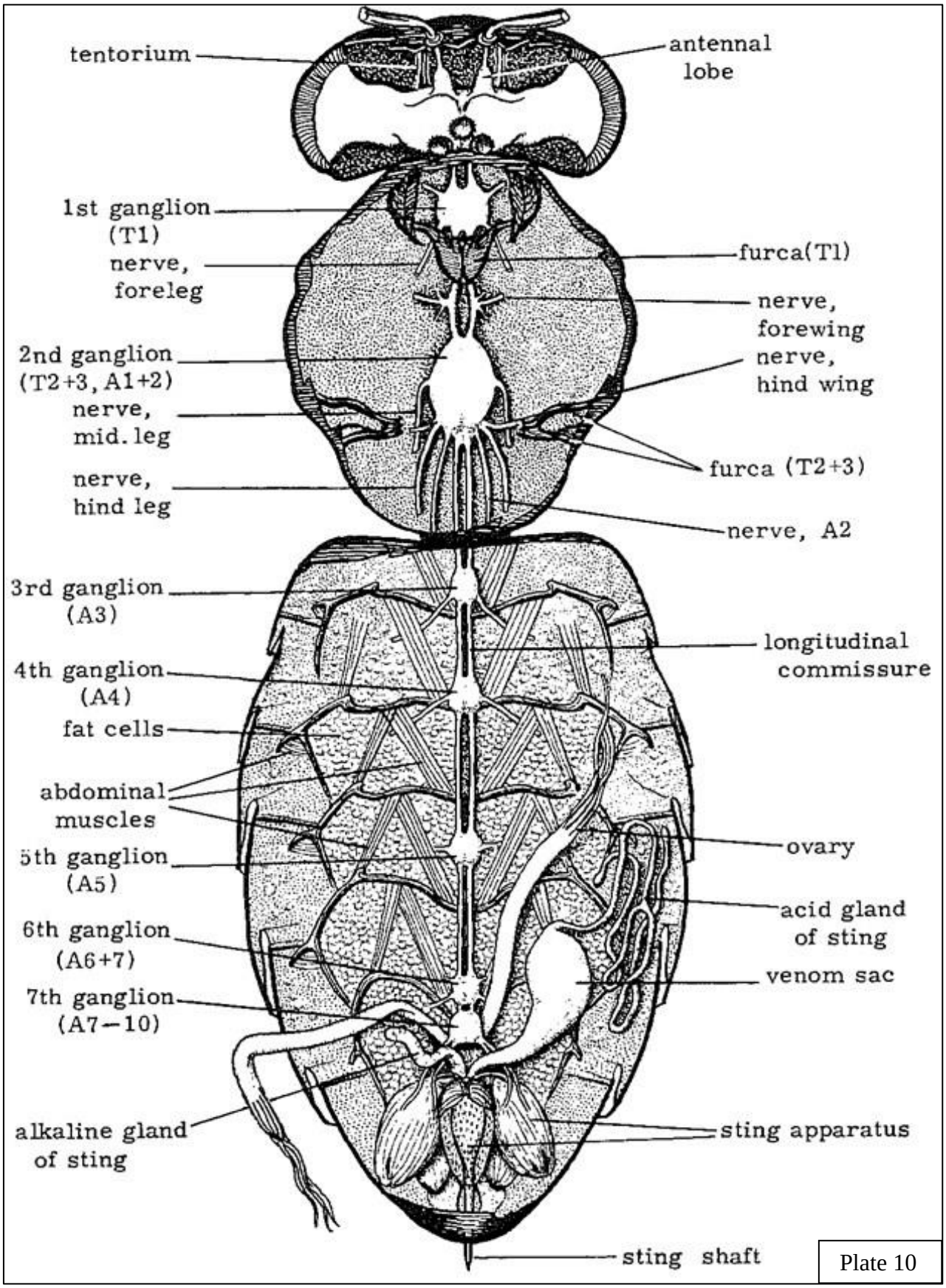
3.2.2.1. Exposing the viscera (Plates 1, and 8 A and B)

To open the abdomen:
1. Steady the dish with the left hand, and the scissors by resting them against the left thumb on the edge of the dish (Plate 1).
2. Insert one point of the scissors under the overlapping edge of the tergite of A5, on the right side of the body.
3. Cut through the body wall.
4. Continue snipping through the right side, working forwards towards the thorax.
Keep the inner blade of the scissors as far as possible parallel with the side of the insect, thus avoiding thrusting it in deeply and damaging the viscera.
5. Turn the dish clockwise to suit the scissors hand when the corner at the front end of the abdomen is reached.
6. Cut across the broad front of the abdomen to the opposite corner.
7. Then again turn the dish and work down the left side.
8. Turn the dish again when the tergite of A6 is reached.
9. Cut across the tergite, taking great care not to damage the soft organs underneath, and so complete the circuit at the beginning of the first incision.
10. Lift off the roof of the abdomen gently with the point of a needle; it should be free and easily removed.
If it resists, one or more of the infolded parts of the tergites have not been severed. These uncut parts must be found and cut through, using the inner scissors point as a probe while gently lifting the roof with the needle. When it is clear that the roof is free, do not pull it off roughly, but lift it gently with two needles. There may be slight resistance from tracheae, but these will break without doing any damage. If, however, it seems that the internal organs are being pulled out or disturbed, take the roof by its edge with the fine forceps, and with the needle in the other hand break the tracheae, which will show as fine threads stretching between the roof and the organs below. Finally lift off the roof and turn it over. If the work has been done neatly, it will come off in one piece.
11. Examine the underside of the roof (Plate 8C) as it lies in the dissecting fluid.
Observe:
11.1. the heart, with its closed posterior chamber and its ostioles (there are five pairs, but the anterior pair may have been lost), in the mid-line of the roof;
11.2. the dorsal diaphragm, transparent, but clearly visible, and its attachments to the apodemes of the tergites;
11.3. the pericardial fat cells, large numbers of small, creamy bodies clustered against the heart;
11.4. the dorsal sheet of the fat body forming a pad between the heart and the body wall;
11.5. some of the abdominal muscles may be seen as flat, nearly transparent bands stretched across the tergites.
In preserved bees the heart and dorsal diaphragm occasionally adhere to the viscera and thus tear away from the roof. Having examined all these organs, lay aside the roof, and
12. look at the contents of the abdomen.
12.1. The appearance of the undisturbed viscera is very variable, depending on the state of the alimentary canal (Plate 8, A and B). In a bee which has been confined to the hive for some time, or a young bee which has not yet flown, the rectum is greatly distended by accumulated faeces, the bulk of which are yellow pollen husks (A). If the rectum has been damaged by instruments during the opening operations, some of the faeces will have escaped, and will litter the dissection. If the bee has just returned to the hive after a flight, the rectum will be empty and shrunken to very small proportions; if she has brought home a load of nectar or water, the crop (honey stomach) will be

expanded into a large, transparent globe (B); if it is empty it will appear as a small, semi-opaque, pear-shaped body;
12.2. part at least of the ventriculus will be visible as a broad, corrugated tube;
12.3. a loop of the small intestine will be found connected to the forward end of the rectum; its other end, which joins the ventriculus, may not be visible;
12.4. the slender, tangled threads which spread all over the abdomen are the Malpighian tubules;
12.5. in a freshly killed bee, the tracheal sacs will be seen as
large bags, silvery with included air (which escapes when a needle point is inserted), obscuring parts of the other organs. In preserved bees the sacs are almost invisible, filmy membranes, the air having been dissolved by the preserving fluid. When air-filled sacs obscure the view, they should be pulled out with forceps.
12.6. Tracheae in large numbers appear as silvery tubules in all parts of the body.
13. Clear away debris (faeces, fragments of tissues, etc.) which collects in the abdominal cavity from time to time during dissection.
This is done by irrigation with clear dissecting fluid, a jet of which is directed into the cavity with the pipette.

3.2.2.2. Displaying the alimentary canal (Plate 9)

1. Take a needle in each hand.
2. Pass them under the rectum and ventriculus
3. Lift up the alimentary canal, gently tearing away the network of investing tracheae in which it hangs.
4. Lay it over to the left side, as shown in Plate 9.
5. Carefully tease out the tracheae and Malpighian tubules to permit the canal to lie loosely, showing all its parts.
6. Identify and examine the parts. Notice:
6.1. the six rectal pads, which appear as whitish bars on the wall of the rectum;
6.2. the small intestine, as a narrow coiled tube with six longitudinal pleats;
6.3. at its junction with the ventriculus, about one hundred Malpighian tubules are inserted. This is the pyloric region of the canal;
6.4. the ventriculus, in which food masses in the course of digestion can usually be seen, showing as dark areas where the corrugations of the ventriculus are smoothed out. If the ventriculus is torn with a needle, this food mass will exude as a brownish gelatinous substance.
6.5. the proventriculus, which will be visible through the walls of the crop if it is full of nectar. In any case, tear open the wall of the crop, using needles, and turn up the proventriculus so that its four triangular lips may be clearly seen. If they are closed, the lips meet to form a cross. If they are partly open, an aperture like a four pointed star is seen;
6.6. the forward end of the crop, which narrows into
6.7. the oesophagus, which enters the thorax through the petiole.



3.2.2.3. The underlying organs (Plate 10)

1. Grasp the alimentary canal with forceps.
2. Stretch it.
3. Cut through the oesophagus with scissors.
4. Treat the rectum in the same way, cutting through it as

far back as possible.
5. Carefully cut away the remaining small triangle of the roof which was left at the tip of the abdomen.
6. Lower the side walls with scissors, giving a better view of the floor.
7. Flush out the cavity with the pipette.
8. Compare with Plate 10.
9. Identify and examine the parts. Notice:
9.1. the ventral diaphragm, which may not be noticed at first, but closer inspection will reveal it as a transparent film which very slightly obscures the view of the chain of ganglia and other underlying features. In a later dissection, the diaphragm may be studied more carefully; it is attached to the apodemes of the sternites; its anterior end extends into the thorax and is attached to the furca of T2 and T3, while its posterior end is anchored to the spiracle plate of A8.
10. Tear out the diaphragm with the fine forceps, taking care not to damage other structures in the process.
11. Observe the now more clearly visible organs lying on the floor of the abdomen:
11.1. the chain of five ganglia is the most conspicuous, it is connected by twin longitudinal commissures. The last, the 7th ganglion, is attached to the sting apparatus, and comes away with the latter when it is torn out of the worker's body after stinging;
11.2. the main lateral nerves which spring from the ganglia can be seen running out to right and left; those of the 7th may be seen passing to the muscles of the sting;
11.3. the fat body spreads widely over the floor of the abdomen, being particularly well developed over the wax glands of the sternites of A4 to A7. Smaller clusters of fat cells occur along the sides of the abdomen. The fat body is highly developed in young bees and winter bees, where the cells are large and plump, but in old foragers they are shrunken;
11.4. the abdominal muscles show clearly, some of the larger sets being very conspicuous as broad V-shaped pairs of bands stretching between the thickened forward margins of adjacent sternites;
11.5. the ovaries are difficult to see, and since they encircle
the alimentary canal they are torn away when it is lifted out. To prevent this, after removing the roof of the abdomen,
11.5.1. lift the alimentary canal slightly from the right-hand side,
11.5.2. look sideways under it, the right ovary with its oviduct will be seen as an almost transparent, narrow, flat tube running to the root of the sting,
11.5.3. gently disengage the ovary from the tracheae
which tie it down and attach it to the other viscera,
11.5.4. repeat this operation from the other side, thus freeing the left ovary,
11.5.5. go on with the dissection, removing the canal.
The ovaries will then be seen lying or floating in the abdominal cavity, their oviducts disappearing behind the sting, their distal ends separated. In the undisturbed abdomen, the tips of the ovaries are joined and attached to the heart (Plate 17).

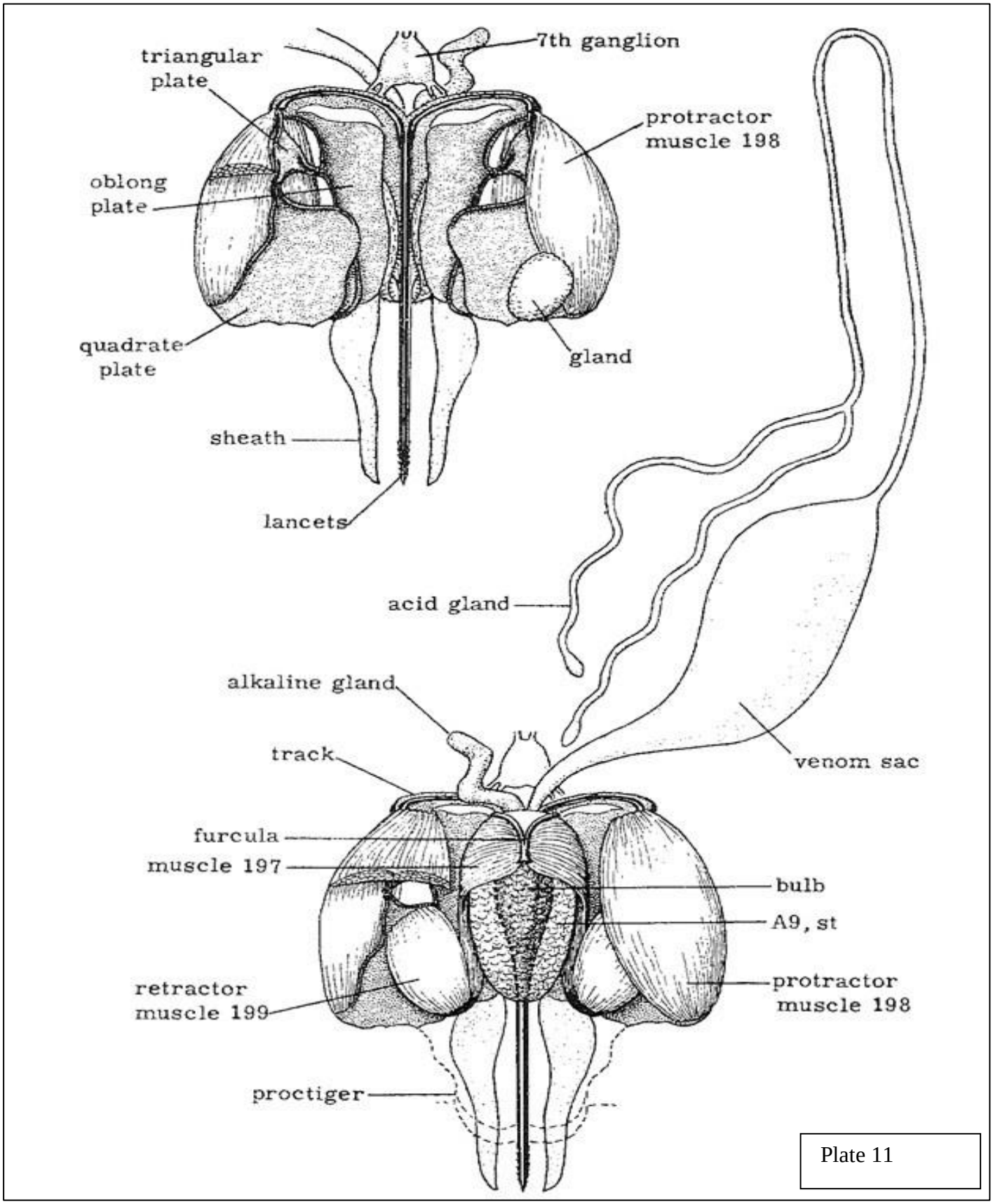

triangular plate
7th ganglion
protractor muscle 198
oblong plate
quadrate plate
gland
sheath
lancets
acid gland
alkaline gland
track
venom sac
furcula
muscle 197
bulb
A9, st
retractor muscle 199
protractor muscle 198
proctiger

Plate 11

11.6. the sting, if not wholly visible, can be examined in situ by removing more of the wall of the abdomen at the tip (Plate 10).
Identify the parts flagged in Plate 11, dorsal aspect. The whole apparatus can be removed intact very easily by passing needles below it and lifting it out, the small muscles which hold it giving way without offering noticeable resistance. The extracted apparatus can now be turned over, as it lies in clear fluid, and its ventral aspect (Plate 11) can be examined. Very rarely, the sting apparatus is laterally reversed, the only evidence of this being that the positions of the venom gland and the alkaline gland are reversed. The powerful muscles of the sting apparatus conceal the plates which constitute the system of levers actuating the lancets. The plates can be exposed by removing the muscles by maceration. Note that the sting apparatus is arched; it can be flattened by tearing away the proctiger, which is firmly attached to the oblong plates, and it is then easier to examine and also to mount as a microscopical preparation.

3.2.3.1. Exposing the flight muscles (Plates 8A and 12)

The roof of the thorax is best taken off with the knife:
1. Insert the extreme point only, as shown in Plate 1.
2. Make a short slit in the body wall by an outward and forward stroke.
3. Continue this along the dotted line in the Plate, all round the domed roof of T2.
4. Make a longitudinal slit along the mid-line.
Usually the roof is very firmly attached to the flight muscles, and must be detached, again with the point of the knife.
5. Keep the blade in a horizontal position, pass its point under the body wall, through the longitudinal slit, and separate the roof from the muscles by small forward movements, gradually working the point further under the body wall. When the first half of the roof is nearly free, steady it with forceps while completing the separation. If this is done carefully, the muscles will be undisturbed and undamaged, and will have the appearance shown in Plate 8.
6. Remove the other half of the roof.
7. Take off the remainder of the roof, along the second dotted line in Plate 1.
This is not attached to the muscles, and will come off easily.
8. If necessary, remove more of the side walls of the thorax, down to the level of the wings.
The indirect flight muscles are now exposed.

3.2.3.2. Oesophagus and glands (Plate 9)

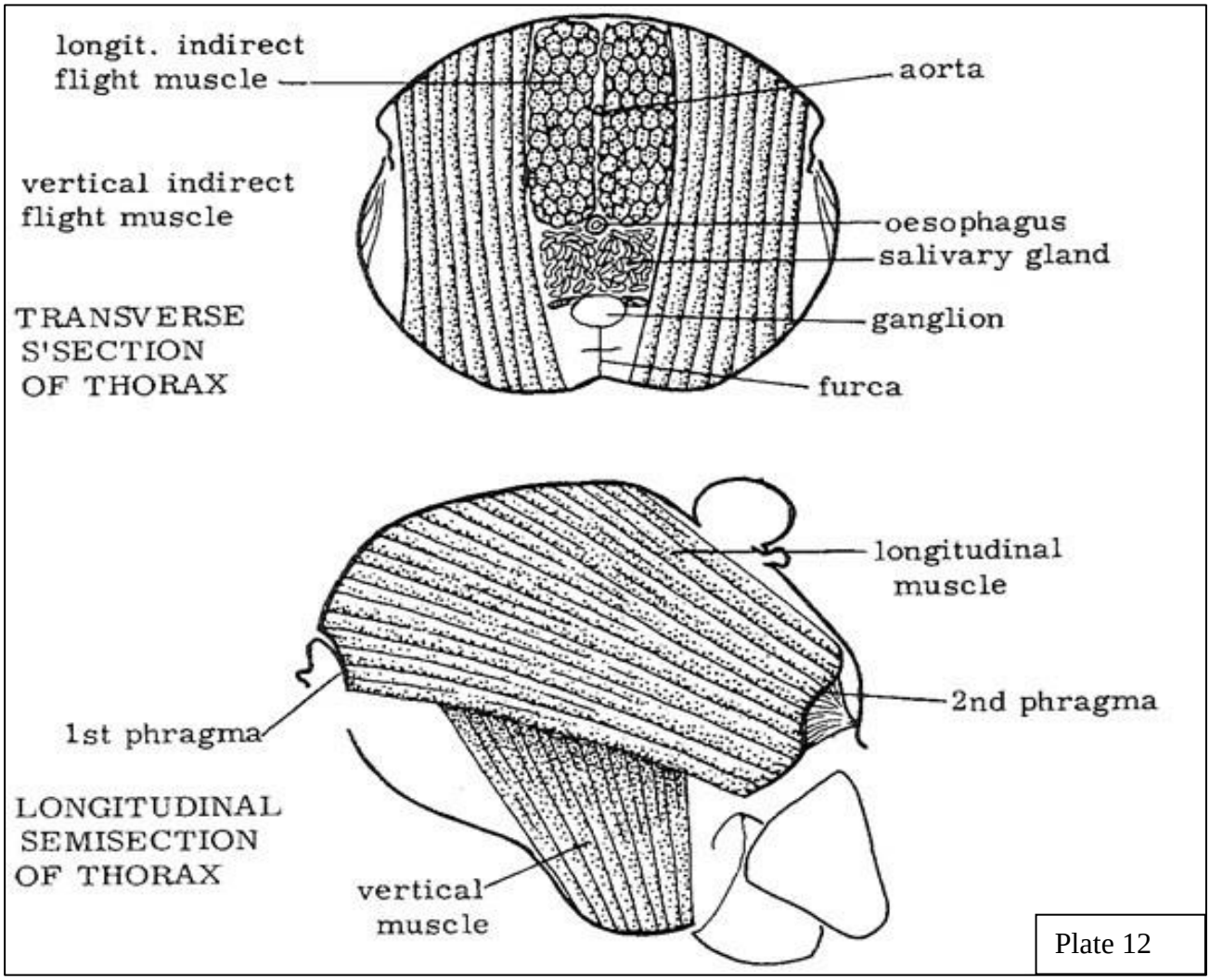
1. Remove the flight muscles: simply grip bunches of them with the forceps and pull them out (this is virtually impossible in a freshly-killed bee).
2. Observe:
2.1. the attachment of the longitudinal muscles to the 2nd phragma, which is an extension inwards of the tergite of the second segment;
2.2. the oesophagus below the longitudinal muscles, it passes from abdomen to head;
2.3. the salivary glands of the thorax (derived from the silk glands of the larva);
2.4. the aorta is a delicate tube which is destroyed by the
removal of the indirect flight muscles; it can be found by
careful lateral dissection.

3.2.3.3. The nervous system in the thorax (Plate 10)

1. Remove the oesophagus and salivary glands with forceps.
2. Observe:
2.1. the combined furcae of T2 and T3, which are now conspicuous.

There is much more; see The COLOSS BEEBOOK: anatomy and dissection. We could also go into mounting the body parts.

Jeremy Quinlan



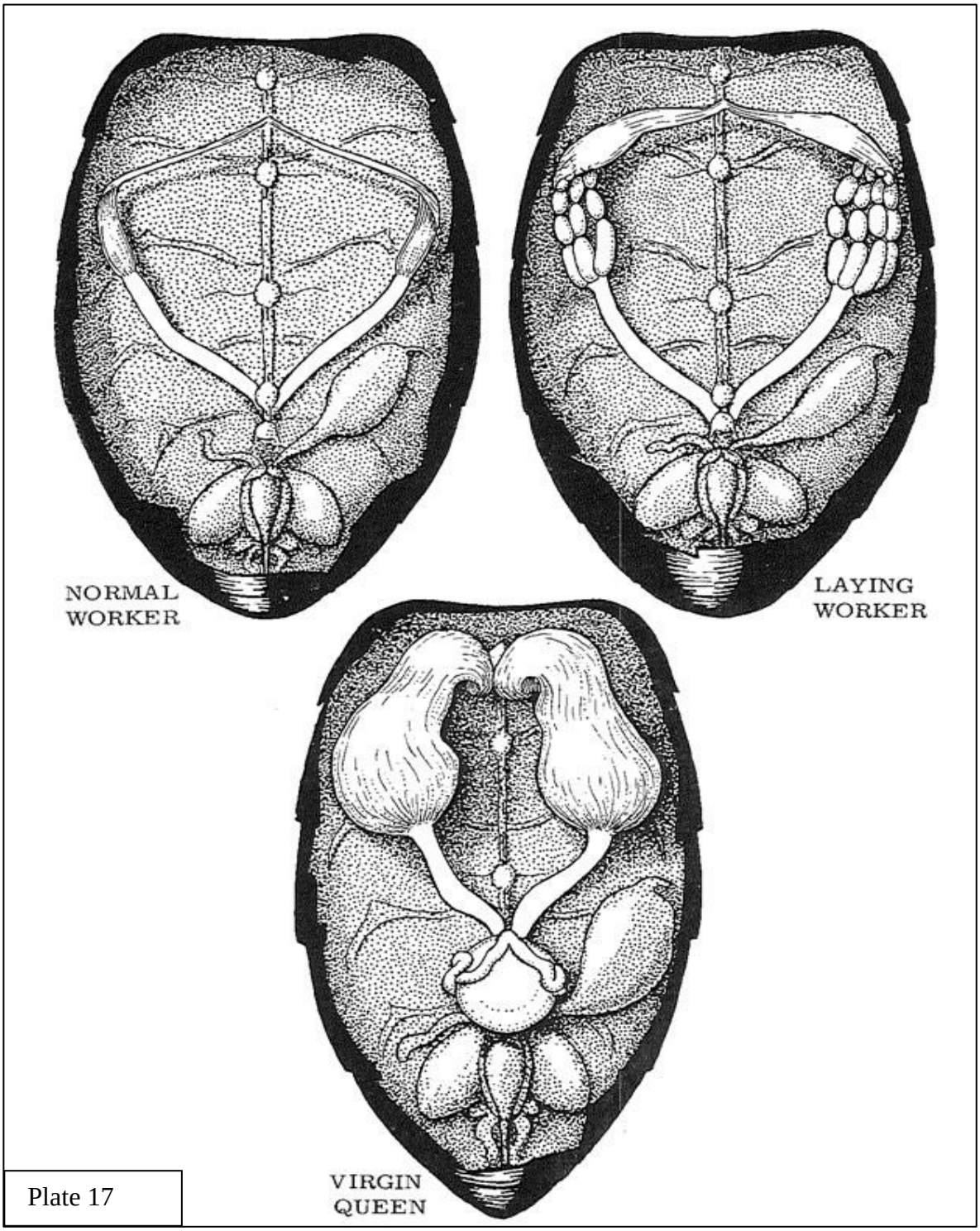


Plate 17