29. DIAGNOSIS OF SNAKE BITE

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INTRODUCTION

Micro techniques are now available for estimating precipitin antibody (Crowle, 1961). This has been used for labelling lizard serum (Rawlinson, 1964) and antibody to albumin in guinea-pig anaphylaxis (Trethewie, 1964). In view of the difficulty often experienced concerning the diagnosis of snake bite this technique has been applied to simplify this.

METHOD

The technique employs slides coated with agar in which small wells are cut — one centrally and a number circularly around at a distance of 0.5 cm. This is illustrated in figure 1. The slides are coated with a preparation of agar 1% Ionagar and 1% NaCl to which is added a 1/10,000 solution of aqueous Merthiolate and distilled water to a volume of 100 ml. This is allowed to soak for 30 minutes and then heated to 98°C in a water bath. 12 ml of this solution is thoroughly mixed and poured into a 10 cm siliconized petri dish, to make a layer 2 to 3 mm thick. Once the agar is set, holes are punched to the desired pattern. This agar “skin” is removed from the petri dish under water with a spatula. It is placed on a slide where it is trimmed to size and lightly touched or covered with filter paper which draws off excess saline. It is left for 24 hours at room temperature to dry. It is sometimes necessary to replace the filter paper after the first seven minutes. When the agar on the slide is dry the filter paper is removed, using distilled water. The stain is prepared from 1.5 g Azocarmine B (red), 500 ml methanol, 100 ml acetic acid, and 400 ml distilled water. It is put in a large petri dish into which the slide is placed and left for about five minutes. Excess stain is wiped off the glass. A washing solution of 1,800 ml ethanol and 200 ml glacial acetic acid is placed in a second petri dish into which the slides are placed for destaining for about seven minutes. The use of tweezers makes the handling of the slides less difficult but care must be taken not to scratch the agar.

PRINCIPLE

It appeared to one of us (E.R.T.) that in view of the difficulty in assessing:

1. Whether a child has been bitten by a snake at all (say in long grass) or
2. The type of snake biting which:

(i) may not have been seen

(ii) seen briefly and insufficiently to diagnose or

(iii) seen by someone uninitiated in knowing the type when seen adequately;

that this technique could be applied to the diagnosis of snake bite.

Australian snakes present a well marked feature of cross and multi-antigenicity. This is so well developed that originally polyvalent tiger antivenene was regarded as the treatment of choice for snake bite by any snake in Australia. Most snakes in Australia are venomous. They are *Notechis scutatus* (Tiger snake), *Oxyuranus scutellatus* (Taipan), *Acanthophis antarcticus* (Death-adder), *Pseudochis porphyriacus* (Black snake), *Denisonia superba* (Copperhead), and *Demansia textilis* (Brown snake). Publishing recently on snake bite I referred to regional distribution in Australia as a guide to treatment (Trethwie, 1966) where the snake biting is not definitely known. This affords additional information as regards administering the specific antivenene.

As regards antivenene treatment and excluding for the moment other local and general measures, we recognize that sera from the Tiger snake, Taipan, Brown snake and Death adder are sufficiently distinct to make them individually useful for treatment rather than the universal use of tiger antivenene.

**Experimental**

When we place a known venom in the centre well of our preparation and differing antivenenes in the five circular wells we find the following distribution of precipitin reactions (Table 1). This we find is adequate to make a diagnosis.

<table>
<thead>
<tr>
<th>Snake or venom</th>
<th>N.S.</th>
<th>O.S.</th>
<th>P.P.</th>
<th>A.A.</th>
<th>D.T.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Notechis scutatus</em></td>
<td>111</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><em>Acanthophis antarcticus</em></td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>111</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudochis porphyriacus</em></td>
<td>11</td>
<td>11</td>
<td>111</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Demansia textilis</em></td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>111</td>
</tr>
</tbody>
</table>

We have compared the use of saline extract of the bitten area, serum from whole blood, and the expression of serum from the bite or injection for diagnosis. Expressed serum is the best material.
In the case of Tiger snake venom placed in a well, or expressed serum from the "bite" (injected) area of the animal (in this case a guinea-pig), marked precipitin reaction occurs against the Tiger antivenene and moderate precipitation against the remaining four (Fig. 1).

In the case of Death adder venom injected into a guinea-pig leg (20 mgm) extract shows marked precipitation against Death adder antivenene, moderate against Tiger, slight against Taipan and Black snake and no significant reaction with Brown antivenene. These appearances are far clearer on the original glass slide preparation (Fig. 2).

With Black snake venom (5 mgm injected into a guinea-pig) likewise marked precipitation occurs with Black snake, moderate with Tiger and Taipan and slight reaction with Brown antivenene (Fig. 3).

In one instance a Brown snake was placed against a guinea-pig and encouraged to bite and in this instance serum expressed from the bite on the guinea-pig’s leg (Fig. 4) showed marked reaction with Brown antivenene, moderate with Taipan, slight with Tiger, and none with Black and Death adder. When live snakes are used, as is projected, reactions may be expected to be more clear cut because dried commercial serum may be faulty.

These findings are summarized in the Table 1 and Fig. 5. It can be seen that if one considers only the two latter columns there is an almost significant separation of the venom designation. When all five antivenenes are used the distinction is confirmed by maximal precipitant forms against the homologous antivenene.

In this way we are able to separate serum obtained from different types of bite or injection in the experimental animal. The reaction time is five or more hours except with Brown snake which is two hours and we are endeavouring to speed up this reaction. We suggest in the first instance to give antivenene according to the information of the snake if available and the geographic region bitten (with reference to the colour of the snake) and in the absence of this information to use Tiger antivenene in the first instance and subsequent injections to follow the diagnostic pattern. It is suggested that development of this technique may obviate the confusion arising from the two problems, 1, was the subject bitten at all? and 2, what was the nature of the snake? We are proceeding with these experiments.

**Discussion**

The above technique shows that material obtained from the site of the bite—either saline injected especially where there is severe thrombosis, or bled material from incision, affords satisfactory diagnosis of the snake-bite when set up in double diffusion.

The advantages of this procedure are obvious especially where specific antivenene is essential for treatment and this is preferable in the case of Australian snake bite as regards Tiger, Brown snake, Taipan and Death adder.

The time required for a positive test — 2 hours in the case of Brown snake and longer with the others, at room temperature — is a practical difficulty, but we have been able to obtain a positive result more quickly at 37°C and this technique is now being standardized. This should give an adequate answer in approximately one hour which is quite suitable for treatment. In Australia an initial injection of Tiger polyvalent antivenene is advised.
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SUMMARY

1. Diffusion techniques with agar plates give a set pattern of reaction for each Australian snake venom against the series of individual antivenenes.

2. Venom injected into a guinea-pig allows the collection of material from the region of the bite for similar analysis.

3. Material from the region of the actual bite of one snake on a guinea-pig also provided adequate diagnostic material.

4. It is considered this technique may be employed in hospitals to ensure accurate snake bite diagnosis.

REFERENCES


Fig. 1 — Reaction to Tiger venom (Centre well) against Death Adder (Aa), Brown Snake (Dt), Black Snake (Pp), and Taipan (Os) antivenene. (Room Temperature).

Fig. 2 — Reaction to material from the region of injected Death Adder venom from a guinea-pig (Centre Well). Outer wells contain Brown Snake (Dt), Tiger Snake (Ns), Taipan (Os), and Black Snake (Pp). (Room Temperature).
Fig. 3 — Reaction to material from the region of injected Black Snake venom from a guinea-pig (Centre well). Outer wells contain — Death Adder (Aa), Brown Snake (Dt), Tiger Snake (Ns), and Taipan (Os). (Room Temperature).

Fig. 4 — Reaction to material from the region of a bite of a Brown Snake on a guinea-pig (Centre well). Outer wells contain — Death Adder (Aa), Tiger (Ns), Black Snake (Pp), and Taipan (Os). (Room Temperature).

**VENOMS**

**N S.**  
(Tiger Snake)

**A. A.**  
(Death Adder)

**P. P.**  
(Black Snake)

**D. T.**  
(Brown Snake)
DISCUSSION

S. Minton: "How long might a precipitin test be obtained after death? In other words, how long does the venom persist in the body?"

E. R. Trethewie: "We had a case of Tiger snake bite where death occurred after four days and at autopsy extract of the skin area bitten killed 100 mice with typical symptoms of Tiger snake envenomation. Allergic antigen stays in the tissue of the skin for several days and may produce a delayed reaction of seven days. Therefore I consider much venom would be left in the bitten area to give a diagnosis by this technique of medico-legal importance for several days."