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An objective approach to antivenom therapy and assessment of first-aid measures in snake bite

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Treatment of systemic envenoming in snake-bite victims has, in the past, depended almost entirely on the individual clinician's experience in assessing the severity of envenoming. The efficacy of treatment is obviously related to the neutralizing potency of the antivenom used, the route by which it is administered and the dose. The development of enzyme immunoassays has permitted a more scientific appraisal, allowing estimation of circulating specific venom and antivenom concentrations at any time after the bite in the patient's blood. It is therefore possible to measure accurately the efficacy of antivenom in the neutralization and clearance of venom antigen. In Brazil, it appears that clinicians treat patients with excessive amounts of highly efficient antivenoms and this results in an unacceptably high incidence of reactions. In Sri Lanka, the use of imported, Indian antivenom is relatively ineffective in neutralizing the venoms of Sri Lankan snakes, demonstrating the real problem of venom variability within individual species. In West Africa, the improved clearance of venom following treatment of *Echis* victims with a monospecific as opposed to a polyspecific antivenom has been demonstrated, and new, smaller fragment, Fab antivenoms have been developed and are now under clinical assessment. Such clinically-based immunological studies should result in more efficient and controlled use of expensive antivenoms for treatment of systemic envenoming and the accurate assessment of newly designed products. Such studies also emphasise the importance of individual countries producing their own antivenoms for treatment of systemic envenoming. Likewise, the use of such objective systems now enables the use of first-aid measures such as tourniquets to be properly assessed.

For many years conventional antivenoms have been prepared by immunizing large animals, usually horses, with an individual venom (monospecific antivenom) or a range of different venoms (polyspecific antivenom) obtained from large venom pools to eliminate intraspecific variation. The antivenoms produced usually comprise a concentrated $F(ab')_2$ fragment of the IgG molecule, separated from the Fc component by pepsin digestion. However, recent developments in immunotherapy include the use of smaller, possibly less reactive Fab fragments prepared from the whole IgG molecule by papain digestion (Smith *et al.*, 1992; Theakston and Smith, 1995). Although this type of antivenom has some theoretical advantages over an $F(ab')_2$ antivenom, there are also some important disadvantages.

Treatment of envenomed victims has, in the past, been carried out without any real scientific criteria as to the optimal dose of antivenom. The dose given to a patient usually still depends on the individual clinician's experience in assessing the severity of envenoming given the clinical signs (e.g. local and systemic haemorrhage, incoagulable blood, neurotoxicity, myotoxicity, local swelling and necrosis and a range of other effects) (Cardoso *et al.*, 1993). More recently, however, more objective methods, involving the use of enzyme immunoassays (EIA), have been de-

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veloped to estimate the course of envenoming and to assess the effects of antivenom therapy, first aid and traditional methods of treatment.

Some Problem Areas of the World

In Brazil, patients bitten by the Jararaca (*Bothrops jararaca*) are each treated with a starting dose of four, eight or more 10-ml ampoules of *Bothrops* polyspecific antivenom (Theakston and Warrell, 1991; Jorge *et al.*, 1995). The exact dose depends on the severity of envenoming as assessed by the individual clinician treating the patient (Cardoso *et al.*, 1993).

The annual incidence of snake bite in Sri Lanka (400 bites and six deaths/100 000) is currently one of the highest in the world (Phillips *et al.*, 1988). The most important species is Russell's viper, *Daboia russelii pulchella*, and systemic envenoming involving haemorrhage, coagulopathy, neurotoxicity and myotoxicity is normally treated with a starting dose of five to 15, 10-ml ampoules of imported, Indian, polyspecific antivenom (Haffkine or Serum Institute of India) (Theakston and Warrell, 1991).

In Nigeria, bites by the carpet viper (*Echis ocellatus*) constitute a major problem in farming communities (Warrell and Arnett, 1976; Pugh and Theakston, 1980). Envenoming by this species results in local and systemic haemorrhage, incoagulable blood and local necrosis. On the basis of these signs, two ampoules (20 ml) of a monospecific *Echis* antivenom (South African Institute for Medical Research) or four ampoules (40 ml) of either a French (Institut Pasteur) or German (Behringwerke) polyspecific antivenom have been recommended as a starting dose in the past (Warrell *et al.*, 1974, 1980).

METHODOLOGY

Techniques Used for Assessing Antivenom Efficacy

The usual method by which the clinician assesses antivenom efficacy is by observing the reversal of systemic signs such as venom-induced coagulopathy or neurotoxicity. The development of EIA for detection of specific venom antigen (Theakston *et al.*, 1977; Ho *et al.*, 1986*b*) and for detection of therapeutic antivenom (Theakston *et al.*, 1992) has enabled a more objective assessment of antivenom dosage and efficacy to be made. It is now possible, using this method, to detect and quantify specific venom in the blood or body fluids at any time after the bite, as well as to calculate the amount of therapeutic antivenom circulating at any time after antivenom administration. Figures 1 and 2 show how the kinetics of envenoming and therapy can be assessed when both a relatively ineffective (Fig. 1) and an effective (Fig. 2) antivenom is used.

Assessment of First-aid Measures

Such techniques are also useful for determining the efficacy of first-aid measures. For example, venom levels can be measured both proximal and distal to the tourniquet both before and after the release of the tourniquet (Ho *et al.*,1986*a*; Tun-Pe *et al.*,1987).

RESULTS AND DISCUSSION

Brazil

In Brazil, the three major polyspecific *Bothrops* antivenoms have been compared clinically in a randomized comparative trial (Theakston *et al.*, 1992; Cardoso *et al.*, 1993). All the antivenoms produced a high degree of protection and a rapid rate of venom clearance after a single dose of the lowest amount of antivenom (i.e. four 10-ml ampoules) recommended for the treatment of moderately envenomed patients (Cardoso *et al.*, 1993). A more recent study has demonstrated that even a dose of two ampoules (20 ml) is adequate in similarly envenomed patients (Jorge *et al.*, 1995). The levels of circulating antivenom were maintained at a high level even after the venom antigenaemia had been abolished, and only finally cleared from the circulation after 37 days (Theakston *et al.*, 1992); this permitted immediate neutralization of any additional venom entering the circulation from the depot at the bite site. The results of this particular study indicated, both clinically and by immunoassay, that the patients had received

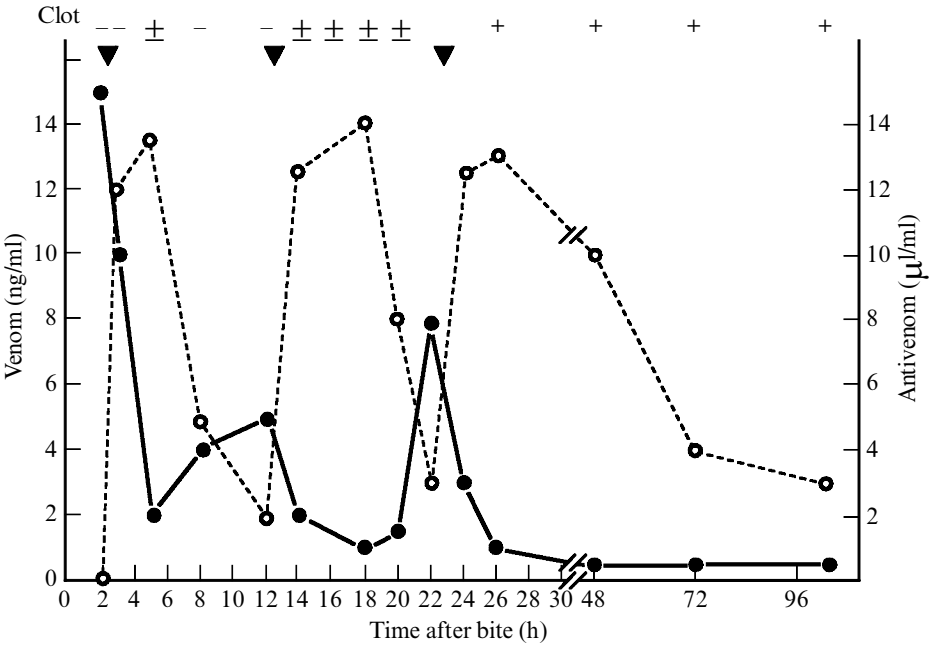

Fig. 1. Venom (●) and antivenom (○) concentrations in the serum of a patient following envenoming by *Daboia russelii pulchella* in Sri Lanka. A dose of five, 10-ml ampoules (50 ml) of Haffkine polyspecific antivenom was given at three times (▼). The varying clot quality of the victim's blood is also shown (−, no clot; ±, defective clot; +, full clot). Note the transient rises in venom antigenaemia causing re-occurrence of clinical signs (e.g. incoagulable blood) and requiring further doses of antivenom.

more antivenom than was actually necessary for neutralizing the circulating venom, especially in the higher-dose ranges (i.e. four, eight or even more ampoules) in cases of moderate envenoming.

Sri Lanka

In Sri Lanka, imported Indian (Haffkine or Serum Institute of India) polyspecific antivenom is used for treating envenoming by Russell's viper (*Daboia russelii pulchella*), saw-scaled viper (*Echis carinatus*), the Indian and Ceylon krait (*Bungarus caeruleus* and *Bu. ceylonicus*, respectively) and the Sri Lankan cobra (*Naja naja naja*). These antivenoms are not very effective against the venom of Sri Lankan *D. russelii pulchella* (Fig. 1) because they are prepared against the venom of Indian *D. russelii russelli* (Phillips *et al.*, 1988; Theakston and Warrell, 1991). The venom from Sri Lankan Russell's vipers contains some different components to the Indian venom, such as a presynaptically-acting, myotoxic phospholipase A_2 (PLA_2), which are not neutralized by the imported antivenom (Phillips *et al.*, 1988).

Nigeria

In northern Nigeria, the best antivenom used in the past has proved to be the monospecific antivenom from the South African Institute for Medical Research (SAIMR), which is raised against the venom of Kenyan *Echis*, probably *E. pyramidum leakeyi* (Theakston and Warrell, 1991). In most cases, venom was cleared from the circulation within 4–8 h of intravenous administration of this antivenom (Fig. 2), with simultaneous, permanent resolution of the clinical signs of envenoming (Pugh and Theakston, 1987*b*; Rugman *et al.*, 1990). The polyspecific German (North and

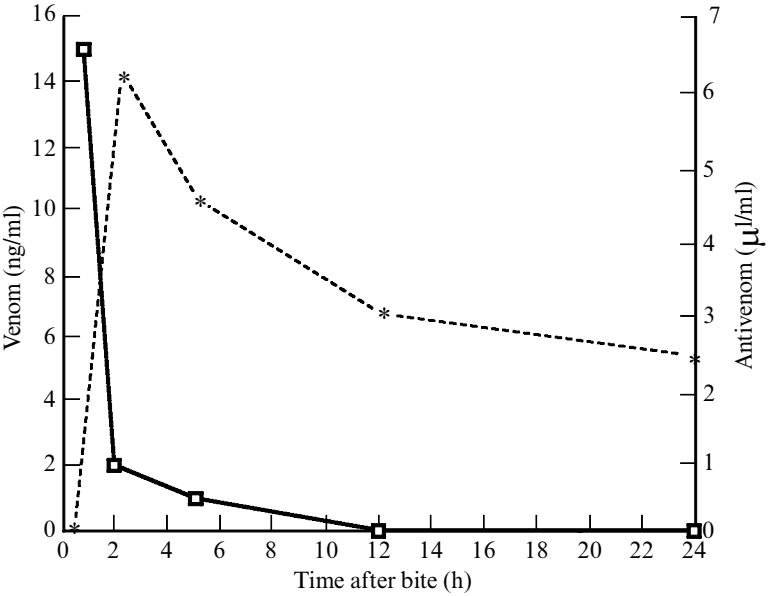

Fig. 2. Venom (□) and antivenom (✻) concentrations in the serum of a patient bitten by a Nigerian *Echis ocellatus* and treated with a monospecific, *Echis* antivenom from the South African Institute for Medical Research. Note the rapid venom clearance, and maintenance of high antivenom levels at the time of complete venom clearance.

West Africa) and French (Pasteur '*Bitis–Echis–Naja*' and Ipser Africa) antivenoms tested were less effective; although their use resulted in an initial decrease in venom antigenaemia and temporary resolution of the clinical signs after admission, this was often followed by an increase in levels of circulating venom and recurrence of clinical symptoms such as incoagulable blood, due presumably to further influx of venom from a depot area at the bite site (Warrell *et al.*, 1974, 1980; Meyer *et al.*, 1997). Owing to an unsustained and inadequate level of antivenom in the circulation, this additional venom was not neutralized and a further dose (or doses) of antivenom was then required. In a case of envenoming by a Tunisian snake of the *Echis pyramidium* complex, a total dose in excess of 300 ml of three different antivenoms (one monospecific and two polyspecific) with activity against *Echis* species failed to neutralize the toxins present in the circulating venom in the patient (Fig. 3), demonstrating the lack of specificity of these antivenoms against this particular venom (Gillissen *et al.*, 1994).

Treatment of Local Venom Effects with Antivenom

The rationale for giving exceptionally high doses of antivenom in Brazil is that some clinicians consider that there may be some beneficial effect in reducing the extent of the local necrosis caused by the cytolytic enzymes present in *Bothrops* (and other) venoms. Both experimental (Iddon *et al.*, 1987) and clinical (R. D. G. Theakston and D. A. Warrell, unpubl. obs.) studies, however, have shown that although a small amount of $F(ab')_2$ antivenom does gain eventual access to the area of local necrosis, this is well after the initial rapid and irreversible changes have occurred. In human victims, who usually arrive at hospital hours or even days after the bite, there appears to be no real effect on local lesions (L. A. Ribeiro and M. T. Jorge, unpubl. obs.).

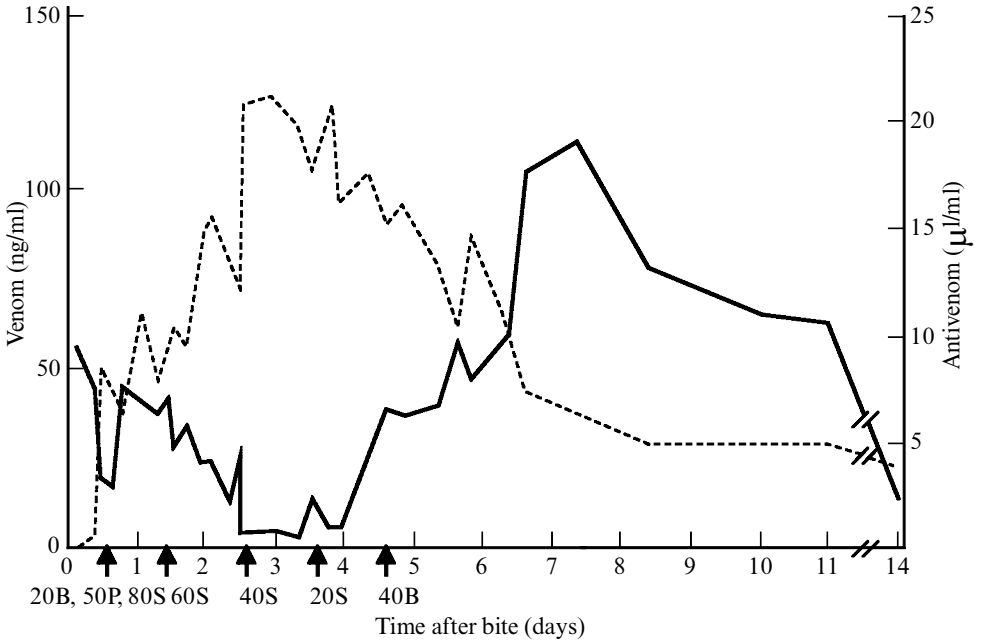

Fig. 3. Complete failure of antivenom therapy in a patient envenomed by a specimen of *Echis pyramidum* from Tunisia. Although there is a fall in venom concentration in the serum (—), the patient failed to respond clinically to a total of 310 ml of three different antivenoms (– – –). The values preceding the letters B (Behringwerke, North and West Africa, polyspecific antivenom), S (South African Institute for Medical Research, monospecific, *Echis* antivenom) or P (Institut Pasteur, *Bitis–Echis–Naja* antivenom) indicate the volume of antivenom (in ml) given on each occasion.

Recent Advances

All the antivenoms discussed so far are $F(ab)'_2$ fragment preparations (Theakston and Warrell, 1991; Theakston *et al.*, 1992; Laing *et al.*, 1992). Recently, attempts have been made to assess a novel, ovine, Fab antivenom, purified by papain digestion, raised against Nigerian *E. ocellatus* venom (Smith *et al.*,1992; Laing *et al.*, 1995; Theakston and Smith, 1995). Preliminary studies have yielded promising results (Meyer *et al.*, 1997). Such an antivenom, being a smaller fragment of the IgG molecule than $F(ab')_2$, has a larger volume of distribution and should theoretically also be less immunoreactive. A major disadvantage of this type of small-fragment antivenom may be that it is cleared more rapidly via the kidney. In a recent study, the elimination half-time of a Pasteur $F(ab')_2$ antivenom was 18.0 h whereas that for an Fab antivenom was 4.1 h (Meyer *et al.*, 1997). However, the latter antivenom may have the advantage of being more potent than other available products because it is a monospecific preparation raised against the venom of local *E. ocellatus*; it may also be more effective against local venom effects because of the relatively small size of the Fab fragment. Further studies, designed to fully assess this type of antivenom both in Nigeria and Sri Lanka, are currently in progress.

Assessment of First-aid Methods

Investigations in Burmese and Thai patients have attempted to assess the effectiveness of tourniquets as a first-aid measure, by estimating venom levels before and after tourniquet release (Ho *et al.*, 1986*a*), venom levels distal

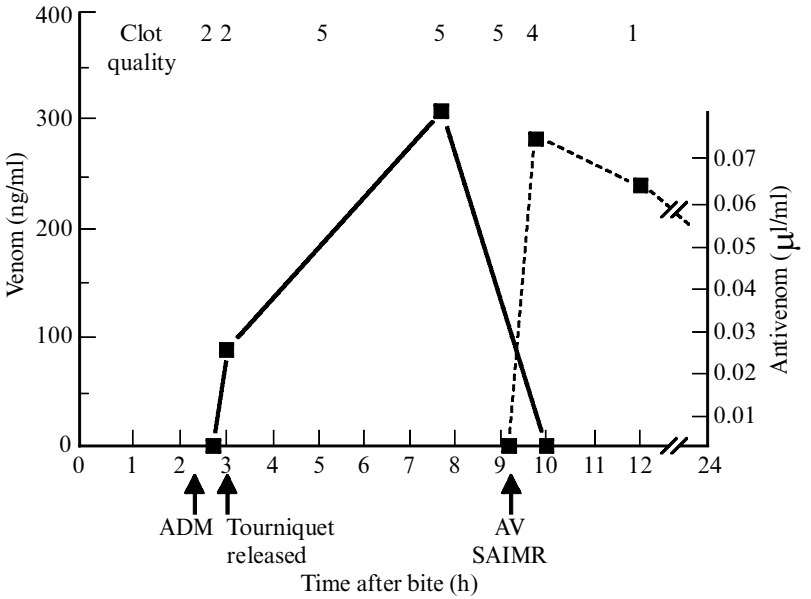

Fig. 4. Apparent success of a properly applied tourniquet in delaying the absorption of *Echis ocellatus* venom into the circulation, showing venom antigen (—) and antivenom (– – –) concentrations in the serum. The varying clot quality of the victim's blood is also shown, according to the grading system of Reid (1967):1, normal clot (fully coagulable blood); 2, slightly defective clot; 4, severely defective clot; 5, completely defective clot (incoagulable blood). The patient had no systemic signs on admission (ADM) but, following release of the tourniquet, signs of envenoming, including incoagulable blood, immediately developed, necessitating the use of 20 ml monospecific, *Echis* antivenom from the South African Institute for Medical Research (AV SAIMR).

and proximal to the tourniquet (Tun-Pe *et al.*, 1987) and admission venom levels in patients admitted with and without tourniquets (Khin Ohm Lwin *et al.*, 1984). Although, in all of these studies, tourniquets did not apparently inhibit spread of venom into the general circulation, it should be borne in mind that, in the field, tourniquets are often not applied or managed correctly. In the Philippines, tourniquets properly applied have been shown to delay the spread of the major neurotoxin present in the venom of the Philippine cobra, *Naja philippinensis*, into the circulation until release in hospital (Watt *et al.*, 1988). Likewise in Nigeria, one patient, admitted to hospital with *E. ocellatus* bite, had no clinical signs and no detectable venom on admission but developed significant clinical signs (incoagulable blood) with associated venom antigenaemia immediately after release of the tourniquet (Pugh and Theakston, 1987*b*) (Fig. 4). Another died, not because of envenoming, but due to pulmonary thromboembolus, preceded by thrombophlebitis, local necrosis and gas gangrene, caused by the late removal of a tight tourniquet which had been in place for 48 h after the bite (Pugh and Theakston, 1987*a*).

The possibility that a smaller Fab IgG fragment, given by the intramuscular route, may be of use in pre-hospital treatment is currently under investigation. If this system works it could represent a major advance in early therapy. Although preliminary results have indicated that absorption (even of an Fab fragment) into the circulation by this route is still too slow to be useful in early neutralization of venom, further, more detailed studies are essential as there is evidence that further

modifications in the antivenom need to be undertaken before such a system could be successful.

Proposed future studies will involve the objective assessment of so-called traditional and other untested remedies [such as the 'black snake stone' or high-voltage/low-current electric shock (Guderian *et al.*, 1986)] and other, somewhat suspect and unproven treatments, using EIA.

CONCLUSIONS

The first important stage in the evaluation of either a new or existing antivenom is its testing in an animal (usually rodent) model, using assessment of protection against the lethal and other venom effects such as haemorrhagic, local necrotising, defibrinogenating and coagulant activities. Tests recommended by the World Health Organization are available for this purpose (WHO, 1981; Theakston and Reid, 1983; Warrell *et al.*, 1986; Laing *et al.*, 1992; Theakston *et al.*,1992). However, it should be stressed that it is dangerous to extrapolate from the results obtained using such animal models to the situation in man, for a wide range of reasons [e.g. unrealistic routes of administration in animals of a pre-incubated mixture of venom and antivenom (Laing *et al.*, 1992; 1995; Theakston, 1991)]. The second stage, which is the only truly meaningful method, is therefore testing the experimentally approved antivenom in envenomed humans using both clinical observations and objective measurements of both venom and antivenom levels.

An effective antivenom (e.g. the SAIMR *Echis* antivenom in Nigeria and the Brazilian *Bothrops* antivenoms) is one in which a high level of active circulating antibody is maintained in the circulation after initial venom clearance in human victims. This will be capable of neutralizing any venom subsequently entering the circulation from a depot. Such conditions are usually obtained when the venom or venoms used for producing the antivenom is obtained from snakes present in the country in which it is proposed to use the antivenom. Venoms, even from snakes of the same species, vary dramatically in composition from region to region (Taborska, 1971; Williams *et al.*, 1988).

The results of the study carried out in Brazil strongly indicate that patients were receiving more antivenom than was necessary, thus resulting in a high incidence (37%–87%) of early anaphylactic reactions requiring urgent treatment with adrenalin and antihistamines (Cardoso *et al.*, 1993). A lower but effective dose should decrease the extent of this problem and also result in a reduction in cost; the latter advantage is of major importance in developing countries. There is no real evidence that a high dose of antivenom is effective in decreasing or eliminating local venom effects such as necrosis (Iddon *et al.*, 1987; Jorge *et al.*, 1995). Both clinical observations and EIA results support these observations.

There is also no convincing, general evidence that tourniquets are effective as a first-aid measure in delaying the absorption of venom into the circulation. As there are so many obvious variables, the results of studies using EIA are, not surprisingly, inconclusive (Khin Ohm Lwin *et al.*, 1984; Ho *et al.*, 1986*a*; Pugh and Theakston, 1987*a*, *b*; Tun-Pe *et al.*, 1987; Watt *et al.*, 1988). Likewise, further, more detailed studies need to be performed on the possibilities of using Fab fragments of lower molecular weight or even smaller antibody components, for early treatment following administration via the intramuscular route.

Using a combination of experimental, clinical and immunological assay systems, it is therefore possible to obtain a highly accurate assessment of the efficacy of antivenom and of current first-aid procedures. The pharmacokinetic differences between $F(ab')_2$ and Fab fragments are obviously connected with the pharmacokinetics of venom. It is therefore possible that the 'ideal' antivenom may be one which combines the less reactive properties, the increased volume of distribution and the more rapid tissue distribution of Fab fragments with the better plasma distribution and longer elimination time of $F(ab')_2$ fragments.

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