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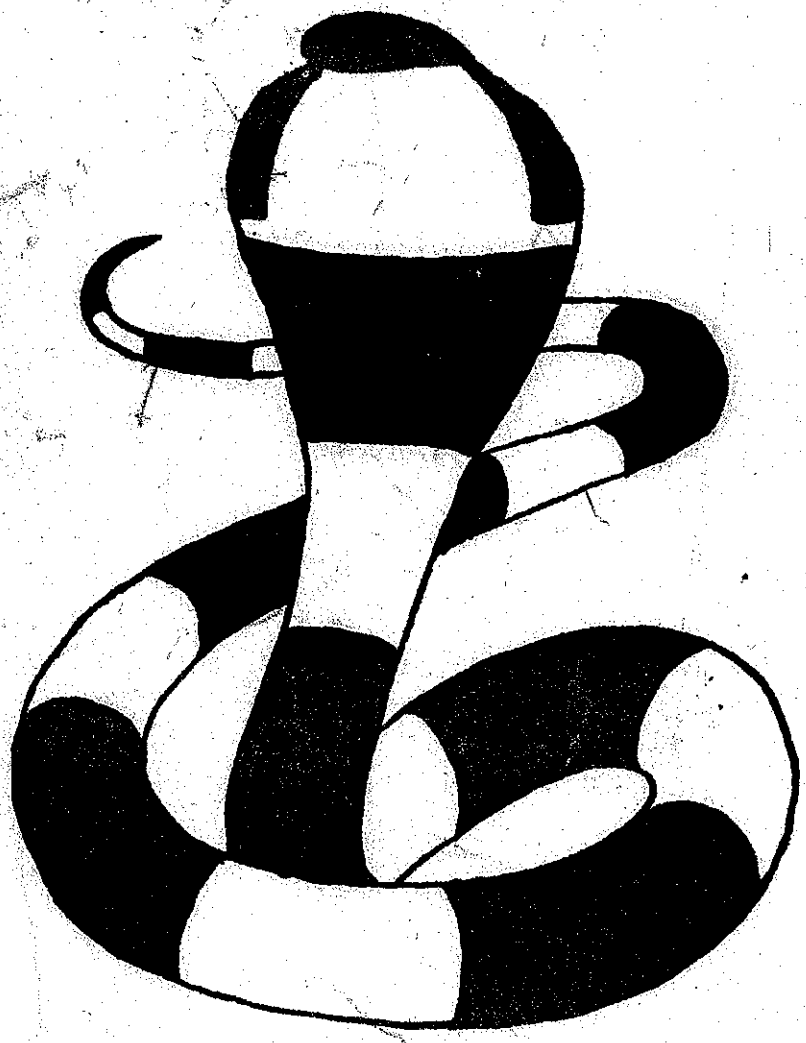
H.A.R.
JOURNAL



THE JOURNAL OF THE HERPETOLOGICAL ASSOCIATION OF RHODESIA.

No. 11 May, 1960.

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JOTTINGS FROM COBRA CORNER

Dear Member,

I am afraid that the Journal appears late once again, due this time to a young Boomslang which bit me on Good Friday and nearly cost me my life. As it was I was hospitalized for 12 days and then convalescent for another 10 days, which put me way behind with my paper work. Fortunately members have responded well to my call for articles and so enabled this Journal to appear, for I have had no time to write up articles myself. Dr. J.H.Mason's excellent article on the preparation of serums will be of particular interest and there will be considerable support for Walter Rose's article.

Good Hunting,

Donald G. Broadley
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PREPARATION OF ANTI-SNAKEBITE SERUM.

By Dr. J.H.Mason

South African Institute for Medical Research, Johannesburg.

The South African Institute for Medical Research issues the following anti-snakebite sera:-

1. POLYVALENT - prepared with the venoms of Naja nivea (flava), Bitis arietans and Hemachatus (Sepedon) haemachates. It is active against the venoms of these snakes and against those of N. haje, N. nigricollis and N. melanoleuca.

2. TROPICAL - the same as polyvalent, but containing the antivenom of B. gabonica in addition.

3. MONOVALENT Echis carinatus venom.

4. MONOVALENT Dendroaspis antivenom.

Horses are used as producers of antiserum because of their size and ease of management. They must be free from detectable disease before being admitted to the stud.

VENOM:- This is "milked" from the snakes in the usual way, is dried in a dessicator over silica gel and stored in stoppered bottles in a cool, dark cupboard. Under such conditions it retains its original potency for many years, for practical purposes, indefinately. When required for immunization purposes, a weighed ammount, dissolved in salt solution, is sterilized by filtration.

PRIMARY IMMUNIZATION:- At one time, the production of a slight initial immunity before the start of hyperimmunization was costly in venom and time. Venom, detoxicated with formalin, was used but many injections of large doses extending over weeks or months was sometimes necessary before the horse would tolerate even small doses of undetoxicated venom. The process was simplified by adsorbing venom on to a suspension of a clay, bentonite. (Adsorption is not absorption. Adsorption implies the firm adherence of one substance to the surface of another). The venom molecules become firmly attached to the surface of the tiny particles of clay and, when the suspension is injected subcutaneously into a horse, the particles are slowly removed from the site of injection and the venom gradually released from them. Thus, the horse is not intoxicated with an ammount that, unadsorbed on bentonite, would have killed it. (The same principle is involved when a tourniquet is applied to the limb of a bitten person). This venom-bentonite treatment is repeated once or twice and, when the serum of the horse is shown to contain antibody, even a trace, hyperimmunization with unadsorbed venom is started.

HYPERIMMUNIZATION:- This lasts, in a "new" horse, for 4 to 5 weeks. Every 2 or 3 days, a mixture of cobra, puff adder and ringhals venoms is injected subcutaneously, the dose being increased as immunization proceeds. When a test on the serum shows that a satisfactory level of immunity has been reached, 2 X 10 litre quantities of blood are removed from the jugular vein, with an interval of 4

days between bleedings..The blood is collected in bottles containing sodium citrate to prevent it clotting and the plasma is separated from the red blood corpuscles.

The horse, after a rest of 5 weeks, is re-immunized, but on this and subsequent occasions the process is speeded up and lasts only for 14 to 18 days.

PURIFICATION AND CONCENTRATION OF ANTIVENOM:- The difference between purification and concentration must be clearly understood. If 5 grams of salt and 5 grams of sugar are dissolved in 500 ml(c.c,) of water, the concentration of both can be increased by reducing the volume by evaporation. But there are still 5 grams of each in the stronger solution. If the sugar can be removed, the solution will have been purified as far as the salt is concerned.

Normal serum contains albumin and globulin as its main protein constituants, and hyperimmune serum, be it diphtheria or tetanus antiserum or anti-snakebite serum, also contains them. The globulin fraction has been so altered by the immunization process that it has become an 'anti'-substance. The concentration of this antibody-globulin can be increased by evaporating the venom to a smaller volume or by precipitating out both the albumin and globulin with salts such as ammonium or sodium sulphate. The precipitate, dissolved in a volume of water smaller than the original volume of serum, will concentrate the antibody but will not increase its purity in the slightest.

Albumin has no value from an 'anti' aspect and if it can be removed from serum, the purity of the antibody will be increased. This can be accomplished by what is called a 'salting-out' process. Globulin is precipitated from solution by a lower concentration of ammonium sulphate than albumin. The solution, dialysed free of sulphate, can be dissolved in a smaller volume of water and this solution forms a concentrated, purified solution of antibody-globulin. But the concentration and purification are low, -- 2-3 times for the first and $1\frac{1}{2}$ - 2 times for the second.

The serum, or serum products of a horse, cannot always be injected into man with impunity. There is a chance that serum sickness (rash, joint and muscle pains) will appear after 8 - 14 days or, in a person sensative to horse dander or in one who has previously received any serum of equine origin, that an immediate shock (anaphylaxis) will occur. Albumin is relatively innocuous in this connexion so that the main benefit accruing from its removal is in concentration rather than in purification.

The globulin in an antiserum contains, for the purpose of this article, 2 parts, one closely associated with the antibody (useful globulin) and the other containing little antibody (useless globulin). The useless globulin is more <u>likely</u> to cause serum reactions and shock than the useful portion but even this is, unfortunately, not free from this disadvantage. Much of the useless globulin can be eliminated by digesting the crude serum with pepsin, the enzyme present in the stomach. The serum is diluted with water, pepsin is added and the pH lowered, i.e. the solution is made acid. When digestion has proceeded for a specified period, the pH is raised, ammonium sulphate is added and mild heat applied. The useless pro-

tein is coagulated and can be removed by filtration. Useful globulin and albumin remain in the solution. Enough ammonium sulphate is added to precipitate the globulin. The precipitate is collected on a filter and the effluent, containing albumin, is discarded. The precipitate, dialysed free of sulphate, is preserved with creosol and sterilized by filtration. By this process, antibody concentrations of 5 - 6 times and purifications of 3 - 3½ times are obtained.

Antisera, purified with pepsin, are usually referred to as 'refined globulins', the word 'refined' connoting 'enzyme'-refined. But there is apparently no law demanding this, so that an antivenom purified without the use of an enzyme may be described as 'refined'. Possibly the words 'enzyme-refined globulins' should be used to prevent misunderstanding.

LABORATORY ASSAY OF ANTIVENOM:- When a method of assay exists, no antiserum should be issued without a statement of potency. For example, diptheria and tetanus antitoxins are issued in 'units per ml'. To say that '10 ml of tetanus antitoxin were injected' is almost meaningless. A unit of potency for Cape Cobra antivenom, but not for ringhals or puff adder antivenom, was established years ago by the Union Department of Health, but even for Cape cobra antivenom, no minimal requirements were laid down. This means that a producer is not required to issue serum of at least 'x' potency. In the interest of users of anti-snakebite serum there is an urgent need for such a potency regulation.

At the South African Institute for Medical Research white mice are used to assay the 3 antivenoms contained in polyvalent antiserum. A dose of venom containing between 10 and 20 minimal lethal doses of venom is mixed with varking ammounts of serum. The mixtures are left for some time at room temperature and are then injected intravenously into mice. A value, based on the neutralising dose of anti-venom, can then be assigned to the serum.

KEEPING QUALITIES:- If serum is stored in a refrigerator its potency remains unaltered for a long time, certainly for 10 years. If held in a cool place when not actually being carried in the veld it will remain potent for at least 3 years, probably much longer. The value of some South African Institute for Medical Research serum that had been stored (sic) for 6 years in the cubby hole of a car that had traversed Southern Africa on several occasions showed no demonstrable drop in value. But although antiserum is 'tough', it should not be needlessly exposed to high temperatures; at 50°C it will become opalescent and at 60°C it will coagulate.

CASE HISTORY OF A PUFF ADDER (Bitis arietans) BITE.

(Extract from Report on Schools' Exploration Society (Matabeleland Branch) Expedition to Tuli.)

By Luchi Balarin

On Monday, 11th May 1959, Simon Holmes-a-Court was bitten by a 2 foot female puffadder. The bite was with one fang and was inflicted on the knuckle of the left thumb. The following report was compiled