Mem. Inst. Butantan
Simp. Internac.
33(2):583-588, 1966

60. CARDIOVASCULAR RESPONSES TO SNAKE VENOMS AND THEIR FRACTIONS

FINDLAY E. RUSSELL

University of Southern California, School of Medicine, Los Angeles, County General Hospital, Los Angeles, California, U.S.A.

It has long been known that certain snake venoms exert a deleterious effect on the cardiovascular system. During more recent years, attempts have been made to determine which fraction or fractions of snake venoms are responsible for these deleterious effects. Some attempt has also been made to define the relationships between the direct effects of the venom on the cardiovascular system and those which may be precipitated by autopharmacological changes initially provoked by the venom. In the course of these various investigations, many important data, which indicated the complexity of the vascular response, have been obtained. The purpose of the present paper is to present a short review of the compendium of knowledge on the cardiovascular effects produced by snake venoms, and to reflect on some experiences which, I feel, are important as guide lines for future research on the pharmacology of these fascinating toxins.

First, I should like to discuss, in a very general way, some relationships between the physiopharmacological activities of snake venoms and their chemistry. We are well aware, as has been pointed out by numerous workers, that snake venoms are complex mixtures, chiefly proteins, many of which have enzymatic activities. There was a time in our thinking when *all* of the deleterious effects of venoms were linked with the enzymatic components of the toxin. We now know, of course, that this is not true, and indeed, the more lethal activities of most venoms — whether they be snake, arthropods or fish — are not generally associated with one enzyme or even with several, although it does appear that certain enzymes may be closely bound with certain non-enzymatic proteins in a venom, and that these combinations can be quite lethal. On the other hand, the enzymes of snake venoms are certainly responsible for some of the deleterious changes provoked by the crude toxin, and perhaps in particular with those changes which appear to occur at the cell membrane, whether the cell be one in the intima of a vessel or in subcutaneous tissues, or even if it be a blood cell. These unusual combinations of multiple enzymatic and non-enzymatic proteins, along with certain non-protein substances, not only indicate very complex organization and physiopharmacological activity, but might appear to reflect upon a series of adaptive mechanisms during the evolution of the venom. On the other hand, snake venom enzymes may be involved in far more complex biological functions, as has long been proposed by Professor Zeller in his many fine papers. I am sure we are all becoming impressed with the fact that, in general, there is a great deal of conformity in the organization of the chemistry of snake venoms, and while certain fractions are indeed quite different, chemically as

well as physiopharmacologically, particularly at the family level, there appear to be more similarities than differences than what we had thought one or two decades ago. This same general impression is also obtained when one considers the marine venoms and poisons, and indeed to some extent even with arthropod toxins.

There is a tendency, perhaps fortunately so, in the well-oiled scientific mind, to gather data into orderly groups for purposes of classification. This is an admirable trait but it can be fraught with some dangers, and perhaps nowhere in biology is this more evident than in our own field. In the absence of reliable data there has been a tendency to arrange snake venoms into loose indefinable classifications. These classifications have, in many instances, been more confusing than helpful. Even such widely used terms as *neurotoxin* and *cardiotoxin* are inadequate and misleading, for they are often applied to the whole venom, and most venoms are complex mixtures having several or many biological properties. It has also been shown that *neurotoxins* can, and often do, have *cardiotoxic* or *hemotoxic* activity, or both; *cardiotoxins* may have *neurotoxic* or *hemotoxic* activity, or both, and *hemotoxins* may have the other activities. The labeling of a venom as a *neurotoxin* is not only confusing but dangerous, for it may lead a physician to make unwise clinical decisions (1).

No one has brought this point closer to focus than H. Alistair Reid, who has recently demonstrated that the principal clinical feature of cobra poisoning in Malaya is local necrosis, and that *neurotoxic* effects in human victims are indeed rare (2). Furthermore, as Dr. Reid points out, and as has been noted by previous workers, the cardiovascular effects of these venoms are often times marked, and in many cases are the cause of death. It would seem, that until the fractions responsible for the deleterious effects of a snake venom have been isolated and studied individually and in combination, we need to exercise extreme care in systematizing data which are based partly on biological assay methods, partly on biochemical studies, partly on clinical observations and partly on intuitive hunches.

There is another weakness in our over-all approach to the study of the physiopharmacological properties of snake venoms. There is a tendency to link specific chemical structures with specific biological activities; i.e., that substance A produces effect A, and that substance B produces effect B, etc. It would appear to me that it would be highly unlikely and unbecoming for nature to have developed venoms in this manner. Particularly, since perhaps one of the most important factors in the evolution of a venom is the role played by the adaption of the prey or the offending animal. But whether or not this concern is founded, the very principle of looking for a specific fraction to exert a specific effect is a dangerous one, for among other things, it may often limit our concept and our experimental approach to determining the mechanism of action of a venom by thinking that it necessarily follows that *one* specific fraction has *one* specific function in or on *one* organ system, although in some cases this may prove to be true.

Some of the so called *neurotoxins* that have been sent to us for further evaluation in cardiovascular preparations during the past five years have had a more marked effect on the cardiovascular system than on the nervous system, and in some cases, in fact, this effect has far out-shadowed that on the latter system. In most of these cases the error in judgement has not weighed with the venom but rather with the experimenter, who, for example, having seen a mouse in convulsions following the injection of the venom has presumed that the

Mem. Inst. Butantan
Simp. Internac.
33(2):583-588, 1966

venom was a *neurotoxin*, when in reality the mouse is convulsing because of cerebral anoxia caused by a markedly reduced blood supply to its brain secondary to systemic hypotension.

There is one other area of cardiovascular research on which I should like to comment. This involves the choice of the proper experimental animal. One must be exceedingly careful in applying data derived from studies in one group of animals to conclusions about the biological effects of a venom in another group of animals or to data on the design, use and adaption of a venom. We are all familiar with the marked differences in the lethality of venoms for different animals and how in some cases these are particularly related to specific ecological problems. Unfortunately, much of our information on the zootoxicological properties of venoms is based on studies with mammals, which, of course, limits their application as far as our understanding the design of the toxin in the animal's armament. On the other hand, it is equally dangerous to apply data obtained on a fish nerve-muscle preparation or the frog heart, or the cockroach heart, to conclusions about the action of the venom on man, or even on a mammal. Certainly the more diversified our studies the more important data that we will obtain, but the application of these data must be guarded zealously.

Even in applying data obtained in mammals one must be exceedingly careful. For instance, data obtained from cardiovascular studies in dogs cannot be liberally applied to humans. It has become increasingly apparent, particularly from studies on shock, that the dog may respond quite differently than the human under similar experimental conditions. The importance of the portal circulation in the dog during various hypotensive crises is far more marked than in the human. In certain cardiovascular conditions the dog must be considered a *portal* animal while man certainly is not. Another difference is in the renal circulation. In the rabbit and cat, as well as in the human, the renal vein is known to dilate quite readily in response to mechanical and certain chemical stimuli. It is far less responsive in the dog, while on the other hand the splenic vein in the latter animal dilates quite easily with specific drugs and stimuli. This probably reflects the high degree of spleen reservoir function in this species. In the cat, rabbit and human the splenic reservoir is much less marked. In certain mammals, particularly the dog, certain venous values play a more important role in the vascular response than they do in the human. The cat, on the other hand, appears to respond to snake venoms in a manner much more like the human than does the dog. In both the cat and in the human the hypotensive crisis evoked by *Crotalus* venom, for instance, is associated with changes in the pulmonary circulation and perhaps in the larger vessels of the chest (1, 3, 4).

These few examples point out the great care one must take in applying data from one group of animals to another, or to humans. One might conclude that perhaps many of the differences noted in the literature between responses to various venoms are more directly related to the choice of animal used than the venom used or even to the technique applied.

Rather than deal with specific data on the cardiovascular effects of snake venoms and their fraction (and of these effects most of us are acquainted), I should like to review some of the basic concepts that one must consider if he chooses to measure and interpret the changes provoked by venoms in the dependent variables of the various parameters of the cardiovascular system.

Fig. 1 illustrates the principle of parallel circuits and resistances in the cardiovascular system. Since the cardiovascular system accomplishes its biological

functions in a mechanical way, it can be seen that any change in one of the parameters will affect changes in the resistance of one, several or all other parameters of the system. These changes will be reflected in the dependent variables, that is in the heart output, and the pressures on the arterial and venous sides of the systemic and pulmonary circulations. The relationships can be plotted as

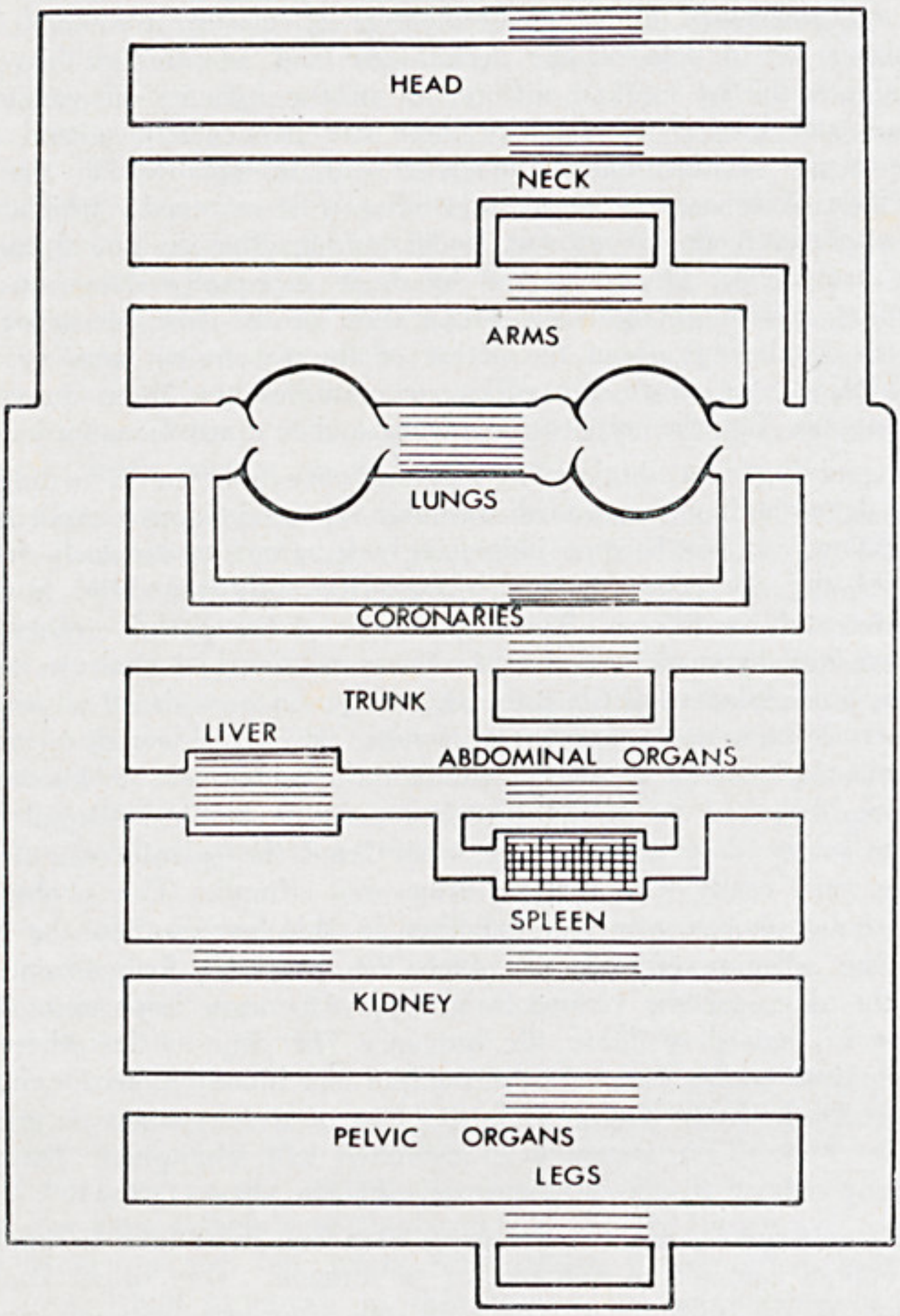

Fig. 1 — Diagram of the circulation showing parameters, including resistances (modified from Wezler and Böger, 1939).

a mathematical formulation, and this has been done by several workers. In 1954, before the days of electromagnetic flowmeters and other flow-pressure monitoring devices, Prof. Van Harreveld and I proposed a model of the circulation (Fig. 2), from which certain equations could be developed and used in defining more carefully the changes provoked in the vascular parameters by various venoms (5).

Mem. Inst. Butantan
Simp. Internac.
33(2):583-588, 1966

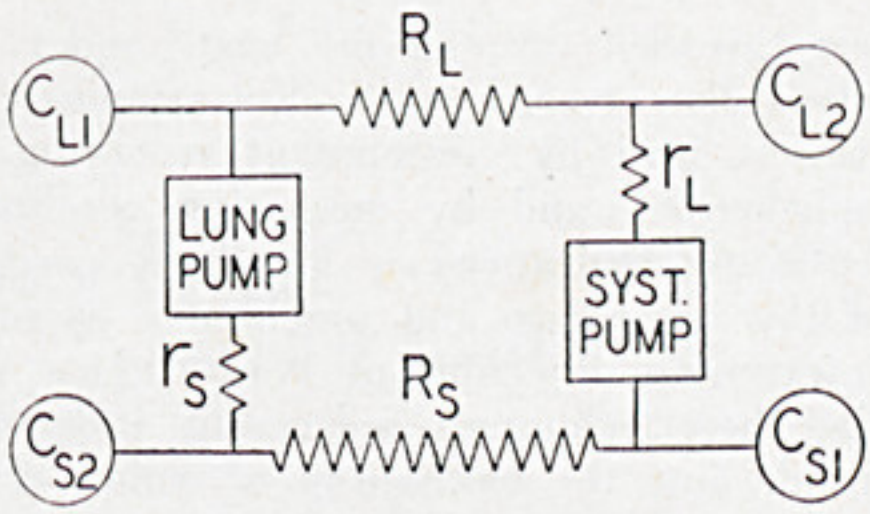

Fig. 2 — Circulatory model.

The relevant equations describing the relations between the parameters and the dependent variables of the circulation may be summarized as follows:

$$I = \frac{V}{C_{S1}\,(R_S + r_S) + C_{S2}\,r_S + C_{L1}\,(R_L + r_L) + C_{L2}\,r_L}$$

$$P_{S1} = \frac{V\,(R_S + r_S)}{C_{S1}\,(R_S + r_S) + C_{S2}\,r_S + C_{L1}\,(R_L + r_L) + C_{L2}\,r_L}$$

$$P_{L2} = \frac{V\,r_L}{C_{S1}\,(R_S + r_S) + C_{S2}\,r_S + C_{L1}\,(R_L + r_L) + C_{L2}\,r_L}$$

In these equations, I is the heart output. P_{S_1} represents the systemic arterial pressure. P_{L_2} indicates the left atrial pressure. V is related, though not synonymous, with the total volume of the circulating blood. R_S and R_L are the resistances in the arterioles and capillaries of the systemic and pulmonary circulations respectively (peripheral resistance). The total resistance in the venous system and all the factors limiting the blood flow into the ventricles are represented by the resistances r_S for the right heart and r_L for the left heart. The largest factor for the value r is the elastical resistance of the ventricular wall against filling. C_{S_1}, C_{S_2}, etc...., are the constants of capacitance (volume change per unit change of pressure) for the various components of the circulatory apparatus. These components are an index of the tone of the larger vessels of the systemic and pulmonary arterial and venous systems.

It can be seen that by varying a parameter one can determine, through measurements in the dependent variables, the pressures and flows in the various components of the cardiovascular system. Today, this has been made more simple by the advent of the electromagnetic flowmeter. It is now possible, with a little care, to measure blood pressure and flow concomitantly in a single vessel, and to carry out this procedure in four or five vessels during a single experiment. The information obtained from such measurements not only gives us valuable data on the changes themselves and their relationships to other vascular phenomena, but it gives us a ready insight into the mechanism of action of the venom.

This might be demonstrated by several recent experiments with *Crotalus* venoms. Using transducers to measure certain arterial and venous pressures on both sides of the systemic and pulmonary circulations (some measurements being

taken through catheters threaded through the heart under fluoroscopy), and by taking simultaneous blood flow records with electromagnetic flowmeters from the same and different vessels, and by concomitant recording of the electrocardiogram and electroencephalogram, and by measuring cerebrospinal fluid pressure and the rate and depth of respirations it has been possible to determine the sequence of events of the immediate and precipitous hypotensive crisis that occurs following the intravenous injection of *Crotalus* venom (3, 4). It has also been possible, using these techniques and certain isolated organ techniques (3, 4), to obtain some insight into the mechanism of action of this venom.

From these studies it has been concluded that the hypotensive crisis is due to changes in the resistance within the pulmonary circuit. These changes lead to a decreased left heart output pressure and flow, which in turn provoke alterations in the flow and pressure within various vessels, changes in peripheral resistance, changes in respiration, cerebrospinal fluid pressure, and in the electrocardiogram and electroencephalogram. It was suggested that the change in pulmonary resistance may be attributed to the pooling of blood in the lungs and larger vessels of the chest, due to post-capillary resistance from either vascular constriction and/or the formation of multiple thrombi. It appears that each of these phenomena may play a part in the crisis. These changes are evident in the human, monkey and cat. They are much less conspicuous in the dog, where the mechanism for the hypotensive crisis may be quite different.

The studies previously noted might serve as a guide for future work on the cardiovascular effects of venoms and venom fractions, for the techniques provide a considerable amount of data not otherwise obtainable, while also permitting a careful check and evaluation of interpretations. But even today new techniques are being developed, and these may give us considerably more insight into the mode of action of toxins than we anticipate. Particularly encouraging is the possibility of measuring definitive vascular changes with tagged venom and blood, although even these tools have obvious limits of usefulness. In all of these studies, however, the critical contribution will still rest with the experimenter. As one studies the progress in our science, as in all sciences, it is obvious that the most significant contributions are fully dependent upon the investigator and not upon his equipment. The cardiovascular effects of venoms and their fractions are slowly being unraveled by scholars and not by gadgets, and we have seen in this meeting a demonstration of the kind of intellectual stimulation and encouragement that will greatly enhance our understanding of the properties of these most remarkable toxins.

References

1. RUSSELL, F. E., in F. A. DAVIS (Editor), *Cyclopedia of Medicine, Surgery and the Specialties,* Vol. II, Philadelphia, 1962, p. 197.

2. REID, H. A., *Brit. med. J.*, **2**, 540, 1964.

3. RUSSELL, F. E., BUESS, F. W., and STRASSBERG, J., *Toxicon,* **1**, 5, 1962.

4. HALMAGYI, D. F. J., STARZECKI, B., and HORNER, G. J., *J. appl. Physiol.* (U.S.A.), **20**, 709, 1965.

5. RUSSELL, F. E., and VAN HARREVELD, A. *Arch. int. Physiol.*, **62**, 322, 1954.