Mem. Inst. Butantan
Simp. Internac.
33(2):461-466, 1966

51. LIBERATION OF PHARMACOLOGICALLY ACTIVE SUBSTANCES FROM MAST CELLS BY ANIMAL VENOMS

E. KAISER and W. RAAB

Department of Medical Chemistry, University of Vienna, Vienna, Austria

In many instances, reactions following contact or parenteral administration of animal venoms are indistinguishable from anaphylactoid reactions. For the first time the analogy between antigen administration in a sensitized animal and the action of a snake venom (*Crotalus adamanteus*) was clearly demonstrated by Arthus (1).

It is now generally accepted that during anaphylaxis several biologically active substances are liberated and activated from precursors (histamine, 5-hydroxytryptamine, heparine, slow reacting substances, kinins) (2, 3, 4).

On the basis of the similarity between the effects of snake venoms and histamine (5, 6, 7), the liberation of histamine from perfused tissues by animal venoms was investigated by Feldberg and Kellaway (8). The release of histamine was first attributed to the phospholipase activity of the venom and to the lysophosphatides formed (9). Rocha e Silva and coworkers (10, 11) demonstrated the liberation of histamine from isolated organs following perfusion with proteolytic enzymes and snake venoms. Besides histamine, slow reacting substance appears in the perfusion fluid of isolated lung under the influence of snake and bee venoms (12). 5-hydroxytryptamine is liberated by animal venoms from platelets (13). Bradykinin can be formed from the globulin fraction of normal plasma (bradykininogen) under the influence of snake venoms or trypsin (14, 15).

In anaphylactic and anaphylactoid reactions (administration of animal venoms) identical mediators are formed and liberated (16). In both types of reactions, tissue mast cells play an important role. Certain chemical substances as well as enzymes produce anaphylactoid reactions by degranulating mast cells and by liberating biologically active substances; concomitantly kinins are activated from precursors.

In Table I, a summary is given of direct and indirect mediators of anaphylactic shock, which are found in animal venoms.

Histamine has been identified in the venoms of **HYMENOPTERA** (17, 18), **DIPTERA** (19), **LEPIDOPTERA** (20), **HEMIPTERA** (21), *Scolopendra* (22) and **OCTOPODA** (23).

5-Hydroxytryptamine is present in the venoms of **ACTINARIA** (24, 25), **GASTROPODA** (26), **CEPHALOPODA** (27, 28) **HYMENOPTERA** (29), **ARACHNIDA** (30, 31) and in skin secretions of **AMPHIBIA** (32, 33, 34).

TABLE I — ANAPHYLACTOID SUBSTANCES IN ANIMAL VENOMS

DIRECT EFFECTORS:
Histamine
5-hydroxytryptamine (5-HT, serotonin)
kinins
INDIRECT EFFECTORS:
(Histamine, 5-HT, kinins)
Enzymes (proteases, phospholipase, esterases, hyaluronidase)
Kinin-producing fraction
Anaphylatoxin-producing fraction
High molecular mast cell depletors of unknown structure
Low molecular mast cell depletors of known structure
Surface active agents

A characteristic *kinin* was found in wasp venom (26, 35, 36, 37). In the venom of *Bothrops jararaca* a bradykinin potentiating factor has been found (38).

It should be mentioned that histamine (39, 40), 5-hydroxytryptamine (41, 39, 40) and kinins (42) exert a mast-cell depleting effect; for this reason, these substances are considered to be indirect effectors of anaphylactoid shock, too.

Several animal venoms contain mast cell depleting (43) enzymes (44) (proteases, phospholipase, esterases, hyaluronidase).

The *kinin-producing fractions* are mainly related to the enzymes of the venoms.

An *anaphylatoxin-producing fraction* was found in Cobra venom (45); it is known that anaphylatoxin degranulates mast cells (46, 47).

High molecular mast cell depletors have been extracted from the jelly fish (48, 49, 50, 51), from the eelworm of swine (52) and from the skin secretions of **AMPHIBIA** (53,34, 54, 55, 56).

Spermine which is known to liberate histamine (57) is found in spider venom (58). *Holothurin*, a saponin-like substance, was isolated from *Holothuria vagabunda* (59). It has been shown that surface active agents are potent mast cell depletors (60).

In Fig. 1, a simplified summary is given on the mechanism which provokes anaphylactoid shock, with special reference to the action of animal venoms.

Morphologically, mast cell degranulation by animal venoms can easily be observed. Biopsies taken at various intervals after injections of animal venoms (*Agkistrodon piscivorus*, skin secretion of *Bombina variegata*) demonstrate the different stages of mast cell degranulation. 3 hours after injection, practically all mast cells are found degranulated. The severe vascular changes at sites of injections seem to result from liberated mast cell substances as well as from direct toxic effects of the venoms (55, 61).

Mast cell degranulation by animal venoms can also be demonstrated in isolated cells by microscopic examination or by biochemical analysis of the suspension fluid (50).

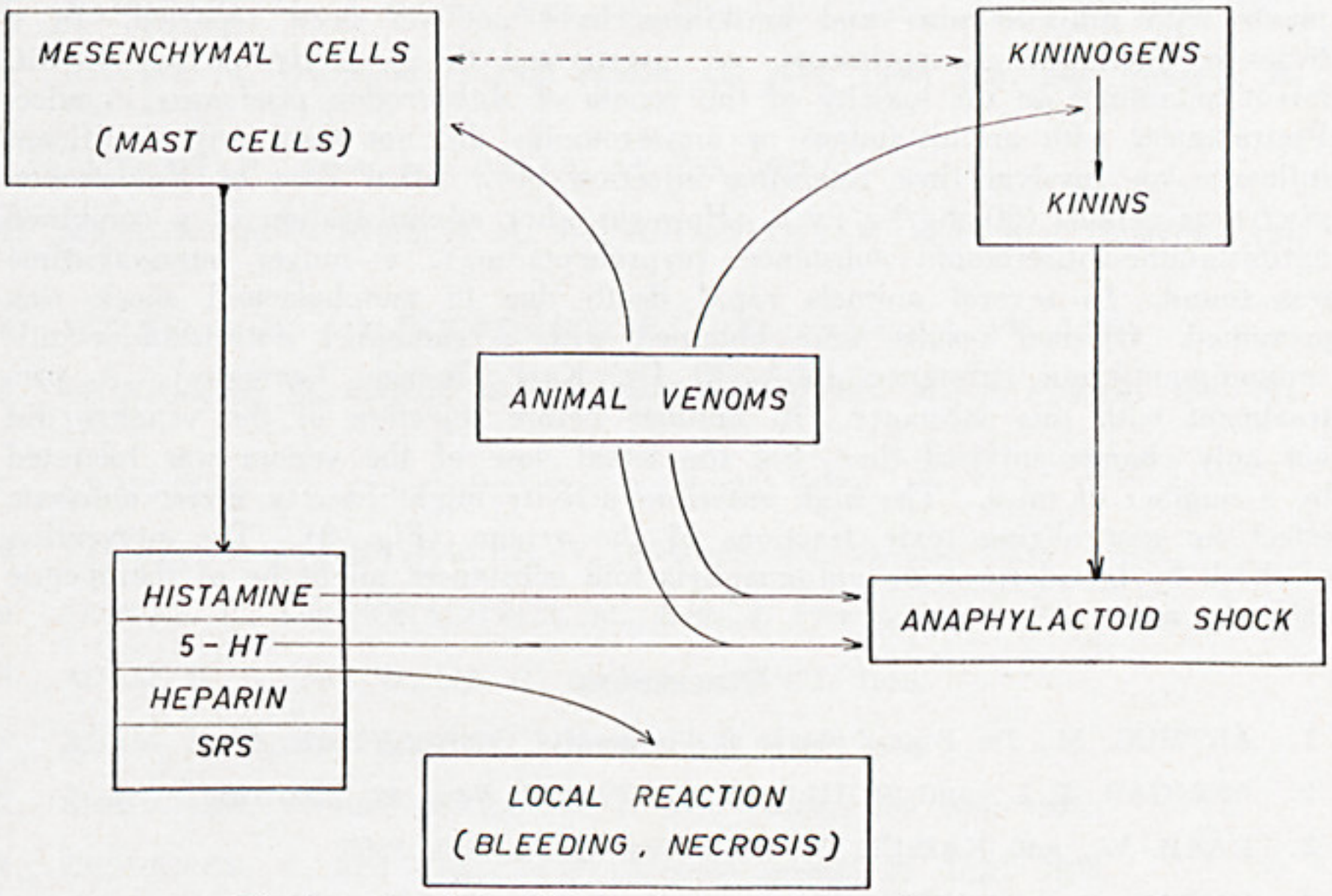

Fig. 1 — Anaphylactoid shock provoked by animal venoms.

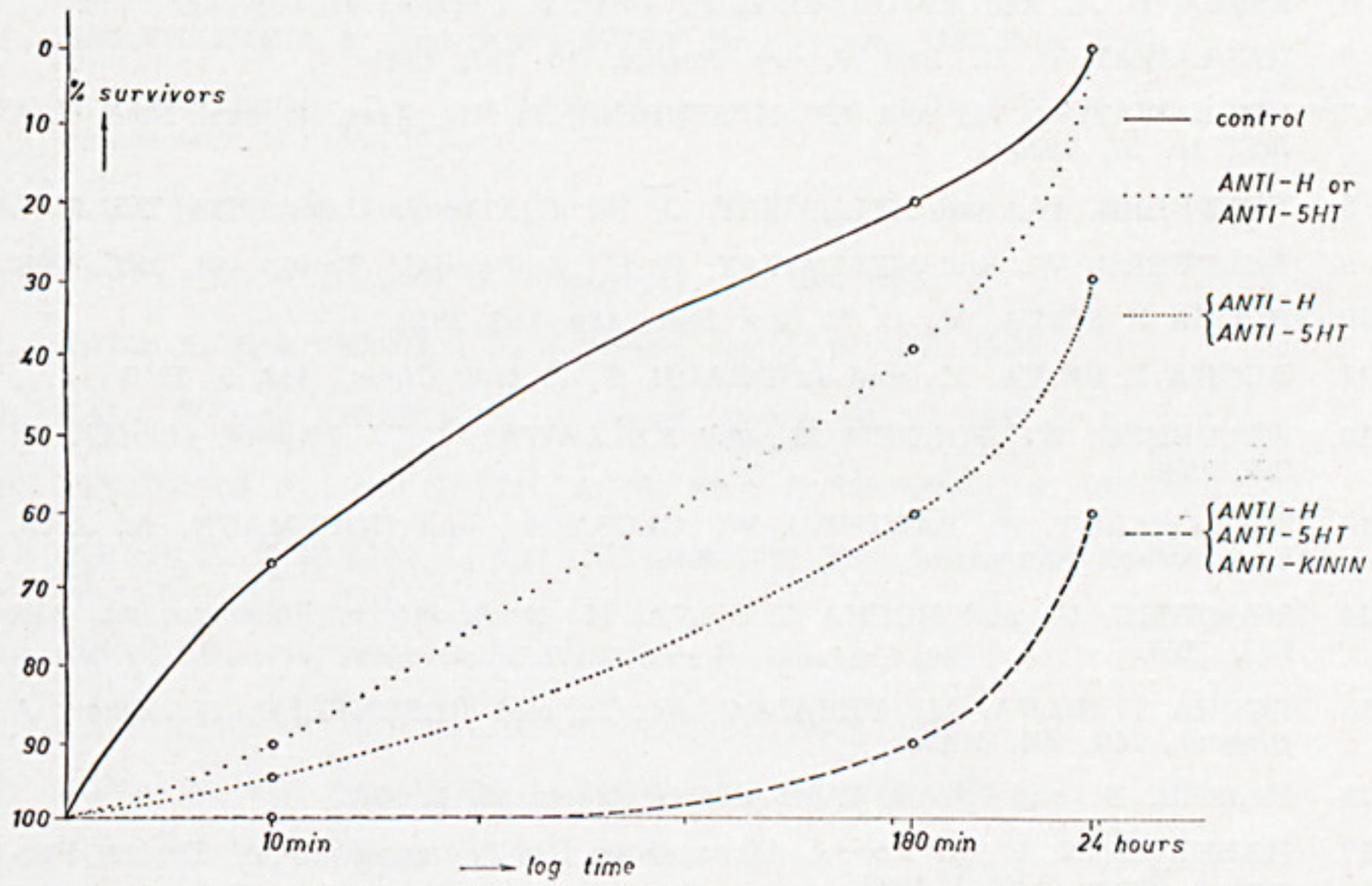

Fig. 2 — Inhibitory effect of antihistamine-, antiserotonin- and antikinin-substances on mortality following snake venom.

Anaphylactoid reactions can be prevented by administration of antamine substances: antihistamines, antiserotonins and antikinins. Antihistamines did not exert any significant influence on toxicity of snake venoms (62, 63), exper

ments with antiserotonins and antikinins have not yet been reported. In a series of preliminary experiments, we investigated the protective action of different antamines on the toxicity of the venom of *Agkistrodon piscivorus* in mice. Pretreatment with antihistamines or antiserotonins did not show any significant influence on survival time following injection of a lethal dose of *Agkistrodon piscivorus* venom (20 mg/kg i.v.). However after administration of a combined antihistamine-antiserotonin substance (cyproheptadine), a longer survival time was found. In several animals rapid death due to anaphylactoid shock was prevented. Optimal results were obtained with a combined antihistamine-antiserotonin-antikinin substance (WA-335 Dr. Karl Thomae, Germany). A pretreatment with this substance (15 minutes before injection of the venom) did not only change survival time, but the lethal dose of the venom was tolerated by a number of mice. The high antikinin activity might exert a direct *antitoxic* effect by neutralizing toxic fractions of the venom (Fig. 2). The prevention of shock by broad spectrum anti-anaphylactoid substances might be of therapeutic value in man.

References

1. ARTHUS, M., *De l'anaphylaxie à l'immunité,* Masson, Paris, 1921.
2. MONGAR, J. L., and SCHILD, H. O., *Physiol. Rev.,* **42**, 226, 1962.
3. RAAB, W., and KAISER, E., *Klin. Wschr.,* **43**, 345, 1965.
4. UNGAR, G., and HAYASHI, H., *Ann. Allergy,* **16**, 542, 1958.
5. ESSEX, H. E., and MARKOWITZ, J., *Amer. J. Physiol.,* **92**, 698, 705, 1930.
6. KELLAWAY, C. H., *Brit. J. exp. Pathol.,* **10**, 281, 1929.
7. KELLAWAY, C. H., and LE MESSURIER, D. H., *Aust. J. exp. Biol. med. Sci.,* **14**, 57, 1936.
8. FELDBERG, W., and KELLAWAY, C. H., *J. Physiol.* (Lond.), **90**, 257, 1937.
9. FELDBERG, W., and KELLAWAY, C. H., *J. Physiol.* (Lond.), **94**, 187, 1938.
10. ROCHA E SILVA, M., *C. R. Soc. Biol.,* **130**, 186, 1939.
11. ROCHA E SILVA, M., and ANDRADE, S., *J. biol. Chem.,* **149**, 9, 1943.
12. FELDBERG, W., HOLDEN, H., and KELLAWAY, C., *J. Physiol.* (Lond.), **94**, 232, 1938.
13. MARKWARDT, F., BARTHEL, W., GLUSA, E., and HOFFMANN, A., *Arch. exp. Pathol. Pharmacol.,* **252**, 297, 1966.
14. HAMBERG, U., and ROCHA E SILVA, M., *Arch. intern. Pharmacodyn.,* **110**, 222, 1957.
15. ROCHA E SILVA, M., BERALDO, W. T., and ROSENFELD, G., *Amer. J. Physiol.,* **156**, 261, 1949.
16. KAISER, E., and RAAB, W., *Z. angew. Zool.,* **52**, 1, 1965.
17. HABERMANN, E., in *Recent Advances in the Pharmacology of Toxins,* Pergamon Press, Oxford, 1965.
18. SLOTTA, K., and BORCHERT, P., *Mem. Inst. Butantan,* **26**, 279, 1954.
19. ECKERT, D., PAASONEN, N., and VARITIANEN, A., *Acta Pharmacol.,* **7**, 16, 1951.
20. VALLE, J. R., PICARELLI, Z. P., and PRADO, J. L., *Arch. intern. Pharmacodyn.,* **98**, 324, 1954.

21. YAMASAKI, M., *J. Orient. Med.*, **34**, 401, 1941; *Chem. Abstr.*, **35**, 8124, 1941.

22. KONISI, M., *Okayama Igakkai Zasshi*, **48**, 1309, 1936; *Ber. ges. Physiol.*, **95**, 528, 1936.

23. BETAZZI, P., *Arch. int. Physiol.*, **18**, 313, 1921.

24. MATHIAS, A. P., ROSS, D. M., and SCHACHTER, M., *J. Physiol.* (London.) **151**, 269, 1960.

25. WELSH, J. H., and MOORHEAD, M., *J. Neurochem.*, **6**, 146, 1960.

26. BHOOLA, K. D., CALLE, J. D., and SCHACHTER, M., *J. Physiol.*, **159**, 167, 1961.

27. ERSPAMER, V., *Arzneimittel-Forsch.*, **2**, 253, 1952.

28. VIALLI, M., and ERSPAMER, V., *Mikrochemie*, **24**, 253, 1938.

29. JAQUES, R., and SCHACHTER, M., *Brit. J. Pharmacol.*, **9**, 53, 1954.

30. ADAM, K. R., and WEISS, C., *Nature*, **178**, 421, 1956.

31. ADAM, K. R., and WEISS, C., *Nature*, **183**, 1398, 1959.

32. ERSPAMER, V., and ASERE, B., *J. biol. Chem.*, **200**, 311, 1953.

33. ERSPAMER, V., and VIALLI, M., *Ricerca scient.*, **22**, 1420, 1952.

34. KISS, G., and MICHL, H., *Toxicon*, **1**, 33, 1962.

35. HERXHEIMER, A., and SCHACHTER, M., *Nature*, **183**, 1510, 1959.

36. HOLDENSTECK, D. J., MATHIAS, A. P., and SCHACHTER, M., *Brit. J. Pharmacol.*, **12**, 149, 1957.

37. SCHACHTER, M., and THAIN, E. M., *Brit. J. Pharmacol.*, **9**, 352, 1954.

38. FERREIRA, S. H., *Brit. J. Pharmacol.*, **24**, 163, 1965.

39. KALZ, F., and FEKETE, Z., *J. invest. Derm.*, **35**, 135, 1960.

40. KALZ, F., and FEKETE, Z., *J. invest. Derm.*, **36**, 37, 1961.

41. FELDBERG, W., and SMITH, A. N., *Brit. J. Pharmacol.*, **8**, 406, 1953.

42. STERN, P., NIKULIN, A., and FERLUGA, J., *Arch. intern. Pharmacodyn.*, **141**, 528, 1962.

43. SELYE, H., *The Mast Cells*, Butterworth, London, 1965.

44. KAISER, E., and MICHL, H., *F. Deuticke*, Wien, 1958.

45. VOGT, W., and SCHMIDT, G., *Experientia*, **20**, 207, 1964.

46. MOTA, I., *Immunology*, **2**, 403, 1959.

47. ROCHA E SILVA, M., and ARONSON, M., *Brit. J. exp. Path.*, **33**, 577, 1952.

48. HÖGBERG, B., SÜDOW, G., THON, I. L., and UVNÄS, B., *Acta physiol. scand.*, **38**, 265, 1957.

49. HÖGBERG, B., THUFVESSON, G., and UVNÄS, B., *Acta physiol. scand.*, **38**, 135, 1957.

50. UVNÄS, B., *Ann. N.Y. Acad. Sci.*, **90**, 751, 1960.

51. UVNÄS, B., *J. med. pharm. Chem.*, **4**, 511, 1961.

52. DIAMANT, B., HÖGBERG, B., THON, I. L., and UVNÄS, B., *Acta physiol. scand.*, **42**, Suppl. 145, 1957.

53. KAISER, E., and KRAMAR, R., *Toxicon*, in press.

54. MICHL, H., and BACHMAYER, H., *Mschr. Chemie* (Wien), **94**, 814, 1963.

55. RAAB, W., and KAISER, E., *Arch. klin. exp. Derm.*, **220**, 374, 1964.

56. BACHMAYER, H., MICHL, H., and ROSE, B., *Toxicon*, in press, 1966.

57. KUTTNER, K., HUBER, G., and JAQUES, R., *Experientia*, **18**, 130, 1962.

58. FISCHER, F. G., and BOHN, H., *Ann. Chemie*, **603**, 232, 1957.

59. YAMANOUCHI, T., *Publ. Sete Marine Biol. Lab.*, **4**, 183, 1955; *Chem. Abstr.*, **50**, 3660, 1956.

60. GROSSBERG, A. L., and GARCIA-AROCHA, M., *Science*, **120**, 762, 1954.

61. RAAB, W., and KAISER, E., *Toxicon*, **3**, 49, 1965.

62. PARRISH, H. M., SCATTERDAY, J. E., and MOORE, W., *J. Am. vet. med. Ass.*, **129**, 522, 1956.

63. SCHÖTTLER, W. H. A., *Amer. J. trop. Med.*, **3**, 1083, 1954.

Discussion

E. *A. Zeller:* "If serotonin, histamine, and other biogenic amines play an essential role in the action of certain venoms, then it should be possible to change the response to venom by changing the metabolism of those biogenic amines. Since amine oxidases, e.g. diamine oxidases (histaminase) and monoamine oxidases can be blocked *in vivo*, I wonder whether anybody has pretreated laboratory animals with inhibitors of the enzymes before the venoms were administered?"

E. Kaiser: "As far as I know substances blocking monoamine oxidase have not been used until now. Preliminary experiments in our laboratory have shown that pretreatment with compound 48/80 (synthetic mast cell depletor) significantly diminishes anaphylactoid reactions following snake venoms."

H. Edery: "Would you please tell us more details about this anti-kinin substance you mentioned. It is specific, it acts in other organ system reactive to kinins?"

E. Kaiser: "I am sorry to say that only very limited information is available on this substance (WA-335, Dr. Thomae, Western Germany). At the present moment I can only tell you that the substance has a high antihistamine, antiserotonin and antikinin activity. I am not informed about pharmacological tests performed by Dr. Thomae."

E. R. Trethewie: "Do you think adenosine and related enzymes released are significant in that cardiac effects? One can select a venom that is simpler in effect, e.g., *Pseudechis porphyriacus* (Australian Black Snake), antihistamine with heparine does reduce its mortality. Polyvinylpyrrolidone will prolong life with the hemotoxic Tiger Snake venom."

E. Kaiser: "Our experiments were restricted to the venom of *A. piscivorus* until now. We have no personal experience on the cardiotoxic effects of the venom."