42. ON THE ACTION OF BUFOGENINS AND ALLIED COMPOUNDS ON THE INTESTINE AND OTHER SMOOTH MUSCLE ORGANS

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Pharmacological studies on the active principles isolated from Ch'an Su, the dried venom of the Chinese toad, revealed some interesting activities. For example, bufalin, a coprostane-type steroid with a six membered unsaturated lactone, has a very strong local anesthetic action and, as Dr. Okada reported in this Symposium, its mode of action differs markedly from that of cocaine and resibufogenin, a steroid with an expoxide in 14-15 position, which has a strong stimulating action on respiration and blood pressure. This paper reports the result of an investigation on the pharmacological actions of bufogenins which were mainly on guinea-pig smooth muscles (intestine, blood vessels and trachea), with the expectation of obtaining some useful data which could throw some more light on their action mechanisms.

Their effects on intestinal tissues were principally studied by using the ileum preparation according to the Magnus perfusion method. The resibufogenin minimum effective doses for intestinal contractions were 5×10^{-8} to 10^{-7}, and the dose-response curve was a sigmoid up to the concentration of 4×10^{-6} (Fig. 1), in higher concentrations, the responses were reduced. All bufogenins studied act similarly upon this smooth muscle preparation.

This contracting action on the ileum preparation is depressed to some degree by atropine (10^{-8}) and morphine (5×10^{-7}), and enhanced by eserine (10^{-9}) (Fig. 2), suggesting the participation of Ach release in the concentration mechanism. As it is not influenced by C6 the Ach releasing effect seems not be ganglionic, but post ganglionic (Fig. 2).

The guinea-pig ileum inhibiting effect induced by atropine is not reproduced in the taenia-coli preparation. In the latter preparation, the contracting actions of the so called Ach-releasers such as nicotine and picric acid were not observed (Fig. 3). This seems to indicate, therefore, that its irresponsiveness seems to result on account of its lack of nerve tissues, which release Ach.

The kymogram of Fig. 4 shows the intestinal contractions produced by bufalin and the blocking effects of atropine. The dose-response curves obtained with the same preparation (Fig. 4) show that bufalin has a much more stronger activity than resibufogenin, and that g-strophanthin is less active than resibufogenin. Though digitoxigenin is more active than resibufogenin the latter is more active than digitoxin. Generally the actions of genins are stronger and faster in appearance than those of the glycosides.

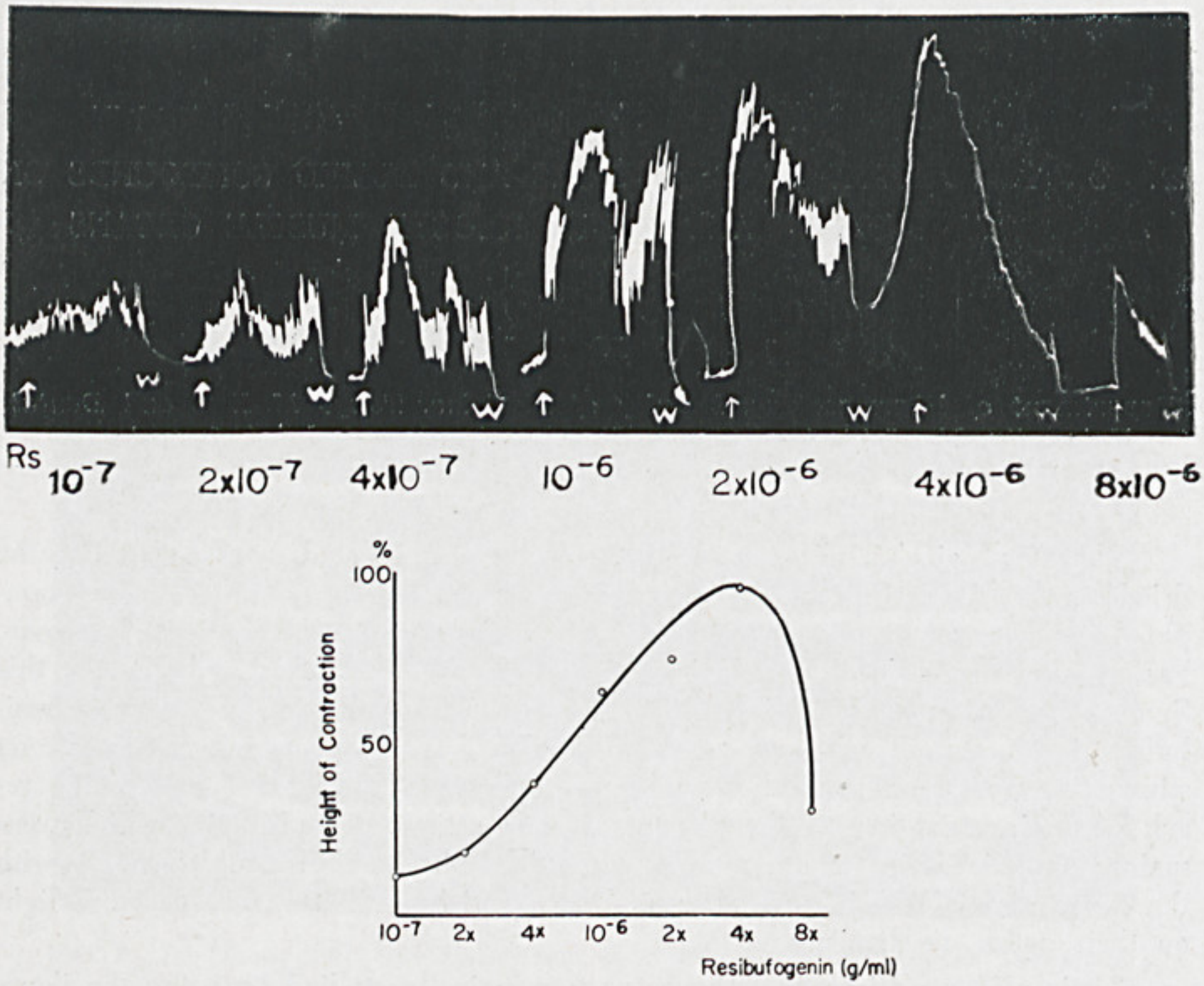


Fig. 1 — Action of Resibufogenin on the small Intestine of Guinea-pig.

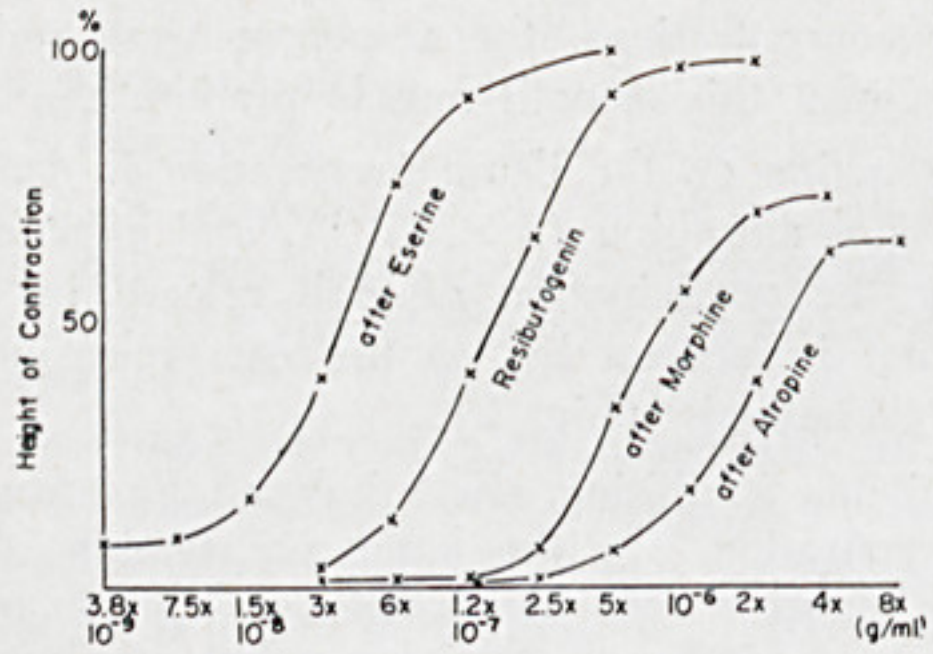


Fig. 2 — Dose-Response Curve of Resibufogenin.

When a comparatively high concentration of resibufogenin is applied to the guinea-pig ileum, relaxation follows contraction considerably faster than with the taenia-coli preparation. Supposing that a probable Ca^{++} release would participate in this mechanism, we studied the effects of nethalide (β-blocker), reserpine and dibenzyline on the mode of relaxation (Fig. 5). There was no remarkable difference of the effects after the pretreatment by these substances.

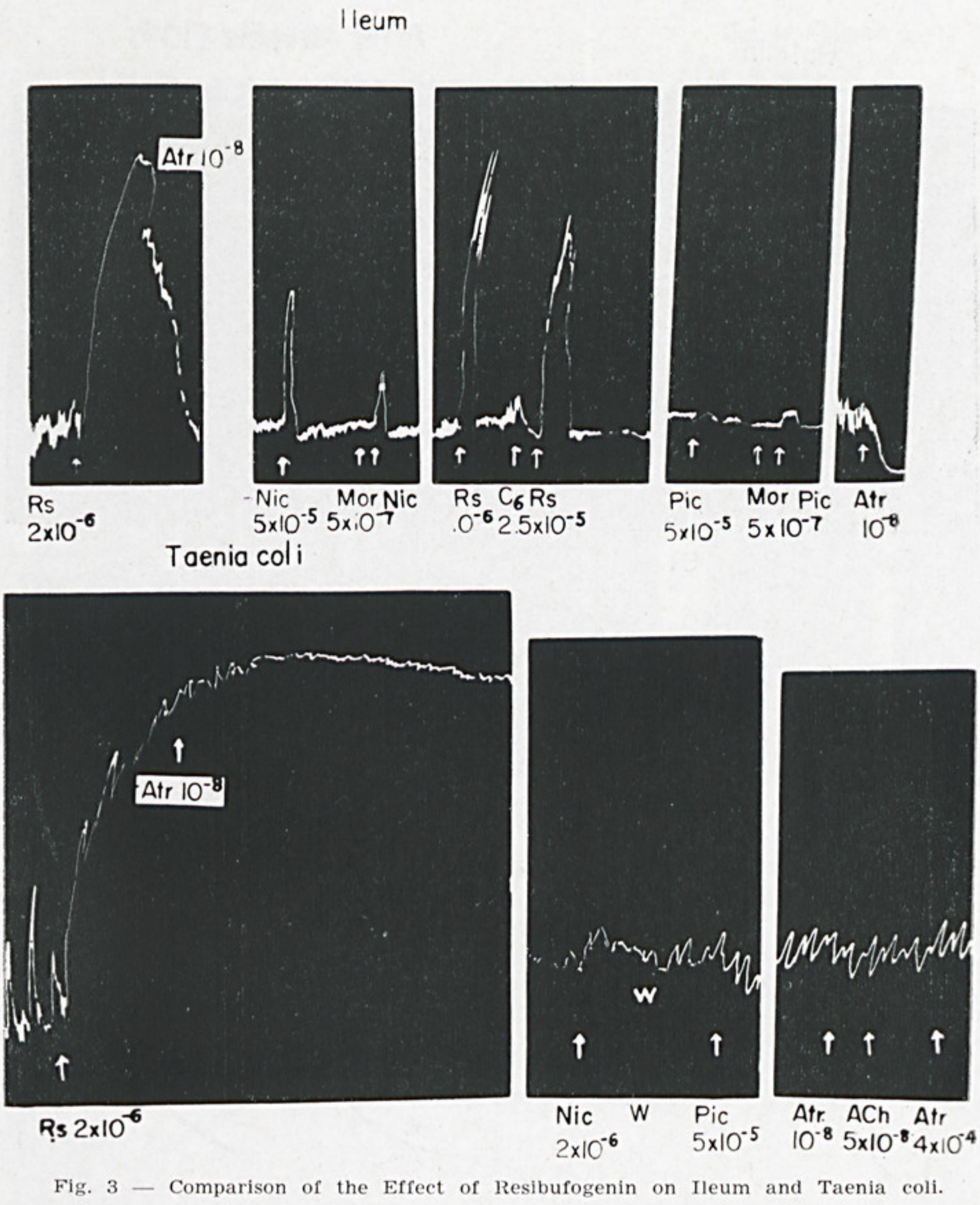
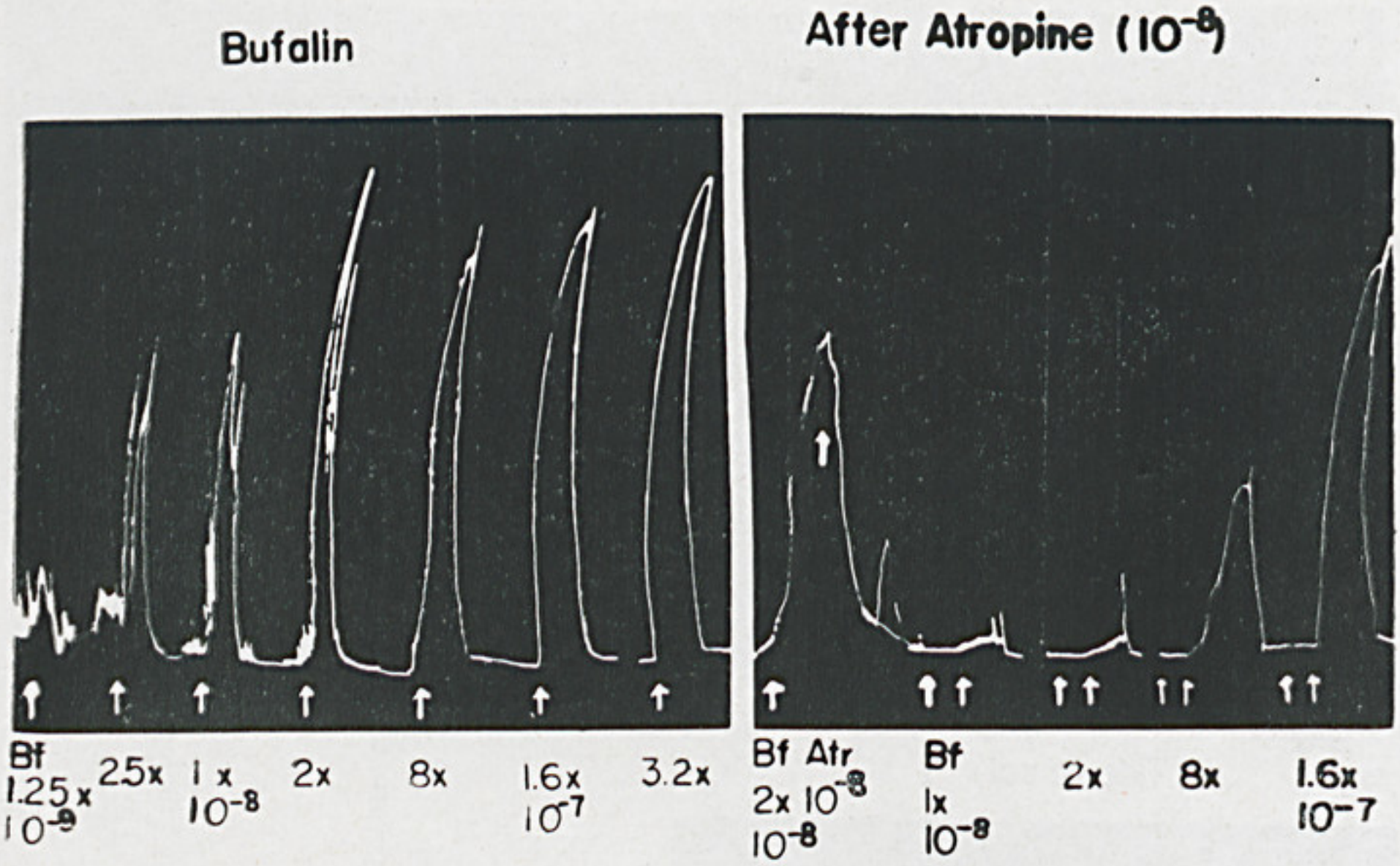


Fig. 3 — Comparison of the Effect of Resibufogenin on Ileum and Taenia coli.

Based on these results, we can assume that Ach release participates from the contracting action of bufogenins, in addition to their direct actions on the excitable membrane of the smooth muscle, as reported by H. J. Schatzmann *et al.* for the taenia-coli preparation. In the phase of relaxation there seems to be no participation of Ca^{++} release, this relaxation seems to depend upon the direct inhibitory effect exerted on the excitable membrane.

It is known that bufogenins and allied compounds, provoke a rise in blood pressure, as a consequence of an augmentation of peripheral vascular resistance resulting from vasoconstriction effects. In the spirally cut aortic strip preparation



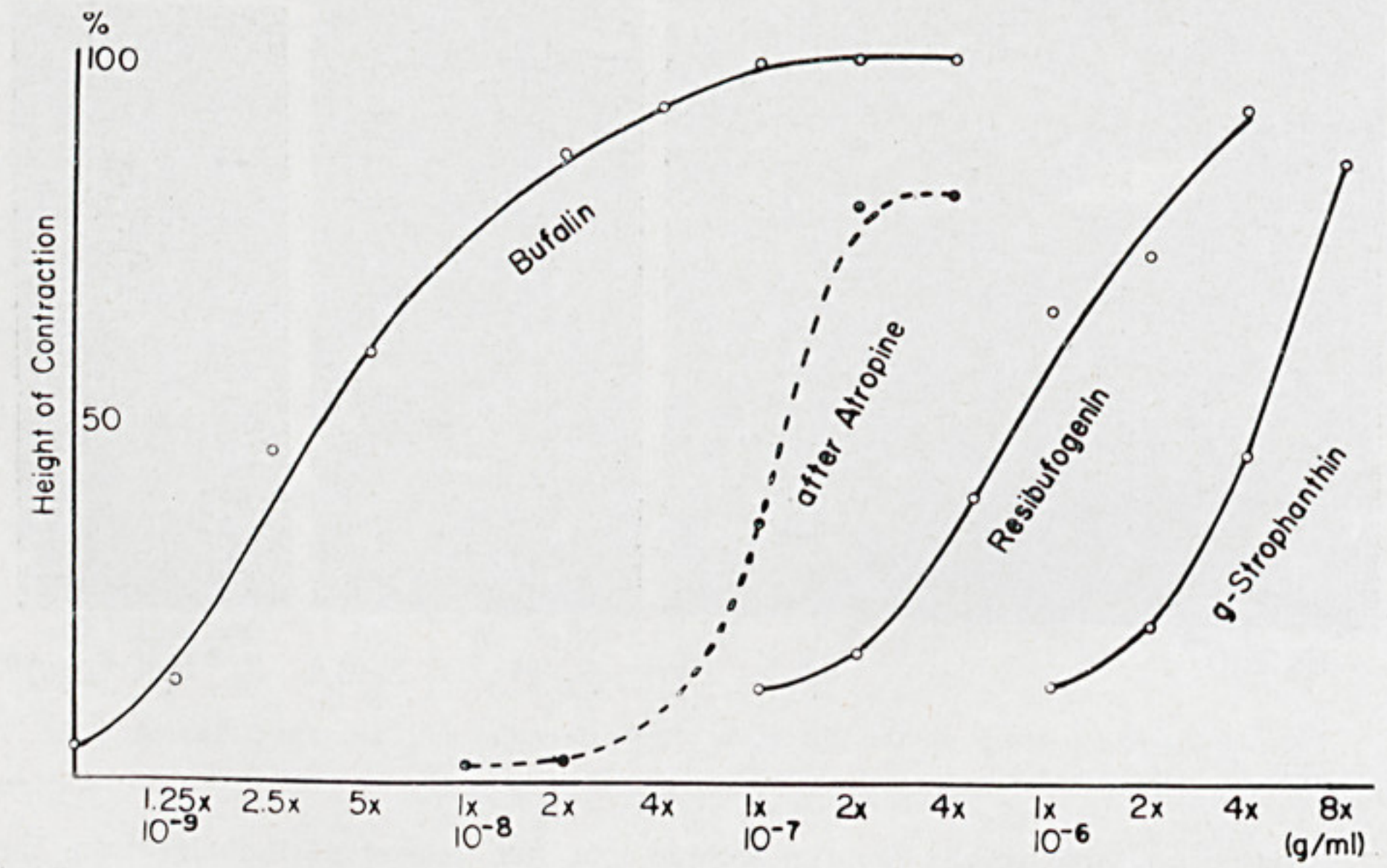


Fig. 4 — Actions of Bufogenins on the small intestine.

arranged according to G. Paterson, bufogenin did not cause such a remarkable contraction as in the ileum preparations, but only a slight rise of muscular tone. However, they potentiated the contraction induced by epinephrine and KCl. These phenomena resembles the effect of Ca^{++} on the K contracture (Fig. 6). The peak of maximum effect is in the 1.5 times concentrated Ca^{++} solution. This

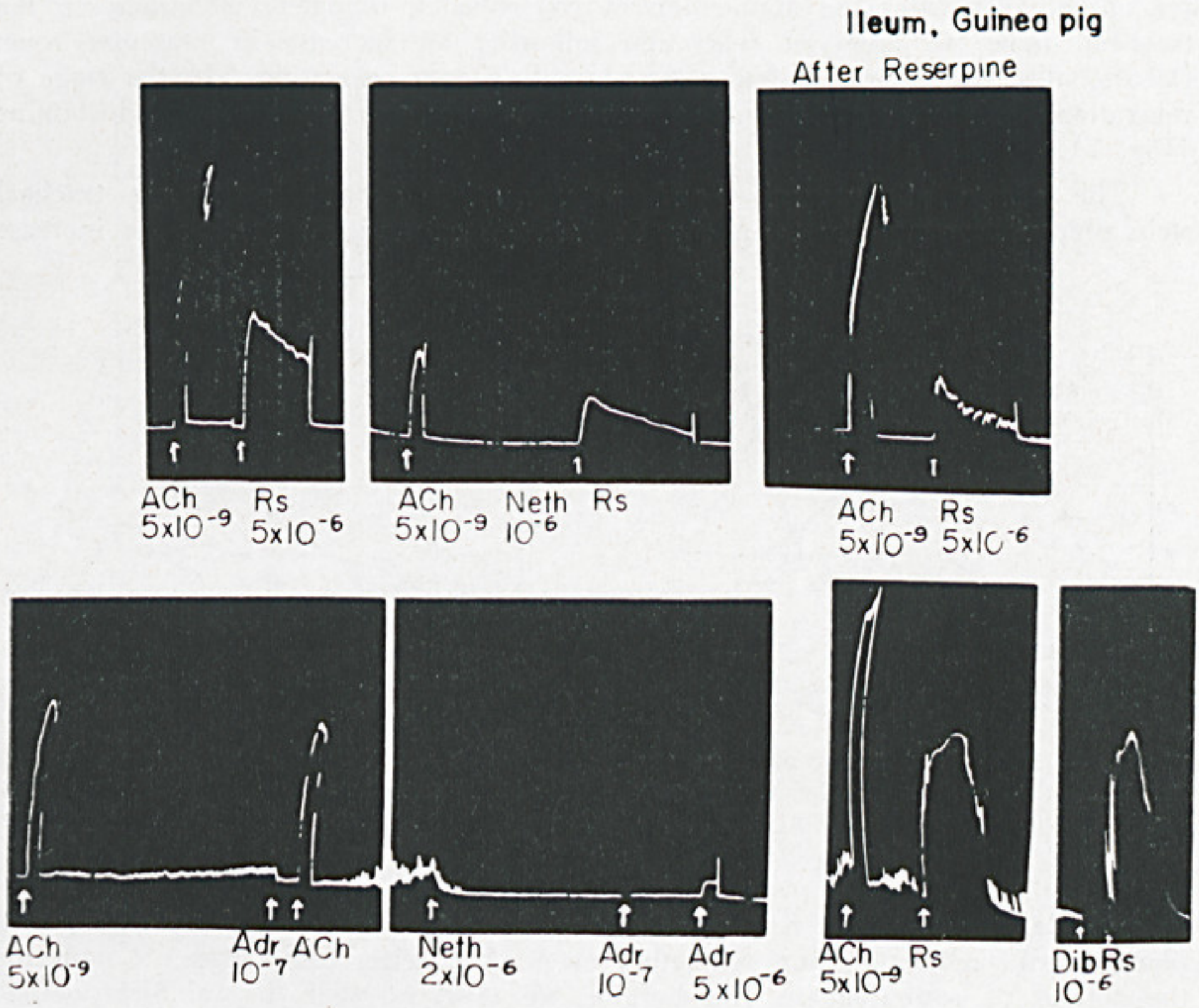


Fig. 5 — Effect of Nethalide and Dibenzyline on the intestinal contraction produced by Bufogenin.

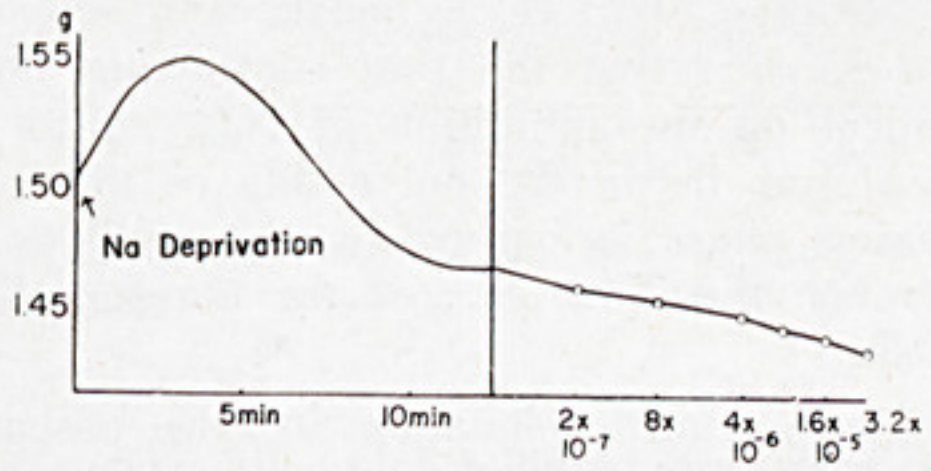


Fig. 6 — Action of Bufogenin of the Tracheal Strip immersed in Na-free Krebs Solution.

concentration of Ca^{++} augmented the contraction produced by K. Other differences, between the actions of bufogenins upon the blood vessels and heart tissue were observed and will be referred to later.

The effects of bufogenins on the tracheal smooth muscle were studied on the spirally cut tracheal strip preparation described by J. W. Constantine. In contrast to their effects on the aortic preparation, where we could not observe any

relaxation, even after the application of concentrated bufogenin solutions, in the tracheal tissue we observed relaxation following an increase in muscular tone. Occasionally we observed a few cases presenting only relaxation. In the stage of relaxation, bufogenin reduced the contractions produced by Ach and histamine (Fig. 7).

The effects of bufogenins and g-strophanthin on the guinea-pig tracheal strip preparations are shown in Fig. 8. Though relaxation following the increase

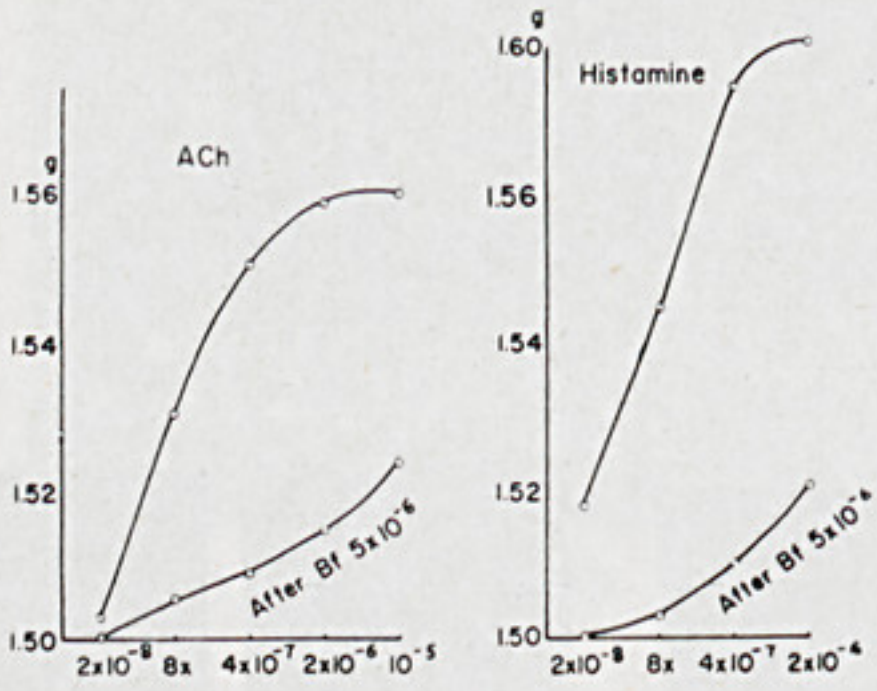


Fig. 7 — Effects of Bufogenins on the Tracheal contractions of the Guinea-pig.

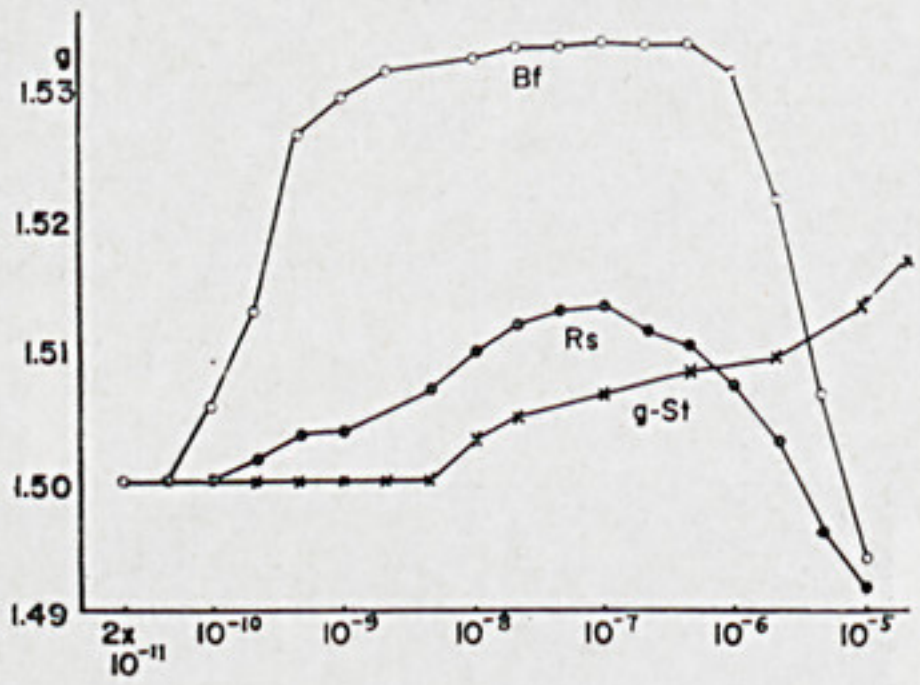


Fig. 8 — Effects of Bufogenins and Allied Compound on Guinea-pig's Tracheal Preparation.

in muscular tone are the responses induced by resibufogenin and bufalin, g-strophanthin did not cause any relaxation even after using high doses. Comparing the actions of resibufogenin and bufalin, we observed that the inhibitory effect of resibufogenin is faster in appearance in spite of its weaker contracting effect.

Studying the effects of ions upon the relaxing action, I found that the increase in muscular tone disappeared in the Na^+ free perfusion, and only the relaxing effect persisted (Fig. 9). H. J. Schatzmann supposed, based on the report of Caldwell and Keynes, that the first contracting phase of the taenia-coli preparation is dependent on the inhibition of Na^+ efflux (Na^+ pump) resulting from the action of oubain on the outer side of the muscle cells membrane, and the second relaxing phase is caused by the inhibition of Na^+ influx resulting from its action on the inner side of the muscle cells membrane after the permeation of the cells.

This hypothesis permits an explanation for the absence of the contracting phase after the perfusion of solutions Na-deprived, and I suppose that the difference of action among resibufogenin, bufalin and g-strophanthin shown in Fig. 9, depends on the difference of the permeative properties, such as shape and size of their molecules.

A comparision of the effects of bufogenins on the various smooth muscle tissues and rectus abdominis muscle of frogs, and their action on the completely depolarized membrane in isotonic K_2SO_4 Krebs solution is shown in Fig. 10.

In the guinea-pig ileum and trachea we could recognize the relaxation after the application of a high concentration of bufogenin. On the other hand, in the aortic strip, heart tissue and rectus abdominis muscle of frogs we observed only the contraction without the relaxation phase. All other tissues than the car-

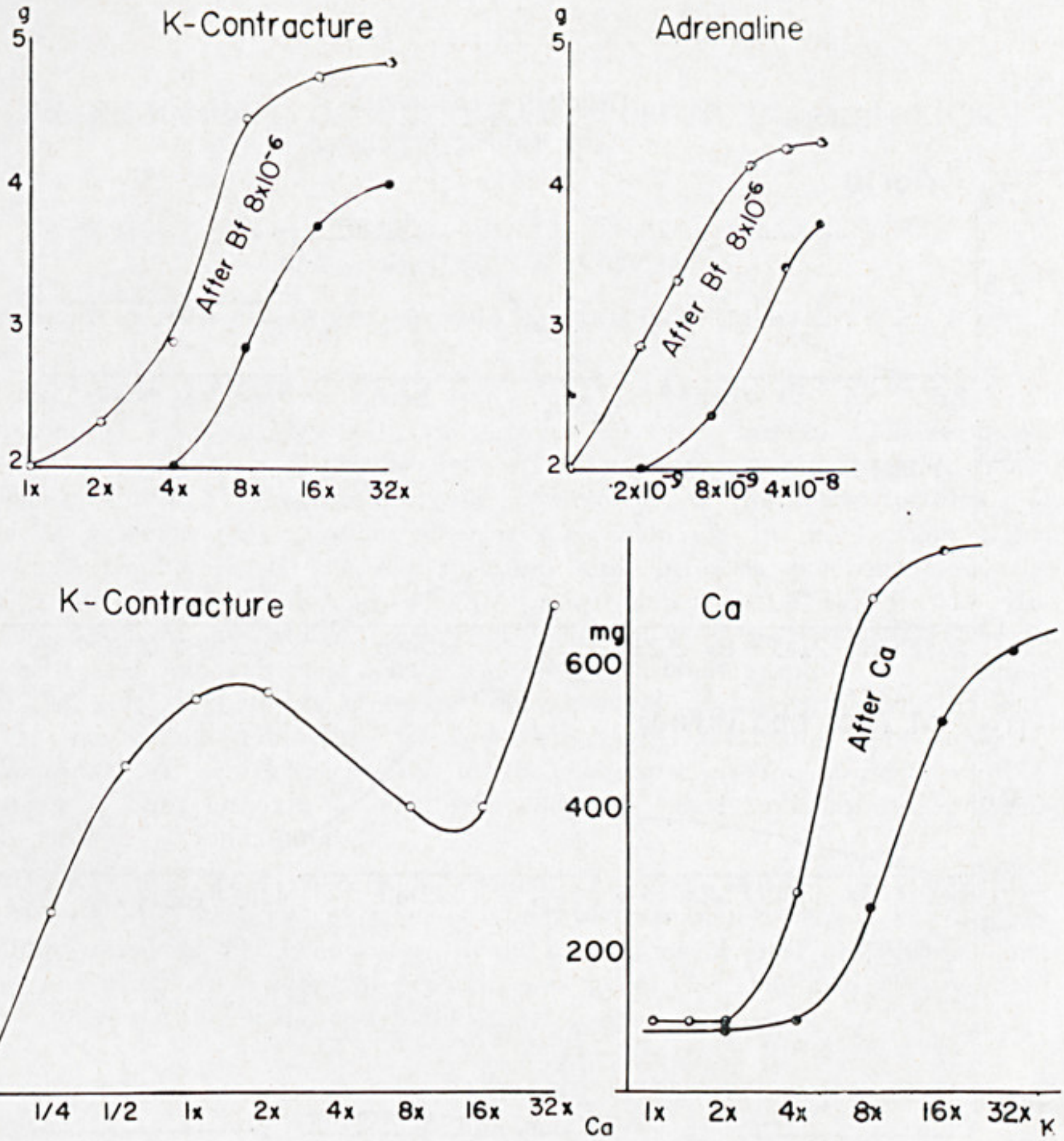


Fig. 9 — Effects of Bufogenins and Ca on the K- and Adrenaline-Contractions of Guinea-pig's and Rabbit's Arterial Strip Preparations.

diac one lost both the contracting and relaxing responses to bufogenins after K induced depolarization. Therefore, the potentiating action of cardic steroid on the smooth contraction depends chiefly on the direct action on the cell membrane, while on the cardiac tissue another mechanism participates than the direct membrane action.

The above mentioned data, regarding my investigations on the actions of bufogenins upon the various smooth muscle tissues, seem to indicate that the comparatively rapid contracting response of the ileum is due to a Ach releasing mechanism. In addition to this direct stimulating action, in the other muscle tissues examined (taenia-coli, blood vessels, trachea and frogs rectus abdominis muscle), the cell membrane seems to be chiefly involved, and there are some differences in the forms the cell membrane is directly actuated by the bufogenins.

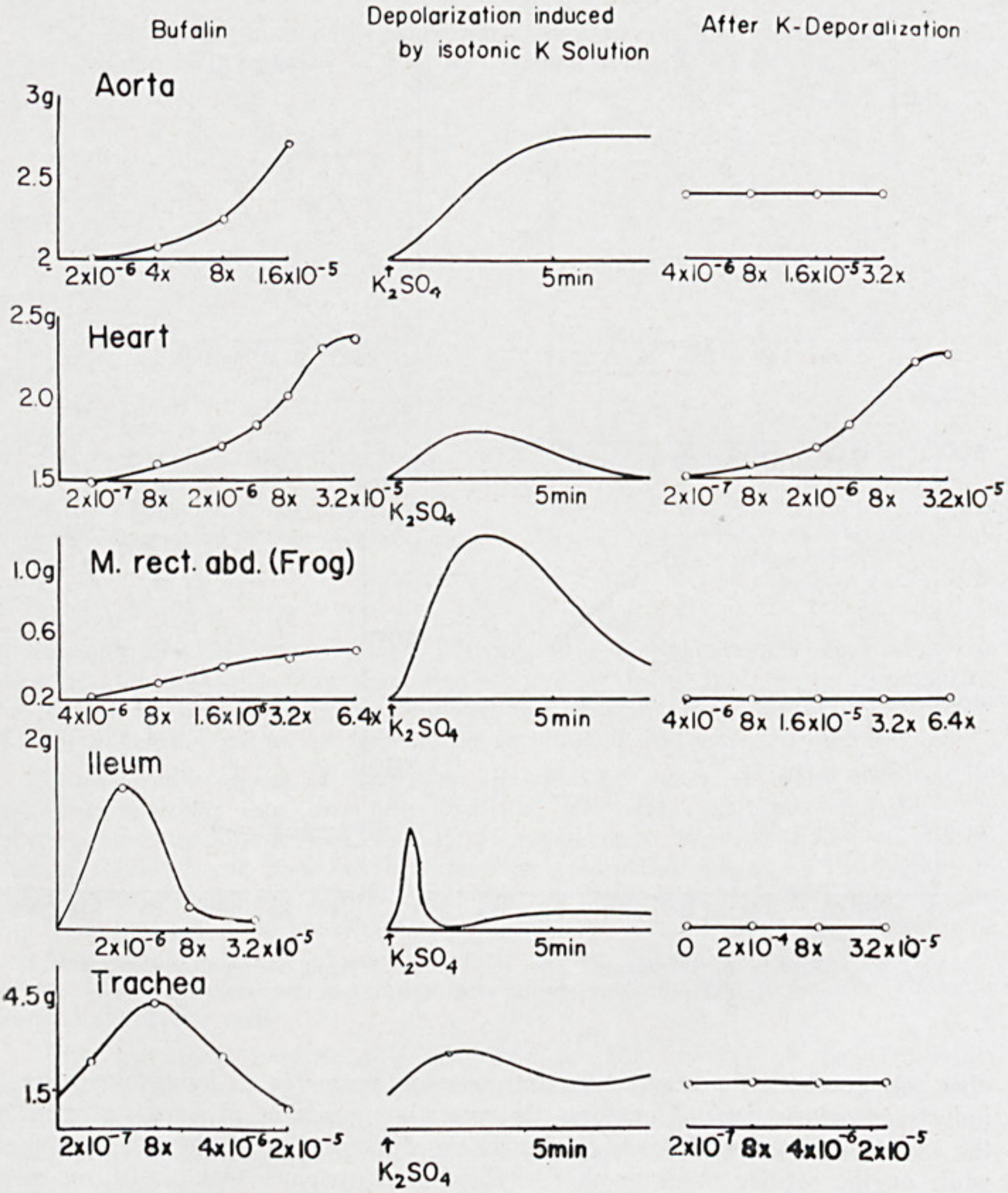


Fig. 10 — Actions of Bufogenin on the Smooth Muscle Tissues and K induced Depolarization.