creases the irritability of muscle(5) but probably inhibits the release of acetyl choline(3). Furthermore, muscle which has been paralyzed by botulinum toxin will contract in response to a dose of acetyl choline(6). The paralytic action of shellfish poison seems to be due primarily to a prevention of the muscle from responding to acetyl choline (curare-like effect), whereas the primary action of botulinum toxin is probably to prevent the release of acetyl choline.

Summary. Shellfish poison has a curare-like action, preventing the skeletal muscles from responding to liberated acetyl choline. Secondarily, it blocks peripheral nerve conduction and decreases the excitability of these muscles to direct stimulation. This material should henceforth be termed a neuromuscular toxin.

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Parallel Inhibition of Granulation Tissue by Cortisone, Hydrocortisone, Dibenamine and Banthine (R).* (20740)

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Hormonal influences upon granulation tissue formation have been well established. Stimulating and inhibiting effects can be observed, depending upon the hormone administered(1). Cortisone and hydrocortisone are preeminent as inhibiting factors and have aroused a great interest because of their clinical utility. In this laboratory it has been demonstrated that the responsiveness of small blood vessels to autonomic nervous stimuli is dependent upon the simultaneous presence of cortisone(2,3). The synergistic and potentiating effects of cortisone and certain neurohumors have been also shown to apply to a number of other phenomena, apparently not related to primary vascular mechanism. These seem to operate in the eosinophile fall(4), in certain aspects of fat transport(5) and other processes under investigation at present. Autonomic blocking agents on the other hand protect experimental animals deprived of their adrenals and subjected to stress from the ensuing shock(6). We have, therefore, investigated the effects of the adrenalins on the one hand, and of dibenamine and banthine on the other, upon granulation tissue formation in order to explore any possible relationship to the action of cortisone. To obtain comparable specimens untreated as well as cortisone and hydrocortisone treated animals were examined in the same manner, and new observations recorded.

Methods. Male, white rats of the Sprague-Dawley strain, weighing about 150 g were used. As in our previous experiments(7), one-half cc of turpentine USP was injected subcutaneously into the region of the right scapula and several animals of each series were killed daily beginning on the first to the 6th day after induction of the abscess. The dry freezing technic of tissue fixation was utilized, hoping that more definite information could be gained in regard to the structure and staining

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TABLE I.

Procedure	No. of animals	Degree of inhibition*	Granulation tissue		
			Fibroblasts	Ground substance	Capillaries
Controls	12	0	Spindle shaped	Ample homogenous, containing small granules	Abundant perpendicular to abscess cavity
Cortisone, 1 mg daily	18	4+	Flat, pyknotic	Sparse, structure same	Sparse, circular to abscess cavity
Hydrocortisone, 1 mg daily	18	4+	Same	Same	Same
Epinephrine/nor-epinephrine, 1 mg daily	9	+	Same as normals	Same as normals	Same as normals
Dibenamine, 1.5 mg/100 g rat bid.	36	4+	Flat, pyknotic	Sparse	Sparse
Banthine, 10 mg/100 g rat bid.	18	4+	Same	Same	Same
Controls, starved	6	+	Same as normals	Same as normals	Same as normals
Adrenalectomized, maintained on 0.3 mg cortisone daily	6	+	Spindle shaped	Slightly reduced	Amt decreased
As above + dibenamine	6	4+	Flat, pyknotic	Sparse	Sparse
As above + banthine	6	4+	Same	Same	Same
Hypophysectomized + STH	8	+	Spindle shaped	Slightly reduced	Ample
As above + dibenamine	8	4+	Flat	Sparse	Sparse
As above + banthine	8	4+	Same	Same	Same

* 0 = no inhibition; + = slight inhibition; 4+ = strong inhibition.

characteristics of the ground substance. One portion of the abscess was fixed in formaline, another portion was immediately frozen according to the technic described by Gersh(8). The results of 17 experiments are summarized in Table I.

Results. In the untreated animals granulation tissue formation around turpentine abscesses proceeded as described before(7). The rich vascularization was again noted beginning on the second day; most of the capillaries grow perpendicularly to the abscess cavity and become abundant on the fourth and fifth day (Fig. 1A). The fibroblasts were numerous, spindle shaped, arranged parallel to the circumference of the abscess. The nuclei contained one of several distinct nucleoli and a fine meshwork of chromatin threads (Fig. 2A). In the frozen preparations the cytoplasm contained 4 to 6 or more granules strongly stained with the Hotchkiss McManus stain. These granules stained even after treatment with salivary amylase or after extraction with fat solvents. They were therefore not glycogen or fat, but considered to consist of muco polysaccharides. The ground substance also stained deeply pink with the Hotchkiss McManus stain; it was in the main homogenous but contained frequently small granules.

The effects of cortisone and hydrocortisone were reinvestigated on 36 animals half of which received a single subcutaneous injection of one mg of cortisone and the remainder one mg of hydrocortisone one day before induction and daily for five days during the development of the abscess. Both steroids had a very similar effect and the results can be discussed together. As before, a marked inhibition of the granulation tissue was observed and considerably fewer newly formed capillaries were present as compared to the response of the untreated animals(9). The direction of growth of the capillaries around the abscess cavity differed from that of the control group. On section many were seen to be cut so that the lumina were visible and fewer seem to grow perpendicularly to the abscess, indicating that the blood vessels were arranged parallel to the cavity, *i.e.* in a circular fashion (Fig. 1B). The fibroblasts differed significantly from the controls, in that they were fewer in number, smaller and containing pykotic nuclei; the

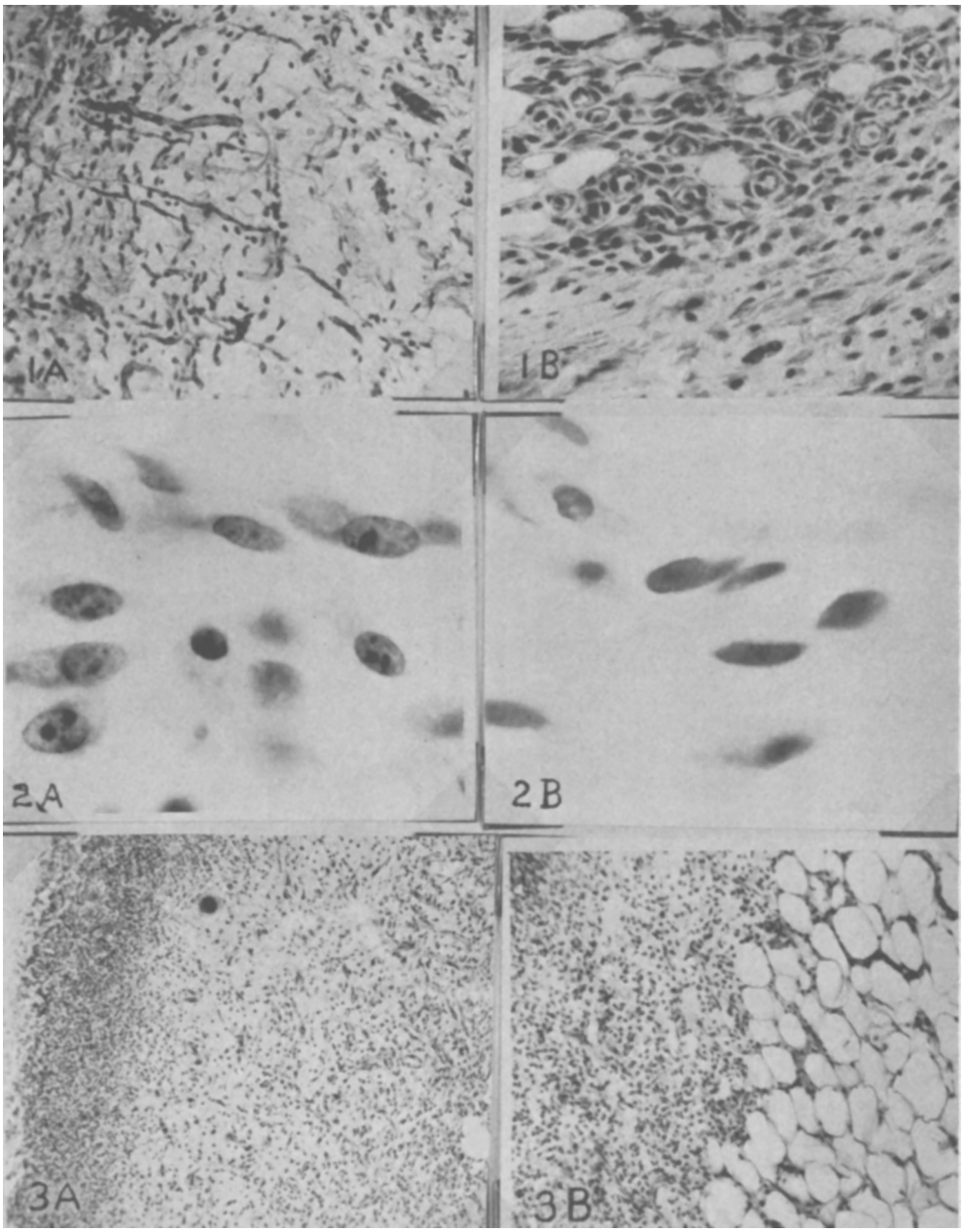

FIG. 1 A: Capillaries in granulation tissue of 5 day turpentine abscess in untreated animal (×192). Note parallel arrangement, perpendicular to abscess cavity.

B: Same in cortisone treated animal (×288). Lumina visible, capillaries arranged circularly to the abscess.

FIG. 2 A: Fibroblasts in granulation tissue of 5 day turpentine abscess in untreated animal. (×1088, oil immersion). Note well defined chromatine structure and nucleoli.

B: Same in cortisone treated animal. Fibroblasts are flat and have pyknotic nuclei.

FIG. 3 A: Turpentine abscess after 5 days in control animal (×80). Leucocytic layer on the left, ample granulation tissue adjacent to it.

B: Same in dibenamine treated animal (×128). Leucocytic layer on left adjacent to fat tissue. No granulation tissue.

chromatin was arranged in denser strands, the nucleoli were less distinct and even invisible in some cells (Fig. 2B). The same number of positive Hotchkiss McManus granules were found as in the control group. The ground substance was sparser, but its finer structure and the intensity of the Hotchkiss McManus stain resembled that of the normal control group. In certain areas, however, containing few cellular elements accumulation of ground substance was noted. Some animals "escaped" the inhibiting effect of one mg of cortisone and hydrocortisone. Possibly higher doses would be necessary to produce the phenomenon with more regularity.

Commercial epinephrine in oil, containing 15-20% of nor-epinephrine, was injected subcutaneously one day before and daily after induction of the turpentine abscess into a series of nine rats. The animals were sacrificed on the 4th, 5th and 6th day. A moderate general inhibition of granulation tissue was observed. No significant changes in the appearance of the fibroblasts or of the ground substance was noted. The general arrangement of the inflammatory tissue was similar to that of the untreated animals. Cortisone and adrenaline were administered together to an equal number of animals. No modification of the inhibitory cortisone effect was noted.

Dibenamine, in amounts of 1.5 mg/100 g of rat, was administered one day before and twice daily after the injection of the turpentine. Thirty-six animals were used in these experiments and the abscesses were examined histologically every day from the first to the sixth day. No changes were observed 1 and 2 days after the induction of the abscess. The amount of necrosis, cellular infiltration and edema was about the same as in the controls. On the following days a marked to complete inhibition of granulation tissue was noted. The leukocytic layer remained unchanged but in many areas it bordered directly on the subcutaneous fat tissue, and no granulation tissue was visible (Fig. 3A and B). Newly formed capillaries were rare and in some areas absent. Fibroblasts were flat, their nuclei pyknotic, the chromatine structure was indistinct and nucleoli were not always visible. The ground substance was reduced, the staining characteristics were the same as in the normal controls. On the whole, the inhibition by dibenamine resembled that following cortisone administration but was even more uniformly obtained.

To another group of 18 animals, banthine was administered in as many injections as in the previous series. The dose was 10 mg per 100 g rat twice daily. Again a profound inhibition of granulation tissue formation was observed, even more pronounced than in the dibenamized animals, the various elements of the granulation tissue were the same as described above.

In further experiments, we attempted to explore some of the possible mechanisms of the dibenamine and banthine inhibition of granulation tissue. First it was possible that animals treated with relatively high doses of the drugs had a poor food intake and therefore, developed inferior granulation tissue. We therefore withdrew any kind of food, except water from 6 rats, while the turpentine abscess was developing. On the fourth and fifth days the absecesses were examined and showed only moderate inhibition of granulation tissue formation, not to be compared with the nearly complete one produced by the autonomic blocking agents. In another series of animals the question was investigated, whether the sympathetic blocking agents might have stimulated the adrenal cortices as non-specific stress agents. Thus, indirectly, via the action of 11 oxysteroids, the granulation tissue formation was inhibited. For this purpose, 18 adrenalectomized animals were divided into 3 equal groups; all the animals received daily doses of 0.3 mg of cortisone hypodermically, an amount which was shown to be necessary to maintain an adrenalectomized rat in good condition (10). Six of these animals were sacrificed on the 4th and 5th day after induction of the abscesses. The granulation tissue was somewhat less well developed than the normal and resembled the one previously observed in equal doses and in the same manner as detailed above. On the 4th and 5th days after injection of turpentine, granulation tissue was profoundly inhibited as seen in the intact animals similarly treated. Six other animals of this group received banthine in the dose applied before. Again strong inhibition of granulation tissue formation was

observed in the absence of the adrenal cortices.

We conclude that the inhibitory influence of dibenamine and banthine is independent of the adrenal cortex and does not follow the release of corticoid hormones resulting from a stress reaction.

Another possible mechanism of action of the drugs was tested; *i.e.* whether their administration would interfere with the production and/or release of anterior pituitary somatotrophic hormone. In previous experiments (9), it was demonstrated that the latter is necessary for formation of granulation tissue. Hypophysectomy resulted in a similar lack of granulation tissue as was observed under the influence of the drugs under investigation. Therefore, the hypothesis had to be tested whether the autonomic nerve inhibitors interfere with the formation of granulation tissue by blocking somatotrophic hormone release. Such a mechanism could be postulated, because it was shown that dibenamine inhibits the release of another anterior pituitary factor, namely the luteinizing hormone(11). On the other hand, ACTH discharge is not influenced by this drug(12). Twenty-four hypophysectomized animals were maintained on daily injections of 500 mg of somatotrophic hormone.† In 8 of these animals turpentine abscesses were examined without any further medication. Two other groups (8 animals each) were given in addition banthine and dibenamine respectively. The first group exhibited fair to good granulation tissue formation indicating that somatotrophic hormone had reestablished the granulation tissue in hypophysectomized animals. Both dibenamine and banthine exerted their strong inhibitory action in the presence of the growth hormone. From these experiments, we conclude that the inhibition of granulation tissue formation by these drugs was not due to lack of somatotrophic hormone and therefore it was improbable that its release from the pituitary gland was interfered with.

Discussion. The inhibitory influence of cortisone and hydrocortisone upon granulation tissue has been reinvestigated and compared with the strikingly similar effect produced by dibenamine and banthine. The latter two drugs were chosen as representatives of sympathetic blocking agents. Diabenamine exerts its action at the ganglionic level only(13). Banthine if administered in high doses has a similar action in addition to the atropine-like effect in the periphery(14). The inhibited granulation tissue shows significant changes of the fibroblasts, and the vascular pattern. The ground substance is only quantitatively diminished. Its staining characteristics and its finer structure, if studied with the dry freezing fixation technic does not differ from the controls, indicating that the chain length of the mucopolysaccharides is not appreciably altered (15).

Some information was obtained concerning the mode of action of the drugs under discussion. They do not act by diminishing the food intake of the experimental animals. Their inhibiting effect is not a non-specific stress effect upon the adrenal cortex and it does not operate by blocking the discharge of somatotrophic hormone, which is necessary for the formation of granulation tissue. The effect then is most likely related to their pharmacologic action upon neurohumors. The observation that commercial adrenaline as administered in our experiments had no—or a slight—inhibitory influence on granulation tissue, is not in contradiction to this assumption, because of the homeostatic regulation which is operating in an intact animal after administration of this drug. As previously described granulation tissue development is only moderately altered in denervated areas(16). It must be remembered, however, that even in such regions circulating neurohumors from distant organs may very well be operating and pharmacological block of the whole animal is more effective than local denervation.

From our experiments no conclusion can be drawn in regard to a relation of the action of the sympathetic blocking agents to the hormones. Granulation tissue under conditions tested here seems to require both hormonal and neurohumoral regulation for its satisfactory development.

Summary. Dibenamine and banthine exert

† Armour preparation of STH Lot No. R 527094 B was used. We are indebted to Mr. Irby Bunding of the Armour Laboratories for his generous supply of the material.

a strongly inhibitory influence upon the formation of granulation tissue of turpentine abscesses in white rats. It resembles the blocking effect of cortisone and hydricortisone upon such tissues. The appearance of the fibroblasts, the vascular pattern and the intercellular substance of the inhibited granulation tissue is the same, regardless of which one of the suppressing agents were administered. Some of the possible mechanisms of action of these drugs were studied.

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Congenital Hypoproconvertinemia.* (20741)

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Congenital hemorrhagic diathesis associated with a prolonged one-stage "prothrombin time" (Quick test) is uncommon. Quick(1) recognized that his test was also dependent upon factor(s) other than the component usually regarded as prothrombin and substantial experimental evidence from many laboratories(2) indicates these non-prothrombin factors to be (A) proaccelerin (labile factor, factor V, AcG, etc.), (B) proconvertin (stable factor, SPCA, factor VII, co-thromboplastin, etc.), (C) fibrinogen, (D) certain inhibitors of coagulation. Additional confirmation comes from study of clinical bleeding disorders and the present communication deals with 2 new cases of congenital proconvertin (SPCA) deficiency, a condition first clearly identified by Alexander *et al.*(3) and further studied by Owren(4).

Case histories. I. A 14-year white school girl (M.T.), who had a bleeding tendency since birth, was seen in 1950. The mother had shown easy bruising and some menorrhagia. A son of one of the mother's sisters was said to be a bleeder. No defect was found in another sister and her children. No information concerning the father could be elicited. M.T. bled from the umbilical cord on the 7th day after birth and during childhood had gum bleeding, easy bruising, hematuria, and attacks of abdominal pain with pallor and jaundice. No epistaxis, hemarthrosis, hematemesis or melena were reported. Menstrual periods, starting at age 14, were frequently excessive, requiring hospitalization and transfusions. Vit. K, including synthetic K_1 (obtained through courtesy of Dr. A. Gibson, Merck & Co.) did not change the

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