Injected intravenously into dogs or cats anesthetized with pentobarbital, a solution of the crystalline material produced a rise in arterial pressure which was augmented in a sympathectomized animal (Fig. 2). In a few animals the response to small doses was depressor, becoming pressor after administration of tetraethyl ammonium chloride. The response after pithing was slightly reduced or unchanged. An isolated ring of rabbit's ileum was sharply contracted by injection of 17 μg into the 30-ml Tyrode solution bath.

The vasoconstrictor activity of the crystalline substance in our assay method employing the perfused isolated rabbit ear preparation (*2*) is more than twice that of an equal weight of commercial epinephrine hydrochloride. Measurable constrictions are obtained by the injection of less than 0.002 μg into the ear vessel preparation.

Work is in progress on the chemical structure of serotonin. A detailed description of the isolation procedure, together with more complete analytical data, will appear elsewhere.

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The Action of Ryanodine on the Contractile Process in Striated Muscle[1]

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While it is unlikely that any chemical agent has a toxic action which is entirely tissue specific, there are many substances that act more rapidly or in lower concentrations in some tissues than in others. Such substances are of particular interest, since they may reveal differences in the enzymatic bases of specific tissue functions. A highly selective action of this kind is indicated by preliminary observations on the mode of action of derivatives of *Ryania speciosa,* which appear to affect specifically the contractile process in skeletal muscle.

Two derivatives of the tropical plant *Ryania speciosa* (Fam. Flacourtiaceae) known as L8A2—a crude extract soluble in alcohol but not in water, and the purified, water-soluble alkaloid, *ryanodine*—were obtained through the kindness of Dr. Ralph Heal, of Merck & Co., Inc. Experiments on intact animals and isolated nerve and muscle were performed to determine the site and mode of action of the toxic agent. The toxic symptoms observed in the animals were similar for both the less active, crude extractives and the pure material.

The LD_{50} has not been determined, but injection of 2–5 γ/gm produced symptoms in insects (*Periplaneta americana, Blaberus craniifer,* and *Platysamia cecropia*), frogs (*Rana pipiens*), and white mice. In the insects, injection of 0.05 ml of an insect saline solution containing 0.1 mg of ryanodine/ml has an entirely depressant effect. After 15 min the insect becomes generally sluggish, and in 25 min is unable to stand. At this point it appears to be partially paralyzed, being capable of making only slow, feeble movements of the appendages. It appears to be unresponsive to stimuli, and during the paralysis period its legs can be placed in any position. Tremors and signs of central excitation are lacking, and feeble, slow reflexes may be elicited throughout the period of poisoning. From this dosage the insect remains partially paralyzed for about 48 hrs and then recovers. Higher doses kill, and lower doses produce a paralysis of less severity and shorter duration.

In the frog, injection of 5 γ/gm intraperitoneally results in complete rigor within 3 hrs. The first effect is flaccidity, occurring within an hour. Shortly thereafter the swallowing movements decrease in amplitude and frequency and eventually cease. Following this a pronounced rigor appears in the forelimbs and proceeds posteriorly.

The oxygen consumption of control and ryanodinized insects was determined in a modification of the Scholander volumetric microrespirometer (*4*). As found earlier in roaches by Chadwick and Hassett (*1*), and in the fiddler crab by Edwards (*2*), the flaccid paralysis of the cockroach (*Periplaneta*) was accompanied by a tremendous increase in oxygen consumption following injection of ryanodine in sublethal doses. With 0.05 ml of 10^{-4} ryanodine by weight O_2 consumption reached a peak of 9.6 times normal in 25 min, the time of onset of paralysis, and gradually decreased thereafter until a twice-normal level was attained in 3–4 hrs. This level of oxygen uptake was then maintained throughout the remaining 45 hrs of paralysis. Injection of 0.05 ml of 10^{-5} ryanodine produced a peak oxygen uptake of 4.2 times normal within 50 min, the paralysis and increased oxygen consumption lasting 24 hrs. A peak of 2.3 times normal oxygen consumption was caused in 75 min by 0.05 ml of ryanodine 10^{-6}. The paralysis and high rate of oxygen consumption lasted 8 hrs. Similar results were obtained with adults of *Blaberus,* diapausing pupae of *Platysamia cecropia,* and with isolated metathoracic legs of *Periplaneta.* In the cases where lethal doses of ryanodine were used, the oxygen consumption rose sharply at the onset of paralysis and then steadily decreased until death occurred. Actually, the only method of determining whether the insect was dead or paralyzed was to measure its oxygen consumption.

In an attempt to determine the site of action, oscillographic studies were made of the effect of the agent on electrical activity in irritable tissues of the cockroach by (a) applying a 10^{-4} solution directly to exposed ganglia and nerves and (b) studying the activity in ganglia and

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nerves of insects previously paralyzed by injection. Ryanodine 10^{-4}, a concentration approximately 50 times the concentration which would result if the effective dose were distributed evenly within the intact insect, failed to cause detectable changes in ganglionic or axonic transmission, spontaneous activity in the nerve cord, or sensory activity in the crural nerve. (For methods see *3*.) Neuromuscular transmission, as indicated by the muscle action potential in the extensor tibiae muscle of the cockroach, also appeared to be unaffected by ryanodine. These results led to the conclusion that excitation and conduction in nerves, ganglia, and muscles are not affected by *Ryania* derivatives in the concentrations used, which leaves the contractile process in muscle as the only possible site at which *Ryania* extracts could act to produce the paralytic action.

TABLE 1

No. of legs used	Injected with:	No. of legs twitching before injection	after 10 min	after 20 min	after 30 min
12	Saline	12	11	9	3
12	Ryanodine	12	0	0	0

This was confirmed by the observation that leg muscles of the roach failed to twitch on indirect or direct electrical stimulation 5–10 min after 10^{-4} ryanodine was perfused through the isolated metathoracic legs, whereas saline-perfused legs responded up to 30 min after preparation (see Table 1). A slow contracture was obtained in some of the poisoned legs with high rates of stimulation (100/sec at 10 v), though twitches to stimuli of 1–20/sec invariably disappeared both in amputated legs injected with ryanodine and in legs removed from previously poisoned roaches. In the same preparations the muscle action potential seemed unchanged at least 1 hr later.

A few experiments with frogs confirmed the general picture. Injection of 5 γ/gm of ryanodine intraperitoneally caused the gradual appearance of a flaccid paralysis which was totally devoid of any excitation or central nervous symptoms. Unlike the situation in roaches, the flaccid stage in frogs was followed shortly by intense and enduring rigor. At the time a marked paralysis appeared in the anterior part of the frog, electrical stimulation of the pectoral and forelimb muscles failed to produce a local response, though the same stimulus reflexly produced flexion of the hind limbs. When the circulation to one leg was stopped by a ligature placed around the thigh prior to injection, electrical stimulation of other regions caused reflex movements in the ligated leg after muscles in the rest of the animal had lost their ability to contract. Thus, it appears that ryanodine affects contractile processes before excitation and conduction are impaired.

In order to investigate the direct action of ryanodine on muscle, rectus abdominis muscles of the frog were immersed in 5 ml of aerated saline containing ryanodine and the contractions recorded as in the method used for acetylcholine assay. Saline containing 10^{-4} ryanodine produced a marked rigor-like contracture of the rectus muscle, which commenced 2 min after the muscle was first placed in ryanodine and reached a maximum in 30 min. With lower concentrations, to 10^{-8} ryanodine, the time of onset of rigor lengthened to 2.5 hrs, the height of contracture decreased, and the slope of the contracture curve became less. In the intact frog the ryanodine effect in the doses used was irreversible, whereas in the intact roach the effect of injection of 0.05 ml of 10^{-4} ryanodine was reversible, recovery occurring within 48 hrs. The rectus muscle of the frog, once it had started contracting, could not be brought back to the relaxed condition when washed with saline within 5 min of application of the ryanodine.

All evidence to this point indicates that ryanodine affects the contractile process before interfering with excitation or conduction. The site of action is then fairly well localized. What is the mode of action? Since the partial paralysis which results from ryanodine injection is tremorless and quite flaccid in the cockroach and frog (followed by rigor in the frog), the striking increase in oxygen consumption suggests that *Ryania* derivatives interfere with either the glycolytic processes or with the high-energy phosphate system in striated muscle. Preliminary experiments are under way to test the perfusate from ryanodinized muscles and to determine the influence of ryanodine on phosphagen and ATP hydrolysis and resynthesis.

Ryanodine solution which has caused rigor in a muscle (perfusate) has a more powerful rigor-producing effect than ryanodine alone. If a ryanodine solution which has caused rigor in a rectus muscle is drained off and applied to a second muscle, rigor begins with little or no latency, and the rate of attainment of complete rigor is more rapid. In one case, the ryanodine solution (10^{-6}) caused rigor to commence in the first muscle within 30 min, reaching a maximum in 135 min. Application of the perfusate from this muscle to a second caused rigor to commence almost immediately, attaining a maximum in 50 min. As is the case with ryanodine, ryanodine-muscle perfusate appears to be heat stable, and its effectiveness depends upon the original ryanodine concentration. The ryanodine perfusate also causes paralysis and a tremendous increase in oxygen consumption when injected into the intact roach, whereas saline-rectus perfusate and saline alone have no effect. There seem to be two possible explanations for this effect. Either contact with the muscle alters the ryanodine, rendering it more toxic, or ryanodine causes the production or release of material from the muscle which is responsible for the genesis of rigor. Though it is only possible to speculate on the nature of this effect, pronounced opacity of the muscles in a poisoned frog suggests that acid production is associated with ryanodine-induced rigor.

Injection of adenosine triphosphate (ATP) 10^{-3} in combination with ryanodine 10^{-4} shortened the interval preceding paralysis in the roach and frog. In the roach the time was decreased from 25 min for ryanodine alone to 6–8 min with ryanodine-ATP. ATP alone caused no apparent symptoms in the intact roach or frog. In the

frog, the ryanodine-ATP brought about an intense rigor within a few minutes, whereas ryanodine alone ordinarily causes rigor in 3–4 hrs. Ryanodine-ATP injections caused a 9-fold increase in oxygen consumption in the roach, and in one case, where the ATP had been warmed to 45° C and then cooled to room temperature before mixing with the ryanodine, the oxygen consumption was increased to 18 times normal following injection. In the intact roach the ryanodine-ATP effect was reversible in the concentrations used. In the intact frog and the rectus preparations the rigor produced was irreversible.

These results strengthen our belief that ryanodine acts specifically on the contractile process in striated muscle and indicate that the mode of action is probably one of interference with the phosphagen-ATP-ADP-actomyosin cycle during contraction.

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The Homing Tendency of Shad

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Two of the important problems in the study of shad populations along the Atlantic coast have been to determine to what extent shad return to the stream of their origin and to determine at what age they reach maturity. Shad are anadromous fish that enter the streams of the Atlantic coast in the spring to spawn, often migrating several hundred miles into fresh water for this purpose. It is known that the resulting young spend the first several months of their existence in fresh and brackish water, feeding and growing, and in the fall leave their fluvial environment for an unknown migration into the ocean, where they stay until maturity. That shad do return to the stream of their nativity has not been demonstrated. The age at maturity has received the attention of a number of investigators. Most have based their conclusions on scale markings, which they have recognized as being difficult to interpret.

Leim (*4*), reading markings believed to be winter rings on the scales from shad taken in the Shubenacadie River, placed the age of mature shad on these spawning grounds at at least 4 years; most he believed to be 5 years old or over, the maximum age being 8 or 9 years. Marks believed to be indicative of previous spawnings were noted on some of the scales.

Borodin (*2*) worked primarily with scales obtained from shad of the Connecticut River system. Because of the lack of distinctness of the annual marks, he counted the number of transverse grooves as well as annual marks —the number of complete transverse grooves divided by two being considered the true age when annuli were unreadable. Borodin concluded that a few male shad enter the rivers as 4-year-old fish, but that most of the males which he examined from the Connecticut River in 1924 were 5–7 years old. The females were determined to be 7 or more years of age. In addition, he pointed out the distinctiveness of the first and fourth annulus, comparing the latter to the "spawn marks" of salmon.

Barney (*1*) confirmed Borodin's observations, using otoliths. In a footnote (p. 57) Barney states that a single 3-year-old buck was taken from the Salmon River, but that this was unusual.

Greeley (*3*) examined scales collected from Hudson River shad and found great variance in the relative distinctness of annuli. He concluded that, though both roes and bucks may mature at 3 years, females in this age group were in the minority, and suggested as probable that "many fish of both sexes, but particularly roe fish, remain at sea during their third year and do not mature until four years old. It is entirely possible that a small percentage of these fish might be immature at an even greater age."

The lack of agreement in the various findings may be due to inherent differences in behavior of populations of the several streams or to differences of interpretation of scale markings for which experimental evidence is lacking. Obviously, the most direct method for interpreting scale markings and for determining age at maturity is to mark the young shad of known age and obtain scale samples from them at known intervals. This marking of young shad was attempted by Robert A. Nesbit and the author on several occasions, but the marking always resulted in the death of the young shad within a few days, presumably because of the injury inflicted in tagging and handling.

In 1941 I was able to tag successfully juvenile hatchery-reared shad by holding them in Ringer's solution, after tagging, until the incisions were healed. To date, three of the tagged fish have been reported. All of these were recaptured within a radius of 10 miles from their point of release, 3, 4, and 5 years after tagging.

The shad were pond reared at the U. S. Fish and Wildlife Service's hatchery located near Edenton, North Carolina. The eggs were collected and fertilized by standard hatchery procedure on April 24, 1941, placed in McDonald jars, and hatched in running water. On April 29, 1941, about 50,000 of the newly hatched fish were placed in a pond of 0.8 acre with a maximum depth of 5′. From then until October the shad were fed with a commercial fish food. On October 10 the pond level was gradually lowered to facilitate seining. The young shad were tagged from October 11 to 15. The fish at this time averaged about 10 cm in length.

The tag used was red celluloid, 20/1,000″ thick, 9/16″ in length, and 3/16″ in width, with ends rounded.

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