primary importance and will be used to illustrate the preliminary results. Table 2 shows the mean yields obtained for Rutgers and Pritchard and the three seed-size classes producing the F_1 and F_2 generations of plants.

The preliminary results for this specific tomato hybrid appear to agree with the proposed hypothesis. It will be noted that the mean yield of the F_2 progenies, produced from the largest seed-size class, is comparable to the mean yield of progenies obtained from all seed sizes of the immediate cross. The smallest seed-size class producing the F_1 generation was, however, lower in production than its two larger seed classes, possibly due to accidental inclusion of a few self-pollinated seeds of the female parent, Rutgers. The fact that early yield in the smallest seed-size class was significantly less than in the other two classes also substantiates the supposition. Had the smallest class been comparable to the other two, the average total production in the F_1 generation progenies would have been 20.3 tons as compared to 19.4 for the largest seed class producing the F_2 generation progenies. More extensive field trials are planned for the coming season.

If a measurable association can be shown to exist between size of seed extracted from F_1 fruits and productivity in the F_2 generation progenies, a new method of producing hybrid seed in volume may result. Breeders of pure line tomato strains may also benefit by being able to select for vigor by seed size, thus eliminating the growing of considerable undesirable material.

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Hypersensitivity in Cold-blooded Animals. II. Salamanders

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In a study of the effect of histamine on intestinal smooth muscle preparations of fish, Dreyer (*2*) observed that histamine produced contractions in teleost but not in elasmobranch smooth muscle. In a subsequent study Dreyer and King (*3*) were able to produce anaphylaxis in several teleost species, using as antigens horse serum and egg albumen injected intraperitoneally. Similar studies using elasmobranch species proved to be impractical because of technical difficulties. A search was then instituted for other cold-blooded animals which would show the same inability to react to histamine stimulation as elasmobranch species and be more suitable subjects in which to attempt protein sensitization.

Previous investigations using frogs as experimental animals have yielded results which are conflicting and difficult to interpret. Friedberger and Mita (*4*) shocked frogs sensitized to sheep serum and noted that the shocked animals displayed a weakness and curious loss of muscle tone. Isolated heart and other organ preparations failed to yield clear-cut results. Arloing and Langeron (*1*) failed to note any signs of hypersensitivity in their series of frogs treated with human serum. Kritchevsky and Birger (*7*) used frogs in which the blood was replaced by a colloid-free salt solution and noted that mammalian serum showed primary toxicity for these animals. Goodner (*6*) failed to produce satisfactory *in vivo* reactions using egg albumen and horse serum as antigens but did notice in the excised heart indications of developing hypersensitization. Friede and Ebert (*5*) demonstrated passive anaphylaxis in frogs and in some instances were able to produce symptoms of hypersensitivity in frogs actively sensitized. It is suggested that these widely divergent results, to which must be added our own negative results (*3*), were perhaps due to differences in the nutrition of the various experimental animals used. In some instances, frogs which had been held all winter without feeding failed to react, whereas frogs caught and used in the summer months showed irregular reactions that possibly might be considered significant.

Because of these variations the American newt (*Triturus viridescens*) was selected as a closely related species, which could be easily kept in the laboratory, and which could be fed without difficulty. *In vitro* and *in vivo* studies of possible hypersensitization were made in these animals. It was soon found that the tiny size of newt organs made them unsatisfactory for kymographic studies, and later studies were carried out using the mudpuppy (*Necturus maculosus*). During the investigation, the newts were kept at room temperature in a closed aquarium jar, in a few inches of water with access to rocks. They were fed three times a week and would accept only live food, taking flies and earthworms readily. With the coming of cold weather they ceased to feed, and the series, which was almost completed, was discarded. The mudpuppies were kept in running water at 18° C and refused to feed when offered earthworms, sliced rabbit liver, or rat meal. They were well fed on arrival and were all used within a month; most of the earlier animals sacrificed for tissue had food in the gut.

Injections were made intraperitoneally at a point midway between the front and hind legs. Horse serum was the only antigen used. This was about 10 months old but had been stored at −20° C without preservative. Sensitizing and shocking doses, which ranged from 0.05 to 0.10 ml for the newts and 0.20 to 0.30 ml for the mud-

puppies, were spaced at irregular intervals, never less than 10 days apart.

In vitro studies were made on "Straub heart" or strips of intestine or stomach, using the usual recording methods for these preparations. All chemicals used in the kymographic studies were made up in frog saline solution.

A series of 14 American newts were injected intraperitoneally with 0.05 ml of horse serum in an attempt to sensitize them. At the same time four other newts were set aside uninjected as controls and were held under conditions comparable to those of the test group. All of the test animals were reinjected, using 0.10 ml of horse serum; two each at intervals of 18, 23, 28, 42, 48, 55, and 65 days after the initial injection. A control animal was injected with the same dose of antigen along with the first four pairs of test animals injected. No demonstrable evidence of hypersensitivity was observed. Similarly, the injections of the control animals produced no results. Two animals died subsequent to injection, but autopsy revealed evidence of trauma that could account for the deaths. Three animals received three injections each at irregular intervals, without reaction.

The injection of test and control animals with 5 γ of histamine phosphate produced no reaction, but 10 γ produced a transitory reaction of obvious distress.

Muscle preparations using a section of the duodenal end of the intestine were disappointing, because the small size of the material rendered kymographic studies difficult. However, it appeared that in three muscle preparations studied, histamine 10 γ, epinephrine 10 γ, and horse serum 0.2 ml produced no demonstrable effect. Barium chloride, 2% solution, produced significant contractions of the gut, showing the ability of the muscle to respond. Heart and stomach preparations were not studied in the newt because of the technical difficulties involved. In view of the results of the organ studies, it was felt that the reaction *in vivo* with 10 γ histamine was an artifact caused by the relatively large size of the dose administered.

Of five mudpuppies obtained, two were sacrificed, without previous attempts to sensitize them, for kymographic studies of heart, stomach, and intestine; and three were injected intraperitoneally with 0.2 ml of horse serum. As this produced no evident reaction, the animals were reinjected four days later, using 0.25 ml antigen in an effort to build up the allergic state. Again there was no immediate reaction, but one animal died two days later; autopsy showed blood in the abdomen, the cause of which was probably the trauma of injection. The remaining two animals were injected again 18 days after the first sensitizing dose and showed no symptoms of shock. Five days later these animals were sacrificed for study of isolated organ preparations.

In kymographic studies of Straub heart preparations of both normal and serum-injected animals, histamine in doses as high as 10 γ and horse serum in 0.2-ml doses failed to exert any effect on the contractility of the heart. Similar doses of histamine and horse serum failed to produce demonstrable responses when administered to stomach or intestinal preparations. One γ of epinephrine produced a marked increase in the amplitude of the myocardial contractions, while the same amount of acetylcholine depressed the contractility of the heart. These reactions were in agreement with those observed using fish Straub heart preparations (*2*) and our unpublished experiments with frog preparations.

As a result of these data it can be concluded that, while teleost fish have the ability to become sensitized to protein antigens, are able to show histamine shock, and can show smooth muscle contractions in the preparations *in vitro* treated with histamine, the American newt and the mudpuppy have none of these characteristics. Acetylcholine depresses the activity of frog, salamander, and fish heart, but seems to play no role in hypersensitization. It is suggested that these data be used as additional evidence of the role of histamine in anaphylaxis.

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Inactivation of Amino Acids by Autoclaving[1]

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Recent work has shown that cystine is the only amino acid partially destroyed by autoclaving casein for 20 hr at 15 lb pressure (*3*). Lysine, arginine, and tryptophan suffer partial destruction when casein (*5*) or soy globulin (*6*) is refluxed for 24 hr in a glucose solution, or when soybean oil meal is autoclaved for 4 hr (*7*). The amounts of aspartic acid, isoleucine, lysine, methionine, and threonine liberated by enzymatic digestion from casein *in vitro* are decreased after autoclaving (*3*), as are all of the amino acids in soybean oil meal (*7*).

Evans and Butts (*1*) found that autoclaving causes two types of inactivation of lysine, one a reaction of the lysine with sucrose to destroy it and the other a reaction with protein to render it unavailable after enzymatic digestion *in vitro*. Moreover, methionine reacts with sucrose or glucose to form a linkage not hydrolyzed by enzymes *in vitro* (*2*).

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