became negative for the nonspecific factor. In previous, unpublished studies with rabbit sera, we found that treatment with zymosan uniformly eliminates the nonspecific factor.

When complement determinations were attempted with a sheep red blood cell system, no activity was demonstrated in one carp, but one pike perch had 5.5 units/ml. These were the only two specimens examined in this manner. The remaining samples were titrated for complement with rabbit red cells (*6*) and all were positive except one of the great northern pike. Both the nonspecific and complement activities (as determined with rabbit cells) were either present or absent from the same sera, except in one northern pike.

Except for previous studies of complement in carp by Cushing (*6*), little is known of the heat-labile serum factor content of fish sera. Such sera may be readily obtained with very little practice by cardiac puncture and the fish may be kept alive for several bleedings. Some 90 ml of blood were procured in separate bleedings on two consecutive days without killing a carp. The eel presented something of a problem because it was difficult to hold, and oozed a considerable amount of oil. We believe, however, that blood, rather than a mixture of blood and oil, was obtained.

It seems reasonable to assume that the heat-labile, antitoxoplasmic system in the fresh-water fish is similar to, if not identical with, that of mammals and birds. Although toxoplasma infections have not been demonstrated in fish, the presence of the heat-labile serum activities is probably a fortuitous biologic coincidence, for it would seem unlikely that they were acquired as a specific response to any infectious agent. Properdin, complement, and Mg^{++} (*2*) make up a portion of the nonspecific system which probably also possesses other as yet unknown components, as well.

The behavior of the great northern pike is of special interest, since two of the three specimens were inactive. In fact, after the first was found to be negative, the other two specimens were acquired to check this initial experience. Since the great northern pike, pike perch, and muskellunge are closely related, it would be of considerable interest to examine the latter. This is to be attempted as specimens become available. Whether the sera of salt-water fish possess heat-labile activities comparable to those of the fresh-water fish also remains to be determined (*7*).

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5. Human "activator" was added to a second control tube of similarly inactivated undiluted serum; the dye reactions were uniformly negative, indicating that the fish sera did not contain specific antitoxoplasma antibodies.
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7. This work was supported by grant E-96(C7) from the National Institutes of Health.

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Chemically Induced Phenocopy of a Tomato Mutant

Abstract. Lanceolate, a spontaneous leaf-shape mutant which fails to produce cotyledons and plumule in the homozygous condition, shows development if supplied with either adenine or a diffusate obtained from normal seeds. Similar development occurs in a different genetic background.

The leaf of the normal tomato, *Lycopersicon esculentum,* is odd-pinnately compound. In contrast, a leaf-shape mutant found by Casseres of Costa Rica has a simple, entire, elongated leaf which we called lanceolate. Associated with the lanceolate leaf shape are small fused cotyledons, early appearance of axillary shoots, which indicate weak apical dominance, leafy inflorescence, and reduced flower size.

On selfing, lanceolate always segregated normal and lanceolate plants approximately in a 1:2 ratio. In addition, about a quarter of the seeds either failed to germinate or appeared as narrow or reduced phenotypes (Table 1). Reduced seedlings never developed beyond a cylindrical mass of green tissue about 5 cm tall and 0.2 cm in diameter, without a trace of cotyledons or plumule. When ungerminated seeds from lanceolate plants were dissected, about one quarter of the embryos were completely devoid of cotyledons and plumule. We assume that such embryos would give rise to reduced seedlings under favorable conditions of germination. Narrow plants, on the other hand, had the appearance of an extreme lanceolate. In contrast to reduced seedlings they had cotyledons and plumule; furthermore, they produced shoots, leaves, and aborted inflorescences, but never flowers. If narrow and reduced phenotypes and the ungerminated seeds are considered to be homozygous lanceolate, the segregation in the upper part of Table 1, which shows the segregation from selfed lanceolate and from the hybrid lanceolate × lanceolate, is not significantly different from a 1:2:1 ratio. That lanceolate is a heterozygote is also clear from the progeny of lanceolate crossed with normal: both lanceolate and normal plants appear in about equal proportions (lower part of Table 1). In effect, the normal allele behaves as a recessive. In accordance with long-established usage, lanceolate is considered to be a dominant allele because it produces an observably different phenotype in the heterozygous condition, and regardless of the fact that homozygous lanceolate may be expressed as an even more extreme phenotype (narrow or reduced).

It is evident that the lanceolate gene has an effect on the growth of cotyledons and leaves. One lanceolate allele reduces the size markedly; with two there is even greater reduction in leaf size (as in narrow) or complete absence (as in reduced). Thus, the lanceolate gene may be responsible for a deficiency of some growth substance. Several authors have identified substances with growth-promoting properties that may have some relevance. Went (*1*) found that there was in the cotyledons of peas a substance that promoted leaf growth. It could diffuse out of germinating seeds and stimulate growth of excised pea leaves in culture medium (*2*). Bonner and Haagen-Smit (*3*) have shown that adenine influences the growth of mesophyll but has no influence on the growth of veins. On the other hand, Went and Thimann (*4*) have shown that auxin increases the growth of veins, but not the growth of mesophyll. Finally, Miller and Skoog (*5*) concluded that the initiation and development of buds from tobacco pith cells in vitro was dependent

Table 1. Segregation in selfed and hybrid cultures of lanceolate.

	Normal	Lanceolate	Narrow	Reduced	No germ.	Total
Lanceolate selfed						
Germinated in soil	22	58	1	0	24	105
Germinated on filter paper	35	68	0	37	0	140
(F_1 lanceolate from lanceolate × broad) × lanceolate	10	33	11	0	0	54
Total	67	159		73		299
Exp. (1:2:1)	75	149		75		299
Lanceolate × normal	19	15				34
Exp. (1:1)	17	17				34

Table 2. Effects of diffusate and adenine on germinating seeds of lanceolate.

Normal	Lanceolate	Reduced	Modified	Total
		Diffusate		
15	25	4	6	50
		Adenine		
	39	8	3	50

Table 3. Effect of various adjuvants on reduced embryos grown in White's medium.

Treatment	Reduced	Modified	Total
(i) Strong diffusate	4	1	5
(ii) Weak diffusate	1	3	4
(iii) Adenine	2	3	5
(iv) Adenine + indoleacetic acid	2	3	5
(v) Indoleacetic acid	5	0	5
(vi) Distilled water (control)	5	0	5

upon the proper balance of adenine and indoleacetic acid (auxin).

Our first success in attempting to obtain growth in the reduced phenotype came by treating germinating seeds of lanceolate with diffusate and adenine; the seeds, of course, contained embryos in the proportion of 1 normal : 2 lanceolate : 1 reduced. Diffusate was prepared in the following manner: 500 normal tomato seeds were soaked in 20 ml of distilled water. After 3 days the water, with diffusible substances, was removed, and distilled water was again added. This procedure was repeated daily for a total of 4 days. The solution removed from the seeds was lyophilized to dryness, and the powder was dissolved in 10 ml of distilled water. Fifty seeds of lanceolate were germinated on filter paper in a Petri dish which contained the 10 ml of concentrated diffusate. Of the 50 resulting seedlings, four were typical reduced plants but six were a new phenotype, which we called "modified" (Table 2). Instead of remaining as a cylindrical mass of tissue, the hypocotyl broadened at the tip to form a structure resembling a cotyledon, at the base of which a bud developed that later grew into a shoot. The mature plant that developed from this shoot was very similar to the narrow phenotype, although the latter always had two cotyledons and a plumule to begin with. A second 50 seeds were treated with 40 parts per million of adenine sulfate; they produced eight reduced seedlings and three of the modified phenotype.

Tomato seeds usually complete their germination in 7 to 10 days; however, it was noted that the normal seeds from which the diffusate had been removed failed to complete germination. The roots developed almost at the normal rate, the hypocotyl elongated only slightly, but the cotyledons never emerged from the seed coat, nor did they show any sign of growth. Thus, a substance or substances required for early cotyledon and shoot growth may diffuse out of the germinating seeds. If the substance or substances are continuously removed from the seeds, normal growth will not take place.

In a second experiment seeds from selfed lanceolate plants were soaked in distilled water for 3 days, then dissected under sterile conditions. Embryos without cotyledons, presumably homozygous lanceolate, were selected. These were placed individually in test tubes on an agar surface. All of the test tubes contained White's medium (*6*) which had been autoclaved with 1-percent agar. To this basic medium the following substances had been added after sterilization by filtration: (i) strong diffusate (1 ml of concentrated diffusate—diffusate from 50 seeds); (ii) weak diffusate (½ ml of concentrated diffusate plus ½ ml of distilled water—diffusate from 25 seeds); (iii) adenine sulfate, 40 parts per million; (iv) adenine sulfate, 40 parts per million, plus indoleacetic acid, 1 part per million; (v) indoleacetic acid, 1 part per million; (vi) distilled water, which served as a control.

Again the modified phenotype appeared when the reduced embryos were treated with either diffusate or adenine (Table 3). It is clear that a substance (or substances) which diffuses out of normal tomato seeds stimulates the development of cotyledon-like structures and buds in the reduced embryos. It is possible that the poorer results obtained at higher concentration of diffusate may be due to toxic substances in the diffusate or to an imbalance of factors. Adenine appears to give the full effect of the diffusate, and its effectiveness is not enhanced by indoleactic acid. Embryos which develop in response to either adenine or diffusate continue to grow when transferred to soil and produce a plant very similar to narrow; that is, the phenotype modified is a phenocopy of narrow.

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Mitotic Arrest by Deuterium Oxide

Abstract. In marine invertebrate eggs, where cell divisions occur without growth, deuterium oxide produces arrest of, or serious delay in, mitosis and cytokinesis. All stages requiring assembly or operation of mechanical structures in the cytoplasm are sensitive to D_2O. The block is reversible in some cells.

Recent investigations of the effect of heavy water on biological systems have emphasized a finding implicit in the early papers—that in concentrations as low as 30 to 40 percent, D_2O arrests or delays cell division, without serious or immediate effects upon growth. Newer experiments on mammals and algae, respectively, are reported by Katz *et al.* (*1*) and by Moses *et al.* (*2*).

This result is of interest for two reasons: first, because the "isotope effect" is so large (see *3*), and second, because interference with cell division by so "simple" an agent offers some possibility of analysis in terms of primary molecular events.

We have undertaken a study of the effect of D_2O on fertilization and cleavage in the eggs of the sea urchin, *Arbacia punctulata,* and the annelid, *Chaetopterus pergamentaceus.* This report is a descriptive summary of the findings. Marine eggs were chosen for this study in order to avoid a source of confusion present in the systems heretofore investigated—that is, simultaneity of growth and division. As explained by Swann (*4*), division occurs in the absence of growth in the early development of the sea urchin. The same is true, presumably, for the egg of *Chaetopterus.*

Lucké and Harvey reported in 1935 (*5*) that cleavage of sea-urchin eggs was completely blocked in 99.5 percent D_2O, while in the same cells there was no evidence of rapid changes in permeability. Hoberman *et al.* (*6*) have found that cleavage is somewhat delayed in sea water containing a 9.1 atom-percent excess of D. Ussing (*7*), studying amphibia, and hampered by the necessity of employing minute volumes of medium, was able to show that even low concentrations of D_2O retard cleavage, and that no cleavage takes place in D_2O concentrations higher than 40 percent.

The experiments under discussion were concerned with the following variables: effect on fertilization compared with that on division, concentration of D_2O, stage of arrest or delay, possibility of reversal, and species.

When sea urchin eggs were inseminated in sea water and in a series of sea waters reconstituted with D_2O, it was found that eggs in media containing up to 25 percent (*8*) of heavy water fertilized normally. The criterion for