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8. Ten seedlings of each kind were used to test each zone of the chromatograms.
9. The preparation methods and the dwarf-maize test exclude substances interfering with gibberellin action in pea seedlings which had been previously found to be present in lima-bean seeds, pea seedlings, and other plant material [D. Köhler and A. Lang, *Plant Physiol.* **38**, 555 (1963)].
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Genetic Influence on Experimental Allergic Thyroiditis in Guinea Pigs

Abstract. *After immunization with low doses of guinea pig thyroid extract, incidence of experimental allergic thyroiditis is greater in the Hartley strain of guinea pig than in strain 13.*

In response to immunization, some strains of animals produce more antibody than others (*1*). These differences in immune responsiveness, apparently genetic in origin, may also affect the incidence of an experimentally induced autoallergic disease. One of these, experimental allergic encephalomyelitis, develops in mice of the BSVS strain more frequently than in other strains (*2*). Similarly, after immunization with central nervous system tissue emulsified in Freund's complete adjuvant, the Hartley strain of guinea pig develops encephalomyelitis in response to doses of the central nervous system antigen which are insufficient to cause the disease in several other strains (*3*). These findings suggested that strain differences might influence the development of another experimental autoimmune disease, allergic thyroiditis. Consequently, two strains of guinea pigs were immunized with selected doses of guinea pig thyroid antigen. The resulting incidence of thyroiditis served as the index of the genetically determined proclivity of each strain to develop this disease.

Random-bred Hartley and inbred strain 13 (*4*) male guinea pigs (*5*) were used for these experiments. They were housed, five to a cage, in one room, similarly cared for, and provided continually with Feed A (*6*) pellets, kale, and carrots. On attaining a weight of 400 to 500 g they were injected with a saline extract of guinea pig thyroid emulsified in Freund's complete adjuvant; the extract was prepared as previously described (*7*) and diluted with saline to the desired concentration, and three volumes were emulsified with one volume of complete adjuvant comprising 80 percent Bayol F, 20 percent Arlacel A, and 4 mg per milliliter of *Mycobacterium tuberculosis* H37Rv. One milliliter of the emulsion was injected intradermally in small portions at approximately 20 sites distributed over the hind feet, legs, and rump. The serial twofold dilutions of the stock thyroid extract were prepared at one time and individually emulsified with the adjuvant; each emulsion preparation was injected into five to ten guinea pigs of each strain.

Six weeks after immunization the animals were killed with ether. The thyroid glands were excised and the right and left lobes were separately fixed in neutral buffered 10 percent formalin and embedded in paraffin. Serial sections were cut at 6 μ through the long axis of each lobe until 0.1 to 0.4 mm of the tissue had been sectioned; the sections were stained with hematoxylin and eosin. The presence of thyroiditis was determined by the appearance of inflammatory reaction in the gland, according to previously described criteria (*7*).

The results (Table 1) show in both strains a relation between the dose of thyroid extract and incidence of disease. All animals given doses of greater concentration than the 1:1280 dilution developed disease, irrespective of strain. As the concentration of thyroid extract decreased, disease incidence diminished in both strains, but did so more slowly in the Hartley strain, until a dose insufficient to produce disease in either strain was reached; this was the 1:40,960 dilution.

Statistical comparison of the frequency of thyroiditis in all the Hartley and strain 13 guinea pigs (injected with the dilutions 1:1280 to 1:20,480, inclusive) indicated that the greater incidence in the Hartley guinea pigs was significant at the 1-percent level, ($p < .01$, by the chi-square test). Similar analysis for each dilution showed that the difference in disease incidence at the three dilutions, 1:1280, 1:2560, and 1:5120, was also statistically significant ($p < .01$).

Many experimental and environmental factors may influence the immune response and could thereby affect the incidence of experimental allergic thyroiditis. Variables such as the nature of the antigen, type of antigen-adjuvant emulsion, immunizing dose, sex, weight, and nutritional status of the animals were controlled. Certain other factors in the environment, such as infection or the normal flora (and their effects on the immune mechanism), are more difficult to regulate; we attempted to control these factors by regulating the housing and care of the animals before immunization and throughout the experiments. Thus, the greater reactivity of the Hartley strain compared with that of strain 13 appears to result from a genetic difference.

Table 1. Incidence of thyroiditis in Hartley and strain 13 guinea pigs immunized with various dilutions of thyroid extract in Freund's complete adjuvant.

Dilution of thyroid extract	Guinea pigs*	
	Hartley	Strain 13
1:640	5/5	5/5
1:1280	10/10	4/10
1:2560	9/10	2/10
1:5120	6/10	0/10
1:10,240	2/10	2/10
1:20,480	1/5	0/5
Total †	28/45	8/45

* Animals with disease/total number of animals.
† Excluding the 1 : 640 dilution.

Genetic influence on the frequency of this disease is not confined to the guinea pig; the experiments of Gorstein *et al.* (*8*) show the incidence of allergic thyroiditis in mice to be greater in the Swiss strain than in the black C_{57} strain. The mechanism of the genetically determined response remains to be elucidated. It may reflect the degree and type of the immune response to a given stimulus, or a differing extent of tissue damage secondary to the same degree of immunity.

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Localization of Calcium-Accumulating Structures in Striated Muscle Fibers

Abstract. *When frog muscle fibers from which the sarcolemma had been dissected away were perfused with a calcium solution and then treated with oxalate, electron-opaque material, probably calcium oxalate, accumulated in the terminal sacs of the sarcoplasmic reticulum. These regions of calcium accumulation were identified with the intracellular calcium sink that controls the relaxation phase of the contraction-relaxation cycle; their proximity to tubules implicated in intracellular stimulus conduction suggests that they might also be regions from which calcium is released to trigger contraction.*

Probably the most generally accepted explanation of the contraction-relaxation cycle in muscle cells is that (i) depolarization of the surface membrane leads to the release of calcium ions from a source within the cell; (ii) this calcium catalyzes interaction of the myofilaments, giving rise to contractile force; (iii) calcium is then removed by an intracellular sink, ending myofilament interaction (*1*). Although the way in which calcium is made available to the myofilaments and then removed from them is not entirely clear, structures formed by a highly differentiated system of internal membranes (Figs. 1*a* and 2*a*) have been implicated in these processes. It has, in fact, been possible to fragment these membranes and form a heterogeneous collection of vesicles and tubules that is capable of accumulating calcium (*2*). Our study was designed to localize specific regions of calcium accumulation within the cell without disrupting the internal membrane system. The general idea was to immobilize calcium *in situ* by forming oxalate deposits which could be detected by electron microscopy (see *3*).

Small bundles of fibers were dissected out of the semitendinosus muscle of the frog, *Rana pipiens*, and covered with paraffin oil. A segment of a single fiber was separated from the bundle and the sarcolemma dissected away, as first described by Natori (*4*). This technique, which did not appear to affect the internal structure of the fiber (Fig. 1*a*), made it possible to apply various solutions directly to cell components without the intervention of the surface membrane.

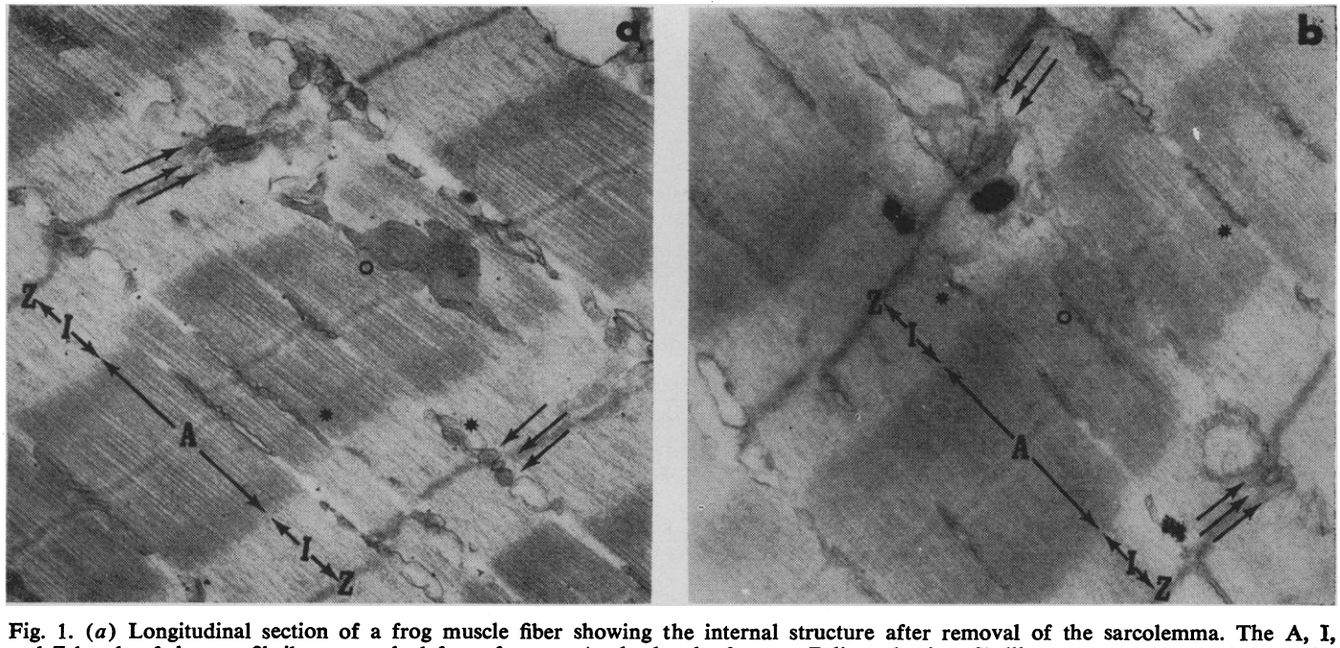

Fig. 1. (*a*) Longitudinal section of a frog muscle fiber showing the internal structure after removal of the sarcolemma. The A, I, and Z bands of the myofibrils are marked for reference. At the level of every Z line, the interfibrillar spaces are occupied by the characteristic group of three elements known as the triad (three arrows) (*13*). The central element of the triad is part of a transverse (or T) system of tubules; the lateral elements of the triad are formed by the terminal sacs of the sarcoplasmic reticulum. Along the A band are longitudinally oriented elements of the sarcoplasmic reticulum (asterisks) which connect terminal sacs at opposite ends of the sarcomere and fuse together to form a flattened cisterna in the middle of the sarcomere (o). Fixed in glutaraldehyde and osmium, stained with lead citrate. (× 24,000) (*b*) Longitudinal section of "skinned" fiber that has been perfused with calcium and then treated with oxalate. The section has not been stained, but there is sufficient contrast to distinguish the main structural features, particularly if one compares this with (*a*) where the same symbols identify similar structures. Electron-opaque material has accumulated in three areas of the sarcoplasmic reticulum, all at the level of the I band, in regions corresponding to the terminal sacs. The general features of deposition are evident: deposits are, at times, only in one corner of the larger terminal sacs (note the largest of the three deposits); some terminal sacs are empty (note lateral elements of triad in lower right corner); deposits are absent from longitudinally oriented elements of the sarcoplasmic reticulum, even though these elements are continuous with terminal sacs, and from the T system. (× 24,000)