Conclusion. Marked differences appeared in strains of mice when they were ovariectomized at birth and then examined in later life. The high tumor JAX dba and JAX C3H strains showed stimulated uterus, vagina and mammary glands. The adrenal glands exhibited extensive nodular, hyperplastic areas. All of these organs of the low tumor JAX C57 black strain remained essentially unstimulated. To ascertain whether the above differences are characteristic of all high and low tumor strains, other strains are being studied.

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Observations on Sensitization of Guinea Pigs with Alum-Precipitated Ragweed Extract.

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Alum-precipitated ragweed pollen extract is a potent antigen for producing anaphylactic sensitization in guinea pigs,[1-4] whereas the watery extract is by comparison a poor one. Alum-precipitated extracts have been successfully applied in sensitizing guinea pigs to house dust[5-8] and cotton linters[5, 6, 9] and in sensitizing dogs to horse serum.[10] The enhanced antigenic response of the alum-precipitated extract is generally attributed to two factors: first, retention of the antigenic substance by the precipitate so that it is only slowly liberated into the system, thus producing a continuous stimulation for the production of antibodies; and second, the local tissue response to the precipitate at the site of injection is said to delay absorption of the antigen. Thus diphtheria toxoid has been detected at the site of injection from a few days to several weeks after administration

1 Harrison, W. T., *U. S. Public Health Reports*, 1934, **49**, 462.
2 Caulfeild, A. H. W., Brown, M. H., and Waters, E. T., *J. Allergy*, 1936, **7**, 451.
3 Zoss, A. R., Koch, C. A., and Hirose, R. S., *J. Allergy*, 1937, **8**, 329.
4 Sherman, W. B., Stull, A., and Hampton, S. F., *J. Immunol.*, 1939, **36**, 447.
5 Coulson, E. J., and Stevens, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 457.
6 Coulson, E. J., and Stevens, H., *J. Allergy*, 1940, **11**, 537.
7 Friedman, H. J., *J. Allergy*, 1939, **10**, 479.
8 Hampton, S. F., and Stull, A., *J. Allergy*, 1940, **11**, 109.
9 Cohen, M. B., Cohen, S., and Hawver, K., *J. Allergy*, 1939, **10**, 561.
10 Waters, E. T., Markowitz, J., and Jaques, L. B., *Science*, 1938, **87**, 582.

of alum-precipitated toxoid.[11, 12] The experiments recorded here were designed to determine whether antigenic constituents of ragweed pollen could be detected in the indurated nodule that forms at the site of injection of the alum precipitate in guinea pigs and persists for several months.

Experimental. An alum-precipitated ragweed pollen extract (1-20 dilution) was prepared in the manner previously described.[6] For sensitizing, 1 ml of the whole alum-treated extract was employed for subcutaneous injection because it was found that the antigenic constituents were not completely precipitated by the alum. Thus precipitates, separated by centrifugation from two 1 ml aliquots of the alum suspension, were suspended in 0.9% sodium chloride and injected subcutaneously into 2 normal guinea pigs. The supernatant solutions, without further treatment, were likewise injected into 2 normal animals. Thirty-four days later each animal received an intravenous injection of 1-100 ragweed extract in 0.9% sodium chloride. The results are shown in Table I.

Each animal sensitized with the alum precipitate was found to be fatally sensitized. At autopsy indurated nodules were found at the site of the sensitizing injection. The animals that had been sensitized with the supernatant solution from the alum-precipitated extract showed symptoms of anaphylaxis but were not fatally sensitized. No nodule was present in either of these 2 animals at the site of the sensitizing injection.

After an incubation period of 30 days guinea pigs sensitized with the whole alum-treated extract regularly respond with anaphylactic shock upon the intravenous administration of ragweed pollen extract. Fourteen animals sensitized in this manner were injected intravenously with 0.1 ml of a 1-100 water extract of pollen. Eight responded with immediate fatal anaphylaxis, 4 with severe anaphylaxis, and 2 with moderate anaphylaxis. Of 29 animals sensitized in

TABLE I.

Sensitization with the Precipitate and Supernatant Solution from Alum-Treated Ragweed Pollen Extract.*

Animal No.	Sensitized with	Test injection,† ml	Symptoms
407	Precipitate	0.2	Fatal anaphylaxis, 3½ min.
409	"	0.2	" " 4 "
406	Supernatant	0.2	Severe anaphylaxis
408	"	0.4	Slight "

*Period between sensitization and shock was 34 days.
†Shock material was 1-100 aqueous pollen extract.

11 Glenny, A. T., Buttle, G. A. H., and Stevens, M. F., *J. Path. and Bact.*, 1931, **34**, 267.

12 Harrison, W. T., *Am. J. Pub. Health*, 1935, **35**, 298.

the same manner and injected with 0.05-0.1 ml of a 1-20 water extract of pollen 23 responded with fatal anaphylaxis and 6 with severe anaphylaxis.

To determine whether antigenic components of ragweed pollen remain unabsorbed in the indurated nodule that appears at the site of injection of the alum precipitate, the following experiments were performed.

Ten guinea pigs weighing between 261 and 347 g were sensitized with alum-precipitated ragweed extract. Thirty-three days later when 2 of the animals were tested for sensitivity by intravenous injections of 0.2 ml of 1-100 pollen extract both responded with fatal anaphylaxis in 2½ and 3 minutes, respectively. The remaining 8 animals were killed by a blow at the base of the skull and the nodules resulting from the sensitizing injection were dissected. Nodules from 2 or 3 sensitive animals were pooled, triturated in 0.9% sodium chloride, and injected subcutaneously into 6 normal guinea pigs. In order to exclude the possibility of passive sensitization 2 of the animals were tested for sensitivity 4 days after injection with the nodules (Table II). Neither of these showed any symptoms of anaphylaxis. Both responded with fatal shock upon injection of pollen extract 28 days later. The 4 remaining animals at the end of the 32-day incubation period were all demonstrably sensitive to the ragweed extract. At autopsy nodules were noted in some of these animals but had disappeared in others.

Discussion. The fact that it is possible to actively sensitize a second guinea pig by the material that is walled in by the cyst which forms at the site of injection of alum-precipitated ragweed extract indicates that the original antigenic substance must be present in practically an unchanged condition and that alum considerably delays the absorption. The continuous stimulus exerted by the slowly absorbed antigen therefore may account for the enhanced antigenic potency of the pollen extract.

TABLE II.
Sensitization with Indurated Nodules.

Animal No.	Sensitized with nodules from animals No.	Incubation period, days	Test injection,* ml	Symptoms
866	760, 788, 789	4	0.2	None
		32	0.2	Fatal anaphylaxis, 3½ min.
870	798, 802, 803	4	0.3	None
		32	0.2	Fatal " 3½ min.
867	760, 788, 789	32	0.2	Slight "
868	791, 792	32	0.2	Moderate "
869	791, 792	32	0.2	Fatal " 3½ min.
873	798, 802, 803	32	0.2	Fatal " 6 "
888	Control		0.5	None

*Shock material was 1-20 aqueous pollen extract.