

that the segmentation of tobacco mosaic virus described by Williams and Wyckoff⁶ was caused in a similar manner.

An effort was made to ascertain whether or not segmentation could be produced in tobacco mosaic virus preparations before shadow casting with gold or in preparations stained with phosphotungstic acid. No evidence for segmentation following prolonged exposure to a high intensity electron beam was obtained in either case. The results confirm the statement by Stanley and Anderson⁷ for unshadowed material that "The fact that a micrograph taken with the first flow of electrons through a specimen does not appear to differ from subsequent micrographs taken after longer exposure to the electron beam makes it seem unlikely that gross changes are caused by the electrons."

The results of the present study indicate that in order to reduce or eliminate granulation and other obvious artifacts, which can

occur when the shadow-casting technic with gold is used, it is necessary to avoid prolonged exposure of the specimen to the intense electron beam.

Summary. Electron micrographs of gold shadow-cast specimens of tobacco mosaic virus show that artifacts, consisting of segmented or even globular particles, are formed when the specimen is exposed to the beam of electrons at high intensity. It is suggested that these are caused by the thin film of gold becoming fluid and forming globules on the surface of the virus rods and supporting membrane. Tobacco mosaic virus not treated with gold did not show any change after having been subjected to the same treatment. The results indicate that gold covered specimens should not be subjected to prolonged exposure to an electron beam of high intensity.

⁶ Stanley, W. M., and Anderson, T. F., *J. Biol. Chem.*, 1941, **139**, 325.

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15795

Hypersensitivity to Egg White in the Rat.*

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Injection of egg white into previously sensitized rats elicits symptoms which have been taken as proof of an anaphylactic reaction.^{1,2} Among these symptoms are hyperemia, cyanosis and edema. One author¹ mentioned that egg white has no primary toxicity.

On the other hand it has been reported³ that egg white administered parenterally is toxic and that sensitized and unsensitized rats reacted with almost identical symptoms. Among the symptoms there is a marked edema of the face, tongue, clitoris and paws preceded by hyperemia.⁴ It seems therefore that the rat is naturally hypersensitive to egg white and that the symptoms attributed to anaphylaxis may be the result of this hypersensitivity. Since hypersensitivity is very seldom observed in animals from the first

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¹ Molomut, N., *J. Immunology*, 1939, **37**, 113.

² Pratt, H. N., *J. Immunology*, 1935, **29**, 301.

³ Parker, J. T., and Parker, F., Jr., *J. Med. Res.*, 1924, **44**, 263.

⁴ Selye, H., *Endocrinology*, 1937, **21**, 169.

contact, this reaction offered an opportunity to approach it experimentally. The purpose of the present investigation is mainly to standardize the conditions under which this reaction will be further studied.

Experimental. In a preliminary experiment 12 male, hooded rats weighing 150-200 g were injected intraperitoneally with a dose of 3 cc of fresh egg white. The animals were examined 30 minutes, 2, 4 and 23 hours after the injection in order to determine when the reaction reaches its maximum. The only symptoms observed consisted of edema of the face, tongue and paws which was preceded by hyperemia noticeable mainly on the nose, ears and paws. The intensity of the edema, read separately for the face and paws, was recorded according to a scale from 0 to 3:0, no reaction; 1, slight but definite reaction; 2, moderate to marked reaction and 3, very marked reaction. The results were as follows: after 30', incidence 15%, severity 12%; after 2 hours, incidence 100%, severity 84%; after 4 hours, incidence 100%, severity 70%; after 23 hours no more edema was visible. We therefore decided, in any further experiments, to examine the animals 2 hours after an intraperitoneal injection of egg white.

When the egg white is injected intravenously the symptoms appear more rapidly. Within a few minutes following injection there is hyperemia, followed by edema, which is maximum after 10 to 30 minutes. In some animals we noticed a cyanosis of the extremities, especially of the paws; in these cases there was no subsequent edema and the animals seemed otherwise normal.

It must be pointed out that this reaction does not apply to all proteins, because the injection of large doses of horse serum was without effect.

Having established that the rat is hypersensitive to egg white we devised another experiment in order to see whether any relationship existed between the dose of egg white injected and the incidence and intensity of the symptoms. Male albino rats weighing 60-80 g were divided into 6 groups of at least 10 animals each. Each group received

TABLE I.
Effect of Dose of Egg White on Incidence and Intensity of the Edema.

Doses in cc	No. of animals	% incidence	% intensity
1.00	9	89	61
0.50	10	100	62
0.25	10	90	45
0.20	10	70	40
0.15	40	57	33
0.10	10	25	9

intraperitoneally one injection of 1 cc, 0.5 cc, 0.25 cc, 0.15 cc and 0.10 cc of fresh egg white respectively. The animals were examined after 2 hours; the results are summarized in Table I.

There is, evidently, a direct relationship between the dose and the response, which is more definite in regard to the incidence than to the intensity. The dose which gives approximately 50% of response is 0.15 cc.

In order to determine the variability of the response in the same animal and to see whether there is acquired tolerance, the animals of the latter experiment were injected, during 9 consecutive days, with the same initial dose of egg white. The responses were read 2 hours later. Since all the animals behaved similarly, we list in Table II only the observations on the first 4 animals of the groups receiving 1 cc, 0.5 cc and 0.10 cc of egg white.

From the results summarized in Table II it can be seen that: (1) the same intensity of edema is not necessarily present at the same time on the face and the paws or vice-versa; one animal may have a reaction of 3 on the face and no edema on the paws. There is no predilection for one area rather than for another. (2) The sensitivity of the animals varies from day to day. (3) On continuous treatment the sensitivity does not necessarily disappear.

Among the other variables, the composition of egg white should be taken into consideration. To eliminate this factor, we lyophilized a large quantity of egg white using it as a uniform stock for all these experiments.

It is known that age and sex have an influence on certain biological reactions. To in-

TABLE II.
Influence of Dose and Duration of Treatment on Production of Edema.

Group	Daily dose, cc	Rat	Day	1st	2nd	3rd	5th	6th	7th	8th	9th
I	1	A		3;3	0;0	2;2	2;0	0;1	0;0	0;0	0;0
		B		3;3	0;0	1;2	0;0	0;1	0;0	0;0	0;0
		C		0;0	1;0	0;0	0;0	0;0	0;0	0;0	0;0
		D		2;2	1;0	0;0	0;3	0;1	1;2	—	0;1
II	0.5	A		2;2	3;3	0;0	0;3	0;2	0;1	1;0	1;2
		B		1;2	0;1	0;0	1;3	0;0	1;0	0;0	0;0
		C		2;3	1;1	0;0	2;2	0;0	0;1	0;0	0;0
		D		3;3	1;1	0;0	3;2	1;0	0;0	0;0	0;0
III	0.25	A		3;2	0;1	0;0	1;0	0;2	0;1	0;0	0;1
		B		1;1	2;2	0;2	0;0	0;1	1;3	0;0	2;2
		C		3;3	0;0	1;0	2;0	0;0	0;0	0;0	1;2
		D		3;3	0;0	0;0	3;3	0;0	0;0	0;2	2;2
IV	0.10	A		0;0	0;0	0;0	0;0	0;0	0;0	1;1	0;0
		B		1;0	0;0	0;0	0;0	0;0	0;0	0;0	0;0
		C		0;0	0;0	0;0	2;0	0;0	1;3	0;0	0;0
		D		1;0	1;1	0;0	0;0	0;0	0;0	0;0	1;2

The first number in each column represents the intensity of edema on the face, the second on the paws.

TABLE III.
Influence of Age and Sex on Production of Edema.

				1st day		4th day		6th day	
				% incidence	% intensity	% incidence	% intensity	% incidence	% intensity
Group I	♂	40-50 g		89	37	25	12	75	33
Group II	♂	70-80 g		62	25	17	3	20	3
Group III	♂	120-140 g		75	37	12	4	29	7
Group IV	♀	120-140 g		37	19	12	4	25	11

investigate this possibility we performed the following experiment. White rats were divided into 4 groups of 8 animals: Group I, male rats weighing 40-50 g; Group II, male rats weighing 70-80 g; Group III, male rats weighing 120-140 g, and Group IV, female rats weighing 120-140 g. All the animals received one intraperitoneal injection of 0.15 cc of egg white on the 1st, 4th and 6th day. The results are summarized in Table III.

Age evidently does not influence markedly the sensitivity of the animals; as far as sex differences are concerned it seems that the females are somewhat less sensitive although experiments with larger groups of animals would be necessary to provide definite proof of this point.

During the course of the present investigation we noticed that a few animals presented disturbances in the vestibular equilibrium apparatus resembling those seen in the

Ménière's syndrome. Since they appear only after injection of egg white, the possibility of spontaneous otitis media can be eliminated.

Discussion. Judged by the present experiments, the egg-white reaction shows the following characteristics: (1) it occurs naturally in rats, (2) it is elicited at the first parenteral injection, (3) there is a direct relationship between dose and incidence or severity of the reaction, (4) the symptoms are always mild and restricted to certain areas: no case of severe reaction or fatal shock has been observed, (5) various areas are not necessarily affected to the same degree, (6) on continuous treatment the sensitivity of the animals varies from day to day; there is no permanent immunity.

It now remains to be determined whether these symptoms should merely be considered as part of a simple toxic reaction or classified among the phenomena of hypersensitiveness.

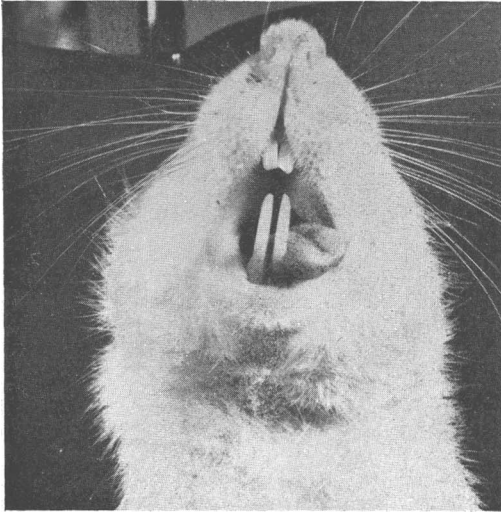


FIG. 1.
Normal control rat.

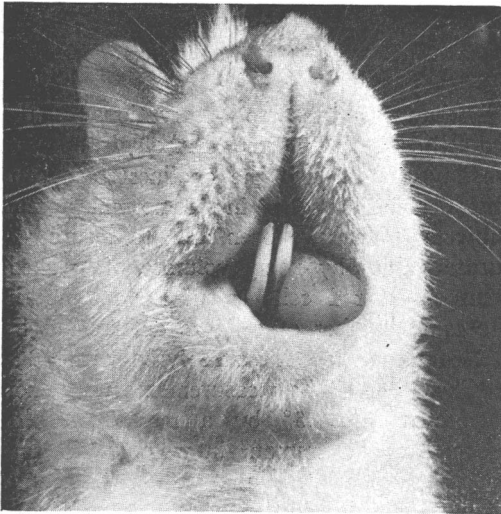


FIG. 2.
Egg-white treated rat. Note edema of tongue, nose and face. The two upper incisors are not visible because of edema and also the medial line of tongue is not as defined as in the control.

One author has already attributed the symptoms following parenteral injection of egg white to the toxicity of the substance.³ Such a possibility is suggested by the relationship existing between dose and response. Against such an interpretation are the facts that even with high doses of egg white the symptoms are always mild and that the incidence and the severity of the reaction vary

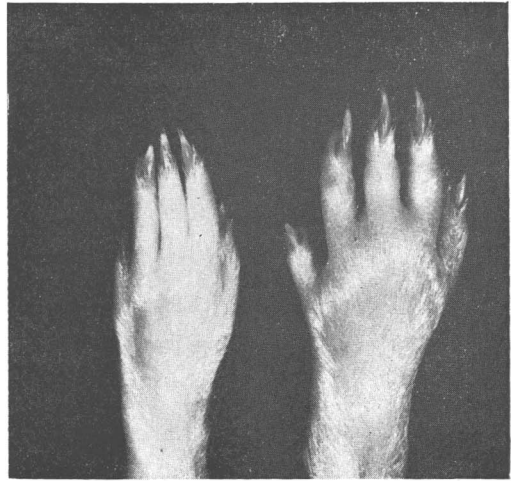


FIG. 3.
Left, paw of normal control rat. Right, edema of the paw after injection of egg white.

from day to day.

The reaction has some similarity with the local anaphylaxis and the symptoms elicited by the local injection or release of histamine. Against the anaphylactic theory is the fact that there is no need for a previous sensitization of the animals, and therefore no explanation can be based on an antigen-antibody mechanism. It seems to the authors that, tentatively, the egg-white reaction can be classified as an anaphylactoid phenomenon which is defined as a group of reactions resembling in their symptomatology those of true anaphylaxis but which cannot be referred to a specific antigen-antibody mechanism.⁵ The inhibition of the egg-white reaction by antihistaminic substances gives some support to this interpretation.⁶

Although anaphylaxis is very difficult to elicit in the rat, the reaction to egg white can readily be obtained in a high percentage of the animals. It can be assumed therefore that different mechanisms are involved.

The symptoms obtained with egg white

⁵ Zinsser, H., Enders, J. F., and Fothergill, L. D., *Immunity. Principles and Application in Medicine and Public Health*, The Macmillan Co., New York, 1941.

⁶ Leger, J., and Masson, G., in press.

are similar to those described for angioneurotic edema in man: edema of the face, lips, buccal cavity associated with vestibular crisis or other neurologic manifestations. Another similarity is the abrupt appearance of the symptoms and their sudden disappearance.

Summary. In the rat a single intravenous or intraperitoneal injection of egg white elicits a reaction characterized by hyperemia, followed by edema of the face, tongue and paws. Although only these regions respond,

they are not necessarily affected to an equal extent. Continued injections of the same dose produce variable reactions in the same animals but there is no acquired tolerance. Age does not play a great influence, but females seem less sensitive than males.

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15796 P

Cholinesterase Content of Erythrocytes in Normal and Pathological States.

I. Content in Normal Humans.*

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Several investigators have recently recently reported changes in plasma, or whole blood cholinesterase content in anemias and leukemia. Values reported were expressed in terms of cholinesterase activity per volume of sample. Sabine¹ has calculated the cholinesterase content per cc of red cells but was unable to secure accurate values by direct measurement.

The red cells contain only specific cholinesterase and none of the nonspecific type found in plasma or serum. It is unlikely, therefore, that the cholinesterase in the red cells is derived from the circulating plasma, but, more probably, their esterase content is produced by the tissues from which they are derived or with which they are closely associated. Any change of esterase content per cell may be important, therefore, in reflecting the activity of the reticulo-endothelial elements of bone-marrow. The production of the esterase by the red cell itself cannot at this time be excluded.

Preliminary to some observations of the red cell content in various pathological states, we have determined the cholinesterase content per million cells in 18 normal human subjects. We have been able to secure accurate values by using volumes of washed, resuspended red cells calculated to contain the same number of cells as determined by cell count. The cells were lysed with 10% NaCl at 2°C. The measurements were then conducted at 37.5° by a modification of the method of Ammon² in which the bicarbonate-Ringers solution and the acetylcholine chloride solution were both placed in the large space and the blood sample in the side cup of the Warburg vessels. Values of cholinesterase activity thus secured are expressed as cmm of CO₂ produced per hour per million red cells. Esterase activity so expressed was not proportional to cell count or total blood hemoglobin. Values for different subjects varied from 3.94×10^{-4} cmm to 7.45×10^{-4} cmm with a mean value for normals of 6.16×10^{-4} cmm.

* Aided by a grant from the Williams-Waterman Fund of Research Corporation.

¹ Sabine, J. C., *J. Clin. Invest.*, 1940, **19**, 833.

² Ammon, R., *Arch. ges. Physiol.*, 1934, **233**, 486.