Table 1. Phosphatase activity in supernatant and precipitate after combination with a constant amount of antiserum in the presence of 0.15*M* sodium chloride. Tubes were centrifuged after 30 to 36 hours at 4°C and the precipitates were dissociated with 0.2*M* carbonate buffer, *p*H 9.8, and brought up to the original volume. Enzyme activity was determined at *p*H 9.8 by measuring the released phenol (*9*). All activities are expressed as micrograms of phenol per milliliter of the enzyme-antiserum combination per 30 minutes. (The sum of the activity of the supernatant and precipitate is higher than the original activity because of an activating effect of serum components other than antibody on the enzyme.)

Enzyme	Initial activity	Substance added	Activity added	Activity supernatant	Activity precipitate
HR	633	Antiserum to HR		165	571
HR	633	Antiserum to HR		135	519
HR	633	Antiserum to HR		109	574
LR	710	Antiserum to HR		823	
LR	710	Antiserum to HR		783	21
LR	710	Antiserum to HR		783	3
LR supernatant	783	HR*	633	1432	40
LR supernatant	823	HR	633	1479	22
LR supernatant	783	Antiserum to LR		32	699
LR and HR	1343	Antiserum to HR		1268	271

* Concentrated HR was added so that change in the volume of the supernatant was negligible.

matically inactive molecule that could react with the antibody to the HR form. In order to determine which of these alternatives was correct, we tested the supernatant to see whether it still contained antibody to HR after reacting with LR material.

High-ratio phosphatase was mixed with the supernatant after centrifugation of the LR and antiserum combination. As shown in Table 1 (lines 7 and 8), the same concentration of HR was used as in the previous experiment in which HR had combined with the antiserum. After 36 hours at 4°C the tubes were centrifuged, and it was found that very little phosphatase had precipitated from the combination of HR with the LR and antibody supernatant. After reaction with the LR material, the antiserum to HR had apparently lost its ability to precipitate the HR enzyme.

It is well known that antigen-antibody complexes are soluble in the presence of excessive amounts of antigen (*8*). Although the activities of the HR and LR forms were adjusted to approximate equality in this experiment, there is a substantial difference in the protein content of the two isozyme preparations, so that the LR tubes contained approximately four times as much antigen protein as those that contained HR. The possibility that the excessive protein or some other factors in the LR experiments inhibits precipitation of an LR and antibody complex has, however, been ruled out by three findings: (i) An enzymatically inactive precipitate was seen when the LR form was combined with the antiserum, indicating that something could be precipitated from this mixture. (ii) When antiserum against the LR form was added to the mixture of LR and antiserum to HR, most of the LR was precipitated (Table 1, line 9), showing that phosphatase can be precipitated from this mixture. (iii) When the same amounts of HR and LR as were used in the previous experiments were combined and then treated with the same quantity of antiserum to HR, a precipitate formed that had phosphatase activity (Table 1, line 10). Since some of the HR antibody would be removed by an inactive cross-reactive component in the LR enzyme preparation, it could be anticipated that less active enzyme would be precipitated from the HR and LR mixture than from the solution that contained only HR, and this is what happened (compare line 10 with lines 1–3 in Table 1). Although these experiments do not rule out the possibility that an excess of antigen exerts some influence on the amount of precipitation formed, the observation that substantial precipitation of phosphatase can occur from this mixture shows that the failure of precipitation of HR with antiserum to HR absorbed with LR (lines 7 and 8 in Table 1) is not the result of an excess of antigen.

We therefore conclude that a molecule that is enzymatically inactive but immunologically similar to HR exists as early as 11 days. The fact that this material is present in the phosphatase peaks eluted from DEAE-cellulose columns indicates that it is not just a small molecule or hapten, but a protein that has the same elution characteristics as the active phosphatase. The possibility that the material is an unstable phosphatase inactivated during purification seems to be ruled out by the fact that 11-day extracts lose very little activity on passage through DEAE-cellulose, and the activity eluted has the same low ratio as the homogenate from which it was prepared. The presence of this inactive form suggests that the appearance of HR beginning at 12 days may be partially caused by an activation of this inactive form.

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X-ray Detection by the Olfactory System: Ozone as a Masking Odorant

Abstract. *The technique of masking was used to test the hypothesis that x-ray detection is mediated by an odorant produced in irradiated air. Rats conditioned to cease licking during exposure to x-ray (conditioned suppression) did not display this conditioned response in the presence of ozone and strong volatile oxidants.*

A role has been established for the olfactory system in the detection of low doses of x-ray by rats. Odorants, the olfactory membrane, nerve, and bulb have been considered (*1*) among possible mediators of this effect. The discovery that alcohol on the nasal mucosa blocks x-ray activation of olfactory neurons (*2*) led to the hypothesis that detection is mediated by the production of odorants within the nasal passage or by direct action of radiation on the olfactory sensory cell.

Because x-ray is known to produce active molecules such as ozone and oxides of nitrogen (*3*), we first under-

took to eliminate gaseous odorants as possible causes of x-ray detection. On the assumption that an odorant, such as ozone, is produced and signals the presence of x-rays, the ability of rats to detect x-ray in the presence of various levels of this gas was tested. The results strongly support the hypothesis.

Twenty-three male Sprague-Dawley rats that weighed 250 to 300 g at the beginning of the experiment were shaped over a period of 3 to 7 days in a Skinner box to lick for sweetened water after 23 hours of daily water deprivation. The Skinner box was a sound-insulated chamber (51 by 34½ by 28 cm) which was provided with a speaker for delivering masking noise. When uniform rates of licking were reached, x-irradiation sessions were begun.

X-rays were obtained from a GE Maximar 200 machine with an 0.75-mm copper filter (half-value layer = 0.93 mm of copper). After the beam had been activated for more than 30 seconds, the exposure was effected by swinging out a 1-cm thick lead shutter designed according to Garcia *et al.* (*4*). During the course of each daily irradiation session, sham exposures were interspersed among the real exposures to control possible effects of shutter movement or machine noise. Only the head was exposed and, despite total doses of more than 600 roentgens, no deleterious effects of the x-rays were detected.

Performance of the animals was monitored by visual observation through closed-circuit TV and by means of operant conditioning apparatus which recorded the licking rate and the time of the shutter movement. The conditioned suppression behavior was established by exposing the animals to x-ray at 12 r/min for 10-second periods during which they ceased licking or received shocks for 0.1 second through a grid which formed the floor of the Skinner box. An important adjustment was to set the shock intensity at a level at which the animals appeared to be punished but were not deterred from a resumption of licking. In this way, control data as well as experimental data could be obtained from the experimental group of animals throughout the course of testing; note the x-ray detection sessions without ozone (*X-S*) scattered throughout weeks 6 to 13 in Fig. 1.

At the beginning of the experiment the rats were randomly divided into three groups: (i) "experimentals," 14 rats habituated to drinking in the presence of various concentrations of ozone and exposed to x-ray concurrently with ozone; (ii) "controls 1," 3 rats tested for x-ray detection throughout the experiments but never exposed to ozone; and (iii) "controls 2," 6 rats habituated to drinking in the Skinner box in the presence of concentrations of ozone equal to those presented to the experimentals. This last group was never exposed to x-ray concurrently with ozone. All groups were treated identically through the second week, at which time habituation to successively increased concentrations of ozone was begun for the experimentals and controls 2. Beginning at the sixth week the experimentals were tested for x-ray detection in the presence of ozone while controls 2 were tested for x-ray detection without ozone. Occasional sessions of x-ray or ozone alone were interspersed through the schedule. Figure 1 illustrates the daily schedule for the experimentals; the control animals were worked on the same days as the experimentals except that their tasks were appropriate for their respective groups.

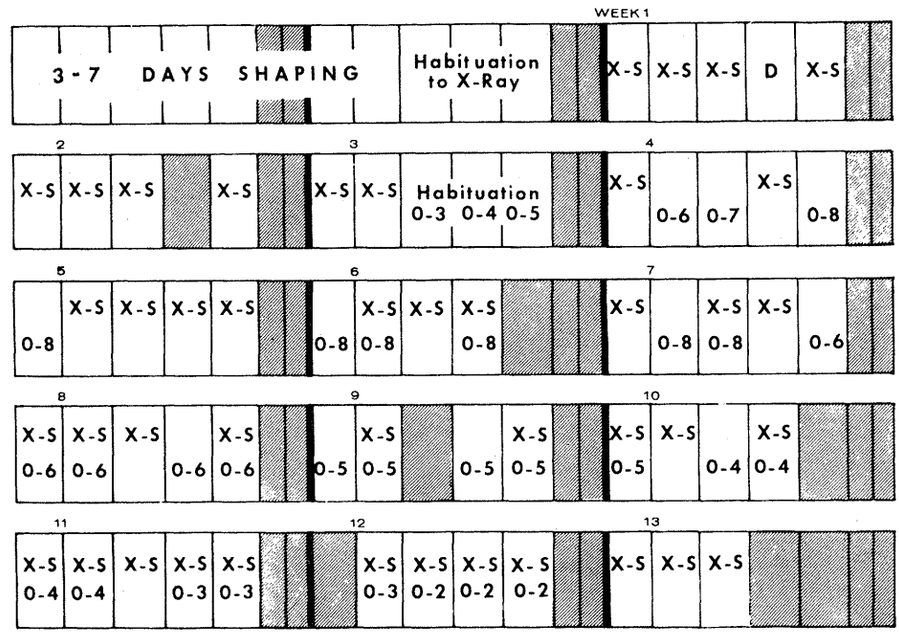
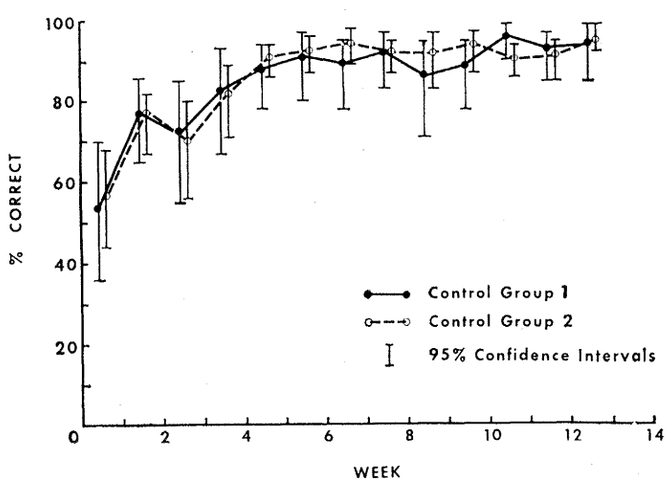


Fig. 1. Training and testing schedule for experimental group of 14 rats. The two control groups were treated similarly except that their tasks were made appropriate for their respective groups (see text). Each block represents a daily session and periods of 1 week are indicated by the heavy black lines. Shaded areas represent days of inactivity. The presence of ozone and its proportionate concentration are indicated by *O-2, O-3*, and so forth, where the numbers represent the ozonizer setting in milligrams per hour. Various types of training sessions include x-ray with possible shock (*X-S*), drinking only (*D*), drinking in presence of ozone only (for example, *O-3*), and x-ray in presence of ozone (for example, *X-S* with *O-3*).

Ozone in concentrations of 2 to 8 mg/hr (manufacturer's rating) was produced by a Sander Ozonizer, Type II, and conveyed to the Skinner box by means of an aquarium pump. At levels of 5, 6, and 8 mg/hr, ozone smelled very pungent and was repulsive to us. Rats showed irritation upon initial exposure to these levels but became habituated in one training session, except at 8 mg/hr for which three sessions were required. Ozone could be detected with KI test paper at the orifice of the ozonizer for the setting of 3 mg/hr. However, we could not detect it subjectively in the Skinner box until a setting of 4 mg/hr (threshold) was used. This latter threshold is in agreement with that for masking as determined from the x-ray conditioned suppression behavior of the rats.

Figures 2 and 3 illustrate the learning curves for the control and experimental groups, respectively. Each point represents the percentage of correct responses for an experimental period for the total number of associations for all members of a group. The variability of each point is expressed in terms of the 95-percent confidence interval for analogous binomial data.



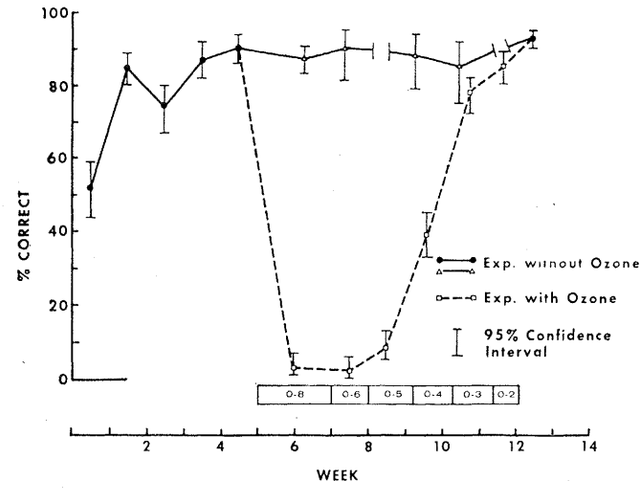


Fig. 2 (left). Acquisition and maintenance of the conditioned suppression response by the control groups. Group 1 consisted of three rats that were never exposed to ozone. Group 2 consisted of six rats that were habituated to ozone but never received x-ray and ozone concurrently. Fig. 3 (right). Acquisition and maintenance of conditioned suppression response by experimental group of 14 rats except when x-ray and ozone were presented concurrently. Ozone was presented at rates varying from 8 to 2 mg/hr and interfered with detection of x-ray at rates greater than 3 mg/hr (broken curve).

The experimental periods used for analysis of the data were 1 week for the control data of Fig. 2 and variable periods for experimental data, as indicated in Fig. 3. Only if the animals stopped drinking for the entire 10-second exposure period was an association counted as a correct response. The conditioned behavior was established at a 90-percent correct level in 4 to 5 weeks with six exposures per day per animal. There was no significant difference in the performance of the two control groups. Control data obtained from the experimentals are plotted in Fig. 3 and do not differ significantly from the controls of Fig. 2. It is also noteworthy that all groups show a characteristic dip in their learning curve before reaching a plateau. This dip probably resulted from an undetermined factor which affected all of the rat colony.

The ozone did not have apparent deleterious effects on the conditioned suppression behavior, the olfactory system, or sensory cells, as is evident from the continued ability of controls 2 and the experimental group to detect x-ray. Controls 2 detected x-ray despite being habituated to drinking in the presence of ozone, and the experimentals gave excellent control responses throughout the weeks of testing in ozone.

During each ozone period shown in Fig. 3, the animals were exposed to x-ray and ozone concurrently for only 3 days of the total period. During the remainder of the time, they licked in the presence of ozone or were tested for x-ray detection without ozone (Fig. 1). The broken curve shows that experimentals could not detect x-ray in the presence of ozone when supplied at 8, 6, and 5 mg/hr. Threshold for masking was between settings of 3 and 4 mg/hr, with the individual animals detecting all the time, occasionally, or not at all. When levels below threshold were reached, the ozone did not seem to interfere with the ability of the rats to detect x-ray with great accuracy. Therefore, it would seem from our results that ozone had a definite masking effect on the sensory cells during irradiation.

Further testing gave additional evidence to support our conclusion that ozone masked an x-ray produced odorant rather than chronically damaged or paralyzed the receptor cells. At the conclusion of the preceding experiment, the animals were tested in an olfactorium in order to determine whether they could detect another odor in the presence of ozone.

The olfactorium was 0.77 m in diameter with a central circular area 25 cm in diameter. By use of radial partitions, the periphery was divided equally into ten compartments equipped with a shocking grid on the floor and mesh cups on the peripheral wall. The rats were deprived of food for 48 hours prior to commencement of training. At the conclusion of their daily runs, they were given 20 g of food which maintained their weights but kept the hunger drive at a level that would insure adequate motivation throughout the experiment. They were placed individually in the central circular area which was enclosed by a mesh barrier 12.7 cm high. From this area the rats could presumably decide by smelling which of the ten cups held the concealed odorant. They then crossed the barrier and grid to obtain the reward. If a rat leaped over the barrier into a wrong compartment, it received an electric shock and its performance was scored as an error. A piece of apple served both as the odorant and as a positive reinforcement for a correct choice. It was randomly placed in the cups on the wall, with the limitations that no cup was successively used twice, nor were the two adjacent cups used in the following run. In this way possible bias resulting from experience or residual odors was reduced.

Thirteen of the experimental animals performed well in the olfactorium, while the remaining animal behaved erratically and consequently was eliminated from the group. When no electric shock was used, the pooled results gave 30 percent correct choices (52 out of 146). After three sessions with negative reinforcement, the behavior of the rats shifted from being primarily exercise and exploration to a purposeful olfactory search pattern; the rats carefully sniffed the various compartments before crossing the barrier. For eight sessions with shock over a period of 4 days, the pooled data gave 80 percent correct choices (104 out of 130). Occasionally an animal was allowed more than one choice per session.

If the rats had been unable to smell, the probability of their making a correct choice would have been 10 per-

cent. The 80-percent value, therefore, is highly significant. It is even more significant than the figures indicate, since the incorrect choices occurred most often in the two chambers adjacent to that with the apple, as would be expected from spread of the odor. Therefore, the rats that had been subjected to prolonged exposure to ozone, with the exception of one, could smell, and their olfactory membranes had not been chronically damaged or paralyzed by the ozone.

The specificity of the ozone block of x-ray detection was tested in two ways. First, it was determined that the olfactory membrane was not acutely paralyzed against detection of another odor in the presence of ozone. This was done by measuring the ability of the rats to find the apple when the olfactorium was filled with ozone at various concentrations. The rats located the apple without difficulty even in a highly pungent environment of ozone supplied at an ozonizer setting of 8 mg/hr.

The second test for specificity was run in the Skinner box where attempts were made to prevent x-ray detection by masking with odors other than ozone. Each potentially masking odor was presented for a total of three sessions over a period of 3 days by placing the liquid odorant in open dishes beneath the shocking grid of the Skinner box.

The approximate concentration of the odorant in solution and the level of detection were: 100-percent oil of wintergreen, 90-percent detection; 20-percent acetic acid, 92-percent detection; 15-percent ammonium hydroxide, 80-percent detection; 5-percent sodium hypochlorite, 6-percent detection; and 30-percent nitric acid, 40-percent detection. The strong odors of oil of wintergreen, acetic acid, and ammonium hydroxide did not block x-ray detection, thus further indicating specificity for the ozone masking. However, the extremely pungent and ozone-like odors of sodium hypochlorite and nitric acid did interfere with detection as might be expected from the stereochemical theory of olfaction (*5*).

Despite their irritant quality, it is very unlikely that these pungent odorants blocked x-ray detection by their action through the trigeminal system. The animals readily became habituated to ozone at high concentrations and performed the licking behavior at ozone settings of 2 to 8 mg/hr with little aberrant behavior; a few animals showed hyperactivity and irritability after testing sessions at 8 mg/hr. If irritation by ozone was a factor in these experiments, it should be noted that it did not interfere with detection of the apple odor. Furthermore, the olfactory bulbs have been demonstrated to be essential for immediate x-ray detection and there is no evidence for a role of the trigeminal system in this function (*1, 6*).

The question of the blocking mechanism is not fully answered by these experiments. Whether ozone masks another odor in the conventional sense of the word "masking," or if it blocks x-ray detection by reducing the intensity discrimination or by adaptation of the olfactory sense, is not settled. Since ozone did not mask apple odor and since the intense odor of oil of wintergreen and the moderately pungent odors of acetic acid and ammonium hydroxide were ineffective, considerable specificity of the blocking agent, ozone, is indicated. This is particularly true in view of the low level of ozone needed to block x-ray detection. These factors, coupled with the known production of ozone by x-ray, lead us to favor either intensity discrimination or adaptation as the blocking mechanism in these experiments, and ozone as the mediating odorant in x-ray detection.

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Genetically Determined Antigen of the Ne Subgroup of Gamma-Globulin: Detection by Precipitin Analysis

Abstract. *A genetic antigen, Gm(n), has been described for the Ne subgroup of gamma-globulin previously devoid of Gm factors. It was detected by precipitin tests with a primate antiserum to Ne-type heavy chains. A relation to the Gm(b) antigens of the Vi subgroup and the Gm(f) and Gm(y) antigens of the We subgroup was apparent. The availablity of genetic antigens for the heavy chains of three subgroups of gamma-globulin with varying relationships in different populations offers an approach to the mapping of the genes concerned.*

There are at least four heavy-chain subgroups of human γG-globulin (*1, 2*). These differ in antigenic, chemical, and biological properties (*3*). Primary attention has been directed to the We (γ_{2b}) and Vi (γ_{2c}) subgroups which contain all of the known genetic antigens (*4*). The Ne (γ_{2a}) subgroup which makes up approximately one-fifth of the γG-globulin has received relatively little attention and no genetic factors have been described.

In the course of studies on the delineation of the Ne subgroup with various antiserums, a primate antiserum was encountered which showed differences among Ne-type myeloma proteins in agar diffusion analysis which were not shown by the other antiserums. Similar differences were found for the Ne-type protein of different normal serums, and the antigen involved was demonstrated to be of genetic origin. This represents the first of the genetic antigens of human γ-globulin to be detected by a direct precipitation system.

Myeloma proteins were isolated and typed with respect to subgroup (*2*). The antiserums utilized for determining proteins of the Ne subgroup were prepared primarily by immunization of monkeys (M) and baboons (B) with Ne-type myeloma proteins (B Ne and B Th) or with the heavy chains prepared from these proteins (M Ne). Two rabbit (R) antiserums, R Ne_1 and R Ne_2, were made against heavy chains of