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

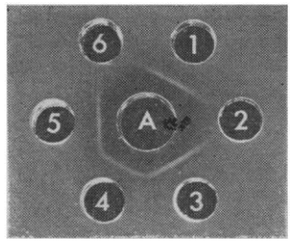

Fig. 1. Precipitin bands given by a Ne-type myeloma protein (wells 1 and 4) and two Gm(n+) normal serums (wells 3 and 5). Two Gm(n−) normal serums (wells 2 and 6) failed to react. The antiserum in the central well A was completely absorbed with the γ-globulin isolated from a Gm(n−) serum.

myeloma protein, Ne, and these antiserums were specific for the Ne subgroup. Many other rabbit antiserums to Ne-type myeloma proteins were produced which failed to distinguish the Ne-type proteins from those of the major We type. All the primate antiserums showed specificity. All immunizations were carried out with complete Freund's adjuvant.

The typing for known Gm factors was carried out by the usual agglutination inhibition procedures with reagents already described (*5*). Serums from different population groups (furnished by A. G. Bearn, L. Märtensson, and F. H. Allen) were kept frozen. Certain serums were also typed for the new factor, Gm(g), described by Natvig (*6*); however, a precipitin method similar to that used for Gm(n) was used. Heavy chains were prepared in the usual fashion from the Ne proteins (*7*), except that the technique for papain splitting had to be modified. It was essential to reduce the proteins with mercaptoethanol before papain digestion in order to obtain Fab- and Fc-fragments; 0.1*M* mercaptoethenol followed by iodoacetamide was utilized before enzymatic splitting.

Antiserums specific for the Ne(γ_{2n}) subgroup of γG-globulin were difficult to obtain in rabbits but were readily obtained in primates. Antiserum B Th proved to be the strongest, and it reacted specifically with 10 of 50 myeloma proteins after absorption with proteins of the We and Vi types. All ten of these proteins had previously been shown to be deficient with antiserums to We-type proteins. Antiserums B Ne, R Ne_1, and R Ne_2 gave similar results and specifically delineated the same ten myeloma proteins. In the case of antiserum B Th, slight differences between the ten myeloma proteins, shown by agar-plate analysis, could be removed by further absorption; individual specificity to myeloma Th and some increased reactivity with the kappa-type proteins were eliminated by absorption with pepsin-treated myeloma Th. This left antibodies only to the Fc-fragment. Immunoelectrophoresis showed that only the Fc-fragment obtained by papain splitting reacted. In the course of this work, we noted antigens, common to the proteins of the Vi(γ_{2c}) subgroup and those of the Ne subgroup, which were not present in We proteins. However, this was only in respect to the antigens of the Fc-fragment. The striking antigens of the Fab-fragments, so characteristic of Vi proteins (*2*), were never observed in the proteins of the Ne subgroup.

The four antiserums described above, after appropriate absorption, were used to detect in normal serums a component which fused in a reaction of identity with the myeloma proteins of the Ne subgroup. However, antiserum M Ne obtained from a monkey immunized with Ne heavy chains gave strikingly different results. Initial bleedings early in immunization showed a single distinct line with some normal serums and a much fainter line with others. Also one Ne-type myeloma protein, Sm, from a Negro, showed a distinctly diminished reactivity which was not observed with the other antiserums. It soon became apparent that this difference was on a genetic basis and that there was a correlation with Gm(f), a genetic antigen of the We group. The early bleedings of this antiserum, although distinguishing between normal serums through intensity of precipitation, did not show good spurs in agar plates, and absorption with the faint-reacting serums and protein removed the line for the strong-reacting serums and myeloma proteins. However, with further immunization over a period of 6 months, antiserums were obtained which showed distinct spurs, as well as clear reactions after complete absorption with normal serums and myeloma Sm. The pattern of reactivity among serums, however, remained the same.

Fusion of the precipitin bands obtained for a Ne-type myeloma protein of the reactive type and the bands for two normal serums that gave strong reactions are shown in Fig. 1. The antiserum was obtained from a later bleeding; it was absorbed with the γ-globulin isolated from a weakly reactive serum. After this absorption, precipitin bands were no longer obtained with such serums. These were termed (n−),

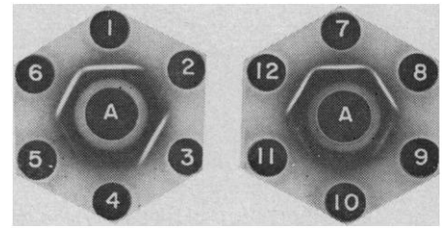

Fig. 2. The reaction of 12 different normal serums with the partially absorbed antiserum used for population screening. The Gm(n+) serums show heavy lines (wells 1, 3, 6, 7, 8, and 12), and the Gm(n−) serums show very faint lines.

and the positive serums were designated (n+). A relation to Gm(f) appeared in the initial studies with serums from Caucasians and Negroes: Gm(f−) serums were always (n−), and Gm(f+) serums were usually (n+); and therefore the term Gm(n) was used. However, absorption with Gm(f+) myeloma proteins had no effect on the Gm(n+) precipitation. Only the absorption with Gm(n+) myeloma proteins of the Ne subgroup removed the precipitation with the n+ normal serums. Quantitative studies (*8*) with other antiserums showed that concentration of Ne protein was not a factor in determining the Gm(n) reaction.

In the tests for population screening (Fig. 2), the antiserum was only partially absorbed with Gm(n−) normal serum as well as myeloma proteins of the We and Vi subgroups. This gave heavier lines and ready discrimination of positive and negative serums. Table

Table 1. Relative numbers of Gm(n+) and Gm(n−) individuals in various populations subdivided according to the known phenotype for the other subgroups.

Subgroup and known phenotype			
n+	n−	We	Vi*
		Caucasian†	
31	9	z a f y	b g
32	8	— — f y	b —
0	42	z a — —	— g
		Negro	
0	22	z a — —	b —
3	2	z a f y	b g
		Japanese	
0	37	z a — —	—
6	0	z a f y	b
		Chinese	
30	0	— a f y	b
17	0	z a f y	b
0	4	z a — —	—
		Asian Indian	
18	12	z a f y	b
4	38	z a — —	—
2	0	— — f y	b
0	4	z a — —	b

* Measurements of Gm(g) were only made in the Caucasian and Negro groups; the Gm(b) reagent detected both Gm(b^4) and Gm(b^3).
† Thirty-two selected Gm(b−) Caucasian serums added to 90 random serums.

1 shows the results of analyses on 321 normal serums from different populations along with the standard phenotypes for the We and Vi subgroups. The striking feature of these data is that all the Gm(f−) individuals among Caucasian, Negro, Japanese, and Chinese groups were also Gm(n−). The only exceptions in this relationship were in the case of four serums from Asian Indians. These were rechecked both for n and Gm(f), and the results were confirmed. Among Gm(f+) Caucasians approximately 21 percent were Gm(n−); but among the Chinese there was complete correlation with Gm(f).

Although at least 20 different genetic factors have been delineated in the human γ-globulin system (*9*), these have all been determined by agglutination-inhibition reactions which involved the use of special anti-Rh serums. This system has had a number of limitations, particularly concerning quantitation. Previous efforts to obtain precipitation systems, particularly with human isoantiserums as well as rheumatoid arthritis serums, have been unsuccessful, possibly because most of the antibodies have been of the 19*S* type and the antiserums have been relatively weak. Recently rabbit antiserums have been successfuly employed in precipitating systems for genetic antigens of the lipoproteins and α_2-macroglobulins (*10*). Such heteroantiserums have also furnished antibodies which detect many of the genetic antigens of γ-globulin by the agglutination-inhibition system (*11*). Most of these antiserums, absorbed with serums negative for the specific genetic factor, do not show precipitin lines. Even so, in certain instances after partial absorption, a differentiation of normal serums on a genetic basis could be obtained. In most instances this differentiation only involved the intensity of the precipitin band, and spurs could not be observed. Such was the case for two genetic factors, Gm(b) and Gm(g), and for the early bleedings of antiserum M Ne which determined Gm(n). However, with persistent immunization this antiserum showed distinct spurs, and Gm(n+) serums showed good lines after complete absorption with Gm(n−) serums. The genetic differentiation, however, could be made with the early antiserums, despite the fact that, in these instances, such absorption removed all precipitin lines.

With the delineation of Gm(n), a genetic antigen has become available in the Ne subgroup of γ-globulin which previously was devoid of genetic factors. Previous studies of the We and Vi subgroups, where a number of genetic antigens are available, have revealed interesting differences in various populations between these subgroup antigens. This has also proved true of Gm(n) in its relation to the genetic antigens of the other subgroups. Of the known genetic factors it most closely paralleled Gm(f) in different populations. A marked exception occurred among Caucasians, where Gm(f+) individuals were Gm(n−) as well as Gm(n+). These three major gene complexes were found in Caucasians for the Vi, Ne, and We subgroups, respectively: $Gm^b\ Gm^n\ Gm^{fy}$, $Gm^b\ Gm^n-Gm^{fy}$, and $Gm^g\ Gm^n-Gm^{za}$. In Negroes the major gene complex was $Gm^b\ Gm^n-Gm^{za}$ which resembles the third Caucasian gene complex except that Gm^g is replaced by Gm^b. One explanation of this difference is that it arose through a crossover event involving the Gm^b and Gm^g genes. The continued association of Gm^n- with Gm^{za} in gene complexes of both racial groups suggests the possibility that the genes involving the Ne and We subgroups remained as a unit, and therefore they may be adjacent. The exact relative position of the genes for the Vi subgroup remains uncertain. Previous studies (*12*) have demonstrated the unusual gene Gm^{fya} in Mongoloid populations, which may have arisen through an intragenic crossover. If this were the case, and in view of the Gm^b and Gm^g relationships mentioned above, the order of the genes might be Vi, Ne, and We. Other findings, including the variation in Gm(n) in Caucasians, do not fit so readily into such a pattern, and further studies are indicated, particularly with regard to distinguishing between the variations stemming from crossover possibilities and those arising through point mutations.

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Lethal Effects of Synthetic Juvenile Hormone on Larvae of the Yellow Fever Mosquito, Aedes aegypti

Abstract. *Emergence of adult mosquitoes is blocked after the addition of 1 part of crude synthetic juvenile hormone to 100,000 parts water. Development is arrested at stages ranging from pupae to fully formed pharate adults incapable of escaping from the pupal exuvium. Fourth-stage larvae just prior to metamorphosis are most sensitive: 40 percent were killed after being exposed for 1 day to 1 part juvenile hormone in 2 million parts water. The active material also inhibits the hatching of mosquito eggs.*

Ten years ago, the promise of juvenile hormone as an insecticide was evident in tests performed on the first hormonally active extracts prepared from male *cecropia* moths. When applied topically to silkworm pupae, the hormone penetrated the unbroken integument and caused lethal derangements of metamorphosis (*1*). Subsequently, these findings have been confirmed in tests of the authentic juvenile hormone of the *cecropia* silkworm, as well as of naturally occurring analogs and synthetic materials showing juvenile hormone activity (*2*).

The most active synthetic material available appears to be a product formed by treatment of ethanolic solutions of farnesoic acid with hydrogen chloride (*3*). This crude synthetic material shows a high degree of juvenile hormone activity when tested on immature insects ranging from the most primitive wingless Thysanura to the most highly evolved Hymenoptera (*4*). We now describe the action of this