cessive generations. That the F_{12} and F_{15} were not much different in sensitivity from the F_1 suggests that trace amounts of carotenoid were made available to the later generations.

We believe that Monroe's diet is free of carotenoid and that the most likely source of carotenoid was microorganisms which either survived efforts to sterilize the eggs or reinfected the adults in each generation. In the latter case, carotenoid supplied to adults by the synthetic activity of microorganisms might then be passed to the next generation in the eggs. These possibilities are being studied.

Alternative explanations of the residual sensitivity can also be proposed. On the basis of present evidence, however, it seems unlikely that the fly has either a visual pigment with a totally different chemistry or the capacity for synthesis of carotenoid *de novo*. There is no precedent for either in animal tissues, and, moreover, should the fly synthesize carotenoid we might reasonably expect to find more than mere traces.

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Specific Inhibition of Antibody Formation by Passively Administered 19S and 7S Antibody

Abstract. *Intravenously administered 7S antibody is more effective than 19S antibody in inhibiting the formation of antibody to bacteriophage φX174. Since considerable amounts of 7S antibody are needed for inhibition, serum antibody formation may act as a "feedback" mechanism to prevent hyperimmunization.*

Primary antibody formation can be prevented by mixing antigen with excess antibody before injection (*1*). Since passively administered diphtheria antitoxin can inhibit completely the primary antitoxin response when injected as long as 5 days after toxoid immunization (*2*) and since it can partially inhibit the secondary antitoxin response when injected prior to secondary immunization (*3*), we suggested that antibody formation may act as a "feedback" mechanism. These experiments (*2, 3*) were performed, however, with large amounts of hyperimmune serum, so that it was not clear whether this proposed mechanism acted only during hyperimmunization. We now report the capacity of antibody obtained at various intervals after immunization to inhibit antibody formation. Guinea pigs were injected intravenously with bacteriophage φX174 and homologous antiserum to this phage. This immunization system appeared advantageous since trace amounts of φX without adjuvants regularly stimulate antibody formation, and the kinetics of the primary 19*S*, primary 7*S*, and secondary 7*S* antibody responses are known and predictable (*4*).

Two preparations of bacteriophage φX174 (*5*) were used. Immunized animals were bled at 1 week and 3 to 4 weeks after primary immunization or 1 week after secondary immunization in order to obtain peak titers of the primary 19*S*, primary 7*S*, and secondary 7*S* responses, respectively. Serum antibody to φX (k) was determined by the phage neutralization assay (*6*); k is the velocity constant with the dimension minute^{-1} of the inactivation of phage by a particular antiserum, and it is a convenient measure of the concentration of neutralizing antibody. Thus, $k \times$ ml is a measure of the quantity of neutralizing antibody. Antibody inactivated by 0.1*M* 2-mercaptoethanol (2-ME) was considered to be 19*S* (γ_{1M-}); antibody not inactivated by 2-ME was considered to be 7*S* (*4*). Actively formed antibody was distinguished from passively administered antibody by also injecting the antiserums used into nonimmunized animals (*7*).

The antiserum to φX used for passive administration was prepared by immunizing guinea pigs with 10^{11} PFU (plaque forming units) of φX intravenously and bleeding at intervals afterwards as follows: for obtaining 19*S* antibody, at 6 or 7 days; for primary 7*S*, at 2 weeks; for secondary 7*S*, at 8 to 10 days after reimmunization. The 19*S* pool had a k value of 15, and less than 1 percent of the k value was due to 7*S* antibody. The primary 7*S* pool had a k of 20 and there was no detectable 19*S* antibody. Three antiserums having secondary 7*S* antibody were used: two with k values of 140 and 500, respectively (designated secondary 7*S* and secondary 7*S*-A, respectively), were each obtained from a guinea pig primarily immunized 1 month earlier; the third serum with a k of 500 (designated secondary 7*S*-B)

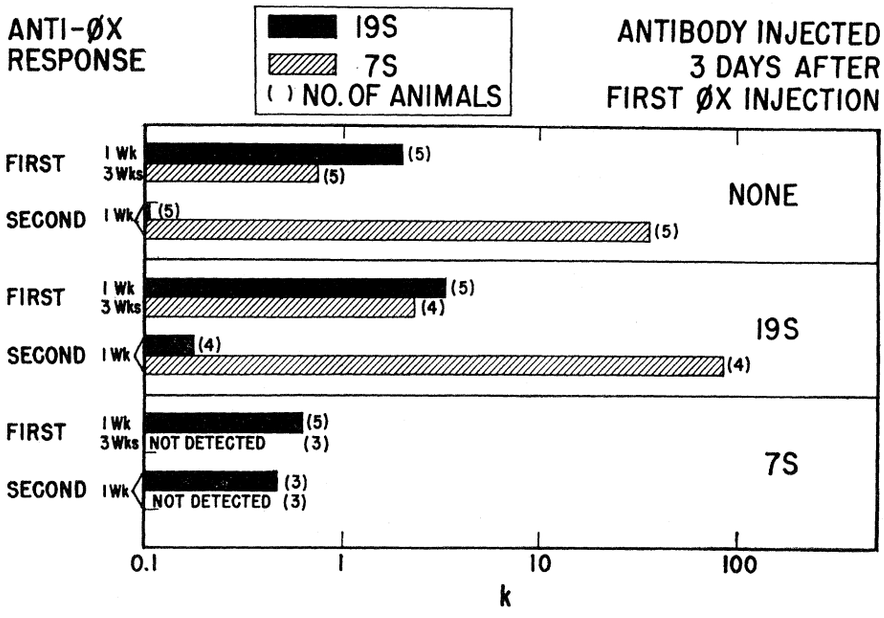

Fig. 1. Inhibition of formation of antibody to ϕX by passively administered 19*S* and 7*S* antibody to ϕX.

was obtained from a guinea pig primarily immunized 1½ years earlier (*8*).

In the first experiment, the effect of 19*S* or secondary 7*S* antibody administered 3 days after immunization with 6×10^9 PFU of ϕX was studied. The equivalent of 1 ml of antiserum with a *k* value of 40 was injected into each animal in order to achieve concentrations approximately equal to the peak titers usually produced after immunization with 6×10^9 PFU of ϕX, that is, 19*S*—*k* of 1 to 2 at 1 week—or 7*S*—*k* of 1 to 2 at 3 to 4 weeks. A control group received only the ϕX immunization. All guinea pigs were challenged again at 4 weeks with 6×10^9 PFU of ϕX.

As shown in Fig. 1, passively administered 19*S* antibody injected 3 days after immunization had no significant effect on the formation of antibody to ϕX, whether it was primary 19*S* at 1 week, primary 7*S* at 3 weeks, or the preparation for a secondary 7*S* response when challenged 1 month later (immunological memory). In contrast, injection of secondary 7*S* antibody at a time that the serum concentration of 19*S* antibody is increasing exponentially (*4*) partially inhibited the primary 19*S* response at 1 week and prevented the development of a primary 7*S* response and 7*S* immunological memory.

This experiment suggests the following conclusions:

1) Three days after immunization there is an extracirculatory transportation of antigen or an antigen-stimulated factor to the cells forming 19*S* antibody. This conclusion is deduced from the facts that at 3 days antigen is no longer in the circulation, administration of 7*S* antibody at this time partially suppresses the formation of 19*S* antibody, and, as previously shown (*4*), the presence of antigen is necessary for continued 19*S* synthesis. Presumably, specific antibody has interrupted this continuous antigenic stimulation by combining with and diverting antigen destined for stimulation of 19*S* antibody-producing cells.

2) The events that determine 7*S* antibody synthesis and 7*S* immunological memory have not been completed by 72 hours; these events are also antigen-dependent. These conclusions are consistent with previous studies (*4*) indicating that below a certain immunizing dose of ϕX, 19*S* antibody formation occurs without subsequent development of 7*S* formation, and that the relative rate of 7*S* formation can be increased by reimmunization with ϕX 9 days after initial immunization.

When 7*S* was administered 10 days before immunization, neither 19*S*, nor 7*S* antibody formation, nor immunological memory to ϕX developed. In contrast, similar administration of 19*S* antibody did not affect any of these three aspects of the subsequent immune response to ϕX.

Additional experiments were performed to confirm the results of the first experiment, and to compare the capacity of 19*S*, primary 7*S*, and secondary 7*S* antibody to inhibit antibody formation. In these experiments, 2×10^{10} PFU ϕX of a second preparation were used which stimulated 7*S* antibody to ϕX at 3 to 4 days in contrast to the lag period of 7 to 8 days ob-

Table 1. Specific inhibition of formation of 19*S* and 7*S* antibody by passively administered 19*S* and 7*S* antibody to øX.

Antibody injected after immunization			Guinea pigs (No.)	Antibody formation to ϕX (serum *k*)*	
Type	Interval † (days)	Amount (k × ml)		19*S* at 1 week	7*S* at 3 to 4 weeks
None			12	8.3 (2.3–19)	24 ‡ (0.95–125)
Secondary 7*S*-A	2	120	6	4.5 ‖ (0.64–11)	0.30 ‖ (<0.04–1.8)
Secondary 7*S*-A	0	20	4	0 ‖ §	7.4 (1.5–17)
Secondary 7*S*-B	3	20	3	8.9 (4.1–12)	3.15 ‖ (0.12–9.2)
Secondary 7*S*-B	0	20	4	1.3 ‖ (0.42–2.3)	14 (0.12–29)
Primary 7*S*	3	20	3	8.6 (7.3–11)	16 (1.4–43)
Primary 7*S*	0	20	5	1.8 ‖ (0.16–4.2)	18 (1.8–32)
19*S*	0,3,6,9	20	2	not done	8.2 (28 and 14)
19*S*	0	20	7	3.2 ‖ (1.0–5.2)	73 ‖ (1.4–230)

* Mean *k* presented; the range of values is shown in parentheses. † Guinea pigs injected with 2×10^{10} PFU of ϕX. ‡ Two animals died. § *k* less than 0.2. ‖ $p \leq .02$.

served with the previous preparation.

The results (Table 1) confirm those of the first experiment, since secondary 7*S*-A injected 2 days after immunization partially suppressed the primary 19*S* and 7*S* responses. The results also indicate that secondary 7*S* antibodies can differ markedly in their capacities to inhibit antibody formation, that 7*S* antibody injected at the time of immunization, rather than 2 to 3 days later, inhibits 19*S* formation more effectively and 7*S* formation less effectively, and that 19*S* antibody does not influence 7*S* formation but can depress 19*S* formation. The capacity of 19*S* antibody to inhibit 19*S* antibody formation may not occur during formation of antibody to ϕX, however, since inhibition was only demonstrated when antibody concentrations usually attained at 1 week were achieved through passive administration at the time of immunization.

It would appear, therefore, that 19*S* antibody is considerably less effective than 7*S* antibody in the inhibition of antibody formation to ϕX. This difference between antibodies may be related to their class of immunoglobulin or to the stage of immunization at which they were obtained or both. That primary 7*S* antibody obtained 2 weeks after immunization was effective as an inhibitor suggests that the class of immunoglobulin is an important factor, but a more definitive test of 19*S* antibody inhibitory capacity would necessitate the use of a different immunization system in which long-term 19*S* synthesis occurs.

There are several possible explanations for the relative inability of 19*S* as compared to 7*S* antibody to inhibit antibody formation, principally: (i) There may be decreased binding affinity for antigen. (ii) There may be a rapid loss of antibody activity. γ_{1M}-Globulin has a half-life in the circulation of only 24 hours (*4*); within reticuloendothelial cells, the half life may also be extremely short, a situation analogous to the rapid loss of 19*S* antibody activity in vitro after treatment with certain enzymes (*9*) and sulfhydryl agents (*10*). (iii) The localization of antibody may differ; intravenously administered 19*S* antibody may not have access to a site of antigen accumulation.

None of these possibilities can be excluded at this time.

The marked differences in inhibitory capacities among antiserums containing 7*S* antibody also suggest that significant inhibition of antibody formation only occurs after a sufficient amount of the proper antibody has been formed. These findings are consistent with the well-known observation that a secondary antibody response can usually be obtained in the presence of a high concentration of serum antibody, but that repeated immunizations become less effective in stimulating antibody formation (*11*). It is suggested therefore that antibody formation "feedback" is one line of immunological defense against hyperimmunization; evidence presented elsewhere (*12*) suggests that induction of immunological tolerance is another.

The site of interaction between inhibitory antibody and antigen that otherwise would have stimulated antibody formation is not known. The studies presented here suggest that the site is extracirculatory. In accordance with current concepts of the essential role of the reticuloendothelial system in "processing" antigen before immunization (*13*) and the demonstration of anatomical continuity between reticuloendothelial cells and lymphoid cells in immunized lymph nodes (*14*), we suggest that this site may be within reticuloendothelial cells. Thus, antibody γ-globulin which also enters these cells may interact with antigen before such antibody undergoes catabolism.

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Hampered Vinyl Polymerization by Embedding Biological Objects

Abstract. *Inhibition of vinyl polymerization by the embedding of biological specimens is due to the presence of biogenic amines. This inhibition is eliminated by acylation of the embedded objects with acid anhydrides.*

Acrylic ester formulations and styrenated unsaturated polyester resins are often used as embedding media for biological specimens (*1*).

Acrylics are also used in the preparation of ultramicrotome cuts and with usual microtome techniques in the preparation of cuts of hard objects like beetles (*2*).

In many cases one can observe zones, with different light refraction, around the object in the innermost part of the casting resin. Such zones tend to develop bubbles after several weeks or months. This behavior is especially well known with molluscs, fish, and isolated organs of higher animals, but practically no embedding process for animal specimens is without this drawback.

The formation of such zones of incomplete polymerization surrounding the embedded objects is due to inhibition, by biogenic amines, of the local vinyl polymerization. Primary and secondary amines (such as those from peptide side chains) interfere, but tertiary amines generally are unreactive in this way, or they accelerate the polymerization. Horner and Schwenk have considered the reactivity (as initiators) of peroxides with amines (*3*).

The inhibition by biogenic amines can be eliminated by acylation and salt formation by means of suitable acid anhydrides. A 10 percent solution of acetic anhydride in anhydrous acetone or anhydrous monomer is an effi-