An analogy with the rabbit T chain (*3*) and J chain (*4*) is suggested by the criteria of electrophoretic mobility, molecular weight, and occurrence in S-IgA; but other characteristics were not determined.

The properties of J chain do not match those of human SP. The latter has a molecular weight of at least 76,000 either free in colostrum or isolated from S-IgA (*13*) and displays amino acid composition (Table 1), electrophoretic mobility, and antigenic determinants which differ distinctly from those of the J chain (Fig. 1B). If SP were composed of two or more J chains, the relative proportions of amino acids should agree. Also, increasingly severe conditions for splitting and dissociation should increase the yield of J chains at the expense of SP. This we did not observe (*12*); SP was not present in IgM.

Since J and L chains resemble each other in ranges of molecular weights only, there are no grounds for assuming that they are related.

The apparent identity of J chains from S-IgA and IgM is interesting in view of their presence in two classes of immunoglobulins, in two body fluids, and in two states of health. The attribute unifying the two immunoglobulins is their polymeric configuration. It may be permissible to speculate that the J chain with its unusually high cysteine content may aid in maintaining the tertiary structure of the polymeric immunoglobulin molecule.

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Stimulation in vitro with Protein Carrier of Antibodies against a Hapten

Abstract. *Rabbits were immunized both with lysozyme and with dinitrophenylated bovine serum albumin. No antibodies against the dinitrophenyl hapten were produced when cell suspensions of their spleens were exposed in vitro to dinitrophenyl-ovalbumin, whereas a positive response was obtained with dinitrophenyl-lysozyme. Moreover, when spleen cells from rabbits previously immunized with dinitrophenyl–bovine serum albumin were exposed in vitro to bovine serum albumin alone, they produced antibodies against the dinitrophenyl hapten.*

In studies of haptenated proteins no secondary response can be obtained with a conjugate of the hapten to an unrelated protein (*1, 2*). A secondary response to the hapten may be obtained when mixtures of cells derived from animals immunized with the initial haptenated protein and other animals immunized with a second protein alone were used (*1, 3*). Moreover, preliminary immunization with the hapten conjugate of one protein, followed by a sec-

Table 1. The effect of different protein carriers on the anamnestic immune response toward the dinitrophenyl (DNP) group. The immune response was tested by [^{14}C]thymidine incorporation and by inactivation of dinitrophenylated bacteriophage T4. Spleen cell suspensions from individual rabbits were cultured on day 0 in the absence (control) or presence of antigen whose concentration varied from 1 to 100 μg per culture tube. After 24 hours the cells were exhaustively washed and then resuspended in M 199 medium supplemented with 20 percent normal rabbit serum. On days 2, 4, 6, and 8 the medium of cell cultures was changed.

Group	Rabbits immunized with	Experiments (No.)	Spleen cells exposed in vitro to DNP conjugated with			
			BSA	Ovalbumin	Lysozyme	BSA
1	DNP-BSA	5	+*	−	−	+
2	Lysozyme DNP-BSA	2	+	−	+	+
3	DNP-BSA lysozyme	5	+	−	+	+

* Positive (+) and negative (−) symbols represent the presence and the absence of a significant (at least tenfold) difference between antigen-stimulated culture and nonstimulated cultures, in terms of both the rate of DNA synthesis (as measured on day 2) and titer of antibodies against the dinitrophenyl hapten (as measured on days 6 and 8).

Table 2. Specificity of antibody response to the dinitrophenyl group in spleen cell cultures boosted by different protein carriers. Spleen suspension was prepared from a rabbit first immunized with DNP-BSA and then with lysozyme. In culture tubes, 10 μg of either DNP-BSA or DNP-lysozyme or BSA was added. Six days after initiation of cultures, supernatants were obtained, and the antibodies against DNP were detected in antigen-stimulated cultures.

Preliminary incubation with*	Number of plaque-forming units obtained after incubation † with				
	Lymphocytes from culture tubes				Buffer only
	Non-stimulated	Stimulated with			
		DNP-BSA	DNP-lysozyme	BSA	
None	338	6	29	12	345
Penicilloyl–aminocaproic acid	302	14	52	11	330
Dinitrophenyl–aminocaproic acid	345	322	341	356	345

* Portions from both stimulated and nonstimulated spleen cell cultures were exposed for 3 hours at 37°C to either $10^{-3}M$ penicilloyl-ϵ-aminocaproic acid or 10^{-3} dinitrophenyl-ϵ-aminocaproic acid. † DNP-bacteriophage T4 (0.2 ml) containing 1700 plaque-forming units per milliliter were incubated for 2 hours at 37°C with 0.5 ml of 1 : 10 dilutions of supernatants and of 0.05M phosphate buffer, pH 7.0, containing 20 μg of gelatin per milliliter (as control).

ond protein, facilitates the formation of antibodies to the hapten upon ulterior immunization with the hapten conjugate of the second protein (*4*). A primary response to a hapten was recently obtained upon exposure to the haptenated protein in vitro of spleen fragments from mice that had been first immunized with the protein carrier alone (*5*).

Using an in vitro system of spleen cell suspension (*6*), we have now confirmed the above conclusions and extended them as follows. Using cells derived from animals that had been immunized with the hapten conjugate of the protein, we were able to stimulate production of antibodies against a hapten upon exposure to the protein carrier alone.

Three groups of rabbits were immunized intradermally with 2 mg of dinitrophenyl (DNP)–bovine serum albumin (BSA) in complete Freund's adjuvant. One of the groups was inoculated 10 days later with 2 mg of hen egg-white lysozyme in complete Freund's adjuvant, whereas another group received lysozyme 10 days before the immunization with DNP-BSA. Both DNP-protein conjugates were prepared according to the procedure described by Eisen (*7*). The conjugates contain 16 and 1.5 DNP residues per BSA and lysozyme molecules, respectively.

Spleen cell suspensions in tissue culture medium M 199 (Gibco) supplemented with 20 percent decomplemented normal rabbit serum were prepared (*6*) 1 month after the first immunization. Suspensions from each individual rabbit were exposed to a variety of antigens in Falcon plastic culture tubes and were incubated at 37°C in an atmosphere containing 10 percent carbon dioxide, 83 percent nitrogen, and 7 percent oxygen. After 24 hours, the cells from control cultures and experimental (antigen containing) cultures were washed first with medium supplemented with 40 percent serum, and then twice with M 199 medium alone. The cell suspensions in M 199 medium supplemented with 20 percent homologous serum were kept at 37°C for 8 days, with the medium being changed every 2 days. The cultures were harvested at 4, 6, or 8 days, and the supernatants were tested for presence of antibodies against the dinitrophenyl group by means of the dinitrophenylated bacteriophage technique (*8*). In each case the incorporation of [^{14}C]thymidine was determined (Table 1) in experimental and control (day 4) cultures (*9*).

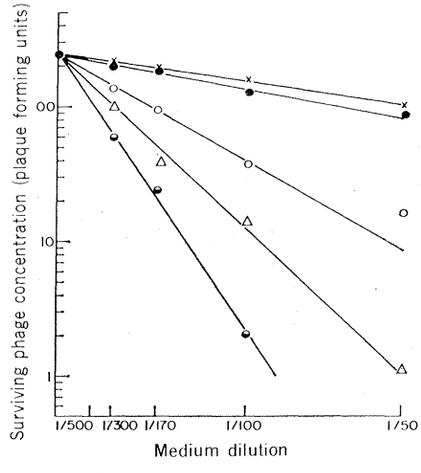

Fig. 1. Inactivation of dinitrophenyl bacteriophage with stimulated cultures. Antibodies toward DNP were quantitatively determined with the use of bacteriophage T4 to which the DNP groups were covalently attached (*8*). Dilutions of spleen cell cultures stimulated with 100 μg of DNP-BSA (◒), DNP-ovalbumin (×), DNP-lysozyme (○), BSA (△) and nonstimulated cultures (●) were incubated in duplicate at 37°C for 2 hours with a DNP–bacteriophage T4 preparation containing 250 plaque-forming units per 0.2 ml. The spleen cell cultures were prepared from a rabbit primed with DNP-BSA as well as lysozyme.

As was expected, the DNP-BSA conjugate gave in all three groups a positive response. This anamnestic immune response was demonstrated by the high rate of DNA synthesis in stimulated cells as well as by the presence of antibodies in supernatants of experimental cultures; these antibodies could inactivate at high dilutions the DNP–bacteriophage T4. When spleen cells from the same animals were exposed to DNP-ovalbumin, neither the uptake of [^{14}C]thymidine nor the inactivation of DNP-bacteriophage was increased as compared of values obtained in control cultures. Incubation with DNP-lysozyme did not lead to an anamnestic response toward the hapten in cell suspensions derived from rabbits that had received DNP-BSA (group 1), but it did lead to a definite positive response to the dinitrophenyl moiety in cultures of spleen cells obtained from animals primed both with lysozyme and with DNP-BSA (groups 2 and 3). No difference between these two groups could be detected.

The detection of antibodies to the dinitrophenyl group is illustrated by a typical experiment in Fig. 1. Spleen cells (10×10^6) from an immune rabbit of group 3 were at day 0 stimulated in triplicate cultures by the appropriate antigens. The tubes were centrifuged on day 8, the supernatants of each type of culture were pooled and then tested for antibodies. The values of [^{14}C]-thymidine incorporation measured on day 4 in the same experiment were 30,000, 600, and 14,000 count/min in cultures stimulated with DNP-BSA, DNP-ovalbumin, and DNP-lysozyme, respectively; in control cultures only 500 count/min were detected.

The specificity of the immune response in vitro to the dinitrophenyl group was defined by means of inhibition of the inactivation of chemically modified bacteriophage (*10*). Supernatants from spleen cells cultured in the presence or absence of antigen were treated with an excess of homologous hapten (dinitrophenyl-ϵ-aminocaproic acid) or unrelated hapten (penicilloyl-ϵ-aminocaproic acid). The ability of supernatants to inactivate the DNP-bacteriophage was completely abolished upon preliminary incubation with dinitrophenyl-ϵ-aminocaproic acid, while no such effect was obtained with pen-

icilloyl-ε-aminocaproic acid (Table 1).

Thus, incubation in vitro with BSA of spleen cell suspensions derived from rabbits immunized with DNP-BSA led to a definite stimulation of the formation of antibodies with specificity directed toward the dinitrophenyl hapten (Table 1 and Fig. 1). The titer of antibodies toward the dinitrophenyl group in cultures challenged with BSA was similar to that obtained with DNP-BSA. The specificity of the antibodies was corroborated by the specific inhibition of modified phage inactivation (Table 2). Moreover, we have some results which show that the anamnestic immune response toward the hapten of cultures stimulated with BSA could be detected also by a modification of the Jerne technique (*11*) in which erythrocytes coated with the dinitrophenyl hapten were used.

Our results agree with those of Dixon and Maurer (*12*), who found an increase of antibodies to human serum albumin upon subsequent immunization with chicken γ-globulin, but not with those of Ovary and Benacerraf (*13*) who reported a lack of effect in vivo of carrier protein injected secondarily on production of antibodies against the hapten used for primary immunization.

The stimulation in vitro with DNP-lysozyme of the formation of antibodies against the dinitrophenyl group by cells derived from animals immunized both with DNP-BSA and with lysozyme confirms previous findings (*1, 3, 4*) and is in agreement with the hypothesis of cell-to-cell interaction. Our observation on the stimulation with the protein carrier alone may be related to the "original antigenic sin" (*14*). The boosting effect of BSA would be, in this case, due to protein areas which share complete determinants with the dinitrophenyl hapten (*15*). On the other hand, the finding reported may mean that an initial stage in the antigen recognition by a cell may involve the intact immunogenic macromolecule, and thus allow an array of different specificities to be stimulated. The recognition at the level of an intact immunogen has been demonstrated also in connection with studies on the role of conformation and net electrical charge in antigenicity (*16*).

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Photosensitivity of the Circadian Rhythm and of Visual Receptors in Carotenoid-Depleted Drosophila

Abstract. Drosophila melanogaster *was raised on aseptic diets, with and without β-carotene. The sensitivity of visual receptors in the carotenoid-depleted flies was lowered 3 log units, but the photosensitivity of the circadian rhythm was not affected. This result suggests that the chromophore of the photopigment which mediates light effects on the circadian rhythm is not a carotenoid derivative.*

Circadian rhythms are affected by light in a variety of ways. They can be entrained to cycles of light intensity, initiated and phase-shifted by single light pulses, inhibited by constant bright light, and changed in free-running period by changes in light intensity (*1*). What is the location and nature of the photopigments that mediate these effects?

The precise location of the photopigment is not known for any organism, but evidence is accumulating that extraoptic sites are involved. Light impinging directly on the brain can entrain circadian rhythms in amphibians (*2*), reptiles (*3*), and birds (*4*); and an extraoptic site—probably the brain—is also implicated in insects (*5, 6*). However, there is evidence that in mammals the eyes might be the photoreceptor (*7*).

The nature of the photopigments that mediate light effects on circadian rhythms is also unknown. Action spectra have been determined for light effects on circadian rhythms in plants (*8*), protozoans (*9*), and insects (*10, 11*), but this approach has not greatly narrowed the choice of compounds.

Another method of determining the nature of the photopigment of insect circadian rhythms is reported here. It takes advantage of the fact that, in all of the chemically known visual systems, the chromophore of the photopigment (rhodopsin) is the carotenoid derivative retinaldehyde (vitamin A aldehyde). Carotenoids are synthesized only by plants, and carotenoid deficiency in insect diets results in impairment and loss of visual sensitivity (*12, 13*). If the chromophore of the photopigment of the circadian rhythm is a carotenoid derivative, we might expect the circadian rhythm of carotenoid-depleted insects to be less sensitive to light than normal. Here we report experiments in which *Drosophila melanogaster* was raised on aseptic diets with and without β-carotene and then tested for both photosensitivity of the compound eye and photosensitivity of the circadian rhythm of adult emergence.

The aseptic diet used was similar to that devised by Sang (*14*), except that β-carotene was added in some cases. The diet (125 ml) was placed in polypropylene flasks and autoclaved; sterile fructose was then added to all of the flasks, and β-carotene, dissolved in ethyl alcohol (0.02 mg/ml), was added to some of the flasks.

Drosophila eggs were collected and sterilized (*14*). They were rinsed with sterile distilled water and added (in