air dried, mounted face-up on microscope slides, and covered with AR-10 stripping film (*10*). After development, photographs were made by phase-contrast microscopy to correlate areas identified by bound antibody with areas of localization of H^3U incorporation.

Of 28 non-nucleate fragments surviving exposure to virus inoculum and subsequent manipulation, 15 bore foci of antigen (Table 1). Of 16 studied by both fluorescent antibody and autoradiographic methods, 11 contained antigen and of these, 7 also showed H^3U incorporation at the same sites (Fig. 1). All of the intact cells which contained antigen incorporated H^3U into cytoplasmic foci of virus growth.

In some experiments, non-nucleate cytoplasmic fragments bore clear-cut foci of antigen but appeared to be rather lightly labeled with H^3U (Fig. 1, *a* and *b*). This was probably due to the autoradiographs being underexposed, since such preparations also showed a minimum amount of labeled cytoplasm in clearly infected intact cells.

In some experiments yielding good autoradiographs, most non-nucleate fragments in which fluorescent antibody fixation occurred were also labeled at the same sites (Fig. 1, *c* and *d*). Those non-nucleate fragments which acquired no antigen were presumed to be uninfected just as some intact cells were, by the same evidence, not infected.

Foci of antigen formation were usually discrete in non-nucleate fragments but were diffuse in the intact cells of the same cultures. The discrete sites in non-nucleate fragments could represent regions in which new virus was synthesized more slowly than in intact cells. Infected cultures containing only intact cells were therefore examined with fluorescent antibody at 0, 2, 3, 4, and 5 hours after the initial period of contact with inoculum. No cells bore foci of antigen in less than 3 hours, but at 3 and 4 hours, many infected cells contained discrete cytoplasmic foci of antigen. These foci and the foci found at 5 hours in non-nucleate cytoplasmic fragments were morphologically alike in shape, size, and intensity of "staining" with fluorescent antibody. It is concluded that synthesis of new viral antigen occurred at a somewhat slower rate in non-nucleate cytoplasmic fragments than in whole cells.

The concurrence of poliovirus antigen and tritiated uridine incorporation in non-nucleate cytoplasmic fragments, under conditions producing results comparable with the process in intact cells, indicates that the poliovirus induced both specific antigen formation and new RNA synthesis in the non-nucleate cytoplasmic fragments and that the cell nucleus did not participate in this sequence of steps in the infectious process.

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Allotypic Specificities of A- and B-Chains of Rabbit Gamma Globulin

Abstract. *The extent to which the A-chain of γ-globulin reacted with antibody against an allotypic specificity controlled by the* b *locus was about that expected from the contamination of A-chain with B-chain; the* a *locus specificity was confined only to A-chain. Thus, data were consistent with complete separation of allotypic specificities,* a *locus with A-chain and* b *locus with B-chain.*

Allotypic specificities (*1*) of rabbit γ-globulin (that is, antigenic specificities each of which is found in some but not in all rabbits) were shown to fall into two genetic groups, *a*, having specificities Aa1, Aa2, Aa3, or *b*, having specificities Ab4, Ab5, Ab6 (*2*)—by the nomenclature of Dray *et al.* (*3*); loci *a* and *b* are not closely linked (*4*). It was observed in 1961 by Oudin (*5*) and confirmed since then (*6*) that specificities of each group *a* and *b* may be carried by a single γ-globulin molecule.

A model of rabbit γ-globulin has been proposed (*7*, *8*) containing two pairs of peptide chains, A and B in the nomenclature of Porter (*7*), which will be followed here or, respectively, H and L in the nomenclature of Edelman and Benacerraf (*9*). Specificities of the *a* and *b* groups have been looked for in A- and B-globulin fractions with the result that B-fraction had only *b* specificities, while A-fraction had specificities of both *a* and *b* groups (*10*). A later report (*11*) suggested that either *b* specificities were actually carried by both chains, or the amount of contamination of the A-fraction would have to be "remarkably great" to account for the heavy precipitate obtained when tested with antiserum of the *b* group. The present report concerns the quantitative measurement of the capacity of the A preparation to combine with antibody against a specificity of the *b* group (Ab4), with the object of finding if this capacity may be explained by a contamination. If rabbit allotypic specificities are due to genetically controlled primary structure variations, one would expect each type of chain to carry only one of the two unlinked locus specificities, as seems the case for human Gm and Inv (hereditary globulin factors) specificities (*12*).

A pool of rabbit serums with Aa1 and Ab4 specificities was precipitated with 1.75*M* ammonium sulfate, and the precipitate was dialyzed against phosphate buffer (0.025*M*, *p*H8) and passed through a diethylaminoethyl (DEAE) cellulose column. The effluent was concentrated by precipitation with 2*M* ammonium sulfate and the precipitate was dialyzed against 1*M*, *p*H8 tris buffer. Only γ-globulin (RGG) was found by starch-gel electrophoresis (*13*). The A- and B-chains were prepared according to Fleischman *et al.* (*14*) by reduction of γ-globulin with 0.2*M* mercaptoethanol followed by *S*-

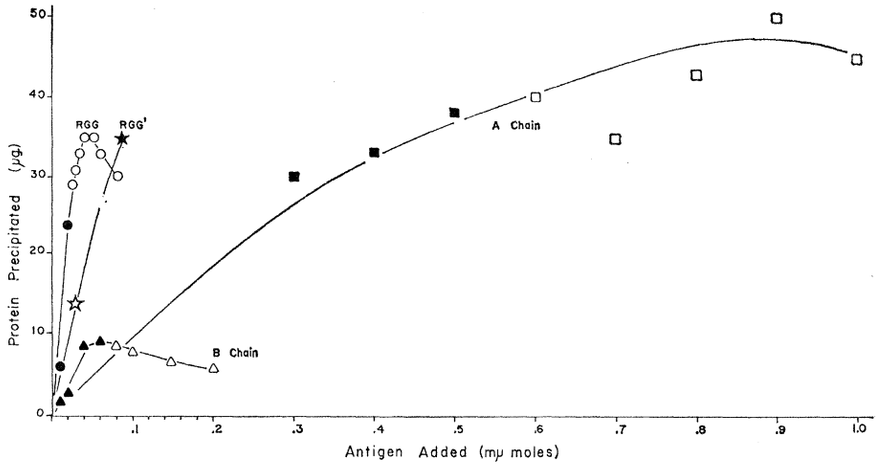

Fig. 1. Precipitin curves of Ab4 antiserum (0.05 ml) versus RGG (circles), RGG′ (stars), A-chain (squares) or B-chain (triangles). Solid symbols, excess Ab4 antibody in supernatants; clear symbols, no detectable excess antibody to Ab4 in supernatants.

alkylation with iodoacetamide. The globulin subunits were resolved by chromatography on Sephadex G-100 in 1*N* propionic acid. Results comparable to those of Fleischman *et al.* (*18*) were obtained, although use of Sephadex G-100 rather than Sephadex G-75 resolved the A-fraction peak into two components, called here A′ and A″. Fractions corresponding to each peak were pooled and dialyzed—first exhaustively against distilled water, then against a mixture of saline (0.85 percent NaCl) and tris (*p*H 8, 0.05*M*). Material of the first peak, A′, was partially precipitated during the final dialysis; from 25 percent to 50 percent of the A′ material (about 20 percent to 35 percent of the total A material) was lost. The A″- and B-fractions remained clear. Preliminary experiments indicated that A′ and A″ were equivalent for the tests to be reported here. Probably the only difference between A′ and A″ was the degree of association in propionic acid (*14*). For the work which follows, a pool of the material of the two peaks A′ and A″ was used as A-chain.

A portion of the reaction mixture (in 1*N* propionic acid) was not passed through the column but was dialyzed directly against water and saline-tris. The material maintained solubility and will be referred to subsequently as RGG′.

Protein concentrations were estimated from the optical density at 280 mμ. For calculations of molar concentrations, the molecular weight of both RGG and RGG′ was assumed to be 150,000, that of the A-chain as 55,000 and of the B-chain to be 20,000. For the tests described, with antiserum to Ab4, material was used after several weeks of aging at 4°C at which time the B-chain reached maximum activity which was then maintained for at least 1 month. With aging the reactions of RGG, RGG′ or A-chain with antiserum to Ab4 did not change. Neither during the aging period nor during the succeeding month was there material lost from any of the fractions by spontaneous precipitation, although the A-material was opalescent and remained so.

The B-fraction gave no visible precipitation in the ring test with antiserum to Aa1, in contrast to RGG, RGG′ and A-fraction. Tested with Ab4 antiserum, A- and B-fractions gave positive reactions, as observed by others (*10*, *11*), the smallest reacting concentrations of the B-fraction being smaller than that of the A-fraction. To obtain more precise quantitative data, 0.05-ml samples of antiserum to Ab4 were mixed with increasing amounts of A-fraction, B-fraction, RGG or RGG′, with the total volume 0.5 ml for each mixture. After 1 hour incubation at 37°C and 2 or 3 days at 4°C, precipitates were collected by centrifugation and washed twice with cold saline. The protein in the precipitate was estimated by the Folin method, with RGG as the protein standard (Fig. 1). All the supernatants were tested by ring test against RGG for antibody excess (solid symbols in Fig. 1). The reaction for antigen excess of the supernatants with antiserum gave less clear results because the antigen dilution was too close to the end point in the ring test, although limited results indicated that the equivalence zones were rather wide. The B-fraction gave smaller amounts of percipitation, probably because of the smaller molecular size and the formation of more soluble complexes. With the A-material preparation, the maximum of the curve was not clearly reached with the highest amount of antigen used; the largest amount of precipitate obtained exceeded the maximum of the RGG curve, probably owing to the aggregated nature of the A material.

In order to compare the capacity of the various fractions to combine with antibody to Ab4, the reciprocal of the least amount (in micromoles) of antigen required to leave the antibody excess region of the precipitation curve was divided by the reciprocal of the least amount (in μmole) of RGG which produced the same effect. The average values obtained with two sets of preparations (one from pooled serum RGG, the other from RGG of a single Aa1,b4 serum) were the following: RGG, 1 (by definition); RGG′, 0.6; two A-chains, 0.1; two B-chains, 0.6.

Two B-chains could thus account for most of the capacity of the whole molecule to combine with antiserum to Ab4. The capacity of the A-fraction to combine with the same serum, on the other hand, was that expected on the basis of a contamination of the order of one or two B-chains for each ten A-chains. The data of Fleischman *et al.* (*15*) from assays of terminal alanine of A-chain and B-chain suggested that one A-chain in ten might still carry a B-chain. The contaminating B-chains had to be associated with large molecules or aggregates to account for the large amounts of specific precipitate with antiserum to Ab4; it has been reported that even free B-chains reaggregate with A-chains upon neutralization (*16*).

These findings are consistent with the hypothesis of a complete separation of allotypic determinants, those responsible for the specificities of the *a* group being on A-chain and those for the *b* group on the B-chain. If there were in the Ab4 pattern two kinds of determinants, one carried by A-chain and one by B-chain, one might expect that each single chain would not completely inhibit the precipitation of antibodies to Ab4 with whole RGG, contrary to the observed result. Furthermore, in the one case tried, the end point of antiserum excess was identical with A-chain or B-chain as supernatant test antigen in place of RGG.

While it would be desirable to prepare A material completely free of B-chain, either by more drastic reduction, or by repeating the reduction of the isolated material, technical difficulties (the poor solubility properties of A-chain) now prevent this last step.

If Ab4 determinants reside only with the B-chain, one is nearly forced to accept the earlier stated assumption that the allotypic specificities (at least of the *b* locus) are due to primary structure changes, since B-chains have less than one carbohydrate molecule (hexose or hexosamine) per protein chain (*14*).

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Daily Sensitivity Rhythm of the Two-Spotted Spider Mite, Tetranychus urticae, to DDVP

Abstract. *Adult females of the two-spotted spider mite* (Tetranychus urticae *Koch), showed a daily rhythm of sensitivity to DDVP (dimethyl 2,2-dichlorovinyl phosphate). When the mites were maintained in alternating light-dark conditions, maximum susceptibility to the chemical occurred 2 hours after dawn and the mites were least sensitive 2 hours after nightfall.*

Although the information on daily rhythms of sensitivity to biologically active chemicals is still meager, the finding of such rhythms has emphasized the basic importance of circadian organization and provides an additional technique for studying it in organisms too small for the usual assays of locomotory activity. Halberg and his coworkers (*1–4*) have found that mice show daily rhythms in their response to a wide variety of chemicals, such as ethanol (*1*), ouabaine (*2*), *Escherichia coli* endotoxin (*3*), and Su-4885, an adrenocortical inhibitor (*4*). Insects and other arthropods are being used extensively in the study of circadian rhythms, and a great deal of attention has been devoted to sensitivity to chemicals, especially insecticides, in this group of animals. Beck (*5*) found that the German cockroach showed a marked rhythm of sensitivity to cyanide, which was phase-related to the daily respiratory rhythm, and also noted daily fluctuations of sensitivity to DDT and Dimetelon. The first finding of a circadian phenomenon in the two-spotted spider mite was of daily rhythms of sensitivity to ether, chloroform, and carbon tetrachloride (*6*). The experiments described herein show that sensitivity in the two-spotted spider mite to DDVP, a commercially used acaricide, also manifests a pronounced daily rhythm.

The mites used were of the Blauvelt strain, whose resistance to acaricides is known to be stable (*7*). They were reared on Fordhook lima bean plants and maintained in a modified refrigerator (*8*) at 25°C (± 2°C) and a relative humidity of about 70 percent. Fresh plants were introduced into the culture every other day between 7 p.m. and 9 p.m. Light was provided by two 20-watt white fluorescent bulbs set about 20 cm above the tops of the plants and connected to a time switch that maintained a light-dark cycle of 14 : 10 (light from 6 p.m. to 8 a.m.; dark from 8 a.m. to 6 p.m.).

Preliminary experiments on the fumigant action of DDVP indicated a daily fluctuation in sensitivity of the mites to the toxicant. We found that more toxicant was required to kill 50 percent of the mites (LD_{50}) during the first hours of the subjective day than around nightfall. We have not, however, found it practical to utilize its fumigant action in a detailed study of the effectiveness of DDVP at different times of day because (i) there are difficulties in determining the exact concentration of the vapor, and (ii) the number of tests necessary to establish an LD_{50} cannot easily be performed simultaneously. Staggering the tests would defeat the point of the study, which was to measure sensitivity at given points in the light cycle. Therefore, we utilized a method in which a single dose of DDVP could be used and equality of treatment in successive tests could be guaranteed.

The method consists of placing adult females on microscope slides (*9*) which are then dipped in a 0.005 percent emulsion of DDVP for 5 seconds. At each treatment time four groups of 25 mites each were tested. The operations of placing the mites on the slides and dipping the preparation in the acaricide for 5 seconds were carried out in dim white light at 25°C. Immediately after treatment the slides were returned to the refrigerator. The resultant mortality was determined 24 hours later. In all, at least 16 groups (each of 25 mites) were tested at each of 12 different points in the cycle, though not more than four of the treatment times could be tested during any one 24-hour cycle.

The results of these experiments are summarized in Fig. 1. The plotted points are the mean mortality figures expressed as percentages of the treated samples. The 95 percent confidence limits are shown. A marked daily rhythm of sensitivity can be seen, with a distinct peak of maximum susceptibility 2 hours after dawn, which falls gradually during the day to reach a

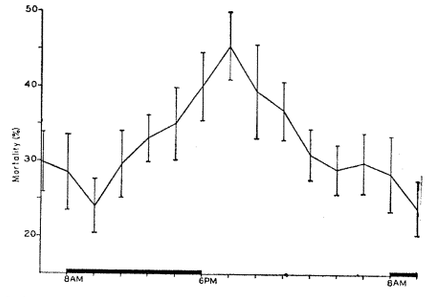

Fig. 1. Percent mortality at different times of day. (The 95 percent confidence limits for each of the test hours are indicated.)