This study of human, mouse, and hamster tissues, both in vivo and in vitro, demonstrated that normal adult, neoplastic, and embryonic hamster tissues have a cellular type of resistance to colchicine that is at least 100 times greater than that of human cells. This degree of resistance for hamster cells in vitro was similar to that previously reported in in vivo toxicity experiments in which the hamster was compared with other mammals (*2*).

The existence of a cellular resistance to colchicine in the hamster will make possible investigation of the effects of high and long-continued doses of colchicine on heterotransplanted tissues, embryonic or neoplastic, with no toxic host reactions. This resistance is an example of a genetically transmitted tolerance, and, if gene-controlled, could be used as a marker in transformation studies upon mammalian cells in vitro (*9*).

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Adsorption of Antibody in vitro and Magnitude of the Schultz-Dale Reaction of Guinea-Pig Ileum

Abstract. Studies show that the adsorption of rabbit antiovalbumin-I^{131} on guinea-pig ileum conforms to a Langmuirian isotherm, and that the physiological response to challenge with antigen depends upon the amount of antibody bound to the tissue.

Recently, Germuth and McKinnon (*1*) showed that antigen-antibody complexes, formed in antigen excess, would produce gross anaphylaxis of varying degrees of severity in the normal guinea pig, and Trapani, Garvey, and Campbell (*2*) demonstrated a quantitative relationship between the antigen-antibody ratio of the complex and the degree of the Schultz-Dale response.

This report concerns the quantitative relationship between the antibody content of the tissue and the magnitude of the specific response. Since active immunization, or even passive transfer of antibodies, could not be expected to provide a reproducible degree of immunological sensitivity, a method of sensitizing tissues in vitro was investigated (*3*). Although sensitization of isolated tissues by exposure to crude antisera has been reported (*4*) we do not know of any studies in which an attempt was made to detect whether antibody was actually adsorbed on the tissue, or of data giving a quantitative relationship between the concentration of antibody bound to the tissue and the magnitude of the Schultz-Dale response.

The present report (*5*) shows that the I^{131}-labeled gamma-globulin fraction of rabbit antiovalbumin can be adsorbed by guinea-pig ileum in proportion to the protein concentration in the *bulk phase* and that the response of the muscle to antigen is a function of the amount of antibody remaining on the tissue.

The antigen used in these studies was four-times-crystallized ovalbumin prepared by the method of Keckwick and Cannan (*6*). The antiserum was prepared by injecting rabbits with ovalbumin for 6 weeks, in accordance with an injection schedule not yet published (*7*). Animals were bled by cardiac puncture, the serum was separated, and the gamma globulin was prepared by reprecipitating the antiserum four times in the presence of ⅓-saturated ammonium sulfate. The preparation was dialyzed against repeatedly changed 1-percent NaCl and dried from the frozen state. The antibody content of the soluble protein was 32.5 percent, as determined by the quantitative precipitin technique; hence, it is likely that the preparation contained other antibodies as well as normal globulins. Antiovalbumin-I^{131} was prepared from a portion of this material by the method of Hughes and Straessle (*8*). The material employed in the studies discussed in section 1, below, contained 0.01 mole of iodine per mole of gamma globulin and had a specific activity of 0.35 μc/mg gamma globulin.

1) To determine the effect of I^{131} gamma globulin concentration and washing upon the residual radioactivity of the tissue, ileal strips measuring 2.5 cm in length when relaxed were prepared from intestines of normal male guinea pigs (350–400 g) according to the method of Feigen and Campbell (*9*). These were immersed in muscle baths, constructed from sintered glass filters, having a capacity of 30 ml, each

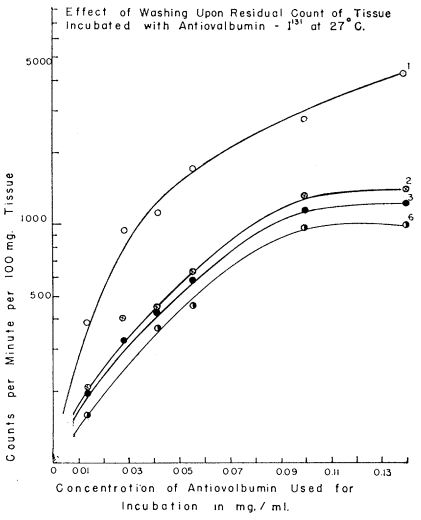

Fig. 1. Residual radioactivity on strips of normal-guinea-pig ileum incubated in various concentrations of an I^{131}-labeled gamma-globulin fraction of rabbit antiovalbumin. Curves 1, 2, 3, and 6 represent residual radioactivity after the first, second, third, and sixth washings.

containing a different concentration of I^{131}-labeled gamma globulin, ranging from 0.014 to 0.140 mg/ml. All tissues were incubated in 30 ml of Tyrode solution for 60 minutes at 27°C and then washed with 30-ml portions of fresh Tyrode solution at 27°C. The radioactivity was measured in a well-type crystal (NaI) scintillation counter. The counting efficiency with an I^{131} standard was 33.3 percent. The tissue was mounted on a glass rod and was immersed in a tube contained in the well of the scintillation counter. The radioactivity of the system was measured in the presence and in the absence of tissue, and the residual radioactivity on the tissue was obtained from the difference in the measurements. The washing and counting operations were carried out for six complete washing cycles. The results, presented in Fig. 1, are mean values for two strips tested at each concentration.

Since, as is shown in Fig. 1, the residual radioactivity of a tissue appears to bear a constant relationship to the concentration of I^{131}-labeled gamma globulin used in the original incubation, and since the radioactivity is not greatly reduced between the second and sixth washings, it appeared possible to test the data of curve 3 for conformance to the Langmuir adsorption isotherm. A convenient form of Langmuir's equation is

$$C/a = (1/\alpha\beta) + (C/\beta) \qquad (1)$$

in which C is the concentration of antiovalbumin in milligrams per milliliter, a is the amount (in milligrams) of adsorbate taken up by 100 mg of tissue, and α and β are constants. Accord-

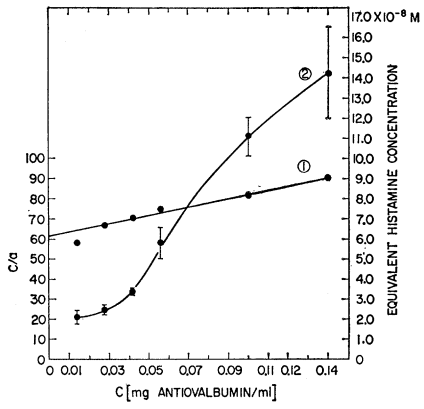

Fig. 2. Curve 1 (left ordinate) is a plot of C/a against C in which C is the concentration of antiovalbumin in the bulk phase and a is the amount (in milligrams) of antiovalbumin remaining per 100 mg of tissue after the third washing. Curve 2 (right ordinate) is a plot of the physiological response to 2 mg of antigen of tissues that had been incubated for 1 hour in antiovalbumin and washed three times before testing. The equivalent histamine concentration, a measure of the physiological response, is obtained graphically from histamine standardization curves carried out for each muscle strip. The vertical bars indicate standard errors of the respective means.

ing to this relationship, a plot of C/a against C should give a straight line having a slope of $1/\beta$ and an intercept of $1/\alpha\beta$. Curve 1 of Fig. 2 shows that the data, in the range between 0.028 and 0.140 mg of I^{131}-labeled gamma globulin per milliliter are adequately described by a line with a slope of 206.7 and an intercept of 61.5, from which the values of β and α are found to be 0.0483 and 3.35, respectively.

2) To determine the dependence of Schultz-Dale response on concentration of antibody used for incubation, tissues were incubated at 27°C for 60 minutes in nonradioactive antiovalbumin gamma globulin, the same range of concentrations being employed as in part 1. These tissues were washed three times at 27°C and then equilibrated at 37°C for 30 minutes before they were challenged with antigen in the Schultz-Dale test. Frontal writing levers were used to obtain kymographic records of muscular response. Each muscle was titrated with at least three doses of histamine before and after challenge with 2 mg of ovalbumin, the second histamine titration being carried out in a dosage range which was closely equivalent to the muscular response to antigen. The histamine equivalent of the specific contraction was obtained by interpolation from the histamine calibration curve of the particular strip of gut.

Curve 2 of Fig. 2 illustrates the dose-response characteristics of challenged muscle strips that had been incubated in the same concentrations of the antiovalbumin preparation that had been used in the experiments with the radioiodinated gamma globulin. Each point is the average of 11 to 17 values, the modal number being 16. As can be seen from the magnitudes of the standard errors, the evaluation of response at the higher protein concentrations is subject to a great deal of error because the sigmoidal dose-response curve with respect to histamine tends to flatten out at higher doses, making interpolation uncertain at near-maximal responses to antigen; for this reason incubation experiments were not made in the range beyond 0.14 mg of antiovalbumin per milliliter. Muscles could not be sensitized in concentrations of antibody lower than 0.014 mg of antiovalbumin per milliliter at 27°C.

Taking 0.014 mg/ml as the limiting value for sensitization, we can, on the basis of certain assumptions, make an approximate calculation of the amount of antibody necessary to set off a minimal reaction. Since preliminary experiments showed that there is no difference in the binding properties of normal and immune radioiodinated gamma globulin, we can assume that the binding of antibody is not preferential—hence, that the amount bound will be determined by the proportion of antibody in the total protein preparation, in this case 32.5 percent. The amount of total protein taken up by 100 mg of tissue from a concentration of 0.014 mg/ml is found to be 2.4×10^{-4} mg; from this finding, a value of 7.8×10^{-5} mg of antibody per 100 mg of tissue is obtained as the minimal ratio for discharge of the Schultz-Dale reaction.

The studies discussed in this report have shown that rabbit antiovalbumin can be bound to the serosal surface of guinea-pig ileum in proportion to the concentration of the antiovalbumin in the bulk phase. Since the relationship conforms to a Langmuirian isotherm, we can infer that these molecules form a monolayer; this does not imply that the combining sites are limited to the cell surfaces or to the superficial layer of cells. The reaction to antigen is also dependent on the amount of antibody bound; whether or not this reaction involves the formation of toxic complexes cannot be determined from the experiments discussed here.

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Variations in Style Number and Other Gynoecial Structures of Lychnis alba

Abstract. Consistent variations in style number (from the typical five), ranging from zero to ten, inclusive, are found in ovaries of *Lychnis alba* flowers. The number of carpels, placentae, and vascular strands of a given ovary usually varied directly with the number of its styles.

Ovaries of the pistillate flowers of *Lychnis alba* Mill. typically bear five styles. However, some descriptions read: "Styles 5 rarely 4" (*1*); "Styles 5 occasionally 4 or rarely 3" (*2*); "Styli 5, rarius 4, v. hinc inde 3" (*3*). Westergaard (*4*), Warmke and Blakeslee (*5*), and Warmke (*6*) published illustrations of artificially induced polyploid hermaphrodite and other flowers of *Melandrium* (*Lychnis*) with ovaries bearing differing numbers of styles but did not comment upon them.

Earlier I had found ovaries of *Lychnis alba* with two, three, four, six, and seven styles. These variations appeared so frequently and consistently that I initiated a statistical survey in 1958 to determine what variations in the number of styles actually occurred in the species. The preliminary results are described in this report.

Flowers were collected from plants (i) in a greenhouse; (ii) near Iowa City and West Liberty, Iowa; and (iii) near Bemidji, Minn. Thirty daily collections were made during May and June 1958 from greenhouse plants (*7*); 29 spaced, individual collections were made outdoors in Iowa, and 31 were made in Minnesota. All pistillate flowers were taken from every plant in every plot studied. Collections were total, and there were no special selections from any plant or plot suspected of producing, or known to produce, ovaries with differing numbers of styles. Collections in Minnesota clover-alfalfa fields covering several