Fig. 1, with 0.05 ml veronal buffer between opposite parts and with enough free horizontal space between adjacent plates to prevent interference. The thickness of the liquid barrier was in all instances about 0.1 mm.

This pile was left for 10 min in a wet chamber. The plates were taken apart rapidly and carefully washed with distilled water and measured after drying. The figures inserted in the diagram indicate the respective loss or gain in A on each treated part. The experiment shows clearly that plate *A* lost material equally on both locations, that plate *C* lost material only on the part opposite the trypsin plate *A*, and that plate *D* adsorbed some material on the part opposite plate *A*. On the other hand, plate *B*, which had the thicker Formvar screen, did not lose a measurable amount of material, and the part of plate *D* opposite *B* is also unchanged. On that part of plate *E*, however, which was opposite plate *B* there is a slight loss of material. There was no change on the two parts of plates *C* and *E* over which cover glasses had been placed. The only plausible explanation for these results is this: Trypsin molecules were able to pass through the thinner Formvar screen on plate *A* and diffused through the buffer solution toward plates *C* and *D*. On plate *C* they penetrated the single Formvar screen and split part of the substrate underneath, which in turn diffused through the screen back into the solution and was removed by washing. On plate *D*, however, the trypsin has been adsorbed. On the other hand, the thicker Formvar screen on plate *B* prevented diffusion of trypsin almost completely, so that no adsorption could be detected on the control plate *D*; but the slight loss on plate *E* indicates that the 4 layers of Formvar still permitted some trypsin molecules to reach the substrate layers on this plate. The cover-slip controls on plates *C* and *E* show that the veronal buffer itself does not remove substrate from underneath the screen. This experiment has been performed several times with essentially the same results.

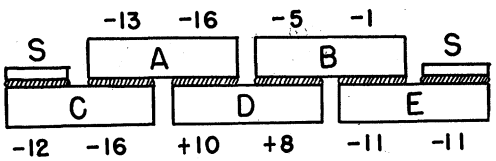

FIG. 2. Action of phosphate buffer.

If the same experiment is done with distilled water instead of veronal buffer, we do not find changes on any of the plates exceeding 2 A–3 A. The standard deviation for different boundary lines was between ± 0.5 A and ± 1.5 A in this set of experiments.

In another experiment, an identical set of plates, as described in Fig. 1, was used. The thickness of adsorbed trypsin on plates *A* and *B* was 28 A. The Formvar film on plate *A* was 46 A thick (2 layers) and on plate *B*, 75 A thick (4 layers). The 3 bovine serum albumin double layers on plates *C* and *E* were 48 A, and the single Formvar layers on top, 16 A in thickness, respectively.

Phosphate buffer was used instead of veronal buffer. After a 10-min period in the wet chamber the plates were rinsed once with veronal buffer and twice with distilled water.

As can be seen from Fig. 2, there is a combined effect of the phosphate buffer on the substrate, as well as on the enzyme. The leaching action on the bovine serum albumin of phosphate buffer through Formvar films, which has been reported previously (*4*), is demonstrated here again. But, furthermore, we find that trypsin is removed through a Formvar film by phosphate buffer. The phosphate action is even stronger than the veronal action, as a comparison of Fig. 1 and Fig. 2 shows.

The difference in loss on plates *C* and *E* can be explained by a double action of trypsin activity and phosphate leaching on plate *C*. The interesting fact that the 2 trypsin plate parts that are opposite plate *D* show a larger loss than the 2 parts opposite the substrate plates (*C* and *E*) can be explained by assuming that the substrate is removed relatively rapidly through the thin Formvar film on plates *C* and *E* and is adsorbed to a certain extent on the opposite parts of plates *A* and *B*. Either this adsorbate interferes somewhat with the diffusion of the trypsin, or it is still present after washing the plate at the end of the experiment. Also, a combination of these two effects seems to be possible and would account for the observed differences.

A detailed discussion of these experiments will be published elsewhere.

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On the Interaction of Protein Films

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In view of two recent notes by H. J. Trurnit (*4*, *5*) on the interaction of protein films, it seems appropriate to state clearly the point of view held in this laboratory.

In previous publications (*1*, *2*), we have shown that thin blankets of barium stearate or of plastic material, coating antigenic layers deposited on metallic slides, do not prevent a specific adsorption of homologous antibodies and, further, that certain enzymes, which would inactivate the adsorbed substrate layers, are capable of inactivating them also in spite of intervening blankets.

In the first of the above-mentioned notes, Trurnit concluded that the observed interaction between antigen and antibody through a blanket can be explained by a simple diffusion process of the antigenic layers. This explanation was based on the fact that the ions of a phosphate buffer are capable of leaching out, through a relatively thin Formvar blanket, a fair amount of bovine albumin

adsorbed as multilayers. Singer (*3*) also had previously concluded that the observed interaction resulted from a simple diffusion process of either the antibody or the antigen or both.

In the second note, appearing on page 329 of this issue, Trurnit postulates that the inactivation by trypsin of adsorbed layers of bovine albumin results from the diffusion of the trypsin molecules through the blanket.

The fundamental point involved is to decide whether a long-range interaction does take place in the observed phenomena. If it can be shown that under the experimental conditions a simple diffusion process is inadequate to explain all the facts now available, *then a long-range action of some kind must be assumed.* Whether the interacting entities eventually come into closer proximity is a secondary issue, if ordinary diffusion forces are insufficient to bring about the closer proximity.

There is a wealth of experimental evidence that seems to be definitely against the simple diffusion of antigenic layers, antibody, or enzyme molecules through a blanket. For instance, a specific adsorption of homologous antibody is still observed to occur through at least 100 A of barium stearate or Formvar when the antiserum is diluted in veronal instead of phosphate buffer. Veronal buffer has no leaching effect whatever by itself. The thickness of antibody that can be adsorbed by slides coated with 1, 2, or 3 double layers of bovine albumin amounts to about 40 A per double layer after a 3-min adsorption period, and is independent of pretreatment of the slides by the veronal buffer alone. The leaching effect by phosphate buffer, which was observed independently by Trurnit, does not occur when 1 double layer of bovine albumin is deposited on the slides; nevertheless, specific adsorption can take place between the double layer of bovine albumin and antibody despite intervening blankets.

Multilayers of bovine albumin can be partially inactivated, as far as their reaction with homologous antibody is concerned, by bombardment with α-particles. A system of 6 such monolayers partially inactivated can still adsorb a layer 80 A thick of homologous antibodies. However, a blanket of Formvar 80 A thick deposited on the layers before or after irradiation prevents any adsorption of antibody, whereas the same blanket would permit considerable specific adsorption if the layers had not been bombarded. These experiments demonstrate that these antibody molecules definitely do not go through by simple diffusion.

The minimum thickness of a blanket necessary to prevent the inactivation of bovine albumin layers by trypsin is a function of the number and the mode of deposition of the layers. The greater the number of layers, the thicker must be the blanket. A Formvar blanket 20 A thick is sufficient to prevent the inactivation by trypsin of 1 deposited monolayer of bovine albumin, whereas more than 600 A of Formvar is necessary when there are 6 underlying monolayers of bovine albumin. Moreover, deposited multilayers of bovine albumin are partially inactivated by heating at 105° C for 10 min. Six "up" layers after such a heat treatment adsorb specifically a layer 100 A thick of antibody, instead of the usual 180 A. Trypsin directly applied on the heated antigenic layers destroys their immunological property just as fast as if they had not been heated, but a blanket of Formvar 130 A thick protects them completely from trypsin. Such a thickness of Formvar would permit complete inactivation of nonheated layers. Finally, the pH of the water upon which the monolayers are formed before transfer on the slide has a considerable effect on the thickness of the blanket necessary to protect them against trypsin. Five monolayers transferred from an "old" water can be inactivated through a screen of Formvar 400 A thick, whereas, if the water has been freshly redistilled, a Formvar blanket of 200 A protects them completely.

In all these cases the "physical state" of the blanket is the same, and, therefore, in none of them can one assume that trypsin goes through by simple diffusion. Trurnit's contention that his control experiments are better than ours is erroneous, because he fails to take into consideration the series of experiments just mentioned.

All these facts clearly show that it is unjustified to envisage the permeability of a blanket as such, either to antigenic layers or trypsin molecules. As we said two years ago (*2*), "If the enzyme molecules do actually diffuse through the blanket, they must then diffuse faster or slower depending on the mode of deposition and number of the antigenic layers underneath, a process which in itself would involve a long-range action." The comparison of a blanket to a sieve with definite-size holes is a misconception; the passage of a molecule of a certain size depends not only on the size of the holes but on the intensity of the fields of force acting through the screen on the molecule.

Finally, the evidence brought forward by Trurnit in his second note, that trypsin molecules go through the blanket by a simple diffusion process, is unconvincing for the following reasons. At no time did Trurnit test his antigenic films with homologous antibody to find out whether the films had lost their immunological property. He is satisfied to consider a small decrease in the thickness of his deposited layers as an indication of trypsin action. Our experience of many years has shown us that, quite often, a small decrease in the apparent total thickness of antigenic layers can occur without being accompanied by a corresponding loss in the amount of antibody that can be subsequently adsorbed; in other words, without loss of immunological reactivity. Consequently, we must regard the few observations offered by Trurnit as insufficient evidence in favor of simple diffusion being the explanation of our observations.

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