lent types I and II antipneumococcus horse serum, obtained through the courtesy of the Lederle Laboratories, Inc. Over 80 per cent. specifically precipitable antibody of type I was isolated by successive precipitations with SSS I in the antibody excess region, and subsequent dissociation of the specific precipitate with 15 per cent. NaCl solution.[5,6] Guanidine hydrochloride, whose action on normal horse serum globulin, GI, has previously been studied,[7] was used as a denaturing agent.

Irreversibly denatured and regenerated antibody were prepared by treatment of a 2 per cent. solution of the native antibody with 8 M guanidine hydrochloride, removal of the denaturing agent by dialysis and separation of the irreversibly denatured fraction by isoelectric precipitation.[7] The regenerated protein, remaining in the supernatant solution, was readily soluble in water and in physiological saline; the highly insoluble irreversibly denatured protein could be dissolved in 2 per cent. NaCNS in saline, to the extent of about 0.3 mg of protein N per ml, at pH 6.5.

After it was found that all fractions were specifically precipitable by the homologous antigen (SSS I), quantitative precipitin titrations were performed, using the method of Heidelberger and Kendall.[8] Comparison of the serological activity of native and regenerated antibody was made in physiological saline solutions, that of native and irreversibly denatured antibody in 0.9 per cent. solutions of NaCl containing also 2 per cent. NaCNS. Representative data relating to the equivalent combining ratios of mg antibody N to mg antigen, R, and to the per cent. of specifically precipitable antibody nitrogen, N, are given in Table 1.

TABLE 1

| Preparation | Solvent | R | Per cent. antibody N specifically precipitable |
|---|---|---|---|
| Native antibody .. | 0.9 per cent. NaCl | 3.3 | 80 |
| Native antibody .. | 0.9 per cent. NaCl + 2 per cent. NaCNS | 2.9 | 37 |
| Irreversibly denatured antibody . | 0.9 per cent. NaCl + 2 per cent. NaCNS | 1.3 | 50–60 |
| Regenerated antibody ..... | 0.9 per cent. NaCl | 6.0 | 70 |

The data reveal no significant difference in the equivalent combining ratio of native antibody in saline and in the presence of NaCNS. When the supernatant of the specific precipitate, obtained at the equivalence point in the presence of NaCNS, was dialyzed against saline, no additional precipitation occurred unless more antigen was added, indicating that NaCNS had shifted the equilibrium between antigen and antibody rather than inhibited the precipitation of the antigen-antibody complex.

The difference in combining ratio between native, irreversibly denatured and regenerated antibody may be ascribed either to a change in effective antibody valence or else to changes in their molecular dimensions, as they have been found to occur when normal horse serum globulin GI is treated with concentrated solutions of guanidine hydrochloride. Molecular weight studies on these fractions are under way.

It is of considerable significance that the denatured and regenerated antibody were serologically active, and the general theoretical and practical aspects of this finding will be discussed elsewhere.

The fact that the regenerated antibody is nearly as fully precipitable by the homologous antigen (70 per cent.) as the native (80 per cent.), and the irreversibly denatured material even more so, when measured under comparable external conditions (50 to 60 per cent. as compared to 37 per cent.), suggests that, contrary to Pauling's hypothesis,[9] the difference between antibody globulin and normal globulin is not merely one of steric arrangement but probably one of amino acid composition. If denaturation of normal serum globulin, followed by regeneration in the presence of an antigen, should give rise to a fraction of a material possessing antibody activity,[10] then, conversely, regeneration of a denatured antibody in the absence of the specific antigen should, in keeping with that hypothesis, yield a material essentially devoid of serological activity. This, however, is not the case.

It remains to be seen whether the denatured and regenerated antibodies are effective in animal protection; requisite experiments are under way. Experiments on the relative antigenic activities of these fractions and on their antigenic relation to native and denatured normal serum globulins will be reported elsewhere.

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## PROTECTIVE EFFECT OF SEPARATE INOCULATION OF SPOTTED FEVER VIRUS AND IMMUNE SERUM BY INTRADERMAL ROUTE

THE purpose of the present work is to investigate the possibility of protecting a susceptible animal against spotted fever by using minute doses of specific

rabbit hyper-immune serum. The immune serum was produced by repeated inoculations of rabbits with both spotted fever vaccine and the virus.[1] The intradermal route of inoculating guinea pigs with virus and serum was used.

Our study is based on the known phenomenon in which the infiltration of the skin in susceptible animals with specific immune serum affords protection against subsequent inoculation with the homologous virus of vaccinia.[2, 3, 4]

Three main factors were considered: the site of inoculation, the time factor and the amount of immune serum.

An area of ¼ square inch of guinea pigs' skin was infiltrated first with 0.4 cc of immune serum, and then two hours later 0.1 cc of virus was injected into this area. These animals remained afebrile and proved immune to subsequent reinfection with spotted fever.

Fifteen guinea pigs were then treated with 0.1 cc of virus and 0.4 cc immune serum simultaneously at the same site. No fever was noted in eleven of them, while four developed spotted fever. Only three of the non-reacting animals showed solid immunity to subsequent reinfection with spotted fever. Of six other guinea pigs injected with virus and serum as above but at *different sites* of the body, five developed typical spotted fever.

In the next series of thirteen guinea pigs smaller amounts of immune serum were used: 0.1 cc, 0.05 cc and 0.025 cc, the serum and 0.1 cc of virus given simultaneously and at the same site. Of these none developed spotted fever. Four weeks later they were all reinfected with a five-fold dose of virus and nine animals showed complete immunity in striking contrast with the control animals.

Inactivation of the virus in the skin was followed by solid immunity even when 0.025 cc of immune serum was injected at the same site and as long as 18 hours following the virus injection. Experiments are now under way to find out the lapse of time subsequent to virus inoculation after which immune serum will still protect the animal.

It is hard to believe that the negligible amount of 0.025 cc of immune serum could afford any degree of passive immunity. We may presume, however, that the virus absorbed by the highly potent anti-serum in the tissue acted as a sensitized vaccine. It is also possible that this amount of serum was not able to neutralize the virus completely, leaving a fraction of the virus to escape. Nevertheless, this negligible quantity of free virus would contribute to the protection of guinea pigs against the reinfection and thus confer a solid immunity. This interpretation clarifies the apparently puzzling phenomenon in which some of the guinea pigs treated with the same dose of virus (0.1 cc) but with larger amounts of immune serum (0.4 cc) did not develop immunity. Under these circumstances the virus was evidently rendered completely innocuous and the antigenic properties altered by the excess serum.

Whatever the intrinsic nature of these phenomena may be, they strengthen the emphasis laid originally by Besredka[5] on the role of the skin in infection and immunity. Due to certain autonomy of the skin as an organ it is possible by intradermal introduction of an attenuated pathogenic agent (bacterial or viral) to create a local immunity which is a step toward the production of a general immunity. In our work a local interception of the virus was achieved in various degrees by minute doses of specific anti-serum. Thus far the interception of the virus is still possible after 18 hours, but further experiments are in progress.

It will be of interest to determine whether it is possible to prevent the disease by the above method when the original tick virus is used.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## OBSERVATIONS ON THE BIOLOGICAL VALUE OF A MIXTURE OF ESSENTIAL AMINO ACIDS[1]

IN a series of convincing experiments, Rose[2] established the conception, now generally accepted, that only nine amino acids are essential for the growth of the rat, but that a tenth, arginine, must be supplied to attain maximal growth. The technique employed by him consisted in eliminating amino acids one at a time from a dietary mixture containing both essential and non-essential amino acids. Impairment of growth occurred only when one or another of the ten "essential" acids was eliminated.

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