Pyridoxamine acts like histamine, while aliphatic diamines activate rather than inactivate the tissue-ATP-ase and ophio-ATP-ase.

By these results histamine and ATP, which have been connected with the mechanism of shock production, are brought in a close relationship.

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Failure to Produce Lesions or Auto-Antibodies in Rabbits by Injecting Tissue Extracts, Streptococci and Adjuvants.*

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Schwentker and Comploier[1] demonstrated the formation of complement fixing antibodies for homologous kidney tissue in rabbits which had been inoculated with a mixture of kidney tissue extract and staphylococcal or streptococcal toxin. Cavelti and Cavelti[2] subsequently reported that an antibody for kidney tissue could be demonstrated by the collodion particle-agglutination technic in rabbits and rats which were inoculated with a mixture of homologous tissue and Group A streptococci. The latter workers also reported that rats immunized in this fashion developed acute and chronic glomerulonephritis. Recently, Cavelti[3] described the development of antibody for homologous heart tissue in rats injected with heart extracts and streptococci, and also demonstrated the appearance of inflammatory myocardial lesions in these animals.

Kabat, Wolf and Bezer[4] and Morgan[5] have recently confirmed the earlier observation of Rivers and Schwentker[6] that the immunization of monkeys with extracts of homologous brain tissue leads to the formation of demyelinating lesions of the central nervous system which resemble those seen in multiple sclerosis. These lesions were brought about in greatly accelerated fashion by the use of the adjuvants described by Freund,[7] consisting of "Falba" or "Aquaphor", paraffin oil, and killed tubercle bacilli.

In the present study, the effect of inoculating rabbits with homologous heart and kidney tissue in combination with streptococci and Freund's adjuvants was studied.

Material and methods. Rabbits. Hybrid brown and grey male rabbits, weighing between 1.8 to 2.3 kg each, were used in all experiments.

Tissue Suspensions. Heart and kidney tissue were otained from normal rabbits. The organs were thoroughly perfused with physiological saline under sterile conditions before removal, and were either used immediately or stored whole in a CO_2 ice box. Antigens were prepared by grinding portions of each organ with abrasive, suspending in sufficient physiological saline to make a 10% suspension, and partially clearing by centrifugation for 5 minutes at 1500 R.P.M. Bacteriological cultures were made from samples of each suspension to ascertain sterility.

Streptococci. Several strains of beta hemo-

* This work was supported by a grant from the Life Insurance Medical Research Fund.

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2 Cavelti, P. A., and Cavelti, E. S., *Arch. Path.*, 1945, **40**, 158.

3 Cavelti, P. A., *Arch. Path.*, 1947, **44**, 1.

4 Kabat, E. A., Wolf, A., and Bezer, A. E., *J. Exp. Med.*, 1947, **85**, 117.

5 Morgan, I. M., *J. Exp. Med.*, 1947, **85**, 131.

6 Rivers, T. M., and Schwentker, F. F., *J. Exp. Med.*, 1935, **61**, 689.

7 Freund, J., and McDermott, K., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 548.

lytic streptococci were employed: a group C strain (H46A) obtained through the courtesy of Dr. O. D. Ratnoff, and 3 Group A strains (type 19) recovered from the throat cultures of patients with acute tonsilitis (one of whom also had rheumatic fever). Eighteen-hour cultures of these organisms were grown in trypticase-soy broth, after which the streptococci were killed by heating at 56°C for 45 minutes.

Adjuvants. Heat-killed, dried tubercle bacilli, obtained through the courtesy of Dr. Jules Freund, were suspended in paraffin oil to a concentration of 0.4 mg per cc. Three parts of this suspension were transferred slowly, with constant stirring, to 2 parts of melted "Falba". The resultant mixture was immediately combined with other reagents as indicated below.

Preparation of Antigen Mixtures. Equal volumes of tissue suspension and streptococcus culture were mixed together, and 2 volumes of this mixture were added, drop by drop, to 5 parts of the adjuvant, with constant stirring. In control experiments, sterile broth replaced the streptococcus culture. Microscopic examination of each final suspension was made in order to ascertain that uniform globules of small size had been obtained.

Inoculation of Animals. Each rabbit was given 4 injections of 1 cc each, in separate subcutaneous areas. The injections were repeated at weekly intervals for a total of 3 weeks.

Each rabbit was bled from the ear before the first injection, and at 2-week intervals following the final injection, and the serum was stored in the frozen state. Sample animals were sacrificed in each experiment at 2, 4, 6, 8, and 10 weeks following the final injection of antigen, and portions of heart, kidney and other organs were placed in 10% formalin for subsequent histological examination.

Complement Fixation Tests. Tissue antigens for the complement fixation test consisted of 5 or 10% suspensions of heart or kidney tissue in physiological saline, which were made up on the day of testing and clarified by centrifugation at 2000 R.P.M. for 10 minutes. In some experiments, modified antigens were prepared by subjecting the tissue suspensions to a) centrifugation at 12,000 R.P.M. for 30 minutes, b) heating at various temperatures between 45°C and 80°C, and c) exposure to various pH levels between 4.5 and 9.5. The standard complement fixation test was employed, using 0.2 cc of complement (two units), 0.2 cc of the antigen, 0.2 cc of the serum under test, and, after a period of two hours at 37°C, 0.6 cc of sensitized sheep cells consisting of 0.2 cc of rabbit amboceptor (two units) and 0.4 cc of 2% cells. Fixation of complement was interpreted as occurring in those tubes which showed no hemolysis after 30 minutes in a 37°C water-bath.

Collodion Particle Agglutination Test. Collodion particles were suspended in normal rabbit heart and kidney extracts by the method described by Cavelti.[8] The agglutination of particles in varying dilutions of serum was tested by the method of the same author.

Results. Approximately 350 rabbits have been subjected to immunization with homologous tissue suspensions in combination with streptococci and Freund's adjuvants. Group C beta hemolytic streptococci were employed in the antigens used for one third of this group, and Group A streptococci in the remainder. In addition, a smaller number of control animals were injected with tissue suspensions alone, or with the suspensions plus adjuvants.

Antibody Determinations. As has been shown by Kidd and Friedewald,[9] normal rabbit serum contains a substance which causes fixation of complement with tissue suspensions from various rabbit organs. This "antibody", which is readily inactivated by heating at 65°C for 30 minutes, was encountered in most of the sera employed in this study, in titers of 1:16 or 1:32. In no instance, however, was a significant rise in complement fixation titer encountered between the pre-inoculation sera and the sera obtained at any period following

[8] Cavelti, P. A., *J. Immunol.*, 1944, **49**, 365.

[9] Kidd, J. G., and Friedewald, W. F., *J. Exp. Med.*, 1942, **76**, 543.

inoculation, with any of the antigens employed. Heating the sera at 65°C resulted in the disappearance of complement-fixing antibody from all sera tested. Treatment of the tissue antigens by high-speed centrifugation, heating, or exposure to different pH levels did not result in any positive complement fixation reactions.

The collodion particle technic yielded results which were uniformly negative in all experiments. No evidence of an antibody for heart or kidney tissue was demonstrable in the sera of rabbits following immunization with any of the materials employed.

Pathological Studies. No significant cardiac or renal lesions were demonstrable in any of the rabbits which were injected with tissue suspensions, adjuvants and Group C streptococci. The results obtained with Group A streptococci were also negative, with the exception of a single experiment involving twenty-one rabbits. In this experiment, 3 animals received kidney tissue suspension in combination with Group A streptococci and Freund's adjuvants, 3 received the tissue suspension with streptococci but without adjuvants, 3 received the tissue suspension with the adjuvants but without streptococci, and 3 received the adjuvants and streptococci without tissue suspension; a similar group of rabbits were given a suspension of heart tissue in the same combinations. The animals were sacrificed at the end of the sixth week after inoculation. All of the rabbits which received either heart or kidney tissue in combination with streptococci and adjuvants showed extensive areas of acute inflammatory reaction and muscle fiber destruction involving the right ventricle. These lesions did not resemble the characteristic lesion of rheumatic myocarditis. Similar but less extensive lesions were seen in 2 of the rabbits which were given the mixture of kidney suspension and streptococci without adjuvants, and in 2 of the rabbits which received heart suspension and streptococci without adjuvants. No lesions occurred in the hearts of the rabbits receiving tissue suspensions with adjuvants alone, or in the group receiving streptococci and adjuvants without tissue suspensions. No significant kidney lesions were seen in any of the experimental animals.

Many attempts were made to repeat these observations, employing the same strain of streptococcus and, so far as possible, the same materials and experimental conditions. All of these attempts were unsuccessful. No myocardial lesions which were in any way similar to those observed in the 10 rabbits referred to above were encountered again. Occasional small areas of infiltration by round cells were encountered between muscle fibers, but these seen with equal frequency in the heart tissue of normal untreated rabbits.

No explanation can be given for the myocardial inflammatory lesions in the single experiment described above. Although bacteriological cultures of the heart tissue and blood of these animals were negative, and the injection of serum from these rabbits into normal animals failed to produce any evidence of disease, it is considered possible that the myocardial lesions may have been due to an irrelevant infection of these animals by an unknown virus or bacterium. In view of the repeatedly negative results which were obtained when the experiment was duplicated, the significance of the myocardial lesions is doubtful.

Summary. The inoculation of rabbits with suspensions of homologous kidney or heart tissue, in combination with heat-killed cultures of Group A and Group C beta hemolytic streptococci and Freund's adjuvants, did not bring about the formation of detectable antibodies for homologous tissue.

Rabbits inoculated in this manner did not develop myocardial or renal lesions suggestive of rheumatic myocarditis or glomerulonephritis. In experiments involving approximately 350 rabbits, the only significant pathological finding consisted of acute inflammatory lesions in the myocardium of the right ventricle which occurred in ten animals. The latter animals had received injections of homologous heart and kidney tissue in combination with Group A streptococci and the adjuvants. The lesions did not resemble rheumatic myocarditis. Repeated attempts to confirm this observation, under similar conditions, yielded uniformly negative results.