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Effect of Adrenal Hormone Overdosage on Bacterial Removal by the Splanchnic Viscera.* (19781)

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An adverse effect of adrenal hormone overdosage on the course of experimental infections has been reported(1,2). A higher incidence of positive blood cultures was noted in animals receiving cortisone who were infected with Group A streptococci and pneumococci(1,3). Some studies have shown a diminution in the inflammatory response(1,4,6). The cause of the increased incidence of bacteremia has not been shown but may be attributed to the failure of the inflammatory response to localize the pathogen at the site of injection or to the failure of the splanchnic vascular system to remove the bacteria after they have entered the blood stream. Gordon and Katsh(5) have demonstrated that adrenal insufficiency lowers endothelial phagocytosis and decreases the removal of particles in the liver and spleen. This report deals with the effects of overdosage of ACTH and cortisone on the ability of an animal to remove a non-encapsulated organism. There is no difference in the removal rate of such an organism before and after chronic adrenal stimulation.

Healthy 4 to 4.5 kg rabbits were maintained on a mixture of rabbit chow and oats with no periods of fasting. The animals receiving adrenocorticotrophic hormone (ACTH, Wilson's) were given 10 units every 4 hours for 24 hours before study. The animals receiving cortisone were divided into 4 groups. Two groups were studied at 4-5 hours after a single intramuscular or oral dose of 25 mg cortisone. The 2 remaining groups were studied after 4 days of therapy with 25 mg of cortisone a day given intramuscularly or orally, the last dose being given 4-5 hours before study. A bacteremia was maintained by constant infusion into a marginal ear vein of hemolytic *Micrococcus pyogenes var aureus*, coagulase positive. The splanchnic removal rate was determined by passing a catheter into the hepatic vein and comparing the number of bacteria found in cultures of the blood drawn simul-

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TABLE I. Effect of Adrenal Hormone Overdosage on Removal of Bacteria from Blood Stream.

Treatment	Duration	% removal	No. of observations, animals	P
Control	4 hr	61.5 ± 20 *	19 – 4	
Cortisone acute, oral	4 "	71.7 ± 13.6	24 – 3	<.05
" " parenteral	4 "	73.9 ± 16.4	23 – 4	<.05
" oral	4 days	66.6 ± 20	24 – 5	>.05
" parenteral	4 "	57.67± 25.1	36 – 5	>.05
ACTH	24 hr	54.6 ± 26.4	20 – 5	>.05

* Stand. dev.

taneously from the hepatic vein and the heart or inferior vena cava(7). The removal rate is expressed as the per cent of bacteria removed when arterial and venous concentrations are compared. One per cent metycaine was used for local anesthesia.

Results. Table I presents a comparison of the removal rate under the circumstances outlined. The values are expressed as per cent removal with the standard deviation. There is no significant difference between the removal of *Micrococcus aureus* in the control group of animals and in the animals receiving ACTH or cortisone for periods greater than 4 hours. There is a questionable increase in the removal rate when measured 4 hours after administration of a single dose of cortisone.

Discussion. Chronic overdosage with ACTH and cortisone neither increased nor decreased the splanchnic removal of bacteria. These normal rates of bacterial removal suggest that the frequency of blood stream involvement noted by previous experimentation(1,2) does not depend on the failure of removal of organism by splanchnic vascular system. They indicate that the cause of the bacteremia may lie outside the splanchnic removal system and may be attributed to the absence of an inflammatory reaction in the tissues with the result that an infection is not localized and organisms may continually spread into the blood stream. The inflammatory reaction both in the interstitial tissue and in the blood vessels(8) may be of importance in preventing dissemination of organisms. Present studies in progress indicate an alteration in metabolism and function of the cells in the exudate exposed to adrenal hormone.

If the above reasoning is correct, the basic cause of the increased incidence of bacteremia after chronic ACTH and cortisone overdosage is similar to that responsible for the increased incidence of bacteremia in marked leucopenia where removal rates are also normal(9). In the case of ACTH and cortisone, the cells are absent from the lesion because the inflammatory reaction is suppressed; in the case of the animals without white cells, the cells are not available to migrate into the lesion. In both instances, organisms move freely from the local lesions into the blood stream.

The increase on acute administration of cortisone may be a reflection of the heightened phagocytic activity previously described by Dougherty and co-workers(10). The difference between acute and chronic administration calls attention to variations which may arise under these circumstances.

Summary. Chronic administration of adrenocorticotrophic hormone and cortisone had no effect on the removal of *M. aureus var pyogenes* by the splanchnic vascular system. It is suggested that the increased incidence of bacteremia seen after ACTH and cortisone as well as that seen in the leucopenic states is the result of poor localization of the organisms in the tissues. In the former, the reduction in the inflammatory reaction and the change in the metabolism of leucocytes are important. In the latter, white blood cells are not present to migrate into the lesion. There was a questionable increase in removal rate after acute administration (4 hours).

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Counts of Virus Particles by Sedimentation on Agar and Electron Micrography.* (19782)

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The solution of many problems in virology is dependent on the quantitative correlation of number of virus particles or unit virus mass with the biological attributes of the agent. Estimation of mass or particle number in preparations of purified viruses of uniform shape and size can be made simply and fairly accurately by chemical analysis. In other cases, only direct counts of the particles offer assurance of accuracy. One excellent method for this purpose involves the comparison of a known number of latex particles mixed with virus particles in sprayed droplets(1). In another procedure(2), virus is sedimented on a collodion membrane, and counts are made of the particles in a measured area. Success with the first of these methods, however, is dependent on high concentration of virus and, with both, on the absence of interfering extraneous material such as protein or salt.

Recently, a modification of the sedimentation procedure has been developed which retains the high sensitivity demonstrated in the earlier work and, in addition, gives promise of high accuracy with preparations containing protein, salts, and other material which hinder electron micrography or may have a harmful effect on the virus particles when dried with them. This method consists in the sedimentation of the virus onto an agar surface from which it can be removed by means of a collodion pseudoreplica.† In this process, excess protein can be washed away, and the salt remaining diffuses into the agar. It is evident, however, that the applicability of this process, as well as others involving electron micrography, is entirely dependent on criteria for unequivocal identification of the virus particles because of characteristic morphology or a specific means for relating virus activity to the particles counted. Preliminary studies on the use of agar for examination of the virus particles of erythromyeloblastic leucosis have been described(3). In the present paper are given results obtained with the agar technic applied to the enumeration of influenza virus particles in purified preparations and in a comparison of these findings with those observed with other methods of counting.

Materials and methods. Counts were made by sedimentation of the influenza virus on an agar surface; sedimentation of the particles on a collodion membrane; and by the spray technic. The mechanical equipment employed in the sedimentation experiments consisted of an air-driven ultracentrifuge, ordinarily used for analytical work, the rotor of which was fitted with cells specially made for the study.

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† The pseudoreplica is a film which, when stripped off, brings with it the virus particles.