The findings reported here support the conclusions of Suki *et al*(4) that furosemide acts upon the ascending limb of the loop of Henle. These observations also indicate that furosemide and hydrochlorothiazide act in different sites as has been suggested previously(7) and in addition are in agreement with the previous suggestion that furosemide and ethacrynic acid have a common site of action(7).

Summary. Furosemide, 25 mg/kg/hr (25 mg/kg prime) abolished the sodium medullary gradient in the dog kidney. It is concluded that the natruresis produced by furosemide is due, at least in part, to inhibition of sodium reabsorption in the ascending limb of the loop of Henle.

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Response of Reticuloendothelial System to Experimental Allergic Orchitis in a Genetically Susceptible and Resistant Mouse Genotype.* (29847)

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Data recently obtained in our laboratory seem to point to a highly important role of the reticuloendothelial system (RES) in the pathogenesis of experimental autoimmune disease. Experiments with 2 mouse strains, BSVS and BRVR, have shown that the former responded to a preparatory injection of pertussis vaccine followed by 2 intracutaneous injections of unpurified mouse brain in Freund's adjuvant with the development of allergic encephalomyelitis while the latter failed to acquire the disease(1). Both strains exhibited a significant rise of their RES activity regardless of whether this was measured with the carbon clearance test of Halpern *et al* or by counting the acid phosphatase positive cells in liver and spleen(2,3). While either strain showed increased activity in comparison with its untreated controls no statistically significant difference was noted when the treated animals of the 2 strains were compared with each other. In the present study this investigation was extended to a second autoimmune disease, allergic orchitis.

Materials and methods. For immunization, the same schedule was used as in the production of allergic encephalomyelitis. BSVS and BRVR mice received 0.5 ml of a 1:5

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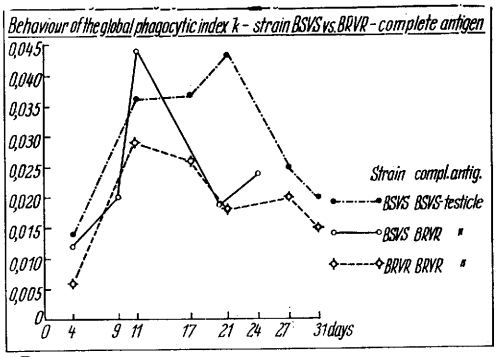
dilution of commercially available pertussis vaccine "Lederle" by intraperitoneal injection followed by intracutaneous injection of unpurified lyophilized BSVS or BRVR testicle in Freund's adjuvant with or without acid-fast bacteria 4 and 11 days later, as suggested by Lee and Schneider(4). One ml of the antigenic mixture was composed of 14 mg lyophilized testicle, 0.5 ml normal sterile saline, 0.2 mg merthiolate, 2.5 mg killed mycobacteria and 0.5 ml Whiterex (Socony Oil). In a second experiment, immunization was done using incomplete adjuvant which did not contain mycobacteria. Throughout the experiment, the animals were kept in cages of 10 and fed Altromin pellets and tap water *ad libitum*.

Activity of the RES was measured as clearance of an intravenously administered test dose of India ink as described by Halpern *et al*(5). Clearance activity is expressed as $k = \frac{\log conc_1 - \log conc_2}{t_2 - t_1}$, where $conc_1$ is the concentration of the injected ink at time 1 and $conc_2$ the concentration at time 2.

Results. Three sets of experiments were designed. In the first one BSVS animals were treated with BSVS testicle. Seventy-five per cent of those mice which had received the antigen in complete adjuvant and 40% of those which had received it in incomplete adjuvant developed orchitis between the 17th and 31st day after the administration of pertussis vaccine. In the second experiment, BRVR mice were challenged with BRVR testicle and in the third one BSVS mice received BRVR testicle. In the second and third experiment no animal developed orchitis.

Fig. 1 describes the behavior of the phagocytic index k after administration of antigen in complete adjuvant (= complete antigen). A significant and lasting stimulation was observed in those BSVS mice which had received BSVS antigen and consequently developed orchitis. A rapid and initially significant rise of RES activity was observed also in those BSVS mice which had been treated with BRVR antigen. Compared with these 2 groups, the BRVR mice which had



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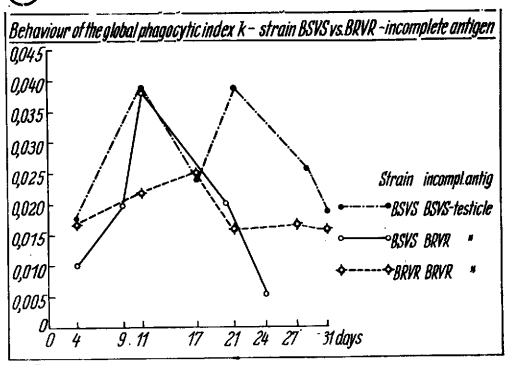

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FIG. 1. Response of RES to i.p. injection of pertussis vaccine followed 4 and 11 days later by an i.c. injection of lyophilized testicle in Freund's adjuvant in mouse strains BSVS and BRVR. Abscissa: RES activity expressed as phagocytic index k. Ordinate: days after injection of pertussis vaccine.

FIG. 2 Response of RES to i.p. injection of pertussis vaccine followed 4 and 11 days later by an i.c. injection of lyophilized testicle in Freund's adjuvant without mycobacteria in mouse strains BSVS and BRVR. Abscissa: RES activity expressed as phagocytic index k. Ordinate: days after injection of pertussis vaccine.

received BRVR testicle exhibited a much smaller rise of activity which, however, was still statistically significant when compared with the untreated controls. While thus the general level of RES activity appeared to be highest in the first experimental group (BSVS mice/BSVS antigen) which was the only one to develop orchitis, the difference between this and the other two groups which failed to develop disease was statistically significant only after day 17.

Fig. 2 shows the behavior of the global phagocytic index k after administration of testicular antigen in incomplete adjuvant

(= incomplete antigen). The experimental group which developed orchitis—*i.e.*, BSVS mice treated with BSVS testicular antigen—responded with the highest increase in RES activity. BSVS mice sensitized with BRVR testicle showed only a brief rise of RES activity. The lowest level, by comparison, was attained by the BRVR mice injected with BRVR antigen.

Discussion. These results indicate that the RES plays a significant role in the pathogenesis of experimental allergic orchitis. They do not support, however, the assumption that RES activity *per se* is the decisive factor in development of autoimmune disease since a significant stimulation occurred both in those mice which developed disease as in those which did not. Since RES stimulation was at least during the second part of the experiment higher in those mice which developed orchitis it is conceivable that the antigen was phagocytized and modified by the RES. The extent of this modification as expressed indirectly by the degree of RES activity might then be the critical factor in the development of autoimmune disease.

Our data provide no clue as to whether serum or cell bound antibodies are instrumental in production of allergic orchitis. In a parallel study of the livers and spleens of all mice tested for RES activity it was found that splenic follicular enlargement, formation of reaction centers and appearance of plasma cells could not be clearly correlated with development of testicular lesions. In general, they seemed to be more prominent in those experimental groups which did not develop orchitis. The only histological change which was loosely correlated with the appearance of lesions in the testis was a shift of phagocytic activity from spleen to liver. This corresponds to our observations in experimental infection with *Salmonella typhimurium* or those made by Howard following the administration of heterologous blood cells to rats (6,7). Our observations are compatible with the view that circulating antibody has no decisive role in the pathogenesis of allergic orchitis.

Summary. 1) BSVS and BRVR mice received an i.p. injection of pertussis vaccine followed 4 and 11 days later by an intracutaneous injection of lyophilized testicle in Freund's adjuvant with or without acid fast bacteria. BSVS mice treated with BSVS testicle developed orchitis, regardless of whether the adjuvant contained acid-fast bacteria or not. BSVS mice treated with BRVR testicle or BRVR mice treated with BRVR testicle failed to develop disease. 2) Development of orchitis was accompanied by a statistically significant rise of RES activity as measured by the carbon clearance test of Halpern *et al*(5). A slower and smaller rise of activity occurred in all those animals which failed to develop orchitis. The smallest rise in activity, by comparison, was observed in BRVR mice treated with BRVR testicle. The group which developed orchitis differed significantly from those 2 which did not, only during the second half of the experiment. 3) A similar response of the RES was noted after administration of antigen in Freund's adjuvant without mycobacteria. 4) It is suggested that the injected antigen undergoes a modification in the RES, the extent of which determines whether disease develops or not.

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