Effect of Castration upon Susceptibility to MEF_1 Poliomyelitis Virus Inoculated Intraperitoneally in Hamsters.* (22679)

CONSTANTIN V. TEODORU.
Department of Microbiology, Mount Sinai Hospital, New York City.

It has been shown that in comparison with normal hamsters inoculated intracerebrally with MEF_1 virus, the castrated animals showed a higher mortality from poliomyelitis while those treated with testosterone or gonadotrophic hormone showed a lower mortality(1). In order to determine whether castration is able to overcome the refractory state of hamsters to intraperitoneal inoculation of virus, the susceptibility to this route of inoculation was investigated at various periods following castration.

Materials and methods. Male Golden hamsters, 25-50 g, were used. A volume of 0.5 ml MEF_1 virus suspension diluted 1/50 in saline (*i.e.*, 800 Mouse LD_{50}) was inoculated intraperitoneally. Bilateral orchiectomy was performed at different intervals before and after inoculation of virus and infectivity in castrated animals was compared with that of non-castrated controls. All paralyzed animals were sacrificed and, in order to confirm the clinical diagnosis, their spinal cords were examined histologically and a suspension of the cord was inoculated into mice. Cortisone (1.5-2.5 mg) in a single intramuscular administration was injected for the study of individual and combined effects of castration and cortisone treatment. To investigate possible protective effects associated with compensatory phenomena following castration, the refractory state to intraperitoneal inoculation was eliminated by means of a single intramuscular injection of cortisone immediately prior to the viral inoculation. The results were analyzed by statistical procedures previously described(1).

Results. Seventeen % of castrated hamsters receiving MEF_1 virus intraperitoneally, 24 hours following orchiectomy, developed paralytic poliomyelitis, confirmed by mouse inoculation and by histological examination of the spinal cord. None of the similarly inoculated but uncastrated control animals developed the disease (Table I). When castration was performed 3 days after the administration of virus, only 1 animal out of 45 developed the disease, which may be explained by our previous observation of the rapid disappearance of intraperitoneally injected virus(2). Recent castration plus small doses of cortisone revealed a synergistic effect in enhancing susceptibility to poliomyelitis virus as compared to either castrated or cortisone treated controls alone (Table I).

The findings with respect to intraperitoneal inoculation of virus 14 days after orchiectomy were similar to those previously reported in hamsters inoculated intracerebrally where it was shown that compensatory phenomena usually occur at that stage following castration substituting a degree of resistance for the initial enhancement of susceptibility induced by testicular suppression(1). To determine whether a similar protective effect may be observed 14 days after castration in peritoneally inoculated animals, 2.5 mg of cortisone was administered intramuscularly followed by MEF_1 virus (800 mouse LD_{50}) inoculated intraperitoneally the same day. A significantly lower proportion of 14-day castrates developed the disease as compared to both 24-hour castrates and uncastrated controls receiving the same dose of cortisone (Table I).

Overdosage of testosterone significantly diminished the mortality of cortisone treated hamsters inoculated intraperitoneally with MEF_1 virus (Table II). However the reduction in paralytic incidence and mortality was of little statistical significance if the animals dying without paralytic symptoms were eliminated from both treated and control groups (Table II).

Discussion. From the above experiments it may be observed that orchiectomy is a conditioning factor in the pathogenesis of experi-

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TABLE I. Effects of Castration and of Cortisone Treatment upon Infectivity by Peritoneal Route in Hamsters. (All groups received a single intraperitoneal inoculation of MEF_1 virus, 800 mouse LD_{50}.)

Groups	Cortisone, mg	No. animals	Paralytic incidence, %	Significance of difference "P"
Non-castrated control	0	60	0	.001
24 hr after castration	0	35	17	
Idem	.5–1.0	58	21	.001
"	1.5–2.5	63	54	
Non-castrated control	1.5–2.5	105	36	.01
14 days after castration	2.5	29	10	

mental poliomyelitis since it enhances susceptibility to intracerebrally inoculated virus(1), and overcomes the refractory state to poliomyelitis virus inoculated peritoneally. With time, however, compensatory mechanisms come into play, and a state of resistance follows initial susceptibility induced by orchiectomy. This biphasic effect of castration raises the question as to whether susceptibility alterations are the result of suppression of testicular function or whether they are secondarily induced by the adrenal alterations that follow orchiectomy. It has been shown that during the initial 5 day period following castration, adrenal hypertrophy and involution of the thymus coincide with enhanced susceptibility to MEF_1 virus, while 14 days later, a progressive adrenal involution and thymus hypertrophy coincide with increased resistance to poliomyelitis(1). The fact that both adrenal hypertrophy and enhanced susceptibility to poliomyelitis follow any traumatic stress(2), suggests that during the initial stage following castration, enhancement of susceptibility was produced as a consequence of operatory trauma rather than by the suppression of testicular function. However, several other observations such as the inverse relationship between testicular maturity and susceptibility to poliomyelitis in relation to age and seasonal fluctuations(3), the protective effect of gonadotropine and of testosterone treatment(1), the association of large testes and resistance to poliomyelitis infection in certain races of hamster (Chinese, Albino), would indicate that testicular hormones have a protective influence against experimental poliomyelitis. Actually it is not possible to determine the individual contributions of adrenal gland and testis in determining the susceptibility level, since in addition to the possible direct hormonal interactions, the two endocrines influence each other indirectly through the mediation of the pituitary gland. Thus, orchiectomy produces polyphasic adrenal alterations which are followed by polyphasic fluctuations in susceptibility to poliomyelitis, while cortisone treatment produces testicular involution which is paralleled by enhancement of both morbidity and mortality from poliomyelitis(1). When cortisone treatment follows castration, their combined effects may be synergistic (initial stage), or

TABLE II. Effect of Testosterone Overdosage upon Mortality in Hamsters Injected with 3 mg Cortisone Intramuscularly and with MEF_1 Virus Intraperitoneally (800 Mouse LD_{50}).

		Total mortality (including animals dying without paralytic symptoms)		Mortality from poliomyelitis (excluding animals dying without paralytic symptoms)			
Preliminary treatment	No. of animals	Mortality, %	Significance "P"	Mortality, %	"P"	Paralysis, %	"P"
Depo–Testosterone* (15 mg i.m.)	249	43	.0002	29	.03	40	.04
Control	245	60		40		51	

* Upjohn Co.

antagonistic (compensatory stage), depending upon the experimental timing. From the effects of these interacting mechanisms it may be concluded that: refractoriness may be converted into susceptibility to poliomyelitis and vice versa, by factors influencing the adrenal-testis equilibrium either by direct action or by interference with pituitary function. The latter mechanism, which seems to be responsible for the effects of certain seasonal and dietary factors upon susceptibility(2,3), should be taken into consideration as one of the prospective means for disease prevention.

Summary. 1. A significant proportion of castrated male hamsters inoculated with MEF_1 virus intraperitoneally developed paralytic poliomyelitis, whereas non-castrated controls were completely refractory to the virus by this route. 2. Enhancement of susceptibility observed immediately after castration was found to be reversed during the compensatory stage 2 weeks later. 3. The effect of castration upon susceptibility to experimental poliomyelitis was synergistic with cortisone during the initial stage, but antagonistic during the compensatory stage.

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Relationship between Behavioral Changes and Brain Cholinesterase Activity Following Graded Intracerebral Injections of DFP. (22680)

RICHARD P. WHITE.* (Introduced by Harold E. Himwich.)
Thudichum Psychiatric Research Laboratory, Galesburg State Research Hospital, Galesburg, Ill.

Previous studies have shown that forced circus movements may be induced by unilateral electrostimulation(1,2,3,4) or ablation (5,6,7,8) of various parts of the neuraxis. Forced circling may also be produced by unilateral intracarotid injections of the anticholinesterase diisopropylfluorophosphate (DFP)(9,10,11,12) as well as by micrometer syringe injections of DFP and CNS stimulants into specific brain structures.† It has been disclosed that the intracerebral administration of 0.01 ml solutions containing from 0.06 to 0.16 mg of DFP does not result in significant spread of this substance to adjacent cerebral gray matter.† This finding opened the possibility to determine (1) the level of cholinesterase (ChE) activity at which stimulation of specific CNS structures occurs and (2) the relationships between the dose of DFP, the level of ChE activity and the production of forced circling. The caudate nucleus was selected for this study because of its comparatively high level of ChE activity(10,12) and the known role it plays in the forced circus movements induced by micrometer injections into this structure.†

Methods. In all experiments DFP was administered into the right caudate nucleus by means of a micrometer syringe attached to a stereotaxic apparatus. Solutions of DFP were made by diluting weighed amounts with water so that injections of 0.01 ml would deliver between 0.0001 mg to 0.32 mg. In order to inject greater doses undiluted quantities of DFP were employed because DFP is immiscible in this volume of water in amounts greater than 32 mg. The dose of undiluted DFP was determined by appropriate adjustment of the micrometer syringe and in all such cases the volume was less than 0.01 ml. The entire dose range of DFP employed was from 0.0001 mg to 10 mg. Controls were obtained by injections of 0.01 ml of physiological saline. Mock injections were also made in order to determine whether DFP was de-

* Present address: University of Tennessee Medical School, Memphis, Tenn.

† In abstract, *Fed. Proc.*, 1956, v15, 199.