(Group II) or sub-lethal doses (Group III) 6 weeks prior to challenge. The only suggested differences were: 1) an almost 2-fold greater number of positive liver and spleen cultures between 16 to 30 weeks in Group III as compared to the other 2 groups; this could probably be attributed to the residual viable organisms from the inoculation made 6 weeks prior to challenge. 2) A relatively higher concentration of organisms in the liver and spleen between 6 to 10 weeks in Group I as compared to the other 2 groups. When compared to the concentrations of organisms observed between 1 to 5 weeks, there was a definite trend downward in the previously infected or "immunized" mice as compared to the relative rise at 6 to 10 weeks in the primary challenge group.

Summary. Yeast phase *H. capsulatum* when inoculated, intraperitoneally, into white Swiss male mice may be recovered frequently from reticuloendothelial tissues as late as 45 weeks after infection. The lungs and heart's blood usually clear within 16 weeks post-inoculation. Mice surviving previous sub-lethal challenge or those previously given 1 dose of heat-killed organisms show little variation in these respects, when challenged, from those receiving primary challenge.

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Inhibition of Immunological Enhancement by Endotoxin in Refractory Rabbits. Immunochemical Study.* (22280)

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When Gram negative bacterial endotoxins are repeatedly administered by vein over short periods of time, resistance to the reactions normally produced by these toxic complexes rapidly develops(1). Quantities of endotoxin originally productive of vigorous systemic adjustments no longer produce untoward reactions(2,3). For example, febrile responses normally produced are either obliterated completely(2) or modified. Hemorrhagic and necrotizing phenomena normally produced in tumor tissue and skin prepared for the local Shwartzman reaction do not occur(3,4) after intravenous injection of toxin in refractory rabbits. The generalized Shwartzman reaction is no longer produced by paired spaced intravenous injections of toxin, and the leukopenia producing action of endotoxin is obliterated(5). In addition, refractoriness to the lethal effect of endotoxins occurs(6). Refractoriness to most of these reactions develops very soon after intravenous injections of endotoxin are begun. Resistance to the lethal effect of toxin may be demonstrated as early as 24 hours following the first intravenous injection(7). Refractoriness to each of the other effects of endotoxin occurs within a few days(2-4). This development has been dissociated from the classical immune response primarily by its lack of specificity(8), rapidity of development(7), shortness of duration(2), and lack of correlation with appearance and level of circulating antibody(9,10). Further, agammaglobulinemic patients unable to produce demonstrable circulating antibody develop refractoriness to febrile and intoxicating actions of endotoxins just as readily as do normal persons(11). On

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the other hand, refractoriness is promptly reversed following intravenous injection of certain colloidal substances(8,12), an effect attributed to the capacity of these colloidal substances to produce "blockade" of the reticuloendothelial system(13). Our recent studies have shown that the reactions produced by administration of Gram negative bacterial endotoxins result in profound disturbances in defense mechanisms of the host(14). Meningococcal endotoxin in quantities which prepare rabbits for the generalized Shwartzman phenomenon decreases the ability of rabbits to clear colloidal materials from the blood presumably by interfering with the function of the reticuloendothelial system(13). Further, endotoxin seemed to enhance certain bacterial infections in rabbits. For example, when toxin was given prior to establishment of a pneumococcal skin infection, bacteremia and death resulted whereas in control rabbits given no endotoxin the organism produced only local skin infection(14). In contradistinction, endotoxin did not enhance susceptibility to infection when injected intravenously in rabbits previously made refractory to the endotoxin(14). Indeed, refractory rabbits appeared to resist pneumococcal and streptococcal infection more vigorously than did controls. These observations provoked investigation of the effect of meningococcal endotoxin on the immune response in rabbits as revealed by immunochemical study. In confirmation of observations of Johnson *et al.* (15) we found that when toxin was given together with bovine serum albumin, enhancement of antibody production against the foreign serum protein resulted(16).

The present study was undertaken to determine whether refractoriness to the febrile, toxic, and necrotizing effects of endotoxin would interfere with the adjuvant effect of this material on the immune response.

Materials and methods. Animals employed were 2 kg albino rabbits obtained from one breeder and maintained throughout the experiment on a diet of Purina rabbit pellets. Endotoxin was prepared from lots of a culture-free meningococcal‡ filtrate of high Shwartzman activity. Different lots were combined, spun at 15,000 rpm, the supernatant quick-frozen and stored at −30°C. Details of preparation have been described previously(17). The refractory state was induced using a regimen which had proved to be effective in quickly producing a high degree of resistance in a short time, including refractoriness to fever, lethal effect, necrotizing action and production of leukopenia. This schedule consisted of 11 daily intravenous injections of 2 cc of endotoxin in increasing amounts, beginning with a dilution of 1:10,000 and extending to a dilution of 1:10 on the 11th day. Toxin solutions were prepared daily from the frozen culture free filtrates and dilutions made up with pyrogen free saline. Attempt to provoke the Shwartzman reaction was used as the test for resistance since it had previously been shown that this was a delicate index of susceptibility to the action of endotoxin. This reaction was ordinarily produced by an intradermal preparatory injection of 0.25 ml of 1:2 dilution of endotoxin, followed in 18 hours by an intravenous injection of the same material. In the stock of rabbits used and with the endotoxin employed, the Shwartzman reaction developed in an incidence between 95 and 100%. All injections, with the exception of the intradermal preparation for the local Shwartzman reaction, were made by way of the marginal ear vein. Crystallized bovine serum albumin (BSA), lot #67009 (Armour and Co., Chicago, Ill.), was employed as the antigen. Immediately prior to injection, solutions containing 15 mg BSA were made up to 2 ml with either pyrogen free saline or 1:100 dilution of the endotoxin depending upon the experimental group. Prior experiments established that this quantity of toxin represents the optimal dose for producing enhancement of antibody formation with the quantities of antigen used. Rabbits were divided into 3 groups; Group I normal, non-refractory rabbits given BSA together with endotoxin intravenously, Group II refractory rabbits given endotoxin and BSA antigen intra-

‡ Strain 44-B, generously supplied by Dr. Gregory Shwartzman, Mount Sinai Hospital, New York.

venously, and Group III normal rabbits given BSA intravenously.

Blood was collected by cardiac puncture on the 11th day following antigenic stimulation, since previous studies had established that at this time circulating antibody to BSA is at its highest level. Clots were broken up after standing 24 hours at 4°C, the tubes centrifuged for 20 minutes at 1000 RPM, the serum decanted into lusteroid tubes, frozen at −70°C, and stored at −30°C until antibody determinations were performed.

Serum antibody content was determined using a modification of the quantitative precipitin method described by Kabat and Mayer(18). The method involves two precipitations. The first, a preliminary run, requires only small quantities of serum and is used primarily to determine the optimal antigen concentration to be used in the final precipitation. With the preliminary precipitation, a surprisingly accurate estimate of antibody content can be made merely by analysis of the supernatant for the presence of excess antigen. Known concentrations of antigen were prepared from standard stock solution of BSA prior to each precipitation. Serum samples were thawed at 37°C for 10 minutes, mixed and centrifuged in the cold (4°C) for 1 hour at 3000 rpm. Aliquots of each serum sample were then precipitated with 4 concentrations of antigen prepared from the standard BSA solution. Precipitin tubes were incubated at 37°C for 1 hour and stored in the cold 7 days to insure complete precipitation with agitation each day. Tubes were then centrifuged at 4°C for 1 hour at 3000 rpm and supernates saved for analysis. Excess antigen was determined by capillary tube precipitation with a potent anti-BSA rabbit serum. The tubes were incubated 1 hour at 37°C, placed in the cold and read for precipitate at 36 hours. Final determinations were performed in a similar manner except that nitrogen content of the precipitates was determined. Precipitation was carried out with antigen concentrations known to fall in the equivalence zone of each serum sample. Precipitates were washed twice with 0.145 molar saline (4°C) and precipitate nitrogen was determined colorimetrically using Heidelberger MacPherson modification of the Folin-Ciocalteau reaction(19). Optical density, read at 650 mμ on a Coleman Universal Spec-

TABLE I.

Group	Experimental procedure	Serum antibody content γ N/ml	t	P
I	2 ml I.V. solution containing 15 mg BSA + 1:100 dilution of meningococcal endotoxin	333		
		201		
		74		
		121		
		94		
		233		
	Mean	176		
II	Resistant rabbits, 2 ml I.V. solution containing 15 mg BSA + 1:100 dilution meningococcal endotoxin	45		
		58		
		<10		
		<10		
		58		
		<10		
	Mean	31.8		
III	Controls—2 ml I.V. solution containing 15 mg BSA only	47		
		<10		
		<10		
		28		
		35		
	Mean	26.0		
I & II			3.47	<.02
I & III			3.65	"
II & III			.47	>.50

trophotometer Model 14, was converted to mg nitrogen using a standard calibration curve prepared with a highly purified human gamma globulin.§ Nitrogen content of the stock solution was determined by the micro-Kjeldahl method.

Results. Resistance was developed to a high degree in rabbits receiving daily intravenous injections of meningococcal endotoxin. Six of the 7 rabbits of Group II showed no evidence of a positive local Shwartzman reaction. Lack of usual febrile responses to a standardized pyrogenic dose of endotoxin was characteristic of the resistant rabbit.

The results of the quantitative precipitin determinations are shown in Table I. When meningococcal endotoxin was given together with BSA, a significant enhancement of antibody formation resulted. The mean level of circulating antibody to the BSA on the 11th day following antigenic stimulation was 6 times that of the control animals of Group III which received BSA alone. The probability that this might result from chance distribution is <0.02 which indicates that there exists a highly significant difference between these groups. The quantity of endotoxin employed, 2 ml of 1:100 dilution, was in that range recently reported to be optimal for producing an adjuvant effect resulting in increased levels of circulating antibody against apparently unrelated foreign serum protein(16).

Resistant animals of Group II received the same antigenic stimulation that resulted in greatly elevated levels of circulating antibody in the rabbits of Group I. However, serum antibody levels of the resistant animals on the 11th day following antigenic stimulation were not elevated. The difference between Groups I and II were also significant while statistical analysis revealed no difference between Groups II and III.

Discussion. It is shown in these experiments that resistance to the adjuvant effect of endotoxin was readily induced by repeated daily intravenous injections of increasing amounts of this material. A solution consisting of bovine serum albumin and meningococcal endotoxin produced significantly more antibody when given to normal untreated rabbits then when injected intravenously into refractory animals.

In these experiments the capacity of endotoxin to enhance antibody production is again demonstrated and it is further shown that this effect is prevented by prior treatment of rabbits with endotoxin according to a regimen designed to produce refractoriness to the other known effects of the toxin. These observations provide evidence that the enhancing effect of meningococcal endotoxin on the production of immune bodies cannot be explained simply as the synergistic effect of two unrelated antigens or by an unsuspected antigenic relationship between BSA and meningococcal endotoxin resulting in enhanced antibody production against the former.

It appears that expression of the "toxic" effect of endotoxin is necessary for the stimulation of antibody production by endotoxin observed in this and previous studies(15,16). The nature of this adjuvant effect remains enigmatic. Such an action could be directed specifically at the mechanism of antibody synthesis at a cellular or subcellular level or might act indirectly by mechanisms involving alteration in vascular or cellular permeability, circulatory dynamics, phagocytic function or central nervous system function. Whatever be the mode of action, the adjuvant effect of endotoxin appears to be real and to depend on the "toxic" properties of the bacterial product.

Summary and conclusion. 1. Using an immunochemical system, inhibition of the adjuvant effect of endotoxin by development of refractoriness to the "toxic" properties of endotoxin is demonstrated. 2. The basis for enhancement of immune body formation by endotoxin is discussed.

§ Generously supplied by Dr. John T. Edsall, Harvard University, Boston.

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Progestational Effectiveness of 19-nor-Ethinyl-Testosterone* by Oral Route in Women. (22281)

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Tullner and Hertz(1) have previously demonstrated that 19-nor-progesterone is 4 to 8 times as active as progesterone by injection in the Clauberg rabbit test. Hertz, Tullner, and Raffelt(2) also showed that 19-nor-ethinyl-testosterone is likewise about 5 times as active as ethinyl testosterone when tested for progestational activity by oral administration in the Clauberg rabbit and that it is also highly active in the ovariectomized monkey pretreated with estrogen(2,3).

This report presents observations on the progestational effectiveness of 19-nor-ethinyl-testosterone† upon oral administration in 4 women.

Case 1: S.J.M. 14-year-old white female; proven by biopsy of ovarian anlagen to have ovarian agenesis (October, 1953). She was maintained on cyclic estrogen therapy with monthly withdrawal bleeding until January, 1954. Then after 23 days on 0.1 mg ethinyl estradiol daily she received 10 mg 19-nor-ethinyl-testosterone† twice daily for 12 days. Suction curettement of the endometrium showed marked progestational effects with numerous polyhedral as well as spindle cells in the stroma. Menses ensued 2 days after cessation of therapy.

Case 2: A. H. 21-year-old white female; ovarian agenesis proven by biopsy of both ovarian anlagen in November, 1953. She was maintained on cyclic estrogen therapy until January, 1954, when she was given 5 mg of 19-nor-ethisterone twice daily in addition to her usual daily estrogen dosage of 0.1 mg ethinyl estradiol for 2 days followed by 12 days more of the nor-ethisterone alone. She began menstruating at this point and all treatment was discontinued for 3 months. This bleeding indicated that although 5 mg of nor-ethisterone twice daily was sufficient to markedly prolong her usual latent period for withdrawal bleeding, nevertheless it was not sufficient to maintain the endometrium. Accordingly, about 2 weeks later another course of estrogen followed by 10 mg of nor-ethisterone twice daily was given for 14 days and a suction curettement performed. The endometrium showed a marked progestational effect with distinct decidual reaction in many areas.

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† Also called: "nor-ethisterone."