THE INFLUENCE OF THE FACTORS OF SEX, AGE, AND
METHOD OF INOCULATION UPON THE COURSE
OF EXPERIMENTAL SYPHILIS IN
THE RABBIT.*

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INTRODUCTION.

Our knowledge of the factors which govern the course of syphilitic infection in the rabbit is still incomplete. Until a systematic study has been made of those likely to influence the reaction of this experimental animal to infection with *Treponema pallidum*, our conceptions of the mechanism by which the infection is controlled are likely to be fragmentary at best. This communication deals with a study of some of the factors which seemed worthy of investigation from the standpoint of their influence upon the course of the syphilitic infection. The particular ones studied were those of age, sex, and method of inoculation.

Stated in more precise terms, the object was threefold: (1) to contrast the character of the disease in a group of females inoculated intradermally with that observed in a group of males inoculated in the same manner; (2) to contrast the character of the disease in a group of males inoculated intradermally with that in a group of males inoculated by the intratesticular route; and (3) to contrast the reaction of a group of young males following intratesticular inoculation with that of a group of older males. Opportunity was also afforded for a comparison of the course of the disease in males in which the primary focus following intratesticular injection had been removed by castration,

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with the course in animals in which no such attempt at interference with the ordinary progress of the disease had been made. In addition, it was possible to observe the course of the infection in animals in which attempts were made to produce chancres by implantation within the scrotum of bits of syphilitic rabbits' testicles. However, the influence of the factors of route of inoculation, age, and sex were the principal ones selected for study.

The experiment involved no new method of inoculation for the study of syphilis in the rabbit. All of the routes chosen have been used in the past, but there are not, to our knowledge, any published reports on a large series of animals inoculated simultaneously in various ways with the same material. So far as we are aware, the three factors studied in this experiment, age, sex, and method of inoculation, have not in the past been similarly studied on a given series of rabbits with identical infecting material. Experience with experimental syphilis in the rabbit has shown conclusively that a given series of animals inoculated at one time cannot be compared directly with a series inoculated at some other time, even when one is working with the same strain of *Treponema pallidum*. In any comparison of behavior of groups of animals toward infection with *Treponema pallidum*, it is essential that the various groups be inoculated at the same time, as well as with equal amounts of the same virus. Failure to observe this elementary rule will yield results which are of little value from a comparative standpoint in the study of experimental syphilis in the rabbit, hence the point is stressed here.

Technique.

The strain of *Treponema pallidum* used in these experiments was the Nichols strain isolated originally in 1912 from the spinal fluid of a case of neurorecidive (1). This strain, now nearly 11 years old, has had many passages through rabbits, and has attained a degree of virulence for that animal of such extent that it will produce primary lesions in 100 per cent, and under favorable conditions, will produce generalized lesions in from 80 to 100 per cent of animals inoculated intratesticularly, although the incidence of such lesions is at times very low. The immediate source of the material was testicular tissue of two rabbits that showed clinical evidence of orchitis 23 days after inoculation. The testicles were excised under ether anesthesia at a period when the syphilitic orchitis was progressing rapidly, portions of the body of the organ were finely minced with scissors, ground up in a sterile mortar with sterile salt solution, and the more fluid portion of the

resulting emulsion, which contained numerous actively motile organisms by dark-field examination, used for injection. Other parts of the body of the testicle were cut into small bits, 2 to 3 mm. in thickness, and these pieces served for scrotal implantation.

Intradermal Inoculation.—This method was accomplished by inserting the needle in the subcutaneous tissue of the sheath of the penis or vulva, at a distance of about 1 cm. from the mucocutaneous border, directing the needle toward the latter and at the same time bringing the top of the needle (bevel up) toward the surface until it lay in the skin just beneath the superficial layer, where it could be easily seen, and carrying it as far as the mucocutaneous border.

Intratesticular Inoculation.—This method needs no description.

Scrotal Implantation.—This method has already been described (2) and need not be discussed at length.

In the case of the intradermal and intratesticular inoculations the same amount of emulsion was used throughout; namely, 0.1 cc. For obvious reasons, the scrotal implants could not be of exactly the same size, but were approximately so.

The animals belonged to a variety of breeds and were kept under observation for a period of 90 days. The selection of this period was based upon the course of events following a unilateral testicular inoculation, since experience with the strain had shown that within this interval the infection would pass through a more or less orderly evolution of primary and secondary lesions, while observation over longer periods would entail the possibility of confusion through the occurrence of relapses. Hence, this period was selected as the most suitable for the comparisons that we had in mind. No attempt was made to demonstrate treponemata in the lesions as they appeared because of the possibility of the trauma exerting an influence on the course of the infection.

In all, thirty-seven rabbits were inoculated, of which number two died from pulmonary disease during the course of the experiment and are excluded from consideration. The remaining thirty-five which are to be reported may be divided into six groups as follows: (A) five adult females (over 12 months) inoculated intradermally; (B) seven adult males (over 12 months) inoculated intradermally; (C) five young males (below 5 months) inoculated intratesticularly; (D) seven adult males inoculated intratesticularly; (E) five adult males inoculated intratesticularly, castrated; (F) six adult males inoculated by scrotal implantation. The inoculations were all made on October 5, 1922.

EXPERIMENTAL.

Unless expressly stated otherwise in the ensuing protocols it will be understood that all primary lesions developed at the site of inoculation.

Group A.

This group comprised five adult females. Each animal received 0.1 cc. of testicular emulsion intradermally at the mucocutaneous border of the vulva.

Rabbit No. 1.—New Zealand red. No lesions of any sort except slight inguinal and popliteal lymphadenopathy.

Rabbit No. 2.—Brownish grey. After 11 days an infiltrated area, 3.0 mm. in diameter, and inguinal adenopathy. Maximum diameter, 4.0 mm., attained on the 15th day, later disappearance, followed by two recurrences on the 40th day.

Rabbit No. 3.—Brown. Infiltrated area on the 10th day, 1.0 mm. in diameter, disappearing on the 12th day. Slight inguinal lymphadenopathy. No recurrence.

Rabbit No. 4.—Brown. Infiltrated area appeared on the 19th day, increased to 1 cm. in diameter on the 52nd day, and ulceration occurred, then the lesion gradually receded. Inguinal and popliteal lymphadenopathy.

Rabbit No. 5.—Black. Indurated area, 3.0 mm. in diameter on the 10th day, reached maximum diameter of 8.0 mm. on the 21st day, then diminished gradually. Inguinal and popliteal lymphadenopathy.

Recapitulation.—Of the five animals in this group, one animal showed no local lesion save the initial reaction following injection, while another showed a small infiltrated area which disappeared within 10 days. In a third animal the local lesion consisted at the most of a papule, 4 mm. in diameter, and in a fourth, the lesion consisted of a papule attaining a maximum diameter of 8 mm. In only one of the group did the lesion develop into a characteristic chancre with necrosis of the epithelium, and ulceration. Not one of the animals showed the presence of generalized lesions (bone, skin, eye) within the period of 90 days following injection. In this group, then, the disease in general manifested itself as an inconstant and relatively mild local reaction without clinical evidence of generalization, except for a comparatively slight and rather uncertain reaction in the inguinal and popliteal lymph nodes.

Because of the absence of any generalized lesions in this group it seemed worth while to determine, by transfer of lymph node material to normal rabbits' testicles, if the organisms had invaded the body in spite of the slight local reaction at the inoculation site. The popliteal lymph nodes were removed from each animal in the group and, after

emulsification in salt solution, were injected into the testicles of two normal rabbits, respectively. Of the ten animals to which node transfers were thus made, all developed an orchitis containing treponemata within a period of not more than 62 days after transfer, demonstrating that in all of the five females, infection and general dissemination had taken place in spite of the relatively slight degree or total absence of lesions at the site of inoculation, and in spite of the failure of production of clinically recognizable generalized lesions.¹

Group B.

This group consisted of seven adult males. Each animal received 0.1 cc. of testicular emulsion intradermally in the sheath of the penis. The individual protocols follow.

Rabbit No. 6.—Brownish grey. Infiltrated area, 1 mm. in diameter, 31st day; disappeared on the 42nd day. Transient and immediate inguinal lymphadenopathy. No popliteal lymphadenopathy.

Rabbit No. 7.—Brown. Papule, 2.0 mm. in diameter, on the 31st day; on the 61st day three papules appeared and increased in size during the period of observation. Right-sided orchitis on the 89th day. Inguinal adenitis developed early.

Rabbit No. 8.—New Zealand red. Infiltrated area, 2.0 mm. in diameter, appeared on the 10th day, increased in size, developed into a chancre with a maximum diameter of 8.0 mm. on the 74th day. Inguinal and popliteal lymphadenopathy.

Rabbit No. 9.—Albino. Infiltrated area appeared on the 6th day, increased in size, and became a typical chancre with maximum diameter of 1.5 cm. on the 74th day. Left-sided periorchitis on the 85th day. Inguinal and popliteal lymphadenopathy.

Rabbit No. 10.—Brown. Infiltrated area appeared on the 10th day, became a typical chancre with a maximum diameter of 1.8 cm., and was increasing in size at the end of the experiment. Skin papule on right foot on the 84th day, bilateral orchitis at same time. Skin lesion on the left foot and lesions of the left tarsus, the left fifth metatarsal, and the left ulna on the 89th day.

Rabbit No. 11.—Brown. Infiltrated area appeared on the 10th day, regressed, then became a chancre with a maximum diameter of 10 mm. on the 61st day. Inguinal and popliteal lymphadenopathy.

¹ This portion of the work was carried out in the Syphilis Division of the Department of Medicine of the Johns Hopkins Medical School, and was participated in by Dr. Jarold E. Kemp, to whom thanks are due.

Rabbit No. 12.—Grey. Infiltrated area appeared on the 10th day, a maximum diameter of 5.0 mm. attained on the 19th day; the lesion then receded. No ulceration. Transient inguinal lymphadenopathy.

Recapitulation.—In this group of seven animals there was one in which the only local lesion was a transient tiny area of infiltration, a second in which the local lesion developed into an indurated area, 0.5 cm. in diameter at its maximum, and a third in which the local lesion consisted of three infiltrated papules. In this latter animal evidence of generalization of the virus was provided by an orchitis appearing on the 89th day following injection. In the remaining four animals characteristic chancres appeared on the prepuce at the site of inoculation, and in two of these animals generalized lesions occurred. Summarizing these results: one animal showed only a slight and transient local lesion; two showed persistent lesions which were in the nature of papules which did not ulcerate; and four developed typical chancres. Generalized lesions, consisting of metastatic orchitis, skin papules, or periosteal thickenings, developed in three of the animals within a period of 90 days; the lesions of skin or bones, however, occurred in only one animal of the group. In this group the primary reaction at the site of inoculation was much greater than in the group of females, and the regional lymphadenitis was more marked. Exclusive of testicular involvement, one of the seven rabbits showed generalized lesions, while none of the females showed such lesions.

Group C.

This group consisted of five young males, all less than 6 months of age. Each received 0.1 cc. of testicular emulsion containing spirochetes, in the right testicle. The individual protocols follow.

Rabbit No. 13.—Brown. Orchitis (right) appeared on the 10th day, scrotal edema on the 24th day, metastatic orchitis (left) on the 46th day, and a scrotal chancre (right) on the 60th day. Skin lesion appeared on the right foot on the 66th day.

Rabbit No. 14.—Brown. Nodular orchitis (right) appeared on the 17th day, scrotal edema on the 24th day, and metastatic orchitis (left) on the 40th day. Skin lesion appeared on the right foot on the 62nd day, and a similar lesion on the left foot on the 73rd day.

Rabbit No. 15.—Brown. Orchitis (right) appeared on the 10th day, a scrotal edema on the 19th day, metastatic orchitis (left) on the 46th day, and a scrotal chancre (right) on the 60th day. Skin lesion appeared on the left foot on the 60th day.

Rabbit No. 16.—Brownish grey. Orchitis (right) appeared on the 10th day; the entire testicle involved on the 24th day, after which the orchitis regressed, and no other lesions appeared.

Rabbit No. 17.—Greyish brown. Orchitis (right) appeared on the 17th day, scrotal edema on the 25th day, and metastatic orchitis (left) on the 48th day. Scrotal chancre (right) appeared on the 52nd day and skin lesions appeared on the right foot on the 70th day. A second scrotal chancre appeared on the 77th day on the right side.

Recapitulation.—Of the five young males injected intratesticularly an initial orchitis occurred in all. In four, or 80 per cent, an orchitis developed in the non-inoculated testicle within an average period of 43.7 days. All of these four animals showed skin lesions within an average period of 64 days. One animal only failed to develop metastatic lesions in the testicle or elsewhere.

The outstanding feature of the condition presented by this group of animals was the occurrence of severe primary lesions and of well developed lesions in the uninoculated testicle, as contrasted with comparatively slight lesions elsewhere, all of which were confined to the skin.

Group D.

This group comprised seven adult males inoculated intratesticularly with 0.1 cc. of testicular emulsion containing treponemata. Only one testicle was inoculated and no attempt was made to interfere with the course of the infection. The individual protocols follow.

Rabbit No. 18.—Himalaya cross. Orchitis (right) appeared on the 10th day, a scrotal chancre (right) on the 42nd day, and metastatic periorchitis on the 46th day. Skin papule appeared on the right foot on the 62nd day.

Rabbit No. 19.—Grey. Orchitis (right) appeared on the 17th day, scrotal edema (right) on the 26th day, followed by massive nodular orchitis. No metastatic lesions were observed.

Rabbit No. 20.—Flemish cross. Orchitis (right) appeared on the 19th day, scrotal edema (right) on the 29th day, metastatic orchitis (left) on the 35th day, and a scrotal chancre (right) on the 66th day. A skin lesion appeared on the right foot on the 49th day. Scrotal chancre appeared on the left side on the 84th day and a skin lesion appeared on the left foot at the same time.

Rabbit No. 21.—Belgian cross. Orchitis (right) appeared on the 10th day, scrotal edema (right) on the 17th day, and metastatic orchitis (left) on the 38th day. Skin lesions appeared on the left foot on the 49th and 60th days. Scrotal chancre (right) developed on the 66th day.

Rabbit No. 22.—Black and Flemish cross. Orchitis (right) appeared on the 10th day, scrotal edema (right) on the 21st day, and metastatic orchitis (left) on the 38th day. Skin lesions appeared on the right foot on the 56th day, and on the left foot on the 64th day; periosteal lesion of fifth left metatarsal on the 60th day. Phlyctenular lesions appeared on the sclerocorneal margin of the left eye at the same time.

Rabbit No. 23.—Greyish brown. Orchitis (right) appeared on the 10th day, scrotal edema (right) on the 27th day, and metastatic orchitis (left) on the 46th day. No other generalized lesions occurred.

Rabbit No. 24.—Brown. Orchitis (right) appeared on the 17th day, scrotal edema on the 19th day, and metastatic orchitis (left) on the 54th day. Skin lesions appeared on the left foot on the 49th and 53rd days, on the right foot on the 55th day, and at the base of the tail on the 60th day. Fusiform swelling of the shaft of the fifth right metatarsal appeared on the 45th day.

Recapitulation.—Of the seven adult males which were inoculated intratesticularly, six, or 85.7 per cent, showed metastatic orchitis within a period of 41.5 days, and five, or 71.4 per cent, had metastatic lesions of other structures (skin and bone) within an average time interval of 54.7 days.

Group E.

This group comprised five adult males which were inoculated intratesticularly with 0.1 cc. of testicular emulsion containing treponemata. Only one testicle was inoculated. On the 11th day following inoculation, when lesions could be detected by clinical examination in all of the animals, the entire right testicle was removed under ether anesthesia and the wound closed with sutures. The individual protocols follow.

Rabbit No. 25.—Albino. Orchitis (right) appeared on the 10th day. Castration (right) on the 11th day. Recurrent lesion of the stump on the 38th day, later becoming a chancre; metastatic epididymitis (left) on the 53rd day. Skin lesions appeared on the right foot on the 84th day and on the left foot on the 90th day.

Rabbit No. 26.—Brown and Maltese cross. Orchitis (right) appeared on the 10th day. Castration (right) on the 11th day. Recurrent lesion of stump on the 38th day, later developing into a chancre. Metastatic orchitis (left) on the 40th day. No other generalized lesions were noted.

Rabbit No. 27.—Orchitis (right) appeared on the 10th day. Castration (right) on the 11th day. Some induration of the stump appeared but no marked lesion developed. Metastatic orchitis (left) appeared on the 50th day, lesion of nasal bones on the 70th day, and a skin lesion of the right foot on the 85th day.

Rabbit No. 28.—New Zealand red. Orchitis (right) appeared on the 10th day. Castration (right) on the 11th day. No metastatic lesions of any sort were observed, nor were there any recurrences at the operative site.

Rabbit No. 29.—Brown. Orchitis (right) appeared on the 10th day. Castration (right) on the 11th day. Recurrence at the operative site on the 29th day. Metastatic orchitis (left) on the 42nd day. Periosteal bone lesions appeared on the fifth right metatarsal on the 52nd day, on the left ulna on the 53rd day, and on the nasal bones on the 63rd day.

Recapitulation.—Of the five adult males which were inoculated intratesticularly and the primary focus removed later by castration, four, or 80 per cent, showed a metastatic orchitis within a period of 46.7 days, and in three, or 60 per cent, metastatic skin or bone lesions made their appearance within 90 days. In this group the metastatic lesions were slower in making their appearance than in the control group (Group D), in which there was no attempt made to interfere with the course of the primary focus. The ultimate incidence of generalized lesions was approximately the same in the two groups. In three of the five castrated animals, pronounced recurrent lesions developed in the operative wound. In a fourth there was a similar although less marked recurrence.

Group F.

This group comprised six adult males. All of the animals in the group were inoculated by implantation, within the right scrotal sac, of bits of syphilitic testicular material containing treponemata. The individual protocols follow.

Rabbit No. 30.—Grey. Scrotal chancre appeared on the 10th day, orchitis (right) was detected on the 31st day. Skin lesions appeared on the left foot on the 49th day, and on the right foot on the 60th day. Periosteal lesions of both radii were observed on the 52nd day, and of the right ulna on the 66th day. The chancre began to heal spontaneously before the 90th day.

Rabbit No. 31.—Albino. Scrotal chancre developed by the 10th day, a skin lesion appeared on the left foot on the 70th day. The chancre healed spontaneously before the 90th day.

Rabbit No. 32.—Albino. Scrotal chancre developed on the 10th day. Metastatic orchitis (left) appeared on the 52nd day, and on the 60th day a subcutaneous nodule of uncertain nature appeared. The chancre, after attaining an enormous size, began to heal before the 90th day.

Rabbit No. 33.—Brown. Scrotal chancre developed by the 10th day; skin lesions appeared on both feet. The chancre healed spontaneously before the 85th day.

Rabbit No. 34.—Brown. Two scrotal chancres developed by the 17th day, one at the site of implant, the other at the scrotal incision. Metastatic epididymitis (left) was noted on the 64th day. No other metastatic lesions were observed, while both chancres healed spontaneously by the 66th day.

Rabbit No. 35.—Belgian cross. Two scrotal chancres developed by the 10th day, one at the site of the implant, the other at the scrotal incision. They coalesced, forming a single chancre by the 20th day. Metastatic orchitis (left) appeared on the 46th day, skin lesions on the left foot on the 49th day, and on the right foot on the 60th day. A lesion of the nasal bones was observed on the 60th day. The chancre, after regressing appreciably, relapsed and was increasing in size at the end of the experiment.

Recapitulation.—Of the six adult males which received implantations of syphilitic testicular material in the scrotum, all developed typical chancres which healed spontaneously. In three, or 50 per cent, metastatic orchitis or epididymitis made its appearance within an average time interval of 54.9 days; and five, or 83 per cent, showed metastatic lesions of other structures in an average of 51.6 days.

GENERAL SUMMARY.

For convenience the results are summarized in tabular form. Table I shows a comparison of the primary reaction in the groups of female and male rabbits inoculated intradermally. In Table II the incidence of metastatic lesions is shown in the various groups of animals. In Table III are shown the character and date of appearance of the generalized lesions (exclusive of orchitis) in the various groups of animals.

The results presented in Tables I, II, and III, together with a study of the individual protocols, permit the following general statements to be made relative to the effect of the factors studied upon the course of experimental syphilis in the rabbit.

Sex.—In the group of females inoculated intradermally the lesions at the site of inoculation were in general less marked than in the group of males similarly inoculated. Moreover, they attained their maxi-

mum size earlier and began to recede earlier than was the case in the males. In none of the females was there clinical evidence of the production of generalized lesions, while in the corresponding group of males metastatic lesions of skin or bones detectible by clinical examination occurred in one instance, or 14.3 per cent. In both groups there was wide variation in the character of the initial reaction in individual rabbits and in the time required for it to reach its maximum size.

TABLE I.

Comparison of Primary Reactions in Male and Female Rabbits Inoculated Intradermally with Treponema pallidum.

Group.	Rabbit No.	Character of primary lesion.	Incubation period.	Maximum diameter of primary lesion.	Time of appearance of maximum size of primary lesion.
			days	mm.	days
A. 5 females.	1	None.			
	2	Indurated papule.	11	4	15
	3	Transient indurated area.	10	1	10
	4	Chancre.	19	10	52
	5	Indurated papule.	10	8	21
Average.....			10	4	19.6
B. 7 males.	6	Transient indurated area.	31	1	31
	7	Three indurated papules.	31	5	?*
	8	Chancre.	10	8	74
	9	"	6	15	74
	10	"	10	18	?*
	11	"	10	10	61
	12	Indurated papule.	10	5	19
Average.....			15.4	9	

* Lesion still increasing in size at the end of the experiment.

Age.—In the five young males inoculated intratesticularly the disease was not greatly different from that observed in the older males similarly inoculated. The initial reactions were slightly slower in making their appearance, but the magnitude of the reaction, as judged by enlargement of the testicles, was somewhat greater. The percentage incidence of metastatic orchitis was almost the same in the two groups. The younger animals showed a slightly greater incidence of

generalized lesions involving structures other than the testicles, although the difference was insignificant, but the lesions were smaller, fewer in number, and confined entirely to the skin, and there was no instance of severe generalized lesions. Moreover, the occurrence of metastatic lesions (skin) in the younger animals was definitely delayed.

Method of Injection.—In the animals inoculated by the intratesticular route the development of the primary reaction and the incidence of generalized lesions involving skin and bone were much greater than in

TABLE II.
Incidence of Metastatic Syphilitic Lesions in Various Groups of Rabbits.

Group and method of inoculation.	No. inoculated.	Metastatic orchitis.			Generalized lesions (bone, skin, eye).		
		No.	Per cent.	Average time of appearance.	No.	Per cent.	Average time of appearance.
Intradermal inoculation.							
A. Adult females.	5			days	0	0	days
B. Adult males.	7	3	42.8	87	1	14.3	84
Intratesticular inoculation.							
C. Young males.	5	4	80	43.7	4	80	64
D. Adult males, not castrated.	7	6	85.7	41.5	5	71.4	54.7
E. " " castrated.	5	4	80	46.7	3	60	69
Scrotal implantation.							
F. Adult males.	6	3	50	54.9	3	50	51.6

the animals inoculated intradermally. Thus, of the latter group only one, or 14.3 per cent, showed a secondary metastatic lesion (skin), while in the group inoculated by the testicular route the incidence of metastatic skin and bone lesions was as high as 71.4 per cent. The difference in the character of the disease exhibited by these two groups of animals inoculated in a different manner is quite striking. Intratesticular inoculation produced a much more violent local reaction and a much greater incidence of generalized lesions than did intradermal inoculation.

Effect of Castration.—In the group of animals with unilateral orchitis in which the infection was allowed to run its course without any attempt at suppression by removal of the initial focus, metastatic lesions appeared more promptly and in slightly greater incidence than

TABLE III.

Character and Date of Appearance of Generalized Syphilitic Lesions (Exclusive of Orchitis) in Various Groups of Rabbits.

Group.	Rabbit No.	Generalized syphilis (exclusive of orchitis and lymphadenitis).	Lesions in order of their appearance.	Recur-rence.
Intradermal inoculation.				
A. 5 females.	1	—	<i>days</i>	
	2	—		
	3	—		
	4	—		
	5	—		
B. 7 males.	6	—	S(89), PB(89), PB(89)	
	7	—		
	8	—		
	9	—		
	10	+++		
	11	—		
	12	—		
Unilateral testicular inoculation.				
C. 5 young males.	13	+	S(66)	
	14	++	S(62, 73)	
	15	+	S(60)	
	16	—		
	17	+	S(70)	
D. 7 males.	18	+	S(62)	
	19	—		
	20	++	S(49, 84)	
	21	++	S(49, 60)	
	22	++++	S(56), PB(60), E(60), S(64)	
	23	—		
	24	+++++	S(49), PB(52), S(53, 55, 60)	

E indicates eyes; S, skin; PB, periosteum and bone.

TABLE III—*Concluded.*

Group.	Rabbit No.	Generalized syphilis (exclusive of orchitis and lymphadenitis)	Lesions in order of their appearance.	Recurrence.
Unilateral testicular inoculation, castration.				
E. 5 males.	25	++	S(84, 90)	+
	26	—		+
	27	++	PB(70), S(85)	±
	28	—		—
	29	+++	PB(52, 53, 63)	+
Scrotal implantation.				
F 6 males.	30	++++	S(46), PB(52), S(60), PB(66)	
	31	+	S(70)	
	32	±	S(60)?	
	33	+	S(60)	
	34	—		
	35	+++	S(49), PB(60), S(60)	

in the group of animals in which the initial local reaction was suppressed by removal of the focus itself through castration. The percentage differences are not very great, but they are constantly in favor of the first group, as is the time interval of appearance. This experiment is not in strict accord with similar experiments observed by Brown and Pearce (3) in the past, and while the reasons for this difference are not clear the fact may be noted that the incidence of generalized lesions in uncastrated animals is somewhat higher than that reported by these authors, also that there was a high percentage of recurrence of primary lesions.

DISCUSSION.

The results are sufficiently clear-cut to permit of a generalization on several points. The difference in the character of the initial local response and in the subsequent production of generalized lesions, exhibited by the methods of intradermal and intratesticular inoculation, is striking, and can scarcely be explained upon the basis of biological variation in groups of animals. One is forced to conclude either that the superficial layers of the skin of the rabbit (the scrotum excluded) constitute a relatively unfavorable site for the active proliferation of

the treponemata when small amounts are introduced, or else that the defensive mechanism at this spot is brought to such a level as almost completely to suppress the initial reaction, without, however, preventing general invasion of the body with lymph gland involvement. By contrast the testicle is a much more favorable site for the setting up of a clinically recognizable lesion. Whether this is due to the presence in the testicle of an abundance of substances favoring growth of the treponemata, or to the absence of conditions that favor the operation of factors of resistance it is impossible to say. Perhaps both influences may be at work side by side. The successful production of primary reactions in the scrotum by implantation of bits of tissue containing treponemata does not in our opinion alter the generalization that the primary reactions obtained by testicular inoculation are greater than those obtained by intradermal inoculation. In the case of the scrotal implantations the inoculum was of necessity much larger than that employed for intratesticular and intradermal inoculation.

The difference in the behavior of the females and the males toward intradermal inoculation is of interest. In the former the local reaction was much less prominent than in the males, attained its maximum size more rapidly, and cleared up more promptly. None of the five females developed generalized lesions of other structures, whereas in two of the seven males an orchitis developed, and in a third there was evidence of generalization of the virus in the shape of skin and bone lesions. The behavior of these five females was different from that of eight normal females formerly reported in a paper (4) dealing with the reaction of pregnant females to inoculation with *Treponema pallidum*. In that paper it was stated that non-pregnant female rabbits reacted to intradermal inoculations of the vulva with the prompt development of characteristic indurated lesions at the site of inoculation. Pregnant animals, however, when inoculated under similar conditions were found to react either not at all or with much less marked clinical phenomena. In the five non-pregnant animals reported upon in this paper the reaction to intradermal inoculation approached more closely that exhibited by the pregnant animals in the foregoing paper. This discrepancy in results is probably explainable on the basis of a difference in virulence or a difference in the severity of the prevailing disease since a difference of the same character was observed in the behavior of the male animals of the two series.

Whatever its explanation, it serves to show that the behavior of any one group of rabbits toward infection with *Treponema pallidum* is by no means indicative of what may be expected subsequently in another group of animals even if infected with the same strain. In our opinion this point has not received sufficient attention, particularly from some of those workers in experimental syphilis who have been attempting to classify strains on the basis of the primary reactions exhibited by the rabbit host. Many of the variations in primary reactions that have been proposed as characteristics for differentiating one strain from another were, to judge from their description, no more marked than those exhibited by the group of female rabbits reported upon in this paper.

It is of interest, also, that in all of the five females that were inoculated intradermally the organisms were able to invade the body and localize in distant lymph nodes in spite of the relatively slight local lesion at the portal of entry or the total absence of the same. This observation parallels similar observations by Neisser in monkeys and is in agreement with previous work (5) in which it was demonstrated that *Treponema pallidum* might make its way through the genital mucosa of rabbits and invade the body, without calling forth any reaction at the site of inoculation.

With respect to the factor of age, the results were not sufficiently clear-cut to warrant any broad generalization. In general, the younger animals reacted with a more violent initial lesion, and were apparently able to keep the subsequent manifestations under control for a slightly longer period of time, as well as to prevent the occurrence of any severe generalized lesions. There was a rather striking uniformity in the manner in which the individual animals in this group reacted to the infection at the site of inoculation.

The effect of removal of the initial focus on the subsequent production of generalized lesions, which in former experiments by Brown and Pearce (3) resulted in a greater incidence of the latter, was not observed in the experiments herein reported. In those animals in which the primary reaction was removed by castration the incidence of generalized lesions was no greater and the time of occurrence was distinctly postponed. The former experiments seemed to point definitely to an influence on the course of the infection exerted by the initial lesion. Procedures calculated to suppress the initial lesion (removal, insufficient treatment with arsenicals) resulted in a greater incidence,

within certain time limits, of generalized lesions in the animals in which the interference with the initial lesion was instituted than in the controls. Upon this basis an hypothesis spoken of as a "law of inverse ratio" was formulated. The experiments herein described would seem to indicate that under conditions as yet unknown there may be exceptions to this principle.

CONCLUSIONS.

1. Rabbits inoculated intradermally with active syphilitic virus are within specified time limits less prone to develop generalized syphilis recognizable by clinical methods of examination than those inoculated intratesticularly.

2. The primary reaction to intradermal inoculation of syphilitic virus was distinctly less marked in females than in males.

3. Young rabbits react to an intratesticular inoculation of the syphilitic virus with a slightly more marked initial lesion than do older animals similarly inoculated, and tend to postpone slightly the appearance of subsequent generalized lesions, which are less severe in character and extent.

4. Suppression of the initial reaction by excision does not always lead to an increased incidence or early appearance of subsequent generalized lesions within an observation period of 90 days.

5. In rabbits inoculated intradermally in which no reaction or only a slight reaction developed at the site of inoculation, general invasion of the body by the treponemata may take place, as evidenced by positive lymph node transfer, although no generalized lesions may be observable.

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