

EFFECT OF CORTISONE ON THE COURSE OF EXPERIMENTAL SYPHILIS IN THE GUINEA-PIG

I. EFFECT OF PREVIOUSLY-ADMINISTERED CORTISONE ON GUINEA-PIGS INFECTED WITH *TREPONEMA PALLIDUM* INTRADERMALLY, INTRATESTICULARLY, AND INTRAVENOUSLY*

BY

KONRAD WICHER AND ADAM JAKUBOWSKI

Department of Dermatology and Venereology, University School of Medicine, Bialystok, Poland

Of the smaller experimental animals, the rabbit is most frequently used for studies on experimental syphilis. The guinea-pig has sometimes been used, but the results have been divergent and the suitability of this animal for such experiments has not been clearly demonstrated.

Kolle and Evers (1926) are of the opinion that the guinea-pig belongs to the group of laboratory animals that does not react symptomatically to infection with *T. pallidum*, Gastinel, Vaisman, and Vaisman (1955) are of the same opinion, and Truffi (1910), Hoffman (1910), Uhlenhuth and Miltzer (1909-14), and other authors state that it has been possible to induce symptomatic changes in the guinea-pig infected with *T. pallidum* in only a small proportion of cases.

Kato (cited by Turner and Hollander, 1957) defined the sensitivity of the guinea-pig to *T. pallidum* as between that of the rabbit and that of those animals (mice, rats) which react asymptotically to infection with *T. pallidum*, but the results of his investigations were not convincing. The problem was also studied by Turner and Hollander (1957), whose experiments suggested that symptomatic changes were frequently observed in the guinea-pig. In order to obtain clearer changes they employed mucin and calcium chloride. They found that the guinea-pig does not display symptomatic changes after infection as does the rabbit and is not so valuable as the hamster for the investigation of experimental syphilis.

Wicher, Lesinski, and Jakubowski (1959), in preliminary experiments on the course of experimental syphilis in the male guinea-pig, found that,

after intradermal inoculation of a suspension of *T. pallidum* into the scrotal region, 60 per cent. displayed symptomatic changes. In later investigations (Lesiński, Wicher, Zajac, and Jakubowski, 1960), they found that 90 to 100 per cent. of guinea-pigs developed lesions after inoculation in the scrotal region with suspensions of the Nichols strain of *T. pallidum* containing 10^6 treponemes/ml. These results indicate that the guinea-pig should not be regarded as reacting asymptotically to *T. pallidum* infection. By administering cortisone an attempt was made to isolate the previously injected *T. pallidum* from the immunity mechanism of the host and to give the micro-organism an opportunity to develop freely; it was expected that extensive changes with a large number of treponemes would be noted. According to DeLamater, Saurino, and Urbach (1952), rabbits infected with *T. pallidum* and inoculated at the appropriate moment with cortisone become an abundant source of treponemes. In the study here presented we examined the effect of the previous administration of cortisone on guinea-pigs infected with *T. pallidum* by various routes.

Material and Methods

39 male and 20 female guinea-pigs were used for these experiments. The animals were divided into three groups according to the method of infection:

Group I.—These animals were infected intradermally in the region of the scrotal fold with 0.4 ml. of a suspension of *T. pallidum* (Nichols strain) containing 2×10^6 treponemes/ml. This group consisted of twenty male animals, fourteen treated with cortisone and six serving as controls.

Group II.—These animals were infected intratesticularly with 0.5 ml. of a *T. pallidum* suspension containing

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2×10^9 /ml. Both testicles were infected. The group consisted of nineteen male animals, thirteen treated with cortisone and six serving as controls.

Group III.—These animals were infected intravenously with 0.5 ml. of a *T. pallidum* suspension containing 2×10^9 /ml. The group consisted of twenty female animals, fourteen treated with cortisone and six serving as controls.

All the animals were infected on the same day with the same suspension of *T. pallidum* obtained from the infiltrated testicles of infected rabbits. The treponemes used for the infection were suspended in a physiological saline solution and the number of treponemes was counted by the Morgan and Vryonis method on a dark-field.

The 11-dihydro-17-hydroxycorticosterone preparation of Continental Pharma (Belgium) was used for the experiments. Cortisone was administered in a suspension by intramuscular injection in doses of 7.5 mg./kg./day. The weight of the guinea-pigs used varied between 330 and 400 g. Cortisone was given in daily doses for 48 days at the same time of day, and each animal received a total of 120 mg. Administration began 7 days before infection. From the 4th day after infection the guinea-pigs were placed under observation until all the changes disappeared, after which they were examined twice weekly; their weight was checked once a week.

The appearance of Wassermann antibodies was examined. Blood was obtained by cardiac puncture, and all sera were examined every 4 weeks. The Wassermann reaction was performed by the classical technique, using Difco cardiolipin antigen. The Citochol test was also performed and the quantitative Kahn test as well, if the result was positive.

50 per cent. of the animals chosen at random from Groups I and II were killed 7 months after infection in order to examine the infectivity of the testicles, inguinal glands, spleen, and liver.

50 per cent. of the animals in Group III were killed after 9 months of observation, and the infectivity was examined of all the previously-mentioned organs, except the testicles. All the organs except the liver were examined as a whole, a piece the size of the guinea-pig spleen being cut out of the liver.

The infectivity was tested as follows:

The various organs were cut with scissors and homogenized in a glass homogenizer with 1.5 ml. saline solution. The suspension was shaken in a glass vessel, placed in a water bath at a temperature of 37°C. for 15 minutes, and then centrifuged. The supernatant fluid was used to infect rabbits intratesticularly and for dark-field examinations. The infected rabbits were examined both clinically and serologically, and if specific syphilitic changes were observed the material was examined by dark-field for the presence of *T. pallidum*. If no specific syphilitic changes were noted, the sera of these animals were tested by the classical serological methods; 4 months after infection, and after clinical and serological control, the animals in which no changes had been observed were once again infected intratesticularly with 1 ml. of a suspension of *T. pallidum* (Nichols strain) containing 10^9 /ml.

Specific infiltration of the testicles observed in the majority of cases 2 weeks after infection would seem to justify the contention that the rabbits were not affected with syphilis after injection of fluid from the tested organ of the guinea-pig.

Results (Table I)

Group I

Reaction in the form of skin lesions was observed between the 16th and 23rd day after infection in eight of the fourteen guinea-pigs of Group I receiving cortisone. The lesions, hypertrophic infiltrated nodules, appeared at the site of injection of *T. pallidum*. The diameter of these lesions varied from 3 to 5 mm. The bases and margins were not particularly marked, their thickness was never more than 2 mm. After 7 to 14 days they disappeared. Fig. 1 (opposite) shows the typical lesion observed in the guinea-pigs of this group.

Changes in the form of hypertrophied infiltrated nodules were observed on the 13th to 16th day after infection in each of the six control guinea-pigs. The diameter of these lesions varied from 8 to 14 mm., the bases and edges of these were clearly marked and, within a week, deep ulceration was noted on

TABLE I
RESULTS IN CORTISONE-TREATED GUINEA-PIGS AND CONTROLS AFTER INFECTION WITH *T. pallidum*

Group	Route of Infection	No. of Guinea-pigs	No. of Symptomatic Infections	Time (days)		Size of Lesions (mm. diameter)
				Incubation	Duration of Lesions	
I	Cortisone	14	8	16-23 (Mean 19.9)	7-4 (Mean 9.5)	3-5
	Control	6	6	13-16 (Mean 12.3)	21-28 (Mean 24.2)	8-14
II	Cortisone	13	No difference in clinical symptoms			
	Control	6				
III	Cortisone	14	No clinical symptoms.			
	Control	6				

the whole surface. The thickness of these lesions varied between 2 and 3 mm., and they disappeared between the 21st and 28th day after their appearance. Fig. 2 illustrates the characteristic lesion observed in this group.



FIG. 1.—Guinea-pig No. 644 from Group I, treated with cortisone, at the time when the symptoms were most noticeable.

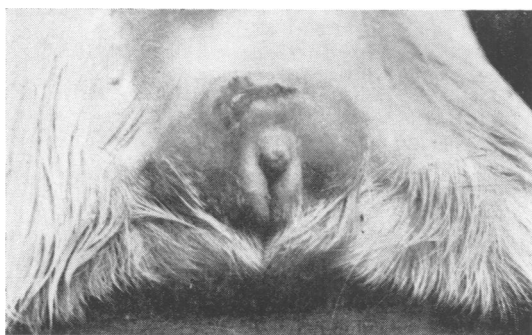


FIG. 2.—Guinea-pig No. 674 from Group I, not treated with cortisone, at the time when the symptoms were most noticeable.

Group II

Irregular recurrent infiltration of the testicles was observed after intratesticular infection in all the guinea-pigs of Group II, including the controls. Macroscopically it was impossible to determine the period of incubation or the duration of the infiltrations. Fluid obtained by testicular puncture performed during the period of testicular oedema, when it was still possible to introduce the testicles into the inguinal canal and during the period of subsiding oedema, was examined by dark-field. No *T. pallida* were found. The clinical observations of this group did not demonstrate any difference between the guinea-pigs treated with cortisone and the control animals.

Group III

12 months' observation of the female guinea-pigs in Group III disclosed neither macroscopic syphilitic

changes nor any difference in the normal development of the animals.

Serology.—Positive serological reactions were noted only in Groups I and II.

Of the fourteen guinea-pigs of Group I to receive cortisone, three were sero-positive in the 7th month of infection. Of the six controls, two were sero-positive 5 months after infection.

Of the thirteen guinea-pigs of Group II to receive cortisone, two were sero-positive but none of the controls. The Wassermann antibody titre of the guinea-pigs examined varied from 1 : 5 to 1 : 20. It should be emphasized that there were fluctuations in the antibody titre in the sera of the guinea-pigs with positive reactions.

In the guinea-pigs of Group III, not one positive Wassermann reaction was noted during the 9 months of serological observation.

Infectivity.—Table II shows the infectivity of the organs of 50 per cent. of animals in all three groups.

TABLE II
INFECTIVITY OF ORGANS IN CORTISONE-TREATED AND CONTROL GUINEA-PIGS AFTER INFECTION WITH *T. pallidum*

Group	No. of Guinea-pigs	Organ Infected				
		Testicles	Inguinal Glands	Spleen	Liver	
I	Cortisone	7	+	+	+	+
	Control	3	+	+	+	+
II	Cortisone	7	+	+	+	+
	Control	3	+	+	+	+
III	Cortisone	7	—	—	—	—
	Control	3	—	—	—	—

+ Infective agent present

— Infective agent absent

The guinea-pigs in Group I which received cortisone and also the control animals had treponemes in the inguinal glands, testicles, spleen, and liver, *i.e.* in all organs examined, and similar results were obtained with the animals in Group II.

No treponemes were found in the organs of any of the animals in Group III.

Discussion

Only in the guinea-pigs from Group I, infected with *T. pallidum* intradermally in the region of the scrotal fold, was it possible to observe the effect of cortisone on *T. pallidum* infection and to note the differences in the period of incubation and in the duration and nature of the changes caused.

In Group II, infected intratesticularly with *T. pallidum*, it was not possible to observe any macroscopical difference in the pattern of infection. Nor did the serological tests and the tests for infectivity

of the organs examined show any differences in the course of infection. It was not therefore possible to determine the effect of cortisone in these animals.

In Group III, intravenously infected, there were no macroscopic changes and the serological tests and organ infectivity tests also gave negative results. (These negative results have been due to the fact that the animals used were females.)

Earlier experiments have shown that intravenous injection of live *T. pallidum* caused infection when administered to male guinea-pigs, since extracts from the organs of these animals injected intratesticularly into rabbits caused specific syphilitic changes. It may also be assumed that the number of organisms intravenously injected was insufficient to cause general infection of the females and that the treponemes were destroyed by the guinea-pig host.

It was not possible to demonstrate any differences between the animals which received cortisone and those which did not, as regards the production of antibodies. Here it should be pointed out that Wassermann antibodies very rarely occur in experimental guinea-pigs. These results support the findings of Lesiński, Wicher, Spett, and Zajac (1959), who studied the occurrence of Wassermann and immobilizing antibodies in the infected guinea-pig.

Other studies show that cortisone has no effect on the spread of *T. pallidum* infection in the guinea-pig or on the development of *T. pallidum* in the various organs. This could be inferred from the time which elapsed before changes appeared in the infected rabbits. All the rabbits infected intratesticularly with extracts from the organs of the experimental guinea-pigs manifested symptomatic lesions (circumscribed orchitis) within 5 to 6 weeks of infection. If, however, a large number of *T. pallida* had been present in any organ, the infected rabbit would have displayed lesions in the form of diffuse orchitis which would have appeared much earlier.

Conclusions

- (1) Cortisone administered to guinea-pigs a week before intradermal infection prolongs the incubation period, shortens the duration of the lesions, and changes the nature of the skin lesions.
- (2) Cortisone injected in the same doses and at the same time into guinea-pigs intratesticularly and intravenously infected, has no effect on the course and nature of the infection.
- (3) Only the intradermal infection of guinea-pigs with *T. pallidum* permits the effect of cortisone on the course of experimental syphilis to be observed.

Summary

The effect was investigated of previous injections of cortisone on the course of experimental syphilis in guinea-pigs infected intradermally, intratesticularly, and intravenously with *T. pallidum*. Cortisone administration was begun on the 7th day before infection, and the guinea-pigs received cortisone in daily doses of 7.5 mg./kg. for 48 days.

It was found that cortisone prolongs the incubation period, shortens the duration of lesions, and affects the character of the changes in intradermally infected guinea-pigs, but the same doses had no effect on animals infected intratesticularly and intravenously. Serological tests and the infectivity of the organs were not affected by the administration of cortisone.

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Effet de la cortisone sur la syphilis expérimentale chez le cochon d'Inde. I.

RÉSUMÉ

On étudia l'effet d'injections préalables de cortisone sur la syphilis expérimentale chez les cochons d'Inde infectés de *T. pallidum* par la peau, les testicules, et les veines. L'administration de la cortisone commença le 7^e jour avant l'infection et continua en dosage quotidien de 7,5 mg./kg. pendant 48 jours.

La cortisone prolongea l'incubation, abrégea la durée des lésions cutanées, et changea le caractère des lésions chez les animaux infectés par la voie cutanée, mais elle n'eut aucun effet chez les animaux infectés par la voie génitale ou veineuse.

La cortisone n'eut aucun effet ni sur les tests sérologiques ni sur l'infectivité des organes.