AN EXPERIMENTAL RESURVEY OF THE BASIC FACTORS CONCERNED IN PROPHYLAXIS IN SYPHILIS

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The prevention of the primary invasion of the male by the syphilis spirochete, as a means of minimizing the loss of effectiveness which is incident to established disease, still constitutes one of the most pressing problems of military medicine. Further, as the organized efforts directed toward the control of this disease in the civil population slowly move forward it becomes increasingly more evident that, sooner or later, a suitable prophylactic method must be worked out for this group if these efforts are to be attended by the degree of success which the gravity of the situation warrants. With the former problem in mind and anticipating the latter, an experimental resurvey of the basic factors in this field has been attempted.

The general theme itself is one of considerable antiquity, the first mention in medical literature being contained in "De Morbo Gallico" published by the great Venetian physician, Fallopus, in 1564 and in which he advocates the application of wine to the exposed area as a means of prevention. The first advocate of the use of mercury is credited to Agato in 1733 as quoted by Kolmer (1). The period of great productive activity in syphilis, which marked the turn of the present century, also witnessed the placing of this subject upon an experimental basis through the work of Metchnikoff and Roux (2) in which the protective value of calomel was demonstrated in experimental animals. From that time until the present the character of the experimental work in prophylaxis has been colored by the current state of the knowledge of experimental infection of animals by the syphilis spirochete. The work of Metchnikoff and Roux, of A. Neisser (3) and of Siebert (4), which lead to a disagreement as to the value of calomel, was carried out with human or passage virus and utilized the higher and lower apes as experimental animals. Berterili (5) and later Parodi (6) demonstrated the susceptibility of the rabbit to the disease and the relative ease with which laboratory strains of the organism could be propagated. Pertinent then to the following study were the reports of Reasoner (7) and of Browne and Pearce (8) which demonstrated the ability of the syphilis organism to penetrate an intact mucous membrane and of Pearce and Browne (9) in which the role played by the lymph glands in experimental disease was portrayed. Then followed the work of Kolle and Evers (10) which demonstrated the remarkably short time interval which is required for the organism to migrate from an abraded skin surface to the regional lymphatic glands. This work has been recently repeated by Tani (11) and the time interval for the migration given as 5 minutes for the rabbit. These latter findings rather tend to obviate the results obtained in previous experimental work in prophylaxis in which scarification methods were utilized.

There thus seems to be available at the present time the experimental knowledge and methods which render possible an acceptable study of the essential factors concerned in the primary invasion by the pallida.

In the present study the first essential step was considered to be the development of an experimental method which would simulate as closely as possible the mechanism which is operative in female to male transmission in the human. This presupposed the maintenance of the intact integument of the exposed area together with whatever mechanical or biological defense mechanisms may be inherent thereto, thus obviating the use of scarification which permits an unnatural freedom of ingress for the organism. Because of these requirements the so-called contact method of experimental infection was considered the most suitable. A detailed description of this method, together with some observations upon the type and character of the primary lesions produced, has been published elsewhere (12), but, due to the frequency with which technical references are made in the following text, the technic will be here reviewed.

The method consists of an exposure of the genital mucosa of the male rabbit by means of a tissue emulsion containing numerous virulent and actively mobile treponema of a laboratory strain. The emulsion is prepared from the testicle of a strain animal in the acute orchitic stage of experimental syphilis. The tissue is ground in a mortar, a small amount of normal salt solution added and the mixture thoroughly agitated. Emulsions containing an average of from 4 to 6 treponema in each darkfield are considered suitable for the production of uniform results. The animal to be exposed is placed upon an animal board and a small pledget of cotton, saturated in the emulsion, is carefully packed into the preputial sac and maintained in position by clamping the fur about the orifice with a hemostat. At frequent intervals during the course of the exposure the clamp is removed and additional emulsion applied to the cotton by means of a medicine dropper. In all manipulations care is exercised to avoid injury to the soft tissues. The exposure may be allowed to continue for any desired length of time.

The lesions produced upon the genital mucosa by this mode of inoculation may differ in many respects from the usual manifestations of experimental syphilis. They may vary in intensity from a typical indurated chancre to an indefinite erythema which may progress to a definite sclerosis or may recede without further change. In point of location the lesions are equally distributed between the penis and the mucous lining of the preputial sac. The confirmation of all diagnosis is based upon the finding of the treponema by darkfield examination. In some atypical lesions a small biopsy is necessary in order to obtain suitable material for the darkfield preparation. The percentage of positive results to be obtained by the technic is high, dependent somewhat upon the number of spirochetes in the emulsion.

In the interpretation of the lesions of the genital mucosa of the rabbit, especially those which do not progress to chancre formation, the possibility of confusion between experimental syphilis and spontaneous spirochetosis of rabbits is always present. The two conditions may simulate each other very closely and the causative organisms cannot be differentiated by visual means. As a safeguard in this respect, in the present

work, all animals were observed for a 30-day period before being used in an experiment and all animals showing any deviation from the normal were excluded. In cases of doubtful lesions following exposure, a small section of the lesion is removed and implanted into the scrotum of a normal animal. If the suspected lesion is spirochetosis no further symptoms develop whereas a chancre is produced if the material contained the pallida.

The contact method thus seems to offer an entirely acceptable vehicle for the study of the factors concerned in prophylaxis.

The second essential step in the general study was considered to be a determination of the time factor in the penetration of the intact mucosa of the rabbit by the syphilis spirochete. Rather exact data upon this point seems necessary to a clear idea of the length of time during which the organism may occupy a vulnerable position upon the surface of the integument and thus be susceptible to the influence of agents applied directly to the exposed area. A portion of the details of this phase of the general study has been previously published (13), but because of their importance to the general theme the work is reviewed in some fullness.

Two experimental approaches were utilized in the study of the penetration time. The first embraced an actual demonstration of the organism in the process of migration through the superficial layers of the mucosa. To accomplish this about thirty animals were used. They were exposed, in groups, in the usual way for time intervals varying from one to four hours. At the completion of the desired length of exposure the animals were sacrificed, the penis amputated and prepared for sectioning. The sections were stained by Warthin-Starry silver method and examined for typical examples of the spiral forms in the superficial tissue structures.

The results of this approach are of interest in several respects. In sections from tissues which were exposed for one and two hours not any acceptable examples of penetrating organisms were found, although not infrequently they were observed upon the surface or in the folds of the mucous membrane. In sections of tissues exposed for three hours some clear examples were found of spiral forms well embedded in the tissue. Plate No. 1 depicts a single spirochete rather deep in the tissue structures and Plate No. 2 shows several spiral forms well into the mucous membrane. Again in this work there is a danger of mistaking the organisms of a latent lesion of rabbit spirochetosis for the penetration of syphilis. This can only be overcome by serial section work and a determination of the extent of the lesion in the case of spirochetosis. Not any acceptable examples of penetrating organisms were observed in those portions of the penis in which the squamous type of surface epithelium was present. The most frequent site of entrance was found to be the extreme distal portion of the penis and the lining of the flaring urinary meatus, in both of which localities a low cuboidal type of cell predominates.

The second approach to the time factor consisted of an estimate of the interval required by the syphilis spirochete to penetrate the

tissue to a sufficient depth to escape the influence of drastic germacides applied at the surface of the exposed area. This was carried out in the following manner.

Seven groups of three animals each, together with suitable controls, were exposed to the virus in the usual way. In representative groups the exposure was terminated at thirty-minute intervals, after the first hour, and the genital mucosa subjected to vigorous disinfection consisting of thorough cleaning with soap and water and successive applications of ether, tincture of iodine and alcohol, 96%. The treatment was as thorough as possible and an effort was made to assist the process by placing tension upon the penis and obliterating the folds of the mucosa. The controls were exposed for one hour and then returned to the cages without further attention. In all cases in which the experimental animals failed to develop evidence of infection, after a suitable period of observation, the animal was sacrificed, and the inguinal and popliteal lymph glands dissected out and implanted into the scrotum of normal animals for the purpose of recovering the strain in cases of symptomless infection. The details of this portion of the work are set forth in Table No. 1.

Table No. 1

Drastic disinfection following varying periods of exposure:-

CONTROL GROUP

Animal No.	Observation period (days)	Results	Gland transfer to animal - no.	Observation period (days)	Result
362	47	Positive			
363	61	"			
364	148	Negative	394	100	Negative
365	40	Positive			
366	28	"			
407	53	"			
408	39	"			
409	39	"			
410	53	"			
411	33	"			

EXPERIMENTAL GROUPS

Exposure time	Animal no.	Observation period (days)	Result	Gland transfer to animal no.	Observation period (days)	Result
Group 1	347	148	Negative	395	87	Negative
1 hour	348	148	"	396	60 (1)	
	349	148	"	397	100	"
Group 2	350	148	"	398	100	"
1-1/2 hours	351	148	"	399	100	"
	352	46	"	(1)	---	
Group 3	353	148	"	400	79	Negative
2 hours	354	148	"	401	100	"
	355	148	"	402	100	"
Group 4	421	154	"	499	130	"
2-1/2 hours	422	44	"	(1)	---	
	423	154	"	500	88	Positive
Group 5	418	53	Positive			
3 hours	419	154	Negative	501	130	Negative
	420	154	"	502	130	"
Group 6	415	59	Positive			
3-1/2 hours	416	148	Negative	498	130	"
	417	90	Positive			
Group 7	412	88	"			
4 hours	413	39	"			
	414	66	Negative			

(1) Intercurrent death.

As indicated in Table No. 1, with one exception all of the control animals developed darkfield positive penile or sheath lesions with an average incubation period of forty-four days, thus attesting to the potency of the emulsions used in the exposures. In the groups of animals which were exposed for less than 2 hours and the exposed area subjected to disinfection, all were apparently protected by the treatment. In the 2-1/2 hour group one animal which failed to develop a local lesion of a degree sufficient for recognition, evidently contracted a symptomless infection as demonstrated by the positive results obtained in the second passage animal. In the 3-hour group one animal developed the disease and in the 3-1/2 and 4-hour groups there was a preponderance of positive results.

From the above it seems logical to conclude that for a period of at least 2 hours, in the majority of instances, the invading organisms occupy

an entirely vulnerable position upon the surface of the mucous membrane and can be directly influenced by prophylactic agent applied to the exposed area. The results obtained in the 2-1/2 and 3-hour groups may indicate some degree of protection. After 3 hours it is evident that a sufficient number of organisms to produce disease have penetrated the superficial layers to a point where they are afforded protection from even severe disinfection, by the overlying tissue structures.

Because of the role played by mechanical cleansing in the practical application of prophylactic methods it seemed desirable to determine the degree of protection which should be expected from this agency alone. Animals in groups of three were exposed in the usual way and individual groups treated at 30-minute intervals up to 4 hours. Ordinary white soap was used and the cleansing process carried out as thoroughly as possible, utilizing an abundance of warm water. The controls for these groups were exposed for 1 hour and returned to the cages without further attention. The details of this portion of the study are shown in Table No. 2.

Table No. 2

The influence of mechanical cleansing following various periods of exposure:-

CONTROL GROUP

Animal No.	Observation period (days)	Result	Gland transfer to animal no.	Observation period (days)	Result
362	47	Positive			
363	61	"			
364	148	Negative	394	100	Negative
365	40	Positive			
366	28	"			
407	53	"			
408	39	"			
409	39	"			
410	53	"			
411	33	"			

EXPERIMENTAL GROUPS
Form of Treatment
Soap and Water

Exposure time	Animal no.	Observation period (days)	Result	Gland transfer to animal no.	Observation period (days)	Result
Group 1	356	148	Negative	403	100	Negative
1 hour	357	148	"	404	72	"
	358	148	"	405	72	"
Group 2	359	148	"	406	100	"
1-1/2 hours	360	64	Positive			
	361	106	Negative	388	100	"
Group 3	432	152	"	503	130	"
2 hours	433	152	"	504	130	"
	434	152	"	505	130	"
Group 4	435	152	"	506	130	"
2-1/2 hours	436	51	Positive			
	437	152	Negative	507	130	"
Group 5	438	51	Positive			
3 hours	439	51	"			
	440		Negative	508	130	"
Group 6	441	78	Positive			
3-1/2 hours	442	92	"			
	443	152	Negative	509	130	"
Group 7	444	51	Positive			
4 Hours	445	51	"			
	446	152	Negative	510	84	Positive

As indicated above, one animal in the 1-1/2 hour group developed a darkfield positive lesion. The 2-hour group apparently escaped infection as no lesions were observed and the second passage group remained negative. A majority of the animals became positive after exposure of 3 hours or longer. Nine of the 10 controls became positive.

From this series it seems evident that use of mechanical cleansing offers a high degree of protection if applied under 2 hours following exposure. This merely confirms a fact which has long been recognized in practical work.

Again reverting to the original theme, the next logical step in

the general study consisted of an evaluation of the prophylactic efficiency of mercury, especially calomel ointment, under controlled experimental conditions. In this phase data were collected along three closely allied lines. The first dealt with the general efficacy of calomel ointment as a preventive agent. The second concerned a comparison of the results obtained when the ointment was applied before the exposure with those obtained by treatment after exposure. The third represented an effort to determine whether the demonstrated efficiency of the drug as a preventive could be attributed to local or systemic spirocheticidal action.

The experimental routine in this phase of the study remained essentially the same. All of the animals were exposed for a period of 1 hour to emulsions rich in treponema. The control groups were given no further attention following the 1-hour exposure. The usual gland transfers were carried out in all cases in which the original animals failed to display evidence of primary infection. The ointment used was a freshly prepared calomel ointment compounded after the original formula of Metchnikoff, (33% in lanolin and petrolatum vaseline). Each treated animal was allotted 4 grams of the preparation and as much of this amount as possible was rubbed into the selected area. A certain percentage of the drug was lost in each case by becoming enmeshed in the surrounding fur. That a large dosage was absorbed was evidenced by the development of toxic symptoms, diarrhea and loss of appetite, in each of the treated animals.

In testing the actual value of the calomel ointment as a preventive agent a large group of animals was used and the results obtained indicated very clearly that a high degree of protection could be attributed to the compound. The details of this phase will not be given as the point is extremely well illustrated in the tables dealing with the remaining two phases under immediate consideration.

As a means of comparison between the administration of calomel ointment prior to and following the exposure, two groups of animals with suitable controls were used. In the first group the genital mucosa was thoroughly treated with the ointment, as much as possible of the allotted 4 grams being rubbed into the penis and sheath. Following the treatment the animals were subjected to the usual exposure for a period of one hour and at the completion of this time interval this group was returned to the cages without further treatment. In the second group the exposure of 1 hour was carried out and the treatment applied to the genital mucosa at the end of this time. As an additional control in this phase a small series of animals were treated by a liberal application of lanolin alone to the genital mucosa, before the exposure. All of the members of this group became positive, indicating that the mechanical presence of this substance did not serve as a deterring factor in the passage of the organism into the superficial layers of the mucosa.

In Table No. 3 the results of this comparison are set forth.

Table No. 3

CONTROL GROUP

Rabbit No.	896	Positive	30 days
	897	Positive	32 days
	898	Positive	44 days
	899	Positive	43 days
	900	Positive	29 days

Genital Mucosa exposed for 1 hour.

Calomel ointment applied prior to an exposure of 1 hour

886	887	888	889	890	891	892	893	894	895	
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Original
973	974	975	976	944	977	978	979	980	981	Gland Transfer
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	

Calomel ointment applied after an exposure of 1 hour

876	877	878	879	880	881	882	883	884	885	
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Original
963	964	965	966	967	968	969	970	971	972	Gland Transfer
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	

As shown in the above table, all of the control group developed darkfield positive penile or sheath lesions with an average incubation period of 36 days. In the group in which the calomel ointment was applied to the genital mucosa prior to the exposure, all of the members seem to have been protected and no evidence of symptomless infection was detected in the second passage animals. In the group in which the treatment was

applied to the genital mucosa following an exposure of 1 hour all of the original animals remained clinically negative during the period of observation. The gland transfer, however, revealed that one member of the group had contracted a symptomless infection as evidenced by the production of a typical chancre in the second passage animal.

The results indicate that only a slight advantage is to be gained by the application of the prophylactic remedy prior to the exposure.

In the effort to gain accurate information in regard to the role played by the systemic action of mercury in prophylaxis, the following experimental routine was carried out. Two series of animals were utilized, together with proper controls. In one group of each series, following the usual exposure of 1 hour to the genital mucosa, the calomel ointment was thoroughly rubbed into the exposed area. In the remaining groups of each series the usual exposure was carried out, but the ointment was applied to a denuded skin area distantly removed from the actual field of exposure. For the inunction an area of the back was selected from which the fur was removed by use of a barium sulphite paste. Gland transfers were made in all cases in which there was no evidence of disease in the original animals.

The details for these groups are embodied in the following table.

Table No. 4

FIRST SERIES

896	897	898	899	900	
Pos	Pos	Pos	Pos	Pos	Original

Control Group. Genital mucosa exposed for one hour. Nichols strain.

876	877	878	879	880	881	882	883	884	885	
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Original
963	964	965	966	967	968	969	970	971	972	Gland
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Transfer

Group No. 1 - Calomel ointment applied to genital mucosa after an exposure of 1 hour.

866	867	868	869	870	871	872	873	874	875	
Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Pos	Original
	954	955		956	957	958	959	960		Gland
	Neg	Neg		Neg	Neg	Neg	Neg	Neg		Transfer

Group No. 2 - Calomel ointment applied to skin of back after an exposure of genital mucosa for 1 hour.

SECOND SERIES

675	676	677	678	679	
Neg	Pos	Neg	Pos	Pos	Original
671		672			Gland
Neg		Neg			Transfer

Control Group - Genital mucosa exposed for 1 hour. Nichols strain.

580	581	582	583	584	585	586	587	588	589	
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Original
673	674	675	676	677	678	624	669	679	680	Gland
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Transfer

Group No. 3 - Calomel ointment applied to genital mucosa after an exposure of 1 hour.

590	591	592	593	594	595	596	597	598	599	
Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Original
581		582	583	584	695	686	687	688	689	Gland
Neg		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Transfer

Group No. 4 - Calomel ointment applied to skin of back after an exposure of genital mucosa for 1 hour.

In Series No. 1 all of the control animals developed lesions of the genital mucosa in material from which the presence of the pallida could be demonstrated. Of the animals in which the calomel ointment was applied directly to the exposed area all of the originals apparently escaped infection. In the second passage group, however, one animal became positive indicating the presence of symptomless infection in one of the experimental group. Of the group in which the calomel ointment was applied to a site distantly removed from the actual point of exposure 7 were protected and 3 developed the disease.

In Series No. 2 only 3 of the control group developed evidence of the disease while two remained negative and were also negative in the second passage. This possibly indicates that the emulsion used was not sufficiently rich in treponema or that the organisms lacked the usual virulence. In the group in which the prophylactic treatment was applied directly to the exposed area all of the members were apparently protected. In the group in which the denuded skin area of the back was used as the site for the application of the ointment, 9 animals were apparently protected and one developed a penile chancre.

As a means of collecting further information upon the subject of the systemic action of mercury in prophylaxis, two additional small groups of animals were added to the general study. In the first of these the exposure was carried out in the usual way but allowed to continue for 2 hours. In the second the exposure was for 3 hours. At the completion of these time intervals the animals were treated by the application of 4 grams of calomel ointment to a skin area of the back from which the fur had been removed. The controls were exposed for 1 hour.

The following table details the results obtained in this group.

Table No. 5

CONTROL GROUP

Rabbit No.	1	Positive	46 days
	2	Positive	32 days
	3	Positive	32 days
	4	Positive	32 days
	5	Positive	32 days

Genital mucosa exposed for 1 hour.

Exposure 2 hours - Calomel ointment applied to skin of back.

6	7	8	9	10	
Neg	Neg	Neg	Neg	D	Original
82	83	84	85		
Neg	Neg	Neg	Neg		Gland Transfer

Exposure 3 hours - Calomel ointment applied to skin of back.

11	12	13	14	15	Original
Neg	Neg	Neg	Neg	Neg	

86	87	88	89	90	Gland Transfer
Neg	Neg	Neg	Neg	Neg	

The findings indicate that after 2 and 3-hour exposures, when the organisms may reasonably be expected to have migrated well into the mucosa, complete protection was accorded the animals by the application of the prophylactic agent to a skin area distant from the site of exposure. There was no indication of symptomless infection.

The results depicted in Tables Nos. 4 and 5 are surely open to the interpretation that a very considerable influence is attributable to the systemic action of mercury in the prevention of the primary invasion of the syphilis spirochete. The difference which exists between the two methods of application of the preventive measure may well be explained by a more ready absorption of the drug by the genital mucosa than by the skin of the back. This supposition is rather supported by the fact that toxic symptoms were much more severe and prolonged in the animals in which the ointment was applied to the mucosa than in those treated by application to the back.

The one case of symptomless infection which was encountered represents an occurrence not infrequently met with in experimental syphilis, but which has no analogue in human disease unless it be invoked to explain the instances of unsuspected disease which are met with in clinical work and in which the patient has not definite knowledge of the date of the original infection. Because of the occurrence of this phenomenon in experimental work the instance cannot be construed as indicating that the animal in question received only sufficient mercury to suppress the primary evidence of infection, but insufficient to cause complete sterilization.

In all of this work the Nichols strain of the pallida was used. The animals were healthy male rabbits of the Chinchilla breed and the period of observation in the original groups averaged 140 days. In the gland transfer animals the observation averaged 100 days. In only one case was it necessary to differentiate between experimental syphilis and spontaneous spirochetosis of rabbits. This animal developed a darkfield positive penile lesion of an extremely atypical nature. A portion of the penis was amputated and implanted into the scrotum of a normal animal where it gave rise to a typical chancre of experimental syphilis.

CONCLUSION

Through the use of experimental methods a resurvey of the basic factors concerned in prophylaxis in syphilis has been carried out.

The so-called contact method of experimental infection has been found to be entirely suitable for the particular problem in that the ingress of the organism is not accelerated, the natural defense forces remain operative and the exposure can be carried out for any desired time interval.

The actual depth to which the syphilis organism may penetrate the intact mucosa of the rabbit within 3 hours after being placed upon the unbroken surface has been shown in photomierograph.

The time limit within which thorough chemical disinfection of the exposed area may exert a protective influence has been established. Drastic disinfection served to prevent disease when applied up to 2 hours exposure, but was ineffective after 3 hours exposure.

Mechanical cleansing of the exposed area with white soap and water afforded a high degree of protection up to 1-1/2 hours exposure, but the effectiveness decreased after 2 hours.

The general effectiveness of calomel ointment has been demonstrated.

There was no striking superiority of pre-exposure application of the preventive agent over its application 1 hour after exposure.

That the preventive efficiency of mercury is due to the systemic spirocheticidal action seems to be indicated by the high degree of protection which was afforded animals in which the ointment was applied to a skin area of the back following exposure of the genital mucosa for 1, 2 and 3 hours.

If these inferences are correct and may be applied to the human there are 2 distinct phases in prophylaxis which must be considered. The first concerns that period of time in which the invading organisms occupy a vulnerable position upon the surface of the exposed area and may be influenced by antiseptic agents applied directly to that area. The second phase beginning from 2 to 3 hours after exposure must concern itself with systemic spirocheticidal therapy and would seem to bring up the question of the effective dosage of the agency used for this particular purpose.

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