

PART II

Strain Source and History

Menstruum of Inoculation

Table II - Summary of Source of Menstruum

STRAIN SOURCE

It was obviously desirable to use the rabbit as the source of *T. pallidum* if found infective. For making the inoculum from the testicle showing acute syphilitic orchitis would permit access to large quantities of the organism more or less at will. The first experiment was designed to test the infectiveness of the material and demonstrate the virulence of the Nichols strain upon parenteral injection. As discussed in the clinical observation section of the paper, comparison of various strains of the organism are recorded. So that in this section only a discussion of the strains and sources will be given.

NICHOLS STRAIN

This strain, more widely utilized than any other for experimental studies in the rabbit, was selected for initial trial for obvious reasons. A tremendous body of experimental knowledge regarding this strain in the rabbit was available, so that an opportunity would thus exist to compare the effects in the rabbit with those obtained in man. This strain was originally inoculated into the rabbit testes on June 30, 1912 by Nichols and Hough (26). It was obtained from the spinal fluid of a patient who suffered a CNS relapse following inadequate arsenotherapy for secondary syphilis. The strain, originally isolated in the Rockefeller Institute Laboratory was supplied to the VDRL in 1924 and maintained there in a manner which will be discussed later.

FREW STRAIN

The donor of this strain, Frew, Hospital No. 177828, was a patient of the VDRL at the USPHS Hospital, Staten Island. On about March 1, 1947, 15 days after the last sexual contact, the patient developed a penile ulcer. No local or systemic treatment had been given prior to admission to the hospital. Upon admission the patient was found to have an indurated, lightly-epithelialized lesion on the dorsum of the shaft of the penis and bilateral inguinal adenopathy. There was no evidence of secondary syphilis. The darkfield examination of serum obtained from the lesion was repeatedly negative, the battery of serologic tests for syphilis done upon admission was positive, and the Kahn titre was 512 units.

On March 24, 1947 under local anesthetic the chancre of 24 days duration was removed, emulsified by grinding in a mortar with sterile sand and normal saline, and the supernate was injected intratesticularly into several rabbits. Following excision the patient was put under penicillin therapy with aqueous solution of penicillin G., 40,000 units every 2 hours for 85 injections, a total of 3,400,000 units. Following therapy the patient was observed to become seronegative by 64 days and to have remained seronegative through 414 days after treatment at the time when he was last observed.

Observation of the animals revealed the development of a typical orchitis in the primary transfer animal, and subsequent transfer animals demonstrated the same clinical response.

History of Strains of T. Pallidum

STREET STRAINS

The inoculum was prepared from the chancres of 3 individuals. Suspension No. 1 was taken from a 22 year old male who had had a penile ulcer of 14 days duration which appeared 5 days after last contact. Physical examination revealed 3 chancres on a long foreskin. Suspension No. 2 was made up of chancres from two individuals. The first, a 19 year old male with a penile ulcer of 24 days duration which appeared 12 days after contact. Physical examination revealed a small firm ulcer in the foreskin which was darkfield positive. The second donor for suspension 2 was a 22 year old male with a penile ulcer of 10 days duration which appeared 7 days after the last contact. There were 3 chancres on a long foreskin which were darkfield positive. Removal of the chancres took place between 1:30 and 3:00 P.M., and the last application of the material to the inoculated patient took place at 6:00 P.M.

All chancres were removed under local procaine anesthetic. The surface of the penis had been cleansed only with sterile saline solution prior to injection of the local anesthetic and no antiseptic of any kind was used during the operation. Immediately following removal of the chancre the individual was placed under penicillin therapy.

The donors were all soldiers belonging to the Army of the Country in which the study was done. Their infection had been acquired by contact with local prostitutes so that the organisms had never undergone animal passage.

HUMAN PASSAGE OF FREW OR NICHOLS STRAIN

From time to time patients originally inoculated with animal-passage material served as donors of chancre material for further experiments. Whenever this procedure was used the lesions, whether from the prepuce especially prepared for potential use as inoculum, or from the forearm, were removed under local anesthesia as described under the preceeding heading STREET STRAINS.

At times treatment was instituted immediately after removal of the chancre; at others, treatment was delayed to study the healing of operative wounds in syphilitic patients. In all patients from whom cutaneous chancres of the forearm or biopsy specimens were removed and in whom treatment was delayed, healing of the operative wound without evidence of secondary infection was observed.

MAINTENANCE OF ANIMALS AND STRAINS

As described elsewhere (7) an animal colony was maintained at the VDRL Staten Island to use in experimental work. The animal colony was in an old outbuilding of the hospital without air conditioning where the animals were kept in separate cages and were under care of a well-trained animal house attendant. To maintain strains of *T. pallidum* transfers from infected animals to uninfected stock were made at biweekly intervals. The technic utilized was that of intratesticular inoculation of an emulsion prepared by mincing of the lesion from the donor animal. Various menstrum were used throughout the years, some of which are described in this paper and which were used during the period this study was in progress.

To supply animals for use in the branch experimental laboratory animals inoculated at Staten Island were sent by Air express within several days of inoculation and were then held in cages at the local laboratory until needed.

CALCULATION OF PASSAGES

Transfers were not always made exactly at the two-week interval for various reasons. Furthermore, since an exact record was not kept in the case of the Nichols strain, no statement can be made as to the exact number of transfers which had been made over the period of 35 years. But if it is calculated that one was made at intervals of every 2 weeks, approximately 900 transfers would have been made.

It was not the custom to keep day-to-day record of strain transfers even for the Frew strain, so that a statement based upon transferring every 2 weeks permits the approximation of the passage-number which is given in strain history and menstrum of inoculation table.

STRAIN HISTORY AND MENSTRUM OF INOCULUM

Experiment Number	Strain	Source	Duration of Infection (in Donor Days)	Previous History	Menstrum (See PP. 44-45 of Introduction)
0101-0103	Nichols	Rabbit	32	{ Isolated in rabbit 1912 Obtained at Staten Island 1924 Transfer every 2 weeks Animal passage only 900 animal transfers	Beef heart infusion broth (Spirochetal broth)
0201-0202	Nichols	Rabbit	33		
0301-0302	Normal	Rabbit	—		Rabbit serum- Normal saline
0401-0404	Nichols	Rabbit	23	Animal passage only	{ Rabbit serum - Normal saline Donor rabbit infected with Nichols strain
0501-0503	Nichols	Rabbit	14	Animal passage only	
0601-0607	Frew	Rabbit	24	10th Animal passage	Rabbit serum- Normal saline Donor rabbit infected with Gilmore strain
0701-0707	Street	Human (3 VD pts.)	10-19	Never in experimental animal	Normal human serum-Normal saline.
0801-0804	Frew	Rabbit	20	12th Animal passage	Normal rabbit serum - Normal saline
0805-0808	Frew	Rabbit	15	12th Animal passage	Normal rabbit serum - Normal saline
0901-0907	Nichols	Human (Experimental penile chancre)	42	First human passage from animal	Normal human serum-Normal saline

Experiment Number	Strain	Source	Duration of Infection (in Donor days)	Previous History	Menstrum
0908-0911	Frew	Rabbit	29	12th Animal passage	Normal rabbit serum - Normal saline
1001-1004	Frew	Rabbit	21-28	16th animal passage	Normal rabbit serum - Normal saline
1005-1006	Nichols	Rabbit	21-28	Animal passage only	Normal rabbit serum - Normal saline
1007-1011	Frew	Human	79	First human passage after 12th animal passage	Normal rabbit serum - Normal saline
1101-1102	Frew	Human	42	2nd human passage after 12th animal passage and first human passage after 16th animal passage	Normal human serum - Normal saline
1103	Frew	Human	42	2nd human passage after 12th animal passage	Normal human serum - Normal saline
1104-1106	Nichols	Rabbit	27	Animal passage only	Normal human serum - Normal saline
1201-1211	Frew Nichols	Human	Frew 62 Nichols 20	Mixture { Frew-2nd human passage after 12th animal passage Nichols-1st human passage out of animal	Normal human serum - Normal saline
1301-1306	Frew	Rabbit	32	22nd rabbit passage	Syphilitic rabbit serum - Normal saline serum - Obtained from animal supplying infected testes.

Experiment Number	Strain	Source	Duration of Infection (in Donor days)	Previous History	Menstrum
1401-1403	Frew	Rabbit	36	23rd rabbit passage	Normal human serum- Normal saline
1501-1503	Nichols	Rabbit	23	Animal passage only	Syphilitic rabbit serum-Normal saline
1504-1507	Frew	Rabbit	18	25th animal passage	Syphilitic rabbit serum- Normal saline
1601-1611	Nichols	Rabbit	20	Animal passage only	Syphilitic rabbit serum- Normal saline
1701-1703	Frew	Rabbit	33	32nd Animal passage	Syphilitic rabbit serum- Normal saline
1704-1705	Nichols	Rabbit	33	Animal passage only	Syphilitic rabbit serum- Normal saline

} Serum obtained from animal supplying infected testes
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MENSTRUM

Date	Experiment Number	Composition
5-13-47	0101-0103	So called "Spirochetal broth" or beef heart infusion broth q.v.
5-14-47	0201-0202	Same as above .5 cc. total for 2 testes
6-28-47	0301-0302	Same as above
8-7-47	0401-0404	Same as above
8-10-47	0501-0503	Mixture of 4 cc. sterile normal saline and 4 cc. fresh unactivated rabbit serum taken from animal infected with Nichols strain for 26 days. 8cc. of mixture used for 1 testicle.
8-24-47	0601-0607	Same as above. Rabbit serum taken from animal having a 31 day infection with Gilmore strain. 8cc. of mixture per testicle, 2 testes used.
8-31-47	0701-0707	Mixture of equal parts of sterile normal saline solution and fresh unactivated non-syphilitic human serum. Suspension No. 1-8 cc. of mixture for 2 chancres. Suspension No. 2-4 cc. of mixture for 1 chancre.
9-12-47	0801-0808	Equal parts of sterile, normal saline solution and fresh unactivated, non-syphilitic rabbit serum. 8 cc. of mixture per testicle. Two testes used - one from rabbit furnishing material for 0801-0804 and one from another rabbit furnishing material for 0805-0808.
9-21-47	0901-0907	Equal parts of sterile, normal saline solution to fresh unactivated non-syphilitic human serum. Total of 8 cc. for chancre removed from human donor.
	0908, 0910, 0911	Equal parts of sterile, normal saline solution and fresh, unactivated non-syphilitic rabbit serum. Total of 6 cc of medium for 1 testicle. (Menstrum decanted after centrifugation)
	0910	2 cc. of emulsion described under 0908, 0910, 0911 was mixed with 2 cc. of a 3% mucin solution prepared as follows: an aqueous solution of 3% mucin prepared from Gastric mucin Granules, Oral, lot 2651 SP - 1518, F. Stearns & Co., Detroit, Michigan, was prepared 4 days before use and kept at refrigerator temperature until 1/2 hour before use. Equal quantities of spirochetal suspension and 3% mucin were then thoroughly mixed so as to give a final concentration of 1 1/2% mucin.
11-30-47	1001-1011	Equal parts of sterile, normal saline solution and fresh unactivated non-syphilitic rabbit or human serum for rabbit or human derived material respectively. The basic emulsions were diluted further with the same diluent to secure the desired concentrations of organisms.
1-11-48	1101-1106	Same as above
1-31-48	1201-1211	Equal parts of sterile, normal saline solution and fresh, unactivated non-syphilitic human serum. Total of 12 cc each of the components (24 cc.) for 3 chancres.
2-8-48	1301-1306	Equal parts of sterile, normal saline solution and fresh unactivated serum taken from the rabbit whose testicles were being used. 16 cc. of mixture for 2 testes.
2-14-48	1401-1403	Same as above
3-19-48	1501-1507	Same as above
5-9-48	1601-1611	Same as above 8 cc. for 2 tests.
7-4-48	1701-1705	Same as above 10 cc of mixture for 2 tests