

NEWBORN TETANUS ETIOLOGY: ISOLATION OF *CLOSTRIDIUM TETANI* FROM UMBILICAL TISSUES

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SUMMARY

Clostridium tetani has so rarely been isolated from the umbilical wound, that the authors' opinions are divided between different microorganisms as the etiological agents of the tetanus neonatorum.

Utilizing Bonnel's medium, agar-blood, and others, we have been able to isolate *Clostridium tetani* in 87.5% of 16 umbilical stumps and in 23% of 13 navels obtained from tetanus-infected patients. We also isolated *Clostridium perfringens* in 13 out of 33 cases, thus demonstrating the existence of an anaerobic condition which favours the growth of anaerobic germs on the umbilical stump.

The identification of *Clostridium tetani* through inoculation of cultures in mice (serum-neutralization test) was positive in 14 cases, while inoculation of ground umbilical stumps proved positive in 8 cases only. The reason for this difference would lie in the low toxin concentration found in the last material.

Pure cultures were obtained in 4 cases (previously obtained by KITASATO only, in 1889) and their morphobiological aspects were studied.

We give added strength to the clostridial theory of the newborn tetanus, which was lacking proof in regard to the problem of isolating *Clostridium tetani*. We point out the preferential location of *C. tetani* in the mummified umbilical stump, which would be the main focus for the toxi-infection.

INTRODUCTION

The etiology of the newborn tetanus has not as yet been satisfactorily explained in spite of the repeated efforts of many research workers, as shown by the large bibliography on the subject. The work already performed led to a tripartition of opinions of the Brazilian specialists in diseases of children: some of them accept the clostridic theory, others deny it, and there still are those which do not give it exclusivity.

The demonstration of *Clostridium tetani* on microscopical examination and on cultures of umbilical wounds and navels, as well as the failure of specific therapy and the good results obtained with penicillin in some cases, have discredited the role of the bacillus. It

was then suggested that it was present in the mummified umbilical stump, which presents favourable conditions for the development of this anaerobius. PINHEIRO^{3,4} has proved this fact experimentally.

In Latin America, in the United States and in Europe (PINHEIRO³) the situation was practically the same, because the few cases of demonstration of the tetanus bacillus in the umbilical wound were statistically overwhelmed by the inverse. This problem, still of great opportunity especially in underdeveloped countries like Brazil, has been discussed at the 4th Brazilian Congress of Pediatrics, and gave us stimulus for the research work whose results we present now.

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MATERIAL AND METHODS

Our material consisted of 20 samples of mummified umbilical stumps (16 infected by tetanus and 4 normal ones) and 13 post-mortem dried navels obtained from tetanus infected patients from the Pediatric Clinics of the Hospital das Clínicas.

The material was ground in a mortar and suspended in a 5 ml saline solution. Smears of this suspension were stained by Gram. Each of 4 mice received subcutaneously 0.2 ml of the suspension. Two of the inoculated animals received previously anti-tetanic serum (600 UI in 0.2 ml).

The remaining of the suspension was heated at 80°C during half an hour and then distributed through the following media: Tarozzi, Bonnel², Brewer blood-agar and Zeissler blood-agar. This last one was placed in an anaerobiosis jar at 37°C. The blood-agar was employed according to the methods introduced by FORTNER and modified by JACOBSTAHL *apud* ¹. Anaerobiosis was obtained by culture of *Serratia marcescens*. All cultures were placed at 37°C and observed after 24, 48, 72 hours and 1 week. The growth in the test tubes and plates was noted, and smears were made and stained by Gram.

A sporulated bacteria grew, as a rule in Bonnel's medium, and was inoculated in mice (4 mice in each experiment; 2 were protected with anti-tetanic serum). The amount inoculated subcutaneously in these animals was of 0.25 ml of the medium. Six cultures, in Bonnel's medium, were subcutaneously inoculated in guinea-pigs, in a total amount of 0.50 ml. One anti-tetanic serum protected animal (1,500 UI, in a total amount of 0.5 ml) was used in each experience.

The ground material was cultivated at 37°C, then heated at 80°C during 30 minutes in order to kill the common bacteria. The material was then maintained at room temperature, so as to favour sporulation and capsule formation. Considering this fact, we periodically made colored smears using Dorner and Wirtz methods for spores, as well as Müller's method modified by MELLO (personnal communication) for capsules.

Thirteen cultures of Bonnel's medium were passed to milk in order to observe

Hall's stormy fermentation. The material obtained from the milk was employed to perform Welch and Nuttal's test in 9 guinea-pigs, through injection of 0.2 ml of the medium by intra-cardiac route. In six cases, using the material obtained from guinea-pigs (blood and liver), we made cultures in Bonnel's medium and, afterwards, Neagle's test was employed (agar-egg).

In 3 cases, titration of the tetanic toxin obtained by means of cultures in Bonnel's medium, as well as in broth with pepsin (a medium used at the "Instituto Pinheiros"), was made "in vivo". By means of the exhaustion method in agar-blood (Fortner's method), after transplanting the Bonnel's medium, we obtained, in 4 cases, pure samples of *C. tetani*, which were partially studied from the biochemical point of view.

RESULTS

Table I shows the results of search for anaerobic germs and identification of *C. tetani* in mummified umbilical stumps and navels of corpses, with and without tetanus.

Gram stained smears of umbilical stumps and exsiccated navels were positive for cocci, as well as form Gram-positive and Gram-negative bacilli, but negative for sporulated forms like *C. tetani*.

In the media of Tarozzi, Bonnel and Brewer, after 24 hours the presence of Gram-positive thin elongated bacilli was noted. After 48 hours there were already Gram-positive bacilli with terminal spores, together with Gram-negative filamentous forms, as well as bacilli resembling *B. subtilis* and *C. perfringens*. After 72 hours the Gram-positive bacilli with terminal spores had increased and a contamination flora was also present.

In blood-agar and in Zeissler's blood-agar, after 48 hours, there was great development in a wide area, with a central condensation, quite a few times smelling like "burnt horns" or else with a "butyric-sulphuric" smell. The plates' flora was like the one observed in liquid and mixed media. Periodical examination of these last ones showed development of sporulated forms, at room temperature.

TABLE I

Result of search for anaerobic germs and identification of *Clostridium tetani* in mummified umbilical stump material and in post-mortem resuscitated umbilicus of normal and tetanic patients.

Cases	Material spread	Number of samples	Positive results for culture of anaerobics					Test of <i>Clostridium tetani</i> characterization	
			Tarozzi	Bonnel	Blood-agar	Blood-agar Zeissler*	Brewer*	Inoculation of triturate	Inoculation of culture (Bonnel)
Tetanic	Mummified umbilical stump	16	14	14	14	12	7	8	14
	Umbilicus	13	3	3	2	—	—	—	—
Normal (controls)	Mummified umbilical stumps	4	—	—	—	—	—	—	—
Total		33	17	17	16	12	7	8	14

* Among the samples positive for other media, there were only 14 spread in Zeissler and 8 in Brewer.

Table I also shows the results obtained with inoculation in mice (after 24 and 72 hours). In two mice "protected" with anti-tetanic serum there was a picture of tox-infection. Since in some mice there was focal necrosis at the inoculation site, we performed also inoculation in guinea-pigs, which are more suited for observation. Independent of the tetanus picture, a gaseous gangrene developed whose exudate showed, through bacterioscopy, capsulated forms of *C. perfringens* and sporulated bacilli of *C. tetani* type. Results obtained in 13 cultures in Bonnel's medium are as follows:

Milk test positive in 9 cases (13 sow-ings)
 Welch & Nuttal test positive in 6 cases (9 inoculations)
 Neagle test positive in 6 cases (6 sow-ings)

COMMENTS

Our present work demonstrates, in an indisputable way, that isolation of *C. tetani* is possible, mainly from the umbilical stump of the tetanic newborn. Using sixteen samples of mummified umbilical stumps of tetanic newborns, in fourteen cases (87.5%) we

isolated *C. tetani*, which was almost always associated with other germs, including *C. perfringens*.

The culture in anaerobic conditions, chiefly in mixed media (Tarozzi) and liquid media (Bonnel and Brewer), enabled a perfect demonstration of that micro-organism. Research for *C. tetani* in the umbilical stumps of 4 normal newborns was negative.

Inoculation of ground umbilical stumps of tetanic newborns, performed in mice, was positive in 8 cases out of the 16 inoculated samples (50%), demonstrating that the culture process is more sensitive than that of inoculation.

The serum-neutralization test in mice enabled the definite identification of *C. tetani*.

Probably, the amount of toxin present in the stump is, in the majority of cases, below the level capable of causing the experimental tetanus in the sensibilized animal. Even then, however, we have demonstrated the presence of the specific germ (*C. tetani*), the umbilical stump being the main focus of the tetanic tox-infection. As for the post-mortem dried umbilicus, the finding of *C. tetani* was less frequent. Thus, in the culture of 13 samples, we could only isolate that micro-organism in three opportunities

(23%), while the inoculation of ground navels in mice was negative.

Analysis of our material permits further comments.

Bacterioscopy with Gram's method, in the examined material, is not a good diagnostic method when tetanus is suspected, because the sporulated bacilli-like organisms, which characterize *C. tetani*, are practically absent.

Bacterioscopy of cultures in liquid and mixed media at room temperature reveals, mainly after a week, a great number of sporulated forms with the morphological and coloration characteristics of *C. tetani*, besides capsulated forms of *C. perfringens*. Other micro-organisms, such as the *Bacillus subtilis*, constitute a frequent microscopic finding in the referred material. We demonstrated, with relative frequency, the presence of *C. perfringens* in the studied material, which proves the existence of favourable biological conditions for the proliferation of anaerobic germs, as a rule, in the mummified umbilical stump.

The study of the toxigenic activity in the isolated samples revealed a low toxigenicity in relation to the standard samples, which are commonly used in the preparation of the tetanic toxin.

RESUMO

Etiologia do tétano do recém-nascido: isolamento do Clostridium tetani de tecidos umbilicais.

O *Clostridium tetani* tem sido isolado tão poucas vezes da ferida umbilical que a opinião dos autores se divide entre vários microorganismos quanto ao agente causal do tétano do recém-nascido.

Utilizando o meio de Bonnel, o ágar-sangue e outros, conseguimos isolar o *C. tetani* em 87,5% dentre 16 côtos umbilicais e em 23% dentre 13 umbigos de pacientes portadores de tétano. Também isolamos o *Clostridium perfringens* em 13 dentre 33 casos, demonstrando assim a existência de uma condição anaeróbica favorável ao desenvolvimento de germes anaeróbios no côto umbilical.

A identificação do *C. tetani* através de inoculação de culturas em camundongos (prova da sôro-neutralização) foi positiva em 14 casos, ao passo que a inoculação de triturados de côtos umbilicais foi positiva em 8 casos apenas. A razão desta diferença residiria na baixa concentração de toxina dêste último material.

Foram obtidas culturas puras em 4 casos (anteriormente conseguidas apenas por KITASSATO, em 1889), estudando-se seus aspectos morfológicos.

Reforçamos a teoria clostridiana do tétano do recém-nascido, que carecia de provas no tocante ao problema do isolamento de *C. tetani*. Chamamos a atenção para a localização preferencial do *C. tetani* no côto umbilical mumificado, que poderia ser o principal foco de toxi-infecção.

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