

## TETANUS PROPHYLAXIS. EXPERIMENTAL ASSAY IN HORSES, WITH PENICILLIN AND TETANUS ANTITOXIN

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### S U M M A R Y

The Authors make a comparative study between the activity of tetanus antitoxin (T.A.T.) produced in sheeps and guinea-pigs, and that of penicillin-benzathine in the prevention of experimental horse tetanus.

An antitoxin dose of 18,000 U., given from 0 up to 12 hours after spore inoculation, does afford total protection, while 7,200,000 U. of penicillin-benzathine did not protect even when injected 12 hours before challenge.

The use of penicillin and of tetanus antitoxin in the prophylaxis of human tetanus is discussed.

### I N T R O D U C T I O N

Following the pioneer works of BRUCE<sup>4</sup> in 1920, the tetanus antitoxin (T.A.T.) was included in the prophylactic measures against the disease, and widely applied throughout the world.

Among other Authors, WILDEGANS<sup>22</sup> has demonstrated the efficiency of T.A.T. in the prevention of tetanus, illustrated by its successful use by the German army in 1939, during the war between Germany and Poland.

On the other hand, VAKIL et al.<sup>20</sup>, MARTINI<sup>12</sup>, PATEL et al.<sup>16</sup>, to name some of the Authors, have put forth papers disclosing unsuccessful results of T.A.T. in tetanus prophylaxis, which might be ascribed to: a) insufficient dosage; b) rather short protection periods granted by the serum; c) a too fast elimination of the antitoxin in patients sensitized by a previous serum injection or, finally, to d) inefficiency of the serum itself.

With NOVAK et al.<sup>15</sup>, em 1949, took place the first assays with penicillin in the pre-

vention of experimentally induced tetanus of mice. These assays were kept up by ANWAR & TURNER<sup>1</sup>, McDONALD et al.<sup>14</sup>, VERNESI & GUIDOLIN<sup>21</sup> and many others, with more or less satisfactory results.

In view of these publications, COX et al.<sup>5</sup> and FILLER & ELLERBECK<sup>9</sup>, do advise the adoption of penicillin instead of T.A.T. in the warding off of tetanus, fundamented in rather insufficient data on the subject, as the following papers on penicillin failures in human tetanus prophylaxis are bound to prove: BOYD<sup>3</sup>, ELLIS<sup>7</sup>, LUCAS & WILLIS<sup>11</sup> and BOTTICELLI<sup>2</sup>.

These controversies have lead us to search the production of an experimental tetanus resembling as closely as possible, the naturally acquired human tetanus, in an animal showing to *Clostridium tetani* a sensitivity comparable to the human's.

Whis this purpose, instead of employing mice and an inoculum consisting of CaCl<sub>2</sub>-associated spores as adopted by many researchers, we followed the method described

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by DESCOMBEY in 1925<sup>6</sup> when he proved the efficiency of the tetanus toxoid in tetanus prevention. DESCOMBEY used the horse as an experimental animal, the horse being very sensitive to natural tetanus (WILSON & MILES<sup>23</sup>); as infection vehicle, a wood splint contaminated with an association of *C. tetani* spores plus secondary infection germs, introduced in the neck musculature, was employed. One hundred per cent infection was thus obtained in the control animals.

Once settled the disease in the conditions above described, we turned to the comparison between the protection granted by penicillin and that afforded by antitetanus sera produced in sheeps and guinea-pigs, heterologous for the experimental animals.

#### MATERIALS AND METHODS

The horses were acquired in the interior of the State of São Paulo, and their weight ranged from to 350 kg. The eventually circulating natural T.A.T. in these animals was checked beforehand and its absence

proved up to 0.01 U./ml levels in the blood serum. The group distribution of the horses was then performed by random assortment.

Sheep anti-serum was obtained by bleeding of animals previously immunized by anatoxin and tetanus toxin produced in our laboratories, while the guinea-pig anti-serum proceeded from animals immunized by alumen-precipitated tetanus anatoxin. Dosages were performed according to ROSENAU & ANDERSON'S<sup>17</sup> method.

The penicillin tested, was the penicillin-G-benzathine available in 2,400,000 U. bottles. The penicillin dosage established, was of 7,200,000 U. per horse, namely, about 24,000 U. per kg of body weight (U./kg) or six times that indicated for human use (1,200,000 U.) and higher than that advised by ENGLISH<sup>8</sup> for animal use (12,000 U./kg). The antibiotic was injected by intramuscular route, 12 hours before or at the moment of infection.

As for the T.A.T., the dose considered by us was of 18,000 U., namely six times the one adopted now-a-days for men (3,000

TABLE I

Penicillin and tetanus antitoxin in the prevention of experimental tetanus in horses

Prophylaxis	Mortality rates upon <i>C. tetani</i> spore inoculation*		Total
	With <i>B. subtilis</i>	With <i>S. aureus</i>	
T.A.T.			
1. Sheep			
1,500 U. — 0 hours .....	—	0/1	
18,000 U. — 0 hours .....	—	0/4	
18,000 U. 12 hours after infection	—	0/2	
2. Guinea-pigs			
14,000 U. — 0 hours .....	—	0/1	0/8
Penicillin-benzathine			
7,200 U. — 12 hours before infection .....		2/2	
7,200 U. — 0 hours .....	2/2	3/3	7/7
Controls .....	3/3	5/5	8/8

\* Number of dead/number of inoculated

U.). Of the guinea-pig T.A.T., only 14,000 U. were available, and we decided to employ them in a single dose, and in an only horse weighing 200 kg.

T.A.T. was always given by subcutaneous route at the moment of the infectious inoculum or 12 hours later.

The *C. tetani* samples employed proceeded from our culture collection, regularly employed for toxin production; these samples were heated for one hour at 80°C for toxin inactivation and destruction of the vegetative forms present.

As associate-organisms, the Wood-46 strain of *Staphylococcus aureus* of our collection, and a *Bacillus subtilis* isolated from a contaminated culture medium — both checked as non-producers of penicillinase — were employed.

The animals were kept under observation for two months.

The results yielded by our experiments are shown in Table I.

#### DISCUSSION

Under the conditions established in the present experiments, 18,000 U. (about 60 U./kg) of T.A.T. produced in sheeps and guinea-pigs, afforded full protection in horses, when give both at the moment of the infective inoculum and twelve hours later.

On the other hand, 7,200,000 U. (about 24,000 U./kg) of penicillin-benzathine did not confer any protection at the same scheme or even if administered 12 hours before challenge.

The data put forth by our experiments lead us to forewarn the medical classes on the dangerous consequences involved in the substitution of T.A.T. by penicillin in the prevention of human tetanus of non-vaccinated subjects. Further and larger-scale judicious researches should be undertaken to provide a safe appreciation of this matter.

We must emphasize here, the importance of passive (serum) and active (vaccine) immunization, as the safest prophylactic means in human tetanus, a standpoint that, in the last few years, has been supported by several

Authors, as TASMAN & HUYGEN<sup>19</sup>, SMITH<sup>18</sup>, MATVEEV & SERGEEVA<sup>13</sup>, FULTHORPE<sup>10</sup>, and many others.

#### RESUMO

*Prevenção do tétano. Ensaio experimental em cavalos, com penicilina e sôro antitetânico*

Os Autores comparam o sôro antitetânico (S.A.T.) obtido de carneiros e cobaios, com a penicilina-benzatina, na prevenção do tétano experimental em cavalos.

O sôro, na dose de 18.000 U., administrado de 0 até 12 horas após a introdução do esporo tetânico, demonstra proteção total.

Quanto à penicilina-benzatina, na dose de 7.200.000 U., não protegeu mesmo quando injetada até 12 horas antes da infecção.

Os Autores discutem o uso da penicilina e S.A.T. na profilaxia do tétano humano.

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#### REFERENCES

1. ANWAR, A. A. & TURNER, T. B. — Antibiotics in experimental tetanus: *in vitro* and *in vivo* studies. *Bull. John Hopk. Hosp.* 98:85-101, 1956.
2. BOTTICELLI, J. T. & WAISBREN, B. A. — Tetanus in an urban community. *Amer. J. Med. Sci.* 242:44-50, 1961.
3. BOYD, J. S. K. — Tetanus: In the African and European theatres of war. *Lancet* 1: 113-119, 1946.
4. BRUCE, D. — Tetanus analysis of 1,458 cases which occurred in Home Military Hospital during the years 1914-1918. *J. Hyg. (Camb.)* 19:1-32, 1920.
5. COX, C. A.; KNOWELDEN, J. & SHARRARD, W. J. W. — Tetanus prophylaxis. *Brit. Med. J.* 2:1360-1366, 1963.
6. DESCOMBEY, P. — Vaccination du cheval par l'anatoxine tétanique. *Ann. Inst. Pasteur* 39:485-504, 1925.

7. ELLIS, M. — Tetanus prophylaxis (correspondence). *Brit. Med. J.* 1:1438, 1964.
8. ENGLISH, P. B. — Antibióticos en medicina veterinaria. *Rev. Med. Vet. (Bs. As.)* 47:27-34, 1966.
9. FILLER, R. M. & ELLERBECK, W. — Tetanus prophylaxis. *J.A.M.A.* 174:1-4, 1960.
10. FULTHORPE, A. J. — The influence of mineral carriers on the simultaneous active and passive immunization of guinea-pigs against tetanus. *J. Hyg. (Camb.)* 65:243-262, 1965.
11. LUCAS, A. O. & WILLIS, A. J. P. — Prevention of tetanus. *Brit. Med. J.* 2:1333-1336, 1965.
12. MARTINI, M. — Prophylactic failure of 1,500 U. of tetanus antitoxin. *Calif. Med.* 82:189-191, 1955.
13. MATVEEV, K. I. & SERGEEVA, T. I. — Epidémiologie et prophylaxie du tetanos en U.R.S.S. *Bull. Org. Mond. Santé* 32:217-223, 1965.
14. McDONALD, T. R.; CHAIKOF, L. & TRUANT, J. P. — An analysis of prophylactic measures in experimental tetanus. *Surg. Gynec. Obstet.* 110:702-706, 1960.
15. NOVAK, M.; GOLDIN, M. & TAYLOR, W. I. — Tetanus prophylaxis with penicillin-procaine G. *Proc. Soc. Exp. Biol. Med.* 70:573-576, 1949.
16. PATEL, J. C.; MEHTA, B. C. & NANAVATI, B. H. — Failure of tetanus antiserum to prevent tetanus. *J. Indian Med. Ass.* 40:443-447, 1963.
17. ROSENAU, M. J. & ANDERSON, J. F. — The standardization of tetanus antitoxin. United States Hygienic Laboratory, Bull. no. 43, 1908.
18. SMITH, J. W. G. — Tetanus prophylaxis. *Brit. Med. J.* 1:373, 1964.
19. TASMAN, A. & HUYGEN, F. J. A. — Immunization against tetanus of patients given injections of antitetanus serum. *Bull. W.H.O.* 26:397-407, 1962.
20. VAKIL, B. J.; MEHTA, A. J.; IYER, S. K. & TULPUL, T. H. — Failure of anti-tetanus serum prophylaxis. *J. Trop. Med. Hyg.* 67:9-10, 1964.
21. VERONESI, R. & GUIDOLIN, R. — Prevenção do tétano: Papel das penicilinas. *Rev. Paul. Med.* 66:281-292, 1965.
22. WILDEGANS, H. — Über das Auftreten von tetanus in Deutsch-Polnischen Feldzug. *Deutsch. Med. Wschr.* 66:869-871, 1940.
23. WILSON, G. S. & MILES, A. A. — Topley and Wilson's — *In Principles of Bacteriology and Immunity*, p.p. 1967. 4th ed. London, Edward Arnold, 1955.

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