

## Observations on penicillin prophylaxis of experimental inhalation anthrax in the monkey

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### INTRODUCTION

Anthrax in the Rhesus monkey (*Macaca mulatta*), induced by respiratory exposure to spores of a virulent strain of *Bacillus anthracis* in doses greater than 100,000, is a rapidly fatal illness, death occurring on the 2nd to 6th post-exposure days. The clinical manifestations are undramatic and inconsistent. Some animals develop fever, others not. Respiratory distress, depression, or convulsions prior to exitus may or may not be manifest. At autopsy, massive bacteraemia is characteristic. Common gross findings are intra-thoracic lymphadenopathy, lung haemorrhages, oedematous mediastinitis, adrenal haemorrhage, splenomegaly, haemorrhagic meningitis, and hydrothorax (Gleiser, Berdjis, Hartman & Gochenour, 1961).

In a series of experiments on prophylaxis of inhalation anthrax in the monkey, Henderson, Peacock & Belton (1956) found that a 5-day course of intramuscular procaine penicillin, 150,000 units daily, initiated 24 hr. post-exposure, merely delayed the times to death of animals so treated when compared to those of non-treated controls. When, however, they supplemented the same regimen of penicillin with two doses of protective antigen (Belton & Strange, 1954), the first at 24 hr. post-exposure, the second 10 days later, all animals survived the infection.

In the course of studies conducted by the authors and their associates (Gochenour, Gleiser, Gaspar, Overholt, Kuehne, Byron & Tigertt, 1961) on respiratory anthrax in sheep, prophylaxis was initiated 24 hr. post-exposure. Five therapeutic regimens were employed: 300,000 units of intramuscular penicillin every 12 hr. for 5 days; the same, plus a single intramuscular 1.0 ml. dose of protective antigen on day 1; 500 mg. of intramuscular tetracycline every 12 hr. for 5 days; the same, plus protective antigen on day 1; and protective antigen alone on day 1. The protective antigen alone had no effect on the infection. All animals in each of the four other groups survived. No evidence of a requirement for administration of protective antigen for successful prophylaxis of respiratory anthrax in the sheep was afforded by this experiment.

The requirement for administration of protective antigen to the monkey for successful prophylaxis of inhalation anthrax and the lack of such requirement for successful prophylaxis in the sheep may be reconciled by either of two hypotheses, both compatible with the observed essentially similar pathogenesis of respiratory anthrax in these two species.

First, unlike the sheep, the monkey may be unable to respond adequately, if at

all, to anthrax antigens elaborated during his infection prior to antibiosis. This might be attributable to failure to recognize and respond to protective antigen in its combined native state, or to a slow rate of immune response to the antigenic stimulus.

Second, the rate of entrance into the lymphatics by the spores and their subsequent germination and invasion of the blood stream in the monkey may be markedly less rapid than in the sheep. Early attainment of bactericidal blood levels of penicillin would, under these circumstances, destroy the bacilli as rapidly as they entered the blood stream; thus depriving the monkey of any significant antigenic stimulus to antibody production. The sheep, on the other hand, well might have had experience with relatively large numbers of blood-borne bacilli and thus have received an adequate antigenic stimulus prior to the attainment of bactericidal blood levels of penicillin. This would result in monkeys vulnerable to infection with anthrax after cessation of antibiotic administration and sheep 'in a continuous state of prophylactic readiness' (Henderson *et al.* 1956).

If the first hypothesis is correct, prophylaxis alone should be unsuccessful regardless of the time of its initiation. If the second is correct, prophylaxis with penicillin alone should be unsuccessful if initiated early and successful if delayed.

The studies reported herein were conducted to test these hypotheses. An effort was made to replicate in so far as possible the conditions under which the experiments of Henderson and his associates were conducted.

#### MATERIALS AND METHODS

The Vollum strain of *B. anthracis* was employed. The lot used, no. 189, was prepared in 1957 and was stored as a phenolated spore suspension ( $4 \times 10^{10}$ /ml.) at 5° C. until used. Spore suspensions were diluted in distilled water and heat-shocked at 60° C. for 30 min. prior to aerosolization. The guinea-pig subcutaneous LD 50 of this suspension has remained constant at < 4 spores.

The device used for respiratory exposure generated a dynamic aerosol cloud at the rate of 20 standard cu.ft. per min.; humidity was controlled at 80 % for all exposures. A Collison generator (Henderson, 1952) was used to disseminate the spores. This fixture produces a predominantly small particle aerosol with a mass median diameter of approximately  $1.5\mu$ . The monkeys were exposed in helmets through which the aerosol flowed at a rate of 25 l. per min., and were calculated to have breathed approximately 1 l. per min. Estimation of aerosol concentration presented to the monkeys was made by examination of samples obtained from all glass impingers (AGI-30's) in the effluent air lines from the exposure helmets.

Twenty *Macaca mulatta*, ranging in weight from 1.6 to 3.1 kg. were used. The drug regimen employed was five single daily doses of 150,000 units procaine penicillin intramuscularly, a total of 750,000 units of penicillin. Drug treatment was started at 24, 48 and 72 hr. Six untreated animals served as controls. Blood cultures were obtained on all animals at the time of initiation of therapy. The control animals were examined for bacteraemia on days 1, 2 and 3.

## RESULTS

Respiratory doses presented to the monkeys ranged from 345,000 to 1,200,000 spores with a geometric mean of 783,000 spores.

The six non-treated control animals succumbed to the infection on days 2, 3 and 4. Five of the six had demonstrable bacteraemia prior to death. All were grossly bacteraemic at death. Fever was noted in only two animals.

Four of five monkeys placed on penicillin prophylaxis at 24 hr. succumbed. Deaths occurred on the 4th (two animals), 8th, and 9th days after cessation of therapy. None of the animals had demonstrable bacteraemia or fever at time of initiation of penicillin. Bacteraemia was not demonstrable prior to death in one animal grossly bacteraemic at death on the 4th day after cessation of penicillin. Fever and bacteraemia were present from 2 to 3 days prior to death in the three others that died. The remaining animal survived, despite fever and bacteraemia from the 10th to 13th post-prophylactic days.

One of five monkeys in the 48 hr. drug group died on the 5th day after cessation of drug. This animal had bacteraemia at the time of the first dose of penicillin. It experienced 2 days of fever prior to death, at which time *Bacillus anthracis* was isolated from blood. The four remaining animals survived. Three were bacillaemic at the time prophylaxis was initiated. One of these had fever at this time. This was the only animal in the group which remained afebrile after cessation of penicillin. The single animal with a negative blood culture at the time penicillin was started, manifested fever from the 11th to the 14th days after drug was discontinued. Bacteraemia was demonstrated on the last day of the febrile episode.

Two of the four monkeys in which prophylaxis was delayed until 72 hr. post-exposure succumbed to the infection while on therapy. Both were bacteraemic at the time of initial administration of the drug and one had been febrile for 2 days. The other two animals were both febrile and had negative blood cultures at the time they received their first dose of penicillin. Both were afebrile thereafter; bacillaemia was not demonstrable.

Significant gross autopsy findings on the animals succumbing are shown in Table 1.

All surviving animals were observed for a period of 31 days after exposure at which time they were inoculated subcutaneously with 5000 heat-shocked spores. The animals at this time had serologically demonstrable antibody against *B. anthracis*, but all survived without ill effect.

## DISCUSSION

The results obtained in this experiment indicate that the monkey, like the sheep, is capable of responding to *in vivo* elaborated anthrax antigens. Under circumstances in which an adequate antigenic stimulus is presented to the monkey, its response is sufficiently rapid to attain within the period of antibiotic cover, a level of immunity sufficient to permit the monkey to cope with the remaining anthrax organisms as they leave the lungs and gain entrance to the body.

It is apparent that the time of initiation of bactericidal therapy is quite critical.

Table 1. *Principal gross autopsy findings on monkeys succumbing to inhalation anthrax*

Principal gross lesions	Penicillin-treated monkeys												
	Non-treated monkeys					Death after Rxt							
	943	947	949	916	938	912	Death during Rx*			946	951		
						950	940	F-19	918	924	946	951	
Intra-thoracic													
Lymphadenopathy	-	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-
Mediastinal													
Oedema	-	-	-	+	+	+	-	-	-	+	+	+	+
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydro-thorax	-	-	-	-	-	+	-	-	-	-	+	+	+
Pulmonary													
Parenchymal haemorrhage	+	-	+	+	-	+	+	-	-	+	-	-	-
Haemorrhagic nodules	-	-	-	-	+	-	-	-	-	-	+	+	+
Meningeal haemorrhage	-	-	-	-	+	+	+	-	+	-	+	+	+
Splenomegaly	+	+	-	-	+	-	-	+	+	-	+	+	-
Day of death (post-exposure)	3	2	4	2	4	4	4	4	13	11	9	14	9
Febrile illness	-	-	-	-	+	+	-	+	+	-	-	+	+

\* Therapy initiated 72 hr. post-exposure.

† Therapy initiated 24 hr. post-exposure except monkey no. 918, 48 hr. post-exposure.

As in the experiments of Henderson and his associates, the monkeys treated at 24 hr. were essentially without benefit of antigenic stimulus and remained vulnerable to infection. Delay until 72 hr. on the other hand permitted this rapidly fulminating infection to pass beyond the point of no return in half the animals so treated.

It is of note that not all of the surviving monkeys attained complete refractoriness to infection after drug cessation. Fever in four, and demonstrable bacteraemia in two, monkeys suggests that the response in some may be barely sufficient to swing the balance in the favour of the animal.

These experiments further suggest that had penicillin prophylaxis been initiated earlier in the above-mentioned studies in sheep, a requirement for the administration of protective antigen for successful prophylaxis might have been demonstrated.

Intra-thoracic lymph node and mediastinal involvement were much more extensive and severe in the animals dying after cessation of early penicillin prophylaxis than in non-treated animals. This, coupled with the higher incidence of fever in the treated group, suggests that such animals more nearly simulate human respiratory anthrax and may serve as appropriate models for study of early diagnosis and therapy.

#### SUMMARY

The result of penicillin prophylaxis of experimental inhalation anthrax in the Rhesus monkey has been shown to be dependent upon the time of its initiation. If begun too early, it is unsuccessful. It may not be too long delayed, or the infection will have passed the point of no return. A brief, critical time period exists, during which successful prophylaxis may be initiated. This favourable outcome is attributed to the elaboration *in vivo* of sufficient antigen or antigens to stimulate an adequate immune response, prior to the initiation of antibiosis.

#### REFERENCES

- BELTON, F. C. & STRANGE, R. E. (1954). *Brit. J. exp. Path.* **35**, 144.  
GLEISER, C. A., BERDJIS, C. C., HARTMAN, H. A. & GOCHENOUR, W. S., JR. (1961) *USAMU Activities Report (3405.05) Part 4*.  
GOCHENOUR, W. S., JR., GLEISER, C. A., GASPAR, A., OVERHOLT, E. L., KUEHNE, R. W., BYRON, W. R. & TIGERTT, W. D. (1961). *USAMU Activities Report (3405.05) Part 2*.  
HENDERSON, D. W. (1952). *J. Hyg., Camb.*, **50**, 53.  
HENDERSON, D. W., PEACOCK, S. & BELTON, F. C. (1956). *J. Hyg., Camb.*, **54**, 28.