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AD NUMBER
AD836232
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AMXFD ltr, 9 Feb 1972

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AD836232

TRANSLATION NO. 1064

DATE: 15 April 1964

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JUL 28 1968

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DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

DETERMINATION OF AMOUNT OF GASES (OXYGEN, NITROGEN,  
AND CARBON DIOXIDE) IN GUINEA PIGS EXPERI-  
MENTALLY INFECTED WITH ANTHRAX

- Argentina -

Following is the translation of an article by  
N. S. Liozaga in the Spanish-language weekly  
Semana Medica (Medical Weekly) published by the  
Institute of Physiology of the Faculty of Medi-  
cal Sciences of Buenos Aires, No 2, 20 August  
1936, pp 491-495.]

Chair Endowed in Honor of Prof. Bernardo A. Houssay  
Appointment Thesis to the Chair of Medical Pathology

It is generally known that wild animals, suffering  
from anthrax present an unostentatious symptomatology and  
that death surprises them abruptly, in general while eating  
or walking.

It is also perfectly well known that autopsy performed  
on these animals reveals the existence of extremely dark  
blood in the heart and the blood vessels, almost of a black  
color, non-coagulated or only with rare and small coagulated  
particles floating freely in its liquid mass.

These items which we had occasion to test in detail  
both in wild animals and in laboratory specimens, inoculated  
experimentally, caused us to suspect within them an altera-  
tion of the hematosiis and to propose to Prof. Dr. Bernardo  
Houssay as a thesis of physiopathology for our appointment  
to the chair of medical pathology of this faculty the inves-  
tigation of the equilibrium of the blood gases in guinea pigs  
infected with anthrax.

Ever since we began our experiments four months ago  
we have had to overcome unforeseen difficulties, inherent in

the technology, the type of animals we had to experiment on and the character of the affliction under study.

For this reason, although we really completed 36 inoculations, we have been able only to complete the study of nine guinea pigs in a fashion which may be considered satisfactory; this is a small number which will force us to be short on conclusions but which undoubtedly will have the value of a preliminary thesis of orientation for the investigations we propose to continue and whose results we will present opportunely to the Faculty of Medical Sciences.

In view of the novelty of the subject matter we had to limit our own ideas because there are no similar studies in the scientific world or we have not been able to locate any.

We have selected the guinea pigs, which, due to its small size, is barely qualified for this type of studies, because among the small laboratory animals it is better than the rabbit for anthrax experiments due to its greater uniformity of reaction to infection and we had to reject sheep due to the difficulty of maintaining them within the faculty and above all of sterilizing the bodies, and the requirement for using a virus of much greater activity; this would increase the risk in view of the difficulty of sterilizing the Van Slike Stadie apparatus used during our calculations.

The experimental technique used was the following: We investigated the carotid artery of the experimental guinea pigs and we extracted by means of puncture with a calibrated syringe 1 cc. of arterial blood; we determined the volume of CO<sub>2</sub>, O<sub>2</sub>, and N<sub>2</sub> in the blood of this healthy animal and injected the anthrax virus subcutaneously. We determined the existence of the state of sickness by means of a local edema formed at the inoculation spot, the degree of animal activity, and the time of death from the virus employed, previously fixed in other guinea pigs. We took a second blood sample from the carotid artery at different moments of the sickness and during the period of agony in order to again determine the volume of the respective gases. We were able to prove each time the existence or absence of bacteria either directly or cultivated, and verified its magnitude.

However, as we stated before, due to the character of the infection, the death of the animal surprised us completely in 27 of the infections despite the fact that we were watching carefully, which proves completely the previous affirmation that death from anthrax occurs with extraordinary

abruptness and without the exterior appearance of the animal revealing its impending death.

For the inoculations we used uniformly an attenuated virus designated C. 15, whose activity is somewhat greater than that of the type of a second Pasteur vaccine. It was cultivated in agar-agar for 24 hours, suspended in a physiological solution of sodium chloride in a normal quantity of 400 cc. The dosage of  $\frac{1}{4}$  cc. was previously shown to be mortal between 48 and 62 hours following subcutaneous inoculation.

The results obtained were the following:

Case History No 1

Guinea pig No 323. Weight: 660 gr. Healthy animal. 23 December 1926, 5:00 P.M.:

1 cc. of right carotid blood

$CO_2, O_2, N_2 = 0.730$  cc.  
 $O_2, N_2 = 0.230$  cc.

Temperature: 25.5° C. Barometric pressure: 756 mm.

At 0° C and 760 mm  $\left\{ \begin{array}{l} CO_2 = 49.04 \text{ cc.} \\ O_2 = 18.82 \text{ cc.} \\ N_2 = 1.49 \text{ cc.} \end{array} \right.$

At 5:00 o'clock: Subcutaneous inoculation:  $\frac{1}{4}$  cc. virus C. 15.

24 December 1926, 7:30 P.M.: Edema at the site of inoculation. Animal sad, somewhat maladjusted

$CO_2, O_2, N_2 = 0.870$  cc.  
 $O_2, N_2 = 0.220$  cc.

Temperature: 25° C. Barometric pressure: 758 mm.

At 0° C and 760 mm  $\left\{ \begin{array}{l} CO_2 = 63.92 \text{ cc.} \\ O_2 = 17.98 \text{ cc.} \\ N_2 = 1.49 \text{ cc.} \end{array} \right.$

Blood sample: No bacilli observed.  
Blood cultures: Negative

25 December 1926, 7:30 P.M.: Death.

In this case the following was observed: For 26½ hours the animal presented edema, it had no bacteremia, there is a small amount of CO<sub>2</sub> = 14.88 cc., O<sub>2</sub> reduction = 0.84 cc., N<sub>2</sub> no change. This determination was made 24 hours before death.

#### Case History No 2

Guinea pig No 298. Weight: 315 gr., healthy animals. 10 March 1927:

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.680 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.200 cc.

Temperature: 27°C and 760 mm. Barometric pressure: 759.

At 0°C. and 760 mm { CO<sub>2</sub> = 46.60 cc.  
O<sub>2</sub> = 16.07 cc.  
N<sub>2</sub> = 1.77 cc.

At 7:00 P.M.: Subcutaneous inoculation with ½ cc. virus C. 15.

2 March 1927: At 8:00 P.M. the guinea pig has a large edema, and although more than 24 hours had elapsed we fear that it will die tonight due to this dosage.

1 cc of left carotid blood.

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.700 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.160 cc.

Temperature: 21°C. Barometric pressure: 757 mm.

At 0°C. and 760 mm { CO<sub>2</sub> = 54.60 cc.  
O<sub>2</sub> = 12.96 cc.  
N<sub>2</sub> = 1.53 cc.

Blood sample: No bacilli.

Blood cultures: Few colonies of anthrax bacilli.

The animal dies during the attempt to remove more blood for other determinations.

From this case we deduce that we surprised the initial instant of bacteremia.

The CO<sub>2</sub> had increased 8 cc. above its initial value.

The O<sub>2</sub> had decreased by 3.11 cc.

The N<sub>2</sub> had decreased 0.17 cc.

### Case History No 3

Guinea pig No 380. Weight: 724 gr. Healthy animal. 19 January 1927, 11:00 A.M.:

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.470 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.190 cc.

Temperature: 28°C, Barometric Pressure: 754.5 mm.

At 0°C. and 760 mm { CO<sub>2</sub> = 26.80 cc.  
O<sub>2</sub> = 14.99 cc.  
N<sub>2</sub> = 1.47 cc.

At 11:00 A.M.: Subcutaneous inoculation with  $\frac{1}{2}$  cc. of C. 15 virus.

20 January 1927 at 11:00 P.M.: Medium edema. Animal appearance, normal.

1 cc. of right carotid blood.

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.600 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.215 cc.

Temperature: 29°C. Barometric Pressure: 754 mm.

At 0°C. and 760 mm { CO<sub>2</sub> = 36.55 cc.  
O<sub>2</sub> = 17.04 cc.  
N<sub>2</sub> = 1.26 cc.

Blood sample: No bacilli found.

Blood culture: Anthrax bacilli.

The animal dies between 36 and 44 hours following the inoculation.

As we see here, the animal does not present any ostensible bacteremia during a direct examination of the blood which indicates that it was light, since the cultures revealed its presence. The gases underwent the following changes: CO<sub>2</sub> increased by 9.75 mm over its normal value. O<sub>2</sub> increased by 2.05 mm over its initial value and N<sub>2</sub> decreased 0.22 cc. over its initial value.

#### Case History No 4

Guinea pig No. 336. Weight: 535 gr. Healthy animal. 9 March 1927:

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.720 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.180 cc.

Temperature: 27°C. Barometric pressure: 759 mm.

At 0°C. and 760 mm  $\left\{ \begin{array}{l} \text{CO}_2 = 52.36 \text{ cc.} \\ \text{O}_2 = 14.29 \text{ cc.} \\ \text{N}_2 = 1.49 \text{ cc.} \end{array} \right.$

At 8:15 P.M.: Subcutaneous inoculation with  $\frac{1}{4}$  cc of C. 15 virus.

10 March 1927: Much edema. Animal appearance normal. The animal runs and eats.

At 4:30 P.M.:

1 cc. of left carotid blood. The right side has a thrombus.

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.730 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.240 cc.

Temperature: 27°C. Barometric pressure: 758 mm.

At 0°C. and 760 mm  $\left\{ \begin{array}{l} \text{CO}_2 = 47.49 \text{ cc.} \\ \text{O}_2 = 19.28 \text{ cc.} \\ \text{N}_2 = 1.49 \text{ cc.} \end{array} \right.$

Blood sample: Anthrax bacilli in a discrete quantity.  
Blood cultures: Abundant anthrax colonies.

At 8:30 P.M.: Animal dead.



The animals was seized by a discrete bacteremia.

The gases presented the following variations with respect to their normal status: CO<sub>2</sub> reduced by 4.87; O<sub>2</sub> increased by 4.99 and N<sub>2</sub> remained unchanged.

The animal died four hours after discrete bacteremia had been verified.

#### Case History No 5

Guinea pig No. 57. Weight: Healthy animal. 18 February 1927, 9:00 P.M.:

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.900 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.210 cc.

Temperature: 29°C, Barometric pressure: 758 mm.

At 0°C. and 760 mm { CO<sub>2</sub> = 65.86 cc.  
O<sub>2</sub> = 16.71 cc.  
N<sub>2</sub> = 1.47 cc.

At 7:30 P.M.: Subcutaneous inoculation of 0.25 cc. C. 15 virus. 20 February 1927, 3:00 P.M.: Medium edema.

At 7:45 P.M.: edema and ganglionic infarct on the same side.

At 8:15 P.M.:

1 cc. of left carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.660 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.220 cc.

Temperature: 29°C. Barometric pressure: 759 mm.

At 0°C. and 760 mm { CO = 42.05 cc.  
O = 17.60 cc.  
N = 1.53 cc.

Blood cultures: Numerous colonies of anthrax.

Death between 48 and 60 hours following inoculation.

The animal had fallen victim to bacteremia.

The gases underwent the following changes with respect to their initial volumes: CO<sub>2</sub> a reduction of 23.81 cc. O<sub>2</sub> an increase of 0.89 cc. and N<sub>2</sub> an increase of 0.06 cc.

#### Case History No 6

Guinea pig No. 379. Weight: 477 gr. Healthy animal. 8 January 1927, 6:00 P.M.:

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.600 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.240 cc.

Temperature: 28°C. Barometric pressure: 759 mm.

At 0°C. and 760 mm  $\left\{ \begin{array}{l} \text{CO}_2 = 40.43 \text{ cc.} \\ \text{O}_2 = 19.44 \text{ cc.} \\ \text{N}_2 = 1.48 \text{ cc.} \end{array} \right.$

At 7:00 P.M.: Subcutaneous inoculation 0.25 cc. C. 15 virus.

10 January 1927, 7:00 P.M.: The animal is motionless on the ground and has dyspnea. Enormous edema.

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.380 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.150 cc.

Temperature: 28°C. Barometric pressure 759 mm.

At 0°C. and 760 mm  $\left\{ \begin{array}{l} \text{CO}_2 = 22.14 \text{ cc.} \\ \text{O}_2 = 11.60 \text{ cc.} \\ \text{N}_2 = 1.48 \text{ cc.} \end{array} \right.$

Blood sample: Numerous anthrax bacilli.  
Blood culture: Numerous colonies of anthrax.

Dead following blood extraction.

The animal was seized with acute bacteremia in a very advanced stage of the illness, almost in agony.

The blood gases had undergone the following changes with respect to their initial volumes: CO<sub>2</sub> reduced by 18.29 cc., O<sub>2</sub> reduced by 7.84 cc. and N<sub>2</sub> remained unchanged.

#### Case History No 7

Guinea pig No 288. Weight 437 gr. Healthy animal 20 February 1927, 9:00 A.M.:

1 cc. of left carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.763 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.230 cc.

Temperature: 28°C. Barometric pressure: 760.5 mm

At 0°C. and 760 mm. { CO<sub>2</sub> = 51.42 cc.  
O<sub>2</sub> = 18.61 cc.  
N<sub>2</sub> = 1.49 cc.

At 10:00 A.M.: Subcutaneous inoculation 0.25 cc. C. 15 virus.

22 February 1927, at 10:45 P.M.: The animal is somewhat sad and presents much edema.

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.620 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.230 cc.

Temperature 27.5°C. Barometric pressure: 760 mm.

At 0°C. and 760 mm. { CO<sub>2</sub> = 37.73 cc.  
O<sub>2</sub> = 18.66 cc.  
N<sub>2</sub> = 1.49 cc.

Blood culture: Numerous anthrax colonies.

Dead 61 hours later and before the 70 hours of inoculation.

The animal has been in a grave state of bacteremia.

The blood gases have undergone the following changes: CO<sub>2</sub> a reduction of 13.69 cc. with respect to its initial volume; O<sub>2</sub> an increase of 0.05 cc. and N<sub>2</sub> unchanged.

Case History No 8

Guinea pig No 303. Weight: 435 gr. 10 February 1927,  
7:00 P.M.:

1 cc. of right carotid blood

$\text{CO}_2, \text{O}_2, \text{N}_2 = 0.835 \text{ cc.}$   
 $\text{O}_2, \text{N}_2 = 0.240 \text{ cc.}$

Temperature:  $29^\circ\text{C}$ . Barometric pressure: 758 mm.

At  $0^\circ\text{C}$ . and 760 mm.  $\left\{ \begin{array}{l} \text{CO} = 56.79 \text{ cc.} \\ \text{O} = 19.30 \text{ cc.} \\ \text{N} = 1.47 \text{ cc.} \end{array} \right.$

At 8:00 P.M.: Subcutaneous inoculation with type V anthrax (virus more attenuated than the second Pasteur vaccine.)

13 February 1927; The animal is sad. It does not have edema. At 8:30 A.M.:

1 cc. of right carotid blood

$\text{CO}_2, \text{O}_2, \text{N}_2 = 0.910 \text{ cc.}$   
 $\text{O}_2, \text{N}_2 = 0.220 \text{ cc.}$

Temperature:  $28.5^\circ\text{C}$ . Barometric pressure 757.8 mm.

At  $0^\circ\text{C}$ . and 760 mm.  $\left\{ \begin{array}{l} \text{CO}_2 = 66.01 \text{ cc.} \\ \text{O}_2 = 17.63 \text{ cc.} \\ \text{N}_2 = 1.48 \text{ cc.} \end{array} \right.$

Blood sample: No bacilli observed.

Blood culture: No bacilli germinating.

Dead 73 hours after the inoculation.

Autopsy. No edema at the inoculation site.

No organic congestion.

Volume of spleen slightly increased, normal color.

One hypertrophic abdominal ganglion with two hardened nodules.

Lungs congested.

Heart: Normal blood color and many coagulations.

Blood culture: Negative.

In this experiment we tried a more attenuated virus than the C. 15 type, expecting to obtain slower progress of the disease but were unable to produce the anthrax infection as substantiated by the negative cultures. But the animal, already ill (hardened ganglia) died after overcoming its infection.

We added this case history because it appears interesting to observe how this animal behaves in its terminal state, which was the inverse of that observed in animals dead from anthrax.

The blood gases also changed in the opposite direction, with respect to their initial state: CO<sub>2</sub> increased 9.22 cc.; O<sub>2</sub> decreased by 1.67 cc. and N<sub>2</sub> increased by 0.01 cc.

#### Case History No 9

Guinea pig No 291. Weight: 712 gr. Healthy animal. 18 January 1927, 6:15 P.M.:

1 cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.570 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.220 cc.

Temperature: 28°C. Barometric pressure: 760.3 mm.

At 0°C. and 760 mm. { CO<sub>2</sub> = 33.74 cc.  
O<sub>2</sub> = 17.72 cc.  
N<sub>2</sub> = 1.48 cc.

Subcutaneous inoculation of 0.25 cc. of C. 15 type virus.

20 January 1927: The animal has a light edema. It is eating.

At 11:00 A.M.:

1. cc. of right carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.580 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.210 cc.

Temperature: 28.2°C. Barometric pressure: 754 mm.

At 0°C. and 760 mm.  $\left\{ \begin{array}{l} \text{CO}_2 = 35.39 \text{ cc.} \\ \text{O}_2 = 16.72 \text{ cc.} \\ \text{N}_2 = 1.47 \text{ cc.} \end{array} \right.$

Blood sample: No bacilli.  
Blood culture: Negative.

20 January 1927, 9:00 P.M.:

1 cc. of left carotid blood

CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> = 0.470 cc.  
O<sub>2</sub>, N<sub>2</sub> = 0.200 cc.

Temperature: 30°C. Barometric pressure: 754 mm.

At 0°C. and 760 mm.  $\left\{ \begin{array}{l} \text{CO}_2 = 23.11 \text{ cc.} \\ \text{O}_2 = 15.66 \text{ cc.} \\ \text{N}_2 = 1.46 \text{ cc.} \end{array} \right.$

Blood sample: No bacilli.  
Blood culture: Negative.

Dead between 41 and 49 hours following inoculation.

Blood culture of the dead animal: Anthrax.

In this experiment we were only able to demonstrate bacteremia in the blood of the dead animal. But the blood gases had shown the following changes with respect to their normal values: 29 hours following inoculation the CO<sub>2</sub> had increased 1.65 cc.; O<sub>2</sub> had decreased 1 cc. and N<sub>2</sub> increased by 0.01 cc. This is in agreement with cases Nos. 1, 2 and 3.

31 hours after inoculation the CO<sub>2</sub> had decreased by 10.63 cc.; O<sub>2</sub> had decreased 2.06 cc. and N<sub>2</sub> decreased by 0.02 cc. This is in agreement with cases Nos. 5, 6 and 7 concerning CO<sub>2</sub> and with No 6 concerning O<sub>2</sub>.

Virus C. 13, % c. c.				Al O <sub>2</sub> @ 700 mm.			Bacteri- mia	f Hours hours	g Observación
a Cambio n°	b Peso gr.	c Edema	d Hours de inoc.	e Estado	O <sub>2</sub> <sup>1</sup>	O <sub>2</sub> <sup>2</sup>			
212	660	— h Pequeño	— 30 ½	Sano Enfermo	49.04 43.92	18.92 17.98	1.49 1.49	No 30 ½	
218	312	— i Grande	— 27	Sano Enfermo	44.40 54.00	18.07 12.00	1.77 1.58	Leve 37	u
219	724	— j Mediano	— 36	Sano Enfermo	24.00 26.33	14.00 17.04	1.47 1.38	o may leve	t Entre 36 y 44
226	533	— Grande	— 42	Sano Enfermo	52.26 47.49	14.26 19.26	1.49 1.49	Leve	66
57	420	— Grande	— 48	Sano Enfermo	65.00 42.03	16.71 17.00	1.47 1.53	p Mediana	Entre 48 y 60
679	477	— Grande	— 49	Sano Enfermo	46.42 22.16	19.44 11.00	1.48 1.48	q Grave	60
680	437	— Grande	— 61	Sano Enfermo	51.42 37.73	18.01 18.00	1.49 1.49	r Estado	Entre 61 y 70
688	433	— No hay	— 72	Sano Enfermo	54.79 64.01	19.20 17.02	1.47 1.48	s No hay	72
691	712	— Leve Grande	— 20 41	Sano Enfermo Enfermo	23.74 25.29 22.11	17.72 16.72 15.00	1.48 1.47 1.49	No hay No hay	Entre 41 y 49

[Legend:] a) guinea pig No; b) weight; gr; c) edema; d) hours following inoculation; e) state of health; f) death, hours; g) remarks; h) small; i) large; j) medium; k) healthy; l) ill; m) no; n) light; o) very light; p) medium; q) grave; r) present; s) none; t) between 36 and 44; u) killed; v) dead four hours after establishing bacteremia; w) agonizing; x) no anthrax; y) anthrax.

From a comparison of the case histories of this small number of experiments shown in the final tabulation, the following may be derived:

1. In experimental anthracic infection, in the early part of the illness an increase of the blood CO<sub>2</sub> occurs in guinea pigs, followed later by a pronounced decrease until the time of death.

2. The O<sub>2</sub> content undergoes pronounced changes, not connected with the CO<sub>2</sub> volume, nor apparently following fixed rules.

3. The N<sub>2</sub> content underwent no appreciable variations.

- END -

- 13 -