

CHEMOTHERAPY OF EXPERIMENTAL *BRUCELLA ABORTUS* INFECTION IN THE GUINEA PIG

(A Preliminary Report)*

Since the advent of the sulfa drugs and more recently of the antibiotics, many reports have appeared on the treatment of experimental brucella infections in animals. The failure to culture brucella organisms from the spleen has been used by some authors as the sole criterion of cure or of the effectiveness of treatment.

This preliminary study has been undertaken in order to find out: (1) If spleen cultures alone are a reliable criterion of the efficacy of treatment. (2) To test the effect of different drugs and combination of drugs on experimental *Brucella abortus* infection in small groups of animals in order to obtain information upon which to base a large scale study. The results of this pilot experiment constitute the subject of this preliminary report.

MATERIALS AND METHODS

Infecting strains and dose.—A typical strain of *Brucella abortus* obtained from Dr. I. F. Huddleson¹ and a *Br. abortus* strain obtained by us from the blood of an acute case of brucellosis (Strain Sablín²) were used. The organisms were grown on tryptose agar for 48 hours and suspensions in sterile 0.9% saline were prepared which matched tube No. 1 of the barium sulphate standard. Guinea pigs were inoculated subcutaneously in the right lower abdomen with 0.1 cc of a freshly prepared suspension.

Diet.—The animals were maintained on a diet of powdered Rockland rabbit feed and water.

Drugs used, dosage and method of administration.—The following drugs were used: Sulfadiazine (Abbott, list number 3452), dihydrostreptomycin sulfate (Abbott, list number 3833), aureomycin hydrochloride (Lederle, capsules for oral administration), chloromycetin (Capsules, Parke, Davis and Co., Lot No. 379) and dimazol or NU 445³ (Control number N 61T-#2). The sulfadiazine and dimazol tablets were ground to a fine powder and thoroughly mixed with the powdered feed to give a drug concentration of one per cent.

Dihydrostreptomycin, aureomycin and chloromycetin were given four times a day (7:00 AM, 10:00 AM, 1:00 PM and 4:00 PM) as follows: (1) Dihydrostreptomycin in aqueous solution, four thousand units in

* With the technical assistance of Carlos Fernández, from the Department of Bacteriology, School of Tropical Medicine of the University of Puerto Rico.

¹ *Br. abortus* #1489 of Huddleson.

² Strain Sablín was obtained by the author from the blood of a case of acute brucellosis. Upon isolation this organism grew only in the presence of 16% CO₂, but after several transplants to tryptose agar it grew easily aerobically.

³ Dimazol is a drug of the sulfonamide group. It was sent to us by Dr. Elmer L. Sevringhaus of the Hoffmann LaRoche Laboratories.

each injection, subcutaneously. (2) Aureomycin in aqueous solution, four milligrams in each feeding. (3) Chloromycetin suspended in water, ten milligrams in each feeding. Aureomycin and chloromycetin were fed to the animals with great facility using a 1 cc tuberculine syringe without needle.

Method of cultivation and culture media.—The animals were bled to death or were killed by a heavy blow on the head. Blood cultures were made using the medium and technique recommended by Castañeda⁴. At the beginning of this work a quantitative method was used to determine the approximate number of bacteria per gram of tissue, but the procedure was too elaborate and time consuming and it was substituted by a simpler and easier method after a comparison of both convinced us that the latter gave results which were sufficiently accurate. The tissues were macerated with sterile sand or cut with scissors and cultures made either by making one streak on the surface of a tryptose agar plate or slant with a loopful of the macerated pulp or with the cut surface of the tissue as the case might be. The inoculated medium was incubated for four days to one week and the amount of growth was recorded. The blood cultures were incubated during two weeks before being discarded. Tryptose agar slants were used to cultivate urine and bile.

EXPERIMENTAL INFECTION WITH *BRUCELLA ABORTUS* IN NORMAL GUINEA PIGS

Twenty-four normal guinea pigs were inoculated, twelve with *Br. abortus* 1489 and twelve with *Br. abortus* strain Sablín. The animals were killed at different intervals and the blood, urine, bile and the different organs and tissues cultured for brucellae. The results are summarized in Table 1. Six animals that died of other infections are not included in this table.

Table 1 does not show marked differences in the infectivity of the aerobic (Sablín) and anaerobic (1489) strains for the different tissues of the guinea pig in this experiment.

The organisms were cultured from the blood from five to seventy-five days after inoculation but not two days after inoculation. Positive cultures were obtained from the lymph nodes in all animals and from the spleen in all except in the two guinea pigs killed two days after inoculation. After the lymph nodes, spleen and blood the bone marrow was the tissue from which the organisms were more frequently cultivated. It must be noted that only the urine from two animals and the bile from one were found positive.

The inguinal, retroperitoneal (lumbar) and axillary lymph nodes were cultured. Cultures from one node could give abundant growth when very few colonies or no growth was obtained from other lymph nodes of the same animal. This fact is important when lymph node

⁴ Ruiz Castañeda, M.: A practical method for routine blood cultures in Brucellosis. Proc. Soc. Exp. Biol. & Med. 64: 114-115, 1947.

TABLE 1
Brucella abortus Infection of Different Organs and Tissues of Untreated Guinea Pigs

G.P. Serial No.	Days after inoculation	Growth from different organs and tissues											
		Strains ^{Sablin (aerobic)} # 1489 (anaerobic)											
		Blood	Brain	Lung	Heart	Liver	Spleen	Adrenal	Kidney	Lymph nodes	Bone marrow	Urine	Bile
28	2	0	0	0	0	0	0	0	0	+++++	0	0	0
16	2	0	0	0	0	0	0	0	0	+++++	0	0	0
19	5	Pos.	0	+++	+++	+++++	+++++	0	+++	+++++	++++	+	0
17	7	Cont.	+++	+++	+++	+++++	+++++	+++++	+++	+++++	++++	0	0
5	8	Pos.	++	++	++	+++++	+++++	0	+	+++++	++	0	X
None													
None													
15	13	Pos.	0	+++	++	++	+++++	++	+	++++	++++	0	X
6	15	Pos.	0	+++	0	0	+++++	0	0	+++++	+	0	0
None													
13	18	Pos.	0	0	0	0	+++	0	0	+++	X	+	0
27	25	Pos.	+++	++++	0	++++	+++++	0	+++	+++++	++++	X	0
12	27	0	0	+++	0	0	+++++	0	+	+++++	+	X	+
23	28	Cont.	0	+	0	0	+++	0	0	+++	+++	0	0
24	28	Pos.	+++	+	0	0	++++	0	0	+++++	+++++	0	0
22	43	Pos.	+++	+++	++++	+++	+++++	+++	+++	+++++	++++	0	0

11	47	Pos.	+	0	0	0	++	0	0	+++	0	0	0
26	51	Pos.	0	+	0	+	+++++	0	0	+++++	+	0	0
8	52	Pos.	0	0	0	0	++	0	0	++	+	0	X
25	73	Pos.	0	+	0	+	+++++	0	0	+++++	+++	0	0
9	75	Pos.	0	+	0	0	+++++	0	0	+++	0	0	0
None													

X = Not done

0 = No growth

Pos. = Positive

Cont. = Contaminated

+ = Very few colonies (one to five).

++ = Few colonies (six to fifteen).

+++ = Some colonies (fifteen to thirty).

++++ = Many colonies (thirty to seventy-five).

+++++ = Abundant growth (more than seventy-five, colonies could be).

++++++ = Very abundant growth (confluent growth).

cultures are used to evaluate the efficacy of treatment. The results recorded in Table 1 are based on cultures of pooled macerated nodes.

TREATMENT OF INFECTED ANIMALS

Eighty-eight guinea pigs were inoculated with a fresh saline suspension of *Br. abortus* 1489. The animals were divided into twenty-two groups of four guinea pigs each and treated as follows:

Treatment⁵

Group No.

- 1 None. Normal diet.
- 2 Streptomycin alone. Started one week after inoculation.
- 3 Streptomycin started one week after inoculation and sulfadiazine two weeks after inoculation.
- 4 Sulfadiazine started one week after inoculation and streptomycin two weeks after inoculation.
- 5 Sulfadiazine alone. Began one week after inoculation.
- 6 Began at the same time one week after inoculation with sulfadiazine and streptomycin.
- 7 Streptomycin started one week after inoculation and dimezol two weeks after inoculation.
- 8 Dimazol started one week after inoculation and streptomycin two weeks after inoculation.
- 9 Dimazol and streptomycin started at the same time one week after inoculation.
- 10 Dimazol started one week after inoculation.
- 11 Aureomycin started one week after inoculation and sulfadiazine two weeks after inoculation.
- 12 Sulfadiazine started one week after inoculation and aureomycin two weeks after inoculation.
- 13 Aureomycin and sulfadiazine started at the same time one week after inoculation.
- 14 Aureomycin began one week after inoculation and streptomycin two weeks after inoculation.
- 15 Streptomycin began one week after inoculation and aureomycin two weeks after inoculation.
- 16 Aureomycin and streptomycin started at the same time one week after inoculation.
- 17 Aureomycin, streptomycin and sulfadiazine started at the same time one week after inoculation.
- 18 Aureomycin, streptomycin and dimazol started one week after inoculation.
- 19 Chloromycetin alone started one week after inoculation.
- 20 Chloromycetin and sulfadiazine started at the same time one week after inoculation.
- 21 Chloromycetin and aureomycin started at the same time one week after inoculation.
- 22 Chloromycetin and streptomycin started at the same time one week after inoculation.

⁵ Experiment lasted three weeks since the date of *Brucella* inoculation.

TABLE 2

Results of Cultures from Spleen and Lymph Nodes of Normal Controls and Treated Guinea Pigs

(Animals were infected 6-14-49. Treatment started one week after infection. Duration of experiment was three weeks. Cultures taken two days after cessation of treatment.)

Group Number	Animals treated with ^a	Number of animals	Cultures from	
			Spleen	Lymph nodes
1 ^r	Normal controls. Not treated	4	++++++	++++++
2	Streptomycin	2 1 1	+++ +++++ ++	+++ +++++ +++++
3	Streptomycin and sulfadiazine	1 1 1 1 (7) ^s	0 0 0 +	0 +++ ++++ ++++
4	Sulfadiazine and streptomycin	4	0	++++
5	Sulfadiazine	1 1 1 1	+ +++ +++ ++++	++++++ +++++ +++ ++++
6	Sulfadiazine plus streptomycin	2 1 1	+ +++ ++	++ +++ +++
7	Streptomycin and dimazol	1 1 1 1	+++ +++++ ++ ++	+++ +++++ ++ +
8	Dimazol and streptomycin	1 1 1 1	+++ + ++++++ 0	+++++ +++ ++++++ +++
9	Dimazol plus streptomycin	2 1 1	X + 0	X 0 ++++
10	Dimazol	2 1 1	++ +++++ ++++	++++++ +++++ ++++++
11	Aureomycin and sulfadiazine	1 1 (7) 1 (11) 1	+++++ ++++++ + ++	0 + 0 +
12	Sulfadiazine and aureomycin	1 (9) 1 (12) 1 (13) 1 (13)	0 +++ +++++ X	++++ ++++ + +++

TABLE 2—Continued

Group Number	Animals treated with ⁶	Number of animals	Cultures from	
			Spleen	Lymph nodes
13	Aureomycin plus sulfadiazine	2 (7, 10) 2 (9, 11)	Cont. +++++	+++++ 0
14	Aureomycin and streptomycin	2 1 (10) 1	0 + X	+ ++++ X
15	Streptomycin and aureomycin	1 (11) 1 1 1	++ + + 0	+ +++ 0 0
16	Aureomycin plus streptomycin	1 (7) 1 1 1	Cont. + + 0	0 + 0 0
17	Aureomycin plus streptomycin plus sulfadiazine	1 (6) 1 (9) 1 (8, 14)	+ +++ +++	++ + 0
18	Aureomycin plus streptomycin plus dimazol	1 (5) 1 (7) 2 (13, 14)	Cont. 0 0	++ X 0
19	Chloromycetin	4	+++++	+++++
20	Chloromycetin plus sulfadiazine	1 1 1 1	+++ + +++ Cont.	+++ +++ ++++ Cont.
21	Chloromycetin plus aureomycin	1 (5) 1 (6) 1 1 (14)	++ Cont. + ++++	++ ++++ +++ Cont.
22	Chloromycetin plus streptomycin	1 (14) 1 1 1	X ++++ ++++ +++	X +++ ++ Cont.

⁶ For details see description of groups given above.

⁷ Each group consisted of four animals.

⁸ The parentheses indicate that the animals died before the completion of the experiment. The figures inside the parenthesis refer to the number of days of treatment at the time of death. Most of the animals that died before the completion of the experiment were being treated with aureomycin. The majority of these deaths were most probably due to the fact that we gave, at the beginning of the experiment, several subcutaneous injections of an aureomycin supplied to us by Lederle (aureomycin hydrochloride parenteral, Lab. No. 7-9315) which was to be administered exclusively by the intravenous route. This preparation is toxic and causes marked destruction of tissue when given subcutaneously. After we became aware of this, oral aureomycin was used.



FIG. 1 "A".—G.P. No. 173: Normal control. No treatment. Killed 23 days after inoculation. (1) Inguinal lymph nodes of the side of inoculation. (2) Inguinal lymph nodes of side opposite that of inoculation. (3) Axillary lymph nodes. (4) Retroperitoneal (lumbar) lymph nodes.

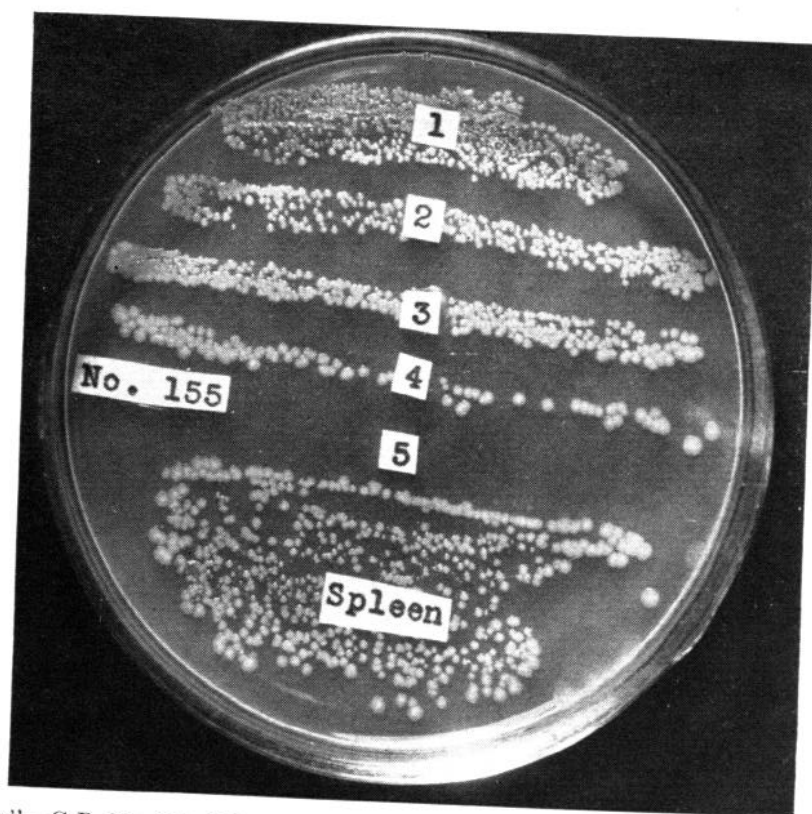


FIG. 1. "B".—G.P. No. 155: *Chloromycetin* alone. Treatment started one week after inoculation and lasted two weeks. Animal killed two days after cessation of treatment. (1) Axillary lymph nodes of the side opposite that of brucella inoculation. (2) Axillary lymph nodes of the side of brucella inoculation. (3) Retroperitoneal (lumbar) lymph nodes. (4) Inguinal gland of the side opposite that of brucella inoculation. (5) Inguinal gland of the side of brucella inoculation.

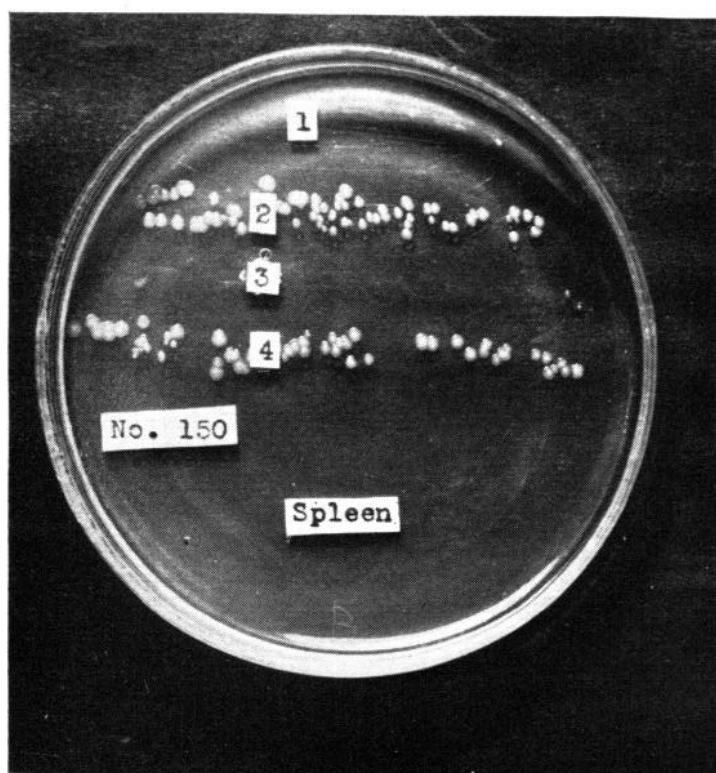


FIG. 2.—G.P. No. 150: *Sulfadiazine* + *Streptomycin* (Group 4). Treatment with sulfadiazine started one week after inoculation and with streptomycin two weeks after inoculation. Animal killed 2 days after cessation of treatment. Experiment lasted three weeks. (1) Inguinal lymph nodes of the side of brucella inoculation. (2) Axillary lymph nodes of the side of brucella inoculation. (3) Axillary lymph nodes of the side opposite that of brucella inoculation. (4) Retroperitoneal lymph nodes.

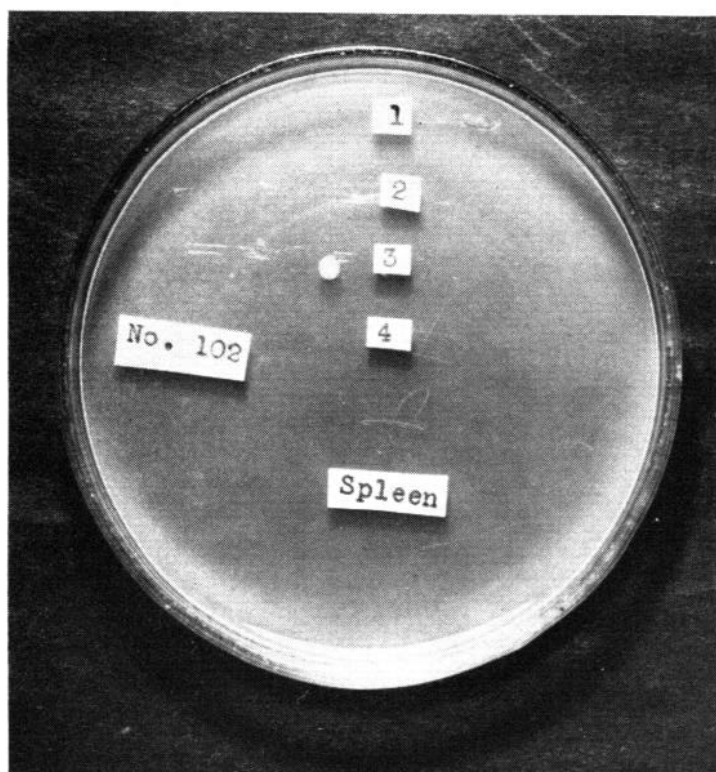


FIG. 3.—G.P. No. 102: *Aureomycin* + *Streptomycin*. Treatment with aureomycin started one week after inoculation and lasted 2 weeks. Streptomycin treatment started 2 weeks after brucella inoculation and lasted one week. Animal killed 2 days after cessation of treatment. (1) and (2) Different retroperitoneal (lumbar) lymph nodes. (3) Axillary lymph nodes of the side of brucella inoculation. (4) Inguinal lymph nodes of the side of brucella inoculation.

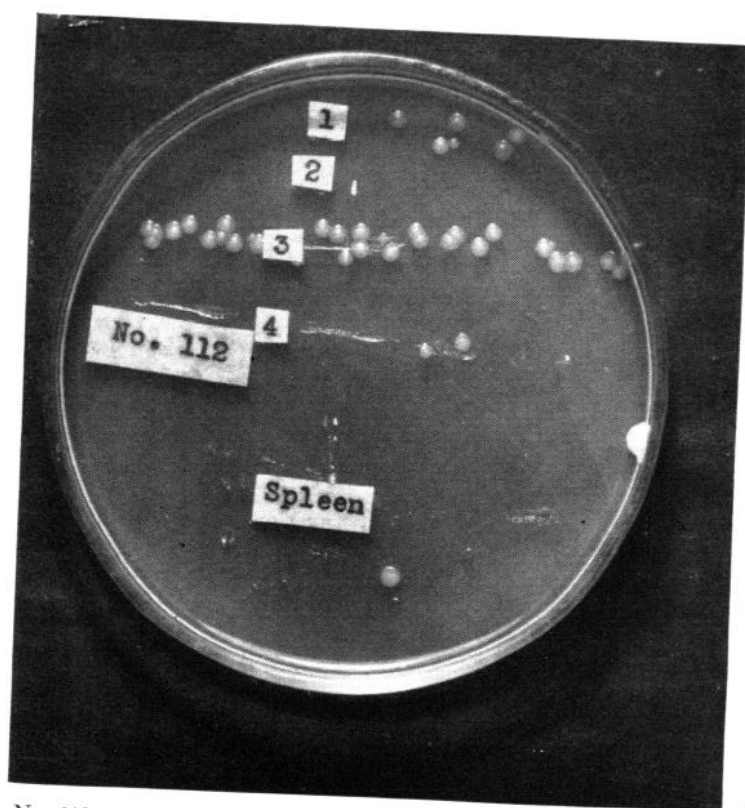


FIG. 4.—G.P. No. 112: *Streptomycin* + *Aureomycin*. Treatment with streptomycin began one week after inoculation and lasted 2 weeks. Treatment with aureomycin started 2 weeks after inoculation and lasted one week. Animal killed 2 days after the cessation of treatment. (1) Axillary lymph node of the side opposite that of brucella inoculation. (2) Axillary lymph node of side of brucella inoculation. (3) Inguinal lymph node of side of brucella inoculation. (4) Retroperitoneal lymph node (lumbar).

The animals were killed two days after cessation of treatment and the spleen and lymph nodes were cultured for *Brucella*. The results are given in Table 2.

We would like to call the attention of the reader to some of the observations made under the conditions of this preliminary experiment.

(1) Chloromycetin failed completely to decrease the number of brucella organisms in the spleen and lymph nodes of the infected guinea pigs, under the conditions of this experiment (See Fig. 1 "a" and "b").

(2) In group 4 (treatment with sulfadiazine started one week after *Brucella* inoculation and continued during two weeks. Treatment with streptomycin started two weeks after inoculation and continued for one week. Cultures made two days after cessation of treatment.) It is interesting to observe that the four animals gave negative spleen cultures but good growth was obtained from the lymph nodes. Figure 2 is illustrative of these findings. It must be noted also in Fig. 2 that cultures of the inguinal lymph nodes on the side of *Brucella* inoculation and of the axillary lymph nodes on the side opposite that of *Brucella* inoculation were negative while good growth was obtained from the retroperitoneal (lumbar) lymph nodes and from the axillary lymph nodes on the side of *Brucella* inoculation. This illustrates the need of culturing both the spleen and the lymph nodes in the evaluation of treatment and of doing cultures of the pooled lymph nodes.

(3) The combination of aureomycin plus streptomycin (groups 14, 15 and 16) was effective in decreasing markedly the number of organisms in the spleen and lymph nodes. Figs. 3 and 4 illustrate two representative cases. All these combinations are being re-tested at present on a large number of animals and at different stages of the infection.

SUMMARY AND CONCLUSIONS

The results of a pilot experiment on the chemotherapy of *Br. abortus* infection in the guinea pig have been presented.

There were no marked differences between one aerobic *Br. abortus* strain (Strain Sablín) and one anaerobic strain (No. 1489) in their infectivity for the different organs and tissues of normal guinea pigs. The splenic pulp and lymph nodes were the only tissues from which *Brucella* were consistently isolated. Therefore only spleen and lymph nodes were cultured for the evaluation of treatment.

The treatment with chloromycetin was completely ineffective when administered orally to guinea pigs. There is the possibility of deficient absorption. Blood levels of the drug were not determined.

Streptomycin dimazol (NU-445) and sulfadiazine when given alone caused only a slight decrease in the number of organisms in the spleen and lymph nodes. However, a combined treatment of streptomycin and sulfadiazine was effective in eliminating the brucellae from the spleen but not from the lymph nodes. This emphasizes the need of culturing both the lymph nodes and the spleen for the evaluation of treatment.

The combined treatment with aureomycin plus streptomycin resulted in a marked decrease or complete elimination of brucellae from the spleen and/or the lymph nodes.

A large scale experiment is already in progress in an attempt to corroborate these preliminary findings and these different drugs and combinations of drugs are being tested at different stages of the experimental brucella infection.

QUIMIOTERAPIA DE LA INFECCIÓN EXPERIMENTAL DE BRUCELLA ABORTUS EN EL COBAYO (*Sumario*)

Se presentan los resultados de un experimento tipo sobre la quimioterapia de la infección de *Brucella abortus* en el cobayo.

No existían diferencias marcadas entre la infecciosidad de la cepa aeróbica Sablín de *Br. abortus*, y la de la cepa anaeróbica No. 1489 para los diferentes órganos y tejidos del cobayo normal. En animales no tratados, solamente se pudieron aislar *Brucellas*, consistentemente, en la pulpa esplénica y en los nódulos linfáticos. Por tal motivo, para evaluar el tratamiento sólo se cultivaron nódulos linfáticos y del bazo.

El tratamiento con cloromicetina resultó ineficaz bajo las condiciones del experimento. La administración de estreptomicina, dimazol (NU-445) y sulfadiazina por separado, produjo sólo una pequeña disminución en el número de microorganismos presentes en estos nódulos. Sin embargo, el tratamiento combinado de estreptomicina y sulfadiazina resultó eficaz para eliminar las *Brucellas* del bazo, aunque no de los nódulos linfáticos. Tal hecho recalca la necesidad de cultivar ambos nódulos para la evaluación de la terapia.

El tratamiento combinado de aureomicina, estreptomicina y dimazol fué el más eficaz.

Actualmente se halla en progreso un experimento en gran escala para tratar de corroborar estos hallazgos preliminares, probándose la eficacia de estas drogas o de esta combinación de drogas en diferentes etapas de la infección experimental brucelósica.

LIST OF DRUGS SCHEDULED TO APPEAR

<i>Latin Names</i>	<i>English Index</i>
Acetarsolum	Acetarsol
Acidum Acetylsalicylicum	Acetylsalicylic Acid
Acidum Ascorbicum	Ascorbic Acid
Acidum Benzoicum	Benzoic Acid
Acidum Hydrochloricum	Hydrochloric Acid
Acidum Hydrochloricum Dilutum	Dilute Hydrochloric Acid
Acidum Nicotinicum	Nicotinic Acid
Aconitinum	Aconitine
Aconiti Tuber	Aconite Root
Adrenalinum (Syn. Epinephrina)	Adrenaline (Syn. Epinephrine)
Aether Anaestheticus	Anaesthetic Ether
Aether Vinylicus	Vinyl Ether
Aetheroleum Chenopodi	Chenopodium Oil
Aethisteronum	Ethisterone
Aethylis Aminobenzoas	Ethyl Aminobenzoate
Aethylis Chloridum	Ethyl Chloride
Amidopyrinum	Amidopyrine
Aminophyllinum	Aminophylline
Amphetaminum	Amphetamine
Amphetamini Sulfas	Amphetamine Sulfate
Amyleni Hydras	Amylene Hydrate
Amylis Nitris	Amyl Nitrite
Apomorphini Hydrochloridum	Apomorphine Hydrochloride
Argenti Nitras	Silver Nitrate
Argentum Proteinicum	Silver Protein
Arseni Trioxydum	Arsenic Trioxide
Atropini Sulfas	Atropine Sulfate
Atropinum	Atropine
Barbitalum	Barbital
Barbitalum Natrium	Barbital Sodium
Barii Sulfas	Barium Sulfate
Belladonnae Herba	Belladonna Herb
Belladonnae Radix	Belladonna Root
Benzylis Benzoas	Benzyl Benzoate
Bismuthi Subcarbonas	Bismuth Subcarbonate
Bismuthi Subsaliicylas (Syn. Bismuthi Salicylas)	Bismuth Subsaliicylate
Bromoformium	Bromoform
Butacaini Sulfas	Butacaine Sulfate
Butylis Aminobenzoas	Butyl Aminobenzoate
Calciferolum	Calciferol
Calcii Gluconas	Calcium Gluconate
Calcii Lactas	Calcium Lactate
Carbacholum	Carbachol
Carbarsonum	Carbarsone
Carbonei Dioxydum	Carbon Dioxide
Carbonei Tetrachloridum	Carbon Tetrachloride
Cascara Sagrada	Cascara Sagrada