



Title	THERAPEUTIC TREATMENTS IN EQUINE INFECTIOUS ANEMIA IV. : THE USE OF NITROMIN AND CHLOROMYCETIN FOR ARTIFICIALLY INOCULATED INFECTIOUS ANEMIA
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**THERAPEUTIC TREATMENTS IN EQUINE  
INFECTIOUS ANEMIA IV.  
THE USE OF NITROMIN AND CHLOROMYCETIN FOR  
ARTIFICIALLY INOCULATED INFECTIOUS ANEMIA**

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**INTRODUCTION**

Although 3 therapeutic experiments<sup>(1,2,4)</sup> already have been made, beneficial, useful results were not obtained at all. In the previous experiments, 4 horses affected naturally with chronic infectious anemia were treated respectively with nitromin, chloromycetin and aureomycin. Clinical, hematological, pathological and biochemical observations were conducted during the whole period of the experiment.

In the present experiment, 3 horses affected with artificially inoculated infectious anemia were used for nitromin and chloromycetin therapies. Comparisons of the findings were made with those obtained in the cases of natural infectious anemia. Clinical, hematological, biochemical and pathological investigations were conducted as was done in the previous experiment.

**METHODS AND RESULTS OF EXPERIMENTS**

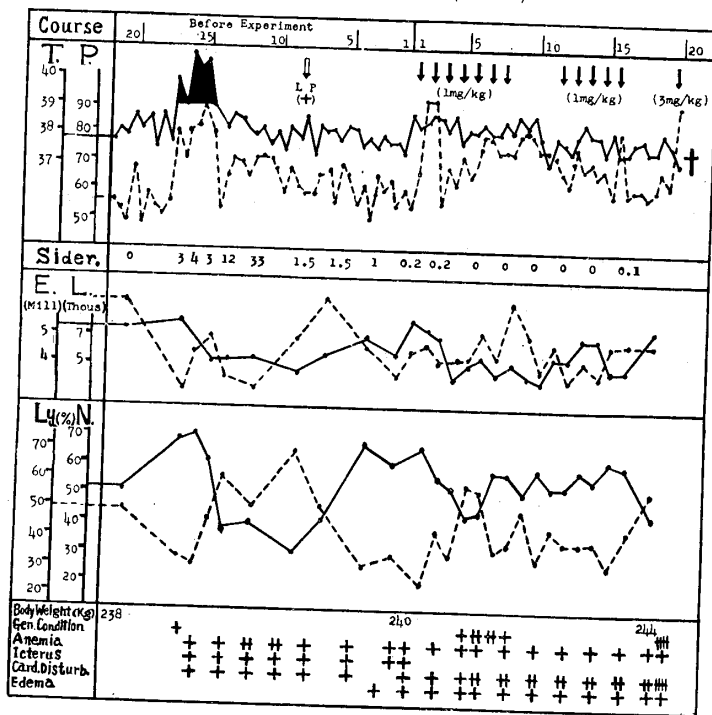
Clinical and hematological observations were made every 1 or 2 days and after slaughter or death of the animals the bodies were subjected to pathological examination. Adequate feed was provided and there was proper care constantly throughout the experiment.

**1. Nitromin Therapy No. 1**

The patient was a mongrel Percheron male 1.5 years old weighing 240 kg and was the same as No. 2 used for the experiment on the transmission of virus by MIURA et al<sup>(3)</sup>. In the transmission experiment, the sickness was exactly recognized as infectious anemia with the clinical, hematological and the pathological findings (by means of liver and spleen puncture).

When the experiment was started, the severity of the disease was not very

CHART 1. Nitromin Therapy in Inoculated Infectious Anemia (No. 1)



Note: L.P. .... Liver puncture.

Sider. .... Siderocyte number per 10,000 leucocytes.

slower than in healthy animals. Erythrocytes were 5.5 millions and leucocytes were 5,500 in number. Siderocytes stained by OGURA'S method appeared at the rate of 0.2 per 10,000 leucocyte cells and the signs of infectious anemia were found by liver biopsy (by means of liver puncture). As noted above, at the commencement of the experiment, the patient apparently possessed such symptoms that the sickness seemed to be chronic infectious anemia.

Nitromin was injected intravenously 1 mg/kg of body weight per day for 12 days as indicated in chart 1. Symptoms found in injection period were almost identical with those of period before experiment, but gradual aggravation in cardiac disturbances was found. The variations in erythrocyte and leucocyte numbers or in differential counts of leucocyte cells were not remarkable in comparison with those of before experiment. Siderocytes appeared only on the 1st, 2nd and on the 15th day at the rate of 0.1~0.2 per 10,000 leucocyte cells. Marked alternations concerning serum protein and glucose, as were showed in table 1, were not found at any time throughout the entire experiment.

With the final injection of 3 mg/kg of body weight, dullness, depression, severe cardiac acceleration, convulsion, increase in breathing and staggering were observed from 1 hour after injection and the animal could not continue standing

violent. The temperature indicated 38.7°C and the pulse rate was 50. On inspection, the general conditions were almost normal and the horse ate heartily. The mucous membranes, especially the ocular mucous membranes, were found to be dirty pale yellow in color, but petechial hemorrhages were not found. Further, in the fetlocks of extremities was observed non-inflammatory edema. On heart examinations, indistinct cardiac murmur, irregularity and cloudiness in sounds, stippling in 1st sound and jugular vein pulse were noted. Heart functions were accelerated easily even by slight irritations or movement, and recovery was

TABLE 1. *Biochemical Findings in Serum*

CASE NO.	MEDICINE	TIME OF EXAMINATION (Day)	PROTEIN (g/dl)				GLOB.-FRAC. (g/dl)			GLUCOSE (mg/dl)	REMARKS			
			Total	Alb.	Glob.	A/G	$\alpha$ -	$\beta$ -	$\gamma$ -					
1	Nitromin	Before	6.9	1.48	4.92	0.40	0.77	2.82	1.33	67	* Severe cardiac disturbance appeared and gradually increased.			
		Injection	3	6.5	1.95	4.65	0.40	0.70	2.28	1.67		60		
			5	6.4	1.55	4.85	0.30	1.05	3.12	1.68		80		
			7*	7.0	1.85	5.15	0.36	1.15	3.94	1.06		92		
		Rest	3	6.5	1.50	5.00	0.30	1.45	2.44	1.11		42		
			10	6.4	1.55	4.85	0.30	0.80	2.90	1.15		70		
		Injection	12	6.5	1.65	4.85	0.34	1.55	2.12	1.18		84		
			16	7.1	1.85	5.25	0.35	0.85	3.45	0.95		98		
		2	Nitromin	Before	7.0	1.50	5.50	0.28	0.98	3.29		1.23	88	** Marked leucopenia and oligocytemia appeared.
				Injection	2	7.2	1.25	5.95	0.23	0.85		4.67	0.43	
5	7.3				1.35	5.95	0.24	0.85	4.67	0.43	50			
9	7.2				1.30	5.90	0.24	0.95	4.38	0.57	25			
After	13**			7.1	1.25	5.85	0.21	1.05	4.10	0.70	47			
	15			7.8	1.50	6.20	0.26	0.80	4.30	1.10	58			
3	Chloro-mycetin	Before	7.2	1.60	5.60	0.29	0.50	4.03	1.07	55	*** Fever attack			
		Medication	2	8.4	1.85	6.55	0.28	1.25	4.25	1.25		43		
			6	7.8	1.50	6.30	0.24	0.90	4.07	1.33		89		
		Rest	8	8.4	1.95	6.45	0.30	1.30	3.87	1.28		56		
		Medication	9	6.3	1.85	4.55	0.40	0.75	2.33	1.47		—		
			12	7.8	2.05	5.75	0.36	0.70	3.32	1.73		85		
		After	14***	6.8	1.50	5.30	0.28	1.00	3.14	1.16		83		
			18***	6.9	1.55	5.35	0.29	0.70	3.57	1.08		47		

from 1.5 hours. Finally, the patient died in 2 hours with agonizing pain.

On the pathological findings in the corpse, although no indications of infectious anemia were recognized, swelling in all lymph nodes, congestion and opacity in liver, catarrhal enteritis and nephrosis were found.

## 2. Nitromin Therapy No. 2

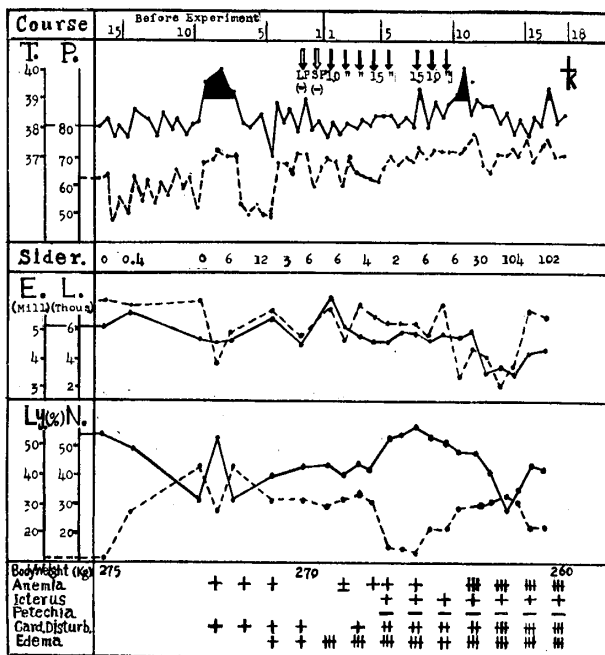
A mongrel Percheron female of 1.5 years was used for the experiment. Body weight was 270 kg. The horse was the same as No. 4 used in the transmission experiment by MIURA et al<sup>(3)</sup>. One or two days before the therapeutic experiment, biopsy findings conducted in liver and spleen showed no signs of infectious anemia, but it was proved beyond doubt that the horse was infected with infectious anemia from the results of clinical and hematological findings.

Nitromin was injected at the rate of 1~1.5 mg/kg body weight per each day for 8 days as indicated in chart 2. A slight fever attack appeared on the 10th day, the second day after terminal injection. On inspection, edema of fetlocks in extremities and emaciation were found to increase gradually by repetition of the

injections. Anemia of visible mucous membranes especially in ocular mucous membranes, was increased. It was associated with dirty and icteric colors, but no petechial hemorrhages were found. On auscultation of heart, cloudiness in sounds, stippling in 1st or 2nd sound were aggravated and cardiac murmur was present from the 3rd day.

From hematological examination it was found that erythrocyte numbers had a tendency to diminish from the 3rd day of experiment; they counted 3.5 millions in the lowest after a slight fever attack. Hemoglobin contents were decreased almost in parallel with those of erythrocytes. Anisocytosis, poikilocytosis and metachromatic conditions were found in red cells, however, no erythroblasts appeared. With repetition of nitromin injections, leucocytes

CHART 2. Nitromin Therapy in Inoculated Infectious Anemia (No. 2)



Note: S P.....Spleen puncture.  
 L P.....Liver puncture.  
 Sider. ....Siderocyte number per 10,000 leucocytes.

decreased gradually in numbers; they showed 3,000 in the lowest on the 13th day. Neutrophils ran almost in parallel to leucocyte numbers and lymphocytes showed a tendency to become antagonistic to them. The characteristic biochemical findings in serum, as indicated in table 1, were the gradual decrease in  $\gamma$ -globulin and glucose. As general conditions were aggravated the observations were discontinued.

Eighteen days after the last injection, the patient was slaughtered. In pathological findings there were proofs, anatomical and histological, that the animal was affected with chronic infectious anemia, however, there still remained some evidence of the activity of viruses.

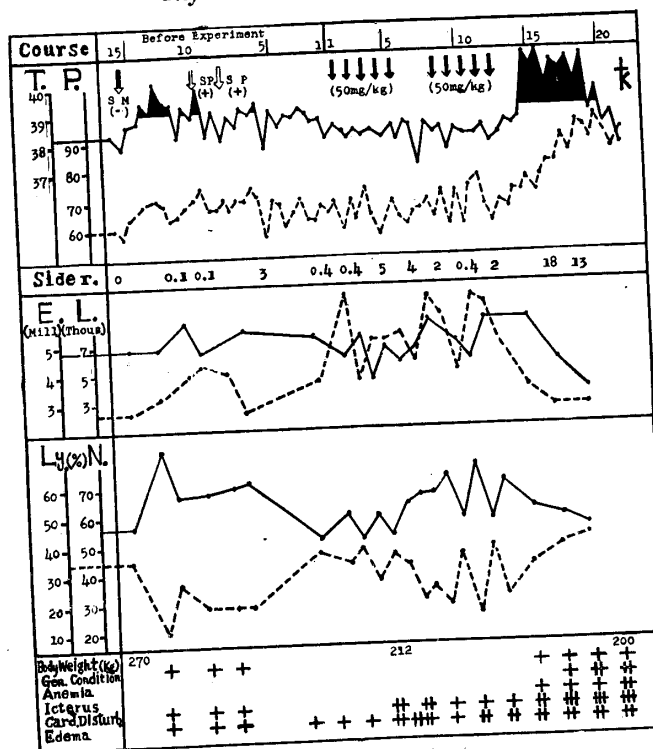
### 3. Chloromycetin Therapy

The patient used for the experiment was the same as No. 3 used for the transmission experiment<sup>(3)</sup>. It was a 1.5 years old mongrel Percheron female weighing 212 kg. It was ascertained to be affected with infectious anemia by clinical, hematological and biopsical examinations using spleen puncture and extripation of submaxillary lymph node.

At the commencement of the experiment, the patient showed 38.5°C temperature and 67 pulse rate, and, except for edema in fetlocks, presented no indications clinically.

Chloromycetin was administered orally 50 mg/kg of body weight per day in 4 doses for 10 days as indicated in chart 3. A serious heavy fever attack occurred on the second to seventh days after administrations were completed: with it were associated depression, anorexia, staggering, anemia and icterus in mucous membranes. Nasal and oral mucous membranes contained petechial hemorrhages varying in size from a mere point to irregular area with the diameter of a lead pencil. Cardiac disturbances appeared from the 4th day and their aggravation was recognized

CHART 3. Chloromycetin Therapy in Inoculated Infectious Anemia



Note: S M.....Extripation of Submax. Ly. Nod.  
 S P.....Spleen Puncture.  
 Sider.....Siderocyte number per 10,000 leucocytes.

from the midst of the fever course.

Erythrocytes seemed to be slightly increased in the later stage of chloromycetin administrations, but showed a marked decrease coincident upon the fever attack. Variations of leucocyte numbers almost ran with those of erythrocytes. Siderocytes per 10,000 leucocyte cells were gradually increased in accordance with repeated administrations. As to biochemical findings in serum, as indicated in table 1, no remarkable changes were noted throughout the whole course.

The experiment was discontinued on the 21st day, the stage of fever lysis, and the patient was slaughtered for pathological examinations. In anatomical findings, swelling in liver, spleen and in all lymph nodes was observed. In histological findings, signs of chronic infectious anemia was observed. Although the diagnosis was taken as chronic infectious anemia, severe reactions against viruses still remained.

#### DISCUSSION

In the previous paper dealing with the therapeutic experiments with nitromin, chloromycetin and aureomycin for 4 horses with naturally infected chronic infectious anemia, the clinical, hematological and the pathological findings were observed and the ineffectiveness of these medicines was described. In the present experiment, 3 horses inoculated with infectious anemia viruses were employed to supplement the earlier experiments with nitromin and chloromycetin. The methods almost identical with those of the previous experiments.

In the present nitromin therapy, characteristic symptoms were cardiac disturbances, leucopenia and aggravation of conditions with repetition of injections as found in the previous experiments. Those alternations are considered to be caused by intoxication of nitromin as we have stated in the previous works. The most interesting facts found in No. 1 were disappearance of siderocytes from the 2nd day of nitromin injections and the entire lack of indications of infectious anemia in autopsy. Considering from these facts, it is doubtful that viruses are rendered inactive by nitromin and it can not be proved that nitromin will lead to recovery from infectious anemia. Further, No. 1 died in 2 hours after injection of 3 mg/kg of body weight on the 19th day with severely aggravated clinical symptoms. The direct cause of death may be considered to be acute intoxication or shock. In postmortem examinations, experimental animals Nos. 2 and 3 were diagnosed respectively as cases of chronic infectious anemia, but still there were found indications of virus activity.

### SUMMARY

Therapeutic experiments in artificial equine infectious anemia with nitromin and chloromycetin were conducted. Comparisons were made with the findings of previous experiments performed in cases of natural chronic infectious anemia. The results thus obtained may be summarized as follows.

1. Nitromin has a serious toxicity and aggravates the diseases. Especially, aggravation of cardiac disturbances are remarkable.

2. Blood cells, especially leucocytes, were decreased in numbers in accordance with repetition of the nitromin injection.

3. One or 3 days after treatments, in experimental horses Nos. 2 and 3, typical fever attacks of infectious anemia were found definitely. In autopsy findings, they were diagnosed as chronic infectious anemia cases.

4. In animal No. 1, siderocytes disappeared from the 3rd day of nitromin injection, but in the other 2 cases they were found usually.

5. While No. 2 showed gradual decrease in  $\gamma$ -globulin and glucose, other 2 cases presented no marked variations in biochemical findings.

6. Characteristic facts found in the present experiment were the disappearances of siderocytes in No. 1 and no indications of infectious anemia in its autopsy findings. However, it is impossible to believe that it could recover even had it not died suddenly.

7. In comparison with artificial and natural infectious anemia, except for No. 1, no differences were found in the therapeutic observations.

As far as shown by above described data, it was indicated that these are no effective agents against the equine infectious anemia.

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