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# THERAPEUTIC TREATMENT IN EQUINE INFECTIOUS ANEMIA

## VI. THE USE OF GREENPOLE, RESOCHIN AND PANS-NO. 610 FOR HORSES ARTIFICIALLY AND NATURALLY AFFECTED WITH INFECTIOUS ANEMIA

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### INTRODUCTION

In the previous papers, the authors have reported that no expected results were obtained in 5 experimental therapeutic treatments for the cases of naturally affected or artificially inoculated infectious anemia. Recently 3 chemicals have been put on the market reputed to be effective for treating infectious anemia horses in the fields. So the present work was conducted for the purpose of examining the effects of the 3 medicines upon horses attacked by the disease. Research was carried out clinically, hematologically and pathologically in the course of the experiment as was done also in the previously reported experiment.

### MATERIALS AND METHODS

General information on the experimental materials and methods are shown in table.

Six young healthy horses were used; 1~2 years, mongrel Percheron, male or female, 280~342 kg. Adequate feed and care were provided constantly throughout the whole course of the work. They were all inoculated intravenously 10~20 ml of serum sampled from acute patients affected with infectious anemia. Typical fever attacks were observed from 7~14 days after inoculations. Anemia and icterus in mucous membranes, cardiac disturbance and edema of fetlocks were noted clinically, and decrease of erythrocyte numbers, appearance of siderocytes were evident in the period after recovery from the temperature. In the pathological findings of biopsies in the liver, no indications of infectious anemia were found. Other horse was naturally affected with the disease; 6 years, female, mongrel Thoroughbred, 470 kg. She had characteristic findings of infectious

TABLE *Experimental Materials and Methods*

GROUP	HORSE NO.	AGE	SEX	BODY WEIGHT (kg)	INFECTION	COMMENCEMENT OF INJECTION	INJECTION METHOD (i. v.)
GP	1	1	♀	310	artificial	end of fever attack	100 mg/day for 5 days, 1 cure
	2	1	♂	342	"	non-febrile period	100 mg/day for 5 days, 2 cures
	3	1	♀	340	"	middle of fever attack	{ 100 mg/day for 5 days, 1 cure 200 mg/day for 5 days, 1 cure
RS	4	2	♂	300	"	"	{ 10 mg/kg per day, divided 3 times, for 2 days, 2 cures
	5	2	♂	280	"	"	{ 7 mg/kg per day, divided 3 times, for 2 days, 3 cures
PS	6	6	♀	470	natural	"	{ 10 mg/kg per day, divided 4 times, for 10 days, 1 cure
	7	2	♂	240	artificial	just after lysis	{ PS; 10 mg/kg per day for 10 days, 3 cures GP; 100 mg/day for 10 days PS; 10 mg/day for 10 days, 2 cures

Notes: GP...Greenpole, RS...Resochin, PS...Pans-No. 610.

anemia on the clinical, hematological and pathological examinations by means of liver puncture.

Medicines were used the 3 kinds of chemicals; Greenpole, Resochin and Pans-No. 610. Greenpole (Sodium-iron-chlorophyllin) was offered by the Nanpo Medical Industry Co., Ltd. It was applied in general for treating the patients of anemic disease. Greenpole was injected intravenously in the amounts of 100 mg or 200 mg per day for 5~10 days for a cure; the injections were made for 1 or 2 cures at 5-day intervals. The commencement of the injections was at the middle and the end of the fever attack or in non-febrile period. Resochin (chloroquine phosphate, 7-chloro-4-(4'-diethylamino-1'-methylamino)-quinoline diphosphate) was offered by the Takeda Pharm. Co., Ltd. made by Bayer Pharm. Co., Ltd. It is stated to have the highest curative qualities against human malaria. In the present experiment, Resochin were applied in an attempt to learn whether the fever attack in infectious anemia may be caused by some protozoa which were discussed by TABUCHI et al. Two horses Nos. 4 and 5 were injected intravenously with Resochin at the rate of 7 or 10 mg/kg of body weight per day divided into 3 times daily for 2 days for one cure and it was repeated for 2 or 3 cures as 5-day intervals. The injections were commenced from the middle of the fever attack.

Pans-No. 610 (4-acethylamino-naphthalein-1-rauloyl-sulfonamide) was offered by the Dainippon Pharm. Co., Ltd. This medicine was used experimentally or practically for treating Japanese encephalitis in man because of having an anti-virus activity. On the basis of the above results, the medicine was applied to 2 horses, Nos. 6 and 7. The medicine was injected intravenously in the amount of 10 mg/kg of body weight 4 times daily for 10 days for each cure.

The injections were commenced from the middle of the fever attack or from just after lysis of the fever.

In the course of the experiments, clinical, hematological and pathological observations (by means of liver puncture) were made.

After slaughter or death of the animals the bodies were subjected to pathological investigation. Especially, siderocytes were examined by method of ISHII's methanol fixation and OGURA's formalin fixation.

## RESULTS OF EXPERIMENTS

### 1. Treatment of Greenpole group

**Horse No. 1** was inoculated intravenously 20 ml of virus serum of infectious anemia. Typical fever attack, anemia and jaundice in mucous membranes and emaciation were observed from the 7th day after inoculation, while no siderocytes were found.

From the histological findings of liver biopsy, the case was diagnosed as infectious anemia. Injections of the medicine were commenced on the 8th day after lysis of the temperature. The course of the experiment is graphed in chart 1.

General condition, anemia, icterus and cardiac disturbance aggravated in accordance with repetition of injections; siderocytes appeared from the 1st day of the injections; leucocyte numbers gradually decreased. From the 3rd day of the injections, cardiac disturbance became more aggravated and the animal died on the 8th day. On the

CHART 1. Treatment with Greenpole, Horse No. 1

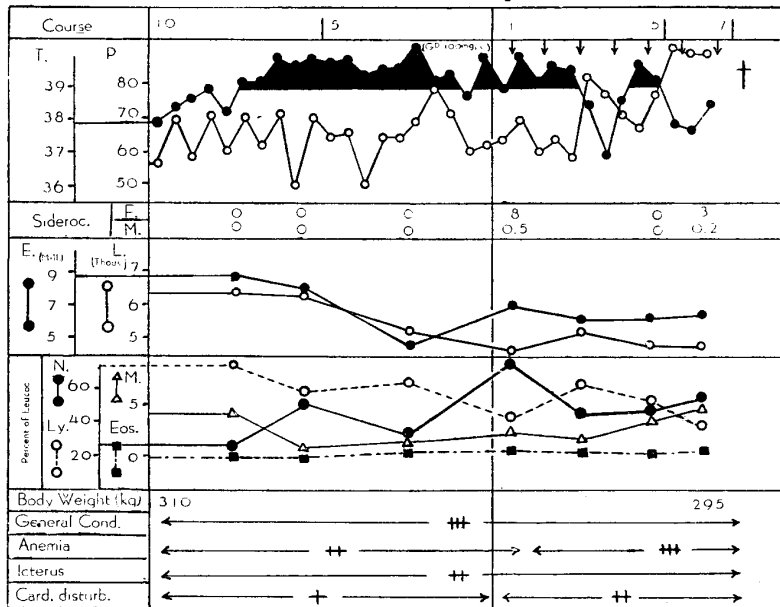
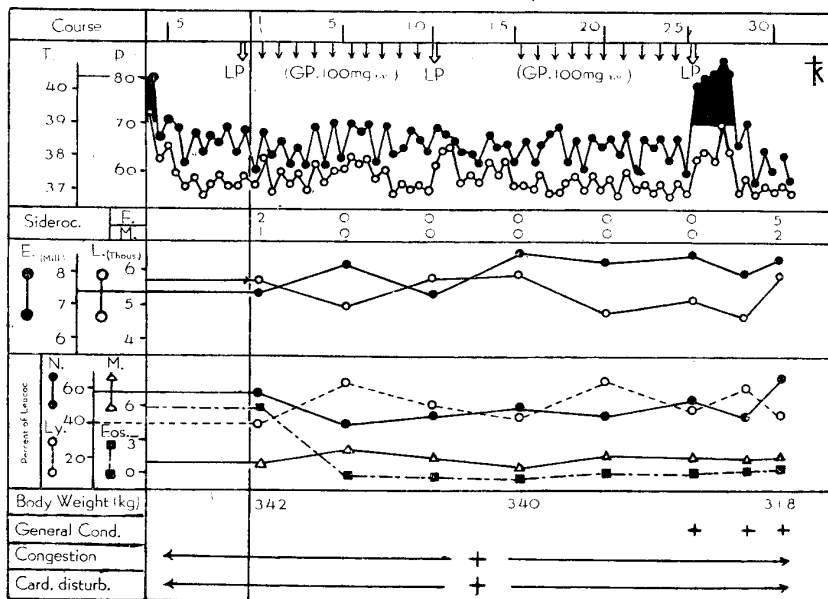


CHART 2. Treatment with Greenpole, Horse No. 2



Note: LP—Liver Punction

post-mortem anatomy, swelling in liver, spleen and lymph nodes was characteristic and the case was diagnosed as having been chronic type infectious anemia.

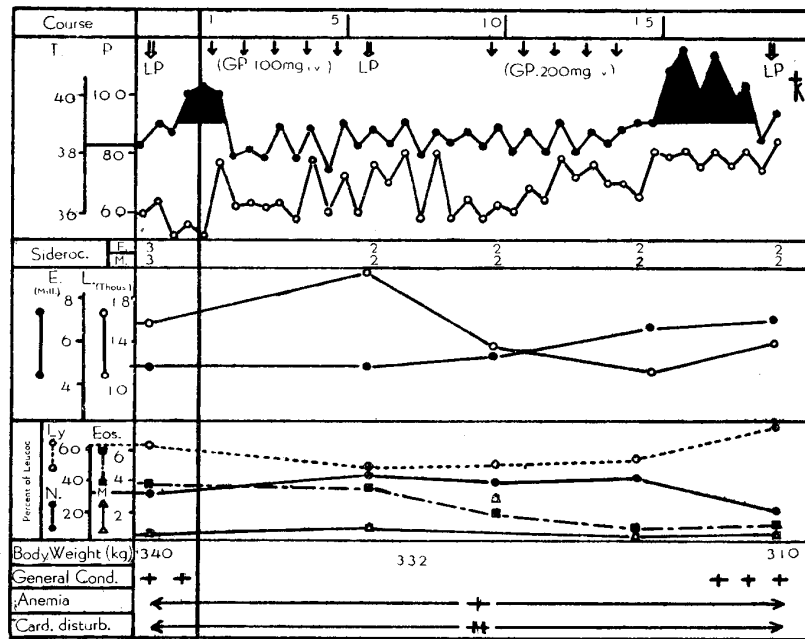
Horse No. 2 showed the 1st fever attack on the 14th day after inoculation of 20 ml of virus serum. Clinically, hematologically and pathologically, the animal was diagnosed as suffering from infectious anemia without doubt. The course of the experiment is indicated in chart 2.

The injection of the medicine was commenced on the 2nd day of the fever attack and

repeated for 2 cures after a 5-day interval. When the injections of the 2nd cures ended, the 2nd fever attack continued 4 days and the patient was slaughtered by reason of aggravation of the disease. In the clinical findings in the course of the experiment, cardiac disturbance, slight emaciation, light anemia in mucous membranes and aggravation of general conditions near the end of the experiment were noted. Erythrocyte numbers gradually increased, while leucocytes decreased in parallel with injections of the second cure. Siderocytes appeared throughout the experiment. Histological findings in liver materials sampled by biopsy at the end of the each cure indicated chronic type infectious anemia. In the post-mortem anatomy, swelling in liver and spleen, enlargement in both ventricles, anemia and edema in whole body were noted. It was diagnosed as surely infectious anemia.

Horse No. 3 was inoculated intravenously 10 ml of virus serum. Fever attack was found on the 10th day and lasted for 4 days. The animal was diagnosed as infectious anemia with the appearance of siderocytes and from the results of liver biopsy. The course of the experiment is shown in chart 3.

CHART 3. Treatment with Greenpole, Horse No. 3



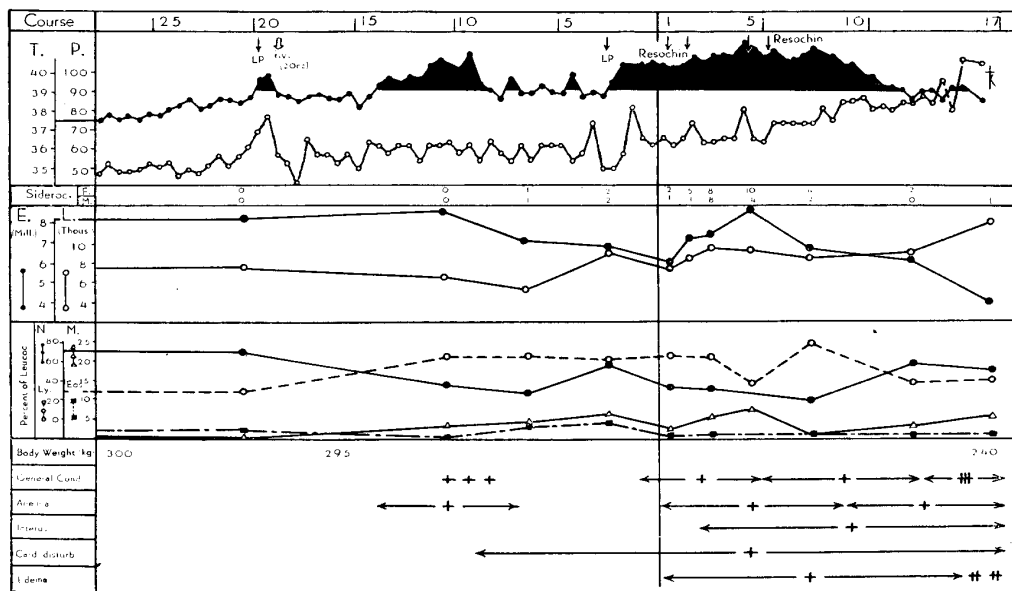
The injection of the medicine was commenced from the 5th day after lysis of the fever and it was succeeded by 2 cures with 5-day interval. Although the general conditions were almost normal and the animal ate heartily, slight cardiac disturbance and emaciation were found in accordance with the progress of the experiment. As the 2nd fever attack lasted for 3 days from the 25th day, the animal was slaughtered on the 33rd day of the injections for the purpose of investigating the pathological features. Erythrocyte numbers were counted to 7.5 million in the early part of the experiment, but they reached to 8.5 million in the end of the experiment. Leucocytes indicated no obvious variations

in numbers throughout the experiment. Siderocytes were examined 7 times during the experiment, however, their appearance was only 1 time in the end of the experiment. In autopsy findings, swelling in spleen, liver and lymph nodes were distinct and infectious anemia was confirmed.

2. Treatment of the Resochin group

Horse No. 4 was inoculated intravenously 20 ml of virus serum on the 20th day prior to treatment. Fever attack was found from the 6th to the 11th day and infection by infectious anemia was clearly recognized by the appearance of siderocytes and by the result of liver biopsy. The course of the experiment is shown in chart 4.

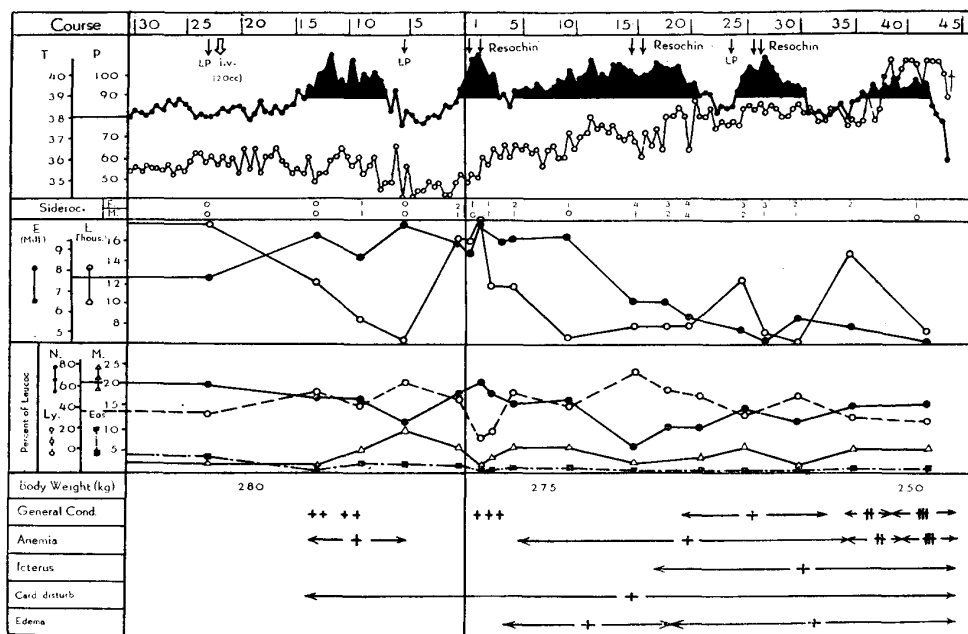
CHART 4. Treatment with Resochin, Horse No. 4



Resochin was injected from the 3rd day of the 2nd fever attack and was continued to 16 days. Although the injections were repeated for 2 cures with 5-day interval, general conditions became worse, while cardiac disturbance, anemia and icterus in mucous membranes, petechiae in conjunctiva and nasal mucous membranes and edema in fetlocks were noticed, the sickness gradually aggravated in accordance with progress of the experiment. So, as the severity of the disease became such as to endanger the life of the animal, he was slaughtered on the 17th day. Siderocytes were found clearly in every examination. Erythrocyte numbers decreased in parallel with the course of the disease, while leucocytes increased distinctly. The findings of post-mortem anatomy were clearly symptoms of subacute infectious anemia.

Horse No. 5 was inoculated with virus serum as was done in horse No. 4. In clinical, hematological and pathological findings, infection by infectious anemia admitted of no doubt. Injection of Resochin was started at the middle of the 2nd fever attack and repeated for 3 cures. The course of the experiment is shown in chart 5.

CHART 5. Treatment with Resochin, No. 5



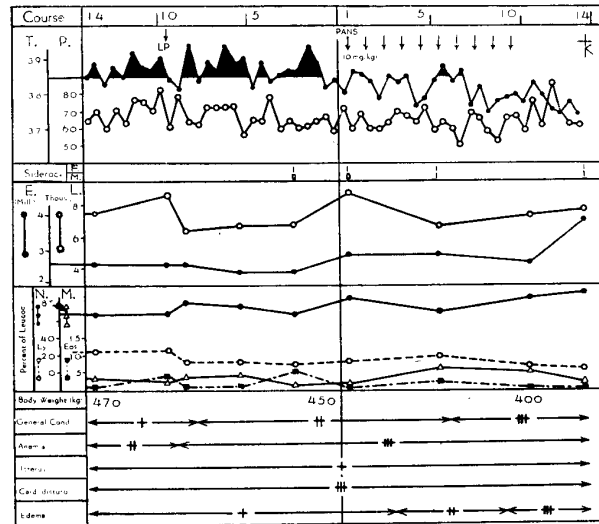
Observing the general aspects, fever attacks were found 4 times during the course of the experiment and the animal died on the 45th day because the severity of the disease was aggravated. In clinical examination, anemia, jaundice and petechiae in mucous membranes especially in conjunctiva and edema in fetlocks were evident. In the heart examinations, acceleration of heart beat, distinct cardiac murmur, irregularity and cloudiness in sounds and jugular vein pulse were noted. A large number of siderocytes appeared in every examination, whilst erythrocytes and leucocytes were decreased in accordance with progress of the experiment. In autopsy findings, serious anemia in the whole body and swelling in liver and spleen were found. Diagnosis, as was to be expected, was concluded as subacute type infectious anemia.

### 3. Treatment of Pans-No. 610 group

**Horse No. 6** was suffering from chronic infectious anemia affected with the disease in the field. The case had turned to acute type since a month before the experiment. Anemia in mucous membranes, indications of infectious anemia in liver and appearance of siderocytes were strikingly conspicuous. After several irregular fever attacks, the medicine was injected 10 mg/kg of body weight per day for 10 days as it is indicated in chart 6. The fever gradually fell with repetition of the injection. Blood cells showed a tendency to increase but an isocytosis of erythrocytes and appearance of siderocytes were conspicuous. Because the conditions, cardiac disturbance, edema and weakness became worse from the termination of the injections, the animal was slaughtered on the 14th day. In the post-mortem anatomy, anemia in whole body and swelling in liver were observed. The case was diagnosed as acute type infectious anemia.



CHART 6. Treatment with Pans-No. 610, Horse No. 6

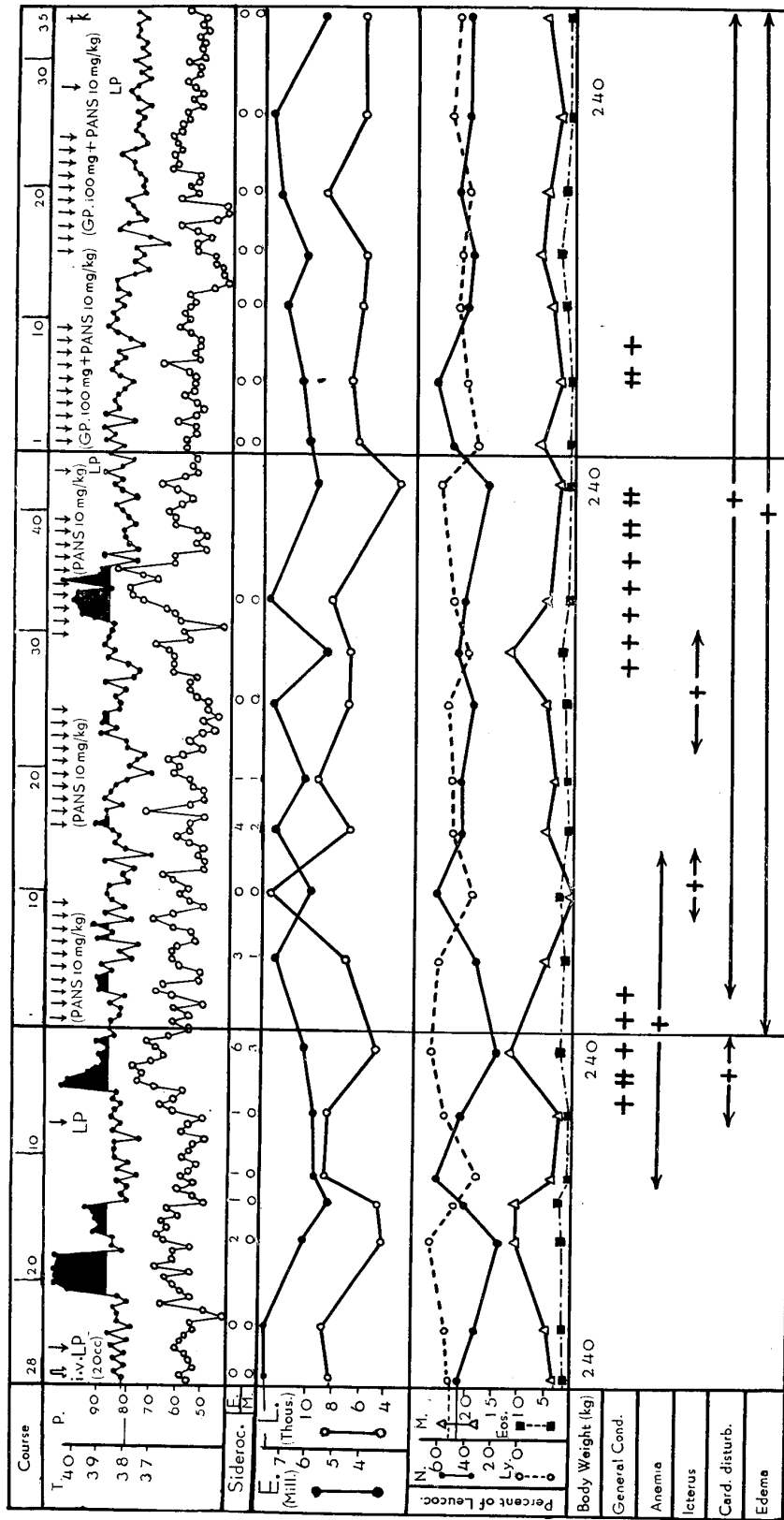


Horse No. 7 was given artificial infectious anemia as shown clinically and hematologically by inoculation of 20 ml of virus serum intravenously. Though the fever attack and appearance of siderocytes were noted, in pathological examination by means of liver biopsy, no signs of infectious anemia were found. In the 1st experiment, the medicine was injected in the amount of 10 mg/kg of body weight per day for 10 days for cure and injections were repeated 3 times with 5-day intervals. Except for typical fever attack during the 3rd cure, no remarkable changes were found in comparison with the pre-injection conditions. Although the pathological findings of the liver in the end of the 1st experiment were negative, siderocytes were present in early 3 examinations. The 2nd experiment was conducted following a rest of 5-days after the 1st experiment. The animal was injected for 2 cures simultaneously with Greenpole and Pans-No. 610. The methods of injection were identical with those applied to horse No. 3 and of the 1st experiment in horse No. 7. The patient indicated no characteristic alteration throughout the 2nd experiment and it seemed to be almost healthy. So, the experiment was discontinued and the animal was slaughtered on the 80th day after the 1st experiment was started. In the morbid anatomy, no findings at all of infectious anemia were found.

CONSIDERATIONS AND SUMMARY

As the authors tried to investigate the suitability of Greenpole, Resochin and Pans-No. 610 for treatment of equine infectious anemia, 6 horses inoculated with infectious anemia viruses and also 1 horse naturally affected with the disease were employed for the experiment. Surveying the above described data, although the methods of medications of each drug may be subject to somewhat divergent view, no therapeutic value in the use of these materials against infectious anemia is recognized.

CHART 7. Treatment with Fans-No. 610 and Greenpole, Horse No. 7



Greenpole was claimed to have anti-anemic activity but in the present experiment, 1 horse died and 2 horses were slaughtered owing to aggravation of sickness despite the fact that the treatment was prolonged.

Resochin treatment was conducted on probational trial to check an opinion by TABUCHI et al. that the fever attack in infectious anemia may be caused by some protozoa. In the present experiment, the sickness was worsened with repetition of injection of Resochin; 1 had deceased and the other 1 animal was slaughtered. Considering from the courses of the treatments in 2 horses, the medicine may be thought to react toxically for the animals under such circumstances.

Pans-No. 610 was used because it has been reported to have anti-virus activity. Horse No. 7 was recognized as a case of infectious anemia clinically and hematologically by inoculation, but in liver biopsy no symptoms of the disease were discovered. However, the animal was treated for 3 cures with Pans-No. 610 alone in the first and simultaneous treatment was followed for 2 cures by combination of Pans-No. 610 and Greenpole. Viewing the course of the experiment, the authors must report that no changes were found clinically, hematologically nor pathologically in the latter half. Especially, in post-mortem anatomy and histology, no figures of infectious anemia were noted. However, no discussion can be offered about the healing of the sickness for the reason blood transmission into healthy horse was not done. On the other hand, an interesting problem in horse No. 7 was the fact that no findings of infectious anemia were noted in liver biopsy nor in post-mortem examinations throughout the whole experiment. There is need for further examination.

Resulting in the gathering of the above described data, the present workers made 3 therapeutic experiments for horses artificially and naturally affected with infectious anemia. The summary of the experiment may be expressed as follows.

1. Greenpole, Resochin and Pans-No. 610 were not found to be effective agents against equine infectious anemia.
2. Particularly, Resochin aggravated the disease in accordance with repetition of the injections and it may be thought to have some toxicity for the patients.
3. On the basis of results of the Resochin treatment, it is difficult to say that the fever attack in equine infectious anemia may be caused by some protozoa as TABUCHI et al. say.
4. Horse No. 7. treated in simultaneously with Pans-No. 610 and Greenpole for a long time, showed no signs of infectious anemia. However, it is impossible to discuss the matter because the virus transmission into healthy horse was not made.

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