

Studies on *Eurytrema coelomaticum* II.

The Anthelmintic Efficiency of Nitroxynil and Praziquantel against *Eurytrema coelomaticum* in Cattle

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Introduction

According to Itagaki^{6,7)}, niclofolan, bithionol, carbon tetrachloride, hexachlorophen, nicrosamide and antimony compounds were used for the therapy of bovine eurytremiasis in the past, but no available evidence on the efficacies of those drugs against pancreatic flukes has been recognized yet. Nosaka et al.¹³⁾ reported that bithionol, bithionol sulfoxide, thiabendazole and piperazine were ineffective against *Eurytrema* flukes in cattle. No drug, therefore, has been established as an effective agent against pancreatic flukes up to date.

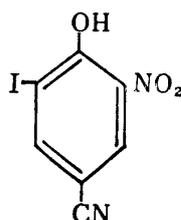
The authors¹⁶⁾ encountered the cattle heavily infected with a number of *Eurytrema coelomaticum* in two pastures situated on the hillside in the northern part of Kagoshima Prefecture, Japan. The therapeutic experiments were attempted, using two drugs for the cattle. In the present paper, the results obtained in the experiment are presented.

Materials and Methods

The 8 infected cattle used in the present experiment were introduced into the above pastures at the same term when they were 6 months old. They were Japanese black cattle, *Bos taurus* var. *domesticus*, aged 20 months, and were extremely emaciated and stunted.

The number of the eggs eliminated in feces was examined every day during a period before treatment, for checking the variation of the number of eggs per gram of feces (EPG) of all of the cattle. Two untreated cattle (Case No. 1 and 2) were dissected as a standard of comparison for checking the condition of the flukes and the pathological findings.

The infected cattle were treated with the doses of 10, 20 and/or 30 mg/kg body weight (b.w.) of nitroxynil accompanied with 50 ml of hepatic drug. The chemical name of nitroxynil is 4-cyano-



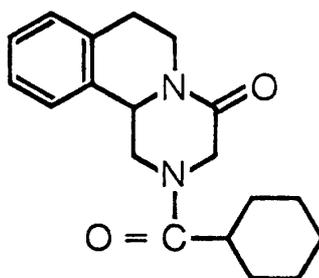
Structural formula of nitroxynil

This work was presented at the 21st World Veterinary Congress, on 1st-7th July, 1979 in Moscow, USSR.

2-iodo-6-nitrophenol, having the following chemical structure. 100 ml of the hepatic drug is composed of 500 mg of arginine monohydrochloride, 1000 mg of taurine, 200 mg of vitamin B₁, 10 mg of vitamin B₂, 10 mg of vitamin B₆, 20 mg of vitamin B₁₂, 300 mg of nicotinic acid amide and 10 g of sorbitol.

2 cattle (Case No. 3 and 4) were injected intravenously a dose of 10 mg/kg b.w. of nitroxylin, and were dissected 4 and 20 days after injection, respectively. A cattle (Case No. 5) was injected twice 30 mg/kg b.w. of the drug at an interval of 30 days, and was sacrificed on the next day of the second injection. 2 cattle (Case No. 6 and 7) were firstly, given 10 mg/kg b.w. of the drug, secondly, given 30 mg/kg b.w. at an interval of 20 days, and thirdly, given the same dose 70 days after the second injection. Those cattle were dissected 90 days later.

A cattle (Case No. 8) was given twice 10 mg/kg b.w. of nitroxylin at an interval of 32 days, but showed no disappearance of the eggs. The cattle was injected subcutaneously, three times, every other day, a daily dose of 10 mg/kg b.w. of praziquantel. The chemical name of the drug is 2-cyclohexylcarbonyl-1, 3, 4, 6, 7, 11b-hexahydro-2-H-pyrazino(2, 1-a)isoquinoline-4-one, having the following chemical structure.



Structural formula of praziquantel

The fecal examination for the eggs of *Eurytrema coelomaticum* was carried out every day in all the cases. The fecal examination for the eggs was carried out as follows: 5 g of feces was stirred with a stainless steel spatula in beaker (300 ml in capacity) containing about 200 ml of tap water until the feces were thoroughly suspended. The fecal suspension was poured, through the sieve of 150 meshes per inch, into the second beaker. The debris remaining in the sieve was washed with small amount of tap water. The filtrate was allowed to be standing for 10 minutes, and then the supernatant was tipped off. Those procedures were repeated three to five times, until the supernatant became clear. The sediment was placed into the measuring centrifugal tube, using a pipette, and was centrifuged. The amount of sediment was adjusted to 1 ml by adding water. The number of the eggs in 0.05 ml of homogeneously mixed content of the tube was counted four times. The total number was represented as the number of eggs per gram of feces (EPG).

All the cattle used were dissected for the checking of the remaining flukes and for the pathological examination. The mounted specimen of the flukes collected from the cattle were prepared, for checking their degenerative changes. All of the organs of the cattle were examined histopathologically. The anthelmintic effect of the drugs was evaluated from the decreasing in the number of the eggs in feces and in the number of the flukes remaining in pancreas.

Results

The cattle used in the present experiment were extremely emaciated and stunted. While

noninfected cattle introduced into the pastures at the same term, were more than 400 kg in body weight, those infected cattle were 193~235 kg. The cattle revealed the clinical signs as follows: general wasting, loss of appetite, severe foamy salivation, weakly peristaltic movement of rumen and intestine and mild diarrhoea. For checking the variation of EPG, the number of the eggs eliminated in feces of all the cattle used was daily examined for a period before treatment. EPG of the cattle fluctuated in a considerable range, as shown figure 1.

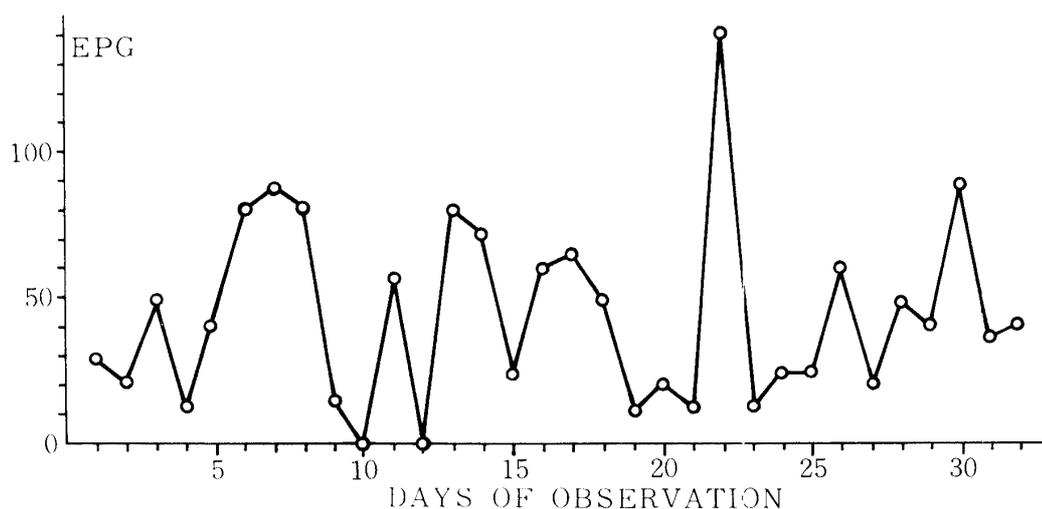


Fig. 1. Variation of EPG in an untreated cattle (Case No. 1) infected with *Eurytrema coelomaticum*.

2 untreated cattle (Case No. 1 and 2) were dissected as control, for checking the pathological changes of their organs and the number and condition of the flukes. About 5 thousand to 7 thousand of the flukes were found harboring in pancreatic duct, and several decads in bile duct and duodenum. There were numerous parasites at all the levels of the pancreatic duct, especially many clumps of the flukes in small-sized interlobular ducts formed cysts which were visible as black clots through the fibrous capsule of pancreas. The histological appearance of the pancreas revealed both thickening of the wall of pancreatic duct containing some parasites and the proliferation of periductal and interlobular connective tissues. The periductal fibrosis extended upon the adjacent acinar tissue which was atropic and degenerative. The clumps of the eggs surrounded with granulation tissues were seen in the connective tissues around the pancreatic ducts. From the above findings, the pancreas was diagnosed as pancreatitis interstitial chronica caused by *E. coelomaticum*.

Case No. 3 and 4 were given 10 mg/kg b.w. of nitroxynil accompanied with 50 ml of hepatic drug. As shown in figure 2, no amount of EPG decreased after the injection. They were sacrificed 20 days after injection. At the postmortem examination, about seven thousand of the flukes were seen remaining in the pancreatic duct, and several decads of the flukes were found from the bile duct, gall bladder and duodenum. The gross and microscopical appearances of pancreas were the same as those of the untreated cases mentioned above.

A single dose of 30 mg/kg b.w. of the drug with 50 ml of the hepatic drug was given to an infected cattle (Case No. 5). As shown in figure 3, EPG increased temporarily 3 days after injection, and then again decreased. No eggs, however, became to be free from feces. The body weight of the cattle decreased temporarily after injection and increased gradually from about 20 days after injection. For checking the anthelmintic action of nitroxynil, the cattle was given again the same

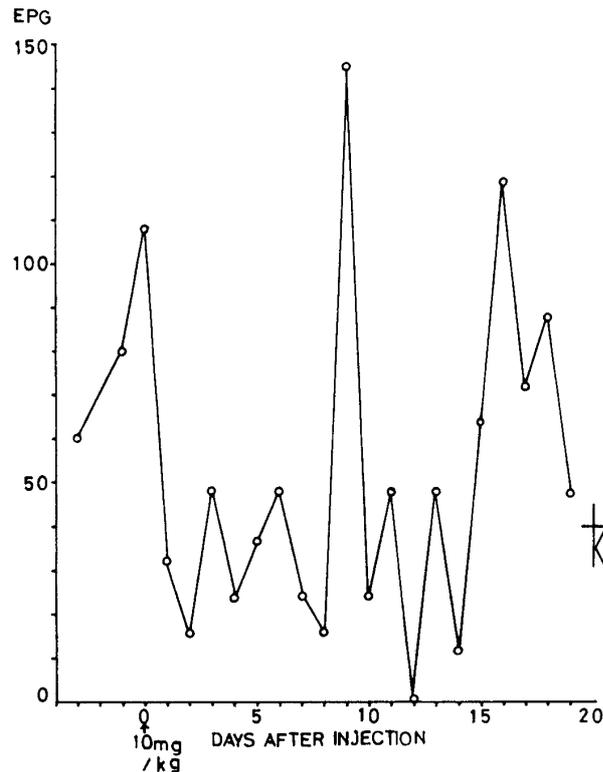


Fig. 2. Effect of one low dose of nitroxylin against *Eurytrema coelomaticum* in cattle (Case No. 4).

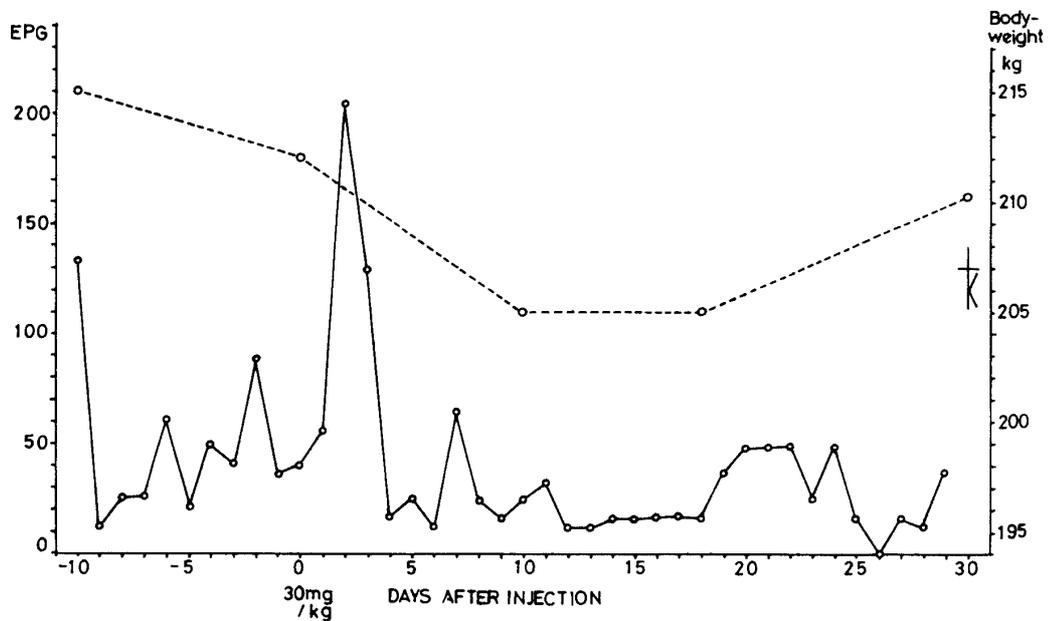


Fig. 3. Effect of one high dose of nitroxylin against *Eurytrema coelomaticum* in cattle (Case No. 5).

dose of the drug 30 days after the 1st injection, and an autopsy was held on the day succeeding the 2nd injection. The massive flukes penetrating in interlobular pancreatic ducts were degenerated, looking like black clots. 553 of the flukes, in which the degenerated flukes were involved at the rate of 76.9%, were found in the pancreatic duct, and 2,874 flukes containing the degenerative ones

at the rate of 57.6% were found in duodenum. Namely, the alive flukes were reddish, while the degenerative ones were discolored. The microscopical examination of the pancreas exhibited the thickening of the pancreatic duct accompanied with the proliferation of periductal connective tissues. The remains of the degenerated flukes were seen in the interlobular pancreatic ducts dilated in cystic form. Periductal and interstitial connective tissues proliferated among the acinar tissues which were atrophic and degenerative.

Case No. 6 and 7 were given, at first, a dose of 10 mg/kg b.w. of the drug, and were given, secondly, a dose of 30 mg/kg b.w. 20 days later. As shown in figure 4, the eggs became free from 23 days after the 2nd injection. Their body weight increased remarkably from 20 days later. The same dose was given to the cattle 70 days after the 2nd injection. The cattle were sacrificed 90 days later. In the postmortem examination, only 1 and 8 parasites were found remaining in each pancreas of the two cattle. The histological appearance of the pancreas exhibited active regeneration of the acinar tissue. No fluke was found in all the levels of the pancreatic duct. The nodules of granulation tissue surrounding the disintegrated parasites with degenerated eggs were seen in pancreatic tissue. Considerable number of so-called globule leukocytes having eosinophilic granules in their cytoplasm were seen occurring among and below the epithelial cells of mucous membrane of pancreatic duct and mast cells in the periductal connective tissues, respectively.

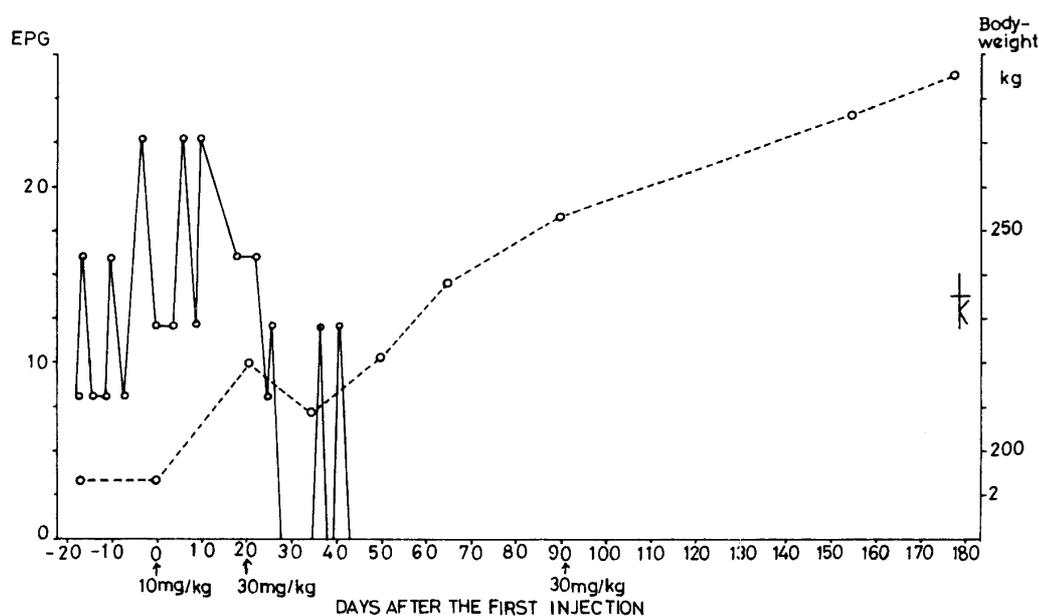


Fig. 4. Effect of three doses of nitroxylin against *Eurytrema coelomaticum* in cattle (Case No. 6).

Case No. 8 was, twice, given a dose of 10 mg/kg b.w. of nitroxylin at an interval of 32 days. However, no EPG showed conspicuous sign of its decrease. Forty five days later, the cattle was given, three times, every other day, a daily dose of 10 mg/kg b.w. of praziquantel. EPG showed a temporary increase for 4 days after the last injection. As shown in figure 5, most of the eggs which were eliminated in feces after the administration, was degenerative. The content of the degenerated eggs showed granular coagulation. Namely, the rates of the degenerated eggs on 1, 2, 3 and 4 days after the first injection were 86.7, 87.9, 82.5 and 93.5%, respectively. The eggs of the flukes disappeared completely from feces 5 days after the last injection.

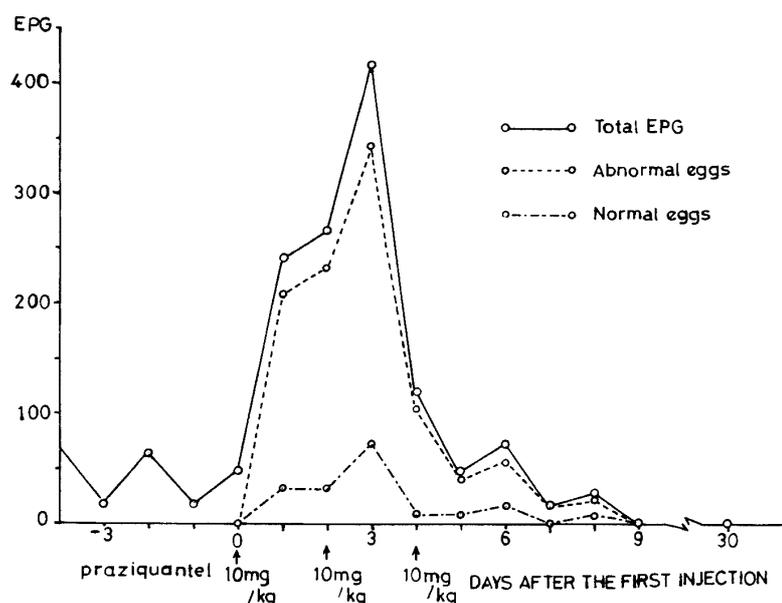


Fig. 5. Effect of praziquantel against *Eurytrema coelomaticum* in cattle (Case No. 8).

Discussion

As mentioned in the preface, there is no available drug exhibiting apparently anthelmintic action against pancreatic flukes belonging to the genus *Eurytrema*. In the present experiments, we were successful in treating the cattle infected with *Eurytrema coelomaticum*, using nitroxynil and praziquantel. Nitroxynil is primarily known as an anthelmintic against liver flukes belonging to genus *Fasciola*. Namely, Colegrave³⁾ gave a dose of 10 mg/kg b.w. of body weight subcutaneously to cattle infected with *Fasciola hepatica*. He observed that the fecal examination for the egg revealed a satisfactory removal of the flukes. Chirol et al.²⁾ who gave the doses of 10 to 20 mg/kg b.w. to 69 cattle, reported rapid clinical improvement and the disappearance of the liver fluke's eggs from feces. As regards the toxicity of nitroxynil, Lucas¹⁰⁾ stated that the maximum tolerated dose in calves was 50 mg/kg b.w., and that higher doses invariably caused some fatalities.

In the present experiment, the infected cattle were injected, once or twice, the doses of 10 or 30 mg/kg b.w. of nitroxynil at an interval of about one month. The satisfactory elimination of the flukes was observed in the cattle (Case No. 6 and 7) given, twice, the drug at a daily dose of 30 mg/kg b.w. This dose rate is near to the maximum tolerated dose reported by Lucas¹⁰⁾. For the prevention of the side effect, we used the drug in combination with a hepatic drug.

Case No. 5 given a single dose of 30 mg/kg b.w. of nitroxynil, showed a remarkable increase of EPG for 3 days after injection. This case was, again, given the same dose 30 days later, and was sacrificed the next day. A number of the flukes containing the degenerated ones at high ratio, were found to be eliminated into duodenum of the cattle. The remarkable increase of EPG was seen, also, in the cattle given praziquantel. Most of those eggs in feces were degenerated. From the above-mentioned facts, it is conjectured that the number of the eggs passed in feces increase temporarily through the collapse of the degenerated flukes eliminated into duodenum. Accordingly, those findings are considered to be an evidence of the anthelmintic action of praziquantel and nitroxynil.

As regards toxicity of praziquantel, Muermann et al.¹¹⁾ reported as follows: The LD₅₀ of praziquantel given orally to mice and rats was between 2,000 to 3,000 mg/kg b.w., and even higher

when injected subcutaneously. On the other hand, the LD₅₀ of nitroxylnil orally given to mice was 330 mg/kg b.w. and that of the drug given subcutaneously was 185 mg/kg b.w. Praziquantel is known as a high effective tapeworm remedy^{14, 15)}. Recently, the schistosomicidal activity of the drug was reported by several investigators^{1, 4, 8)}. Güralp et al.⁵⁾ reported that the drugs were effective against *Dicrocoelium dendriticum* belonging to family "Dicrocoeliidae" which contains genus *Eurytrema*. On the basis of the results obtained from the above experiments, it seems to us that praziquantel is an ideal anthelmintic against *Eurytrema* flukes. Furthermore, the authors consider it to be necessary that the anthelmintic experiment should be made in many cases for the determination of the effective dose of praziquantel against *E. coelomaticum* and *E. pancreaticum*.

Nosaka et al.¹²⁾ reported that specific cells (probably globule leukocytes) were observed in the mucous membrane of pancreatic duct of the cattle infected with pancreatic flukes. In the present experiments, we also observed that a number of globule leukocytes containing eosinophilic granules in their cytoplasm appeared among and below the epithelial cells of mucous membrane lining pancreatic duct in the infected cattle, especially the treated cases (Case No. 6 and 7). As regards the origin of globule leukocytes, investigators proposed mast cells, plasma cells or lymphocytes. Kitagawa et al.⁹⁾ proved immunohistochemically that intraepithelial globule leukocytes were derived from the thymus lymphocytes. From the point of view of host-parasite relationship such as immunological response, we are deeply interested in the appearance of the globule leukocytes in eurytremaiasis.

Summary

Cattle heavily infected with *Eurytrema coelomaticum* were treated with nitroxylnil accompanied with hepatic drug. Subcutaneous injection of the drug at a single dose of 10 or 30 mg/kg body weight (b.w.) was noted to be ineffective against the fluke. Two infected cattle were injected, firstly, 10 mg/kg b.w. of the drug and, secondly, 30 mg/kg b.w. of that 20 days later. The egg became free from feces 23 days after the 2nd injection. The body weight of the cattle increased remarkably from 20 days after the 2nd injection. The cattle were given the same dose 70 days after the 2nd medication, and were dissected 90 days later. Only 1 and 8 of the flukes were found in the pancreas, respectively. The active regeneration of the acinar tissue was seen in the histological examination.

A cattle which was, twice, given a dose of 10 mg/kg b.w., but showed no sign of decrease of EPG. In the cattle given, three times, every other day, a daily dose of 10 mg/kg b.w. of praziquantel, EPG increased temporarily for 3 days after injection, but almost all of the eggs were degenerated, and the eggs disappeared completely from 9 days after the last injection.

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Explanation of figures

All figures are photomicrographs of specimens stained with hematoxylin-eosin.

- Fig. 1. *Eurytrema coelomaticum* in the pancreatic ducts of pancreas of an untreated cattle (Case No. 2). × 32
- Fig. 2. *E. coelomaticum* in the pancreatic duct of pancreas of an untreated cattle (Case No. 2). × 67
- Fig. 3. Pancreas of an untreated cattle (Case No. 1). The acinar tissue (A) round pancreatic duct (D) is atrophic and degenerative. The periductal interlobular connective tissue proliferated remarkably. An arrow shows clump of the eggs. × 67
- Fig. 4. Regeneration of acinar tissue in the pancreas of a cattle (Case No. 7) treated with nitroxylin. × 67
- Fig. 5. Regeneration of acinar tissue in the pancreas of the same cattle. × 170
- Fig. 6. Globule leukocytes (arrows) in the mucous membrane of pancreatic duct of a cattle (Case No. 6) treated with nitroxylin. × 670

