



Title	STUDIES ON THE IN VITRO CULTURE OF STRONGYLUS VULGARIS
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the liver through GLISSON's sheath and were confined within granulation tissue. Degenerations of the liver cells and hyalinization of the blood vessel walls in GLISSON's sheath were observed. After 30 days, these foci were most remarkable. Sixty days after infection, there were no larvae in the liver. The larvae migrated through the blood stream into the lungs and were surrounded by granulation tissue. The larvae were absorbed within 30 days following infection. A slight thickening of the alveolar walls and a slight eosinophilic infiltration around the perivascular connective tissue could still be recognized after 60 days. Similar foci were also established in the lymphonodi and great omentum.

STUDIES ON THE IN VITRO CULTURE OF *STRONGYLUS VULGARIS*

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(Summary of Doctor's thesis written under direction of Dr. J. YAMASHITA)

The author investigated in vitro culture of *Strongylus vulgaris* for the purpose of finding suitable artificial media and of determining the differences in the nutritional requirement of *S. vulgaris* and other nematodes. Both adult and larval *S. vulgaris* were used, the former obtained from the caecum and the latter from aneurysms.

Among the five solutions tested, HANKS' balanced salt solution and LOCKE's solution were found to be valuable in strongyle culture. The two solutions were therefore used as basal media in the experiments. The addition of horse serum was found to be valuable in prolonging the survival, especially of the larvae. The longest survival period (64 days) was obtained when cultured at 28°C in the basal medium with 50 volume % horse serum. *S. vulgaris* had little tolerance for an acid pH, but was almost unaffected by the pH range 6.0~8.0. The optimum pH range was 6.6~7.6. A slight increase in the survival period was observed by addition of glucose. The optimum quantities were 1.0% for the adult and 0.5% for the larvae. By addition of 3~5% of yeast extract, the survival period of adults was slightly increased, but this increase was insignificant. A small amount of suspended blood cells did not affect the survival of the adult females, but larger amounts proved deleterious. Addition of heated and autoclaved horse liver extract prolonged

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the survival of adults. When the extract was added, more than 70% of the worms survived for 40 days (the maximum 53 days). The worms in such media were active and laid eggs for the first 40 days, although, except in the beginning, the eggs were generally abnormal and unfertilized. Raw horse liver extract did not affect the survival. The dialyzable fraction of heat- and autoclave-treated horse liver extract was not destroyed by heating for 10 minutes at 100°C or by autoclaving, and this fraction contained some factor which prolonged the survival of adult females. Various water soluble vitamins appeared to have no effect on survival.

Histochemical examination of surviving worms in media with heat- and autoclave-treated liver extract usually disclosed glycogen in various portions and large quantities of acid phosphatase in the intestinal cells.

The best medium for the adult *S. vulgaris* was composed of HANKS' balanced salt solution, horse serum and 50 volume % heat- and autoclave-treated horse liver extract in proportions of 4.5 : 4.5 : 1. With the larvae good results were obtained with HANKS' balanced salt solution with 50 volume % horse serum.

Thesis for the Masters degree

**OBSERVATIONS OF RUMINAL MOTILITY AND THORACIC
MOVEMENT WITH ARTIFICIAL
RETICULO-DIAPHRAGMATIC ADHESIONS IN SHEEP**

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(Summary of Master's thesis written under direction of Dr. T. SAKAI)

A non-surgical method of recording ruminal motility was attempted in sheep. In five of the cases reticulo-diaphragmatic adhesions were formed, and in one a permanent fistula was formed in the reticulum.

1) Simultaneous wave patterns made by the internal and external recording devices were very similar in the sheep with the permanent fistula.

2) Recording of thoracic movement always showed a large, high amplitude wave (r-wave) in conjunction with regurgitation, and a characteristic wave (e-wave) in conjunction with eructation.

3) The difference between the ordinary ruminal movement (O-wave, generally called primary contraction) and the eructation movement (E-wave, generally called secondary contraction) could be clearly distinguished.