Dept. of Pathol. and Clinical Pathol.,
Fac. Of Vet. Med. Assuit Univ.

PROPHYLACTIC EFFECT OF TANNINS ON VISCERAL LARVAL MIGRANS IN MICE

(With 1 Table and 5 Figures)

By

S. KH. ABD ELGHAFFAR; M.I. ARAFA* and S.A. SOTOHY**

*: Dept. of Parasitology , Animal Health Research Institute, Assuit lab.
**: Dept. of Animal hygiene, Fac. Of Vet. Med. Assuit Univ.

التأثير الوقائى لمادة التنين على هجرة يرقات الديدان فى أحشاء الجرذان

ساري خليل عبد الغفار، محسن ابراهيم عرفه، سطوحي أحمد سطوحي

أولا : تـم دراسة تأثير التنين على حيوية يرقات الباراسكارس اكورم وذلك بأستخدام التركيزات الآتـية (٥٠٠ ، ١٠٠٠ ، ٢٠٠٠ ميكروجرام/ميللتر) حيث قلت حركة تلك اليرقات تدريجيا إلى أن أنعدمت تماما بعد ٤٨ ساعة ' كما بلغ معدل النفوق فى اليرقات ٣٠،١٨،٦% بعد ٢ ، ٢٤ ، ٤٨ ساعة على التوالى عند استخدام ٢٠٠٠ ميكروجرام تنين/ميللتر' بينما بلغ معدل النفوق ٢٠ % بعد ٤٨ ساعة عند استخدام ١٠٠٠ ميكروجرام تنين /ميللتر .

ثانـيا: لدراسة تأثير التنين على العدوى التجريبية للجرذان تم تقسيم ١٢٠ من الجرذان إلى ثلاثة مجموعـات (٤٠ حـيوان فـي كـل مجموعـة) تـم عـدوى المجموعـة الأولى ببويضات التكسوكاراكانس والمجموعة الثانية ببويضات الباراسكارس اكورم والثالثة ببويضات التكسوكارا فيـتلورم . وذلـك بأعطاء كل حيوان ١٠٠٠ بويضة تحتوى كل منها على الطور المعدى وذلك عن طريق الفم وتم تقسيم كل مجموعه الى اربع تحت مجموعـــات(١٠ جرذان لكل واحده) و اعطيـت مـادة التنيـن بالتركـيزات الأتـيه فـي مـاء الشرب (٥٠٠ ، ١٠٠٠ ، ٢٠٠٠ مـيكروجرام/ميللتر) وترك تحت المجموعه الرابعه(١٠ حيوانات) فى كل مجموعة كحيوانات ضـابطة . بـدأ أعطاء التنين قبل العدوى بثلاثة ايام واستمر بعد ذلك لمدة اسبوعين وفى نهاية الـتجربة ذبحـت الحيوانات وأوضح الفحص الهستوباثولوجى للاعضاء الداخلية لتلك الجرذان انخفاض نسبة الأصابة باليرقات المختلفة . ففى حالة التكسوكاراكانس والباراسكارس اكورم كان معدل تثبيط الأصابه فى الجرذان ٦٠% عند استخدام ٢٠٠٠ ميكروجرام تنين / ميللتر ، ٥٠% عـند استخدام ١٠٠٠ ميكروجرام تنين / ميللتر . وفى حالة التكسوكارا فيتلورم كان معدل تثبيط الأصابه ٥٠% عند استخدام ٢٠٠٠ ميكروجرام تنين/ميللتر و٤٠ % فى حالة استخدام ١٠٠٠ ميكروجرام تنين/ميللتر .

SUMMARY

The present study was carried out to evaluate the prophylactic effect of tannins on visceral larva migrans,through both invitro and invivo studies. The invitro study cleared that tannins has a direct effect on the viability of *Parascaris equorum* L_2 (2^{nd} stage larvae), where the motility decreased gradually until they become immobile after 48 hours. Their mortality rate was 6%, 18% and 30% after 2, 24 and 48 hours respectively at 2000 ug tannins/ml and was 20% at 1000 ug tannins/ml after 48 hours. Invivo experimental infection was done by using 120 mice divided into three groups (40 mice each). Group I was infected with *Toxocara canis*; group II was infected with *Parascaris equorum* and group III was infected with *Toxocara vitulorum* larvated eggs. Each mouse was inoculated with 1000 larvated eggs per os. According to the concentrations of tannins used, each group was subdivided into 4 subgroups (10 mice each) given 500, 1000, 2000 ug tannins/ml drinking water, respectively . The last subgroup acted as a control (given drinking tap water only). Tannins were given to animals three days preinfection and remained for 2 weeks, where the animals were sacrificed . Histopathological examination of internal organs of sacrificed mice revealed that **a)** Tannins have a relative inhibitory effect against different species of larvae. **b)** The effect of tannins on visceral larva migrans of *T. canis and P.equoram* was similar where the percentage of inhibition was 50% and 60% with 1000 and 2000 ug tannins/ml, respectively. In case of *T. vitulorum* the percentage of inhibition was 40% and 50% with 1000 and 2000 ug tannins/ml, respectively.

Key words: *Tonnins, Visceral larval migrans, Mice, Histopathtology.*

INTRODUCTION

Visceral larva migrans is refered to invasion of migrating nematode larvae to the visceral tissue of unsuitable host including human and animals (Angus, 1978). There are more than 36 species of nematode larvae known to occur in human causing damage in many organs and tissues. Depending on the number of larvae and their location,the damage includes eosinophilia, pulmonary lesions, hepatomegaly and even death specially when they reach the brain (Gerald and Larry, 1989).

Frequent treatment with anthelmintic drugs has led to the wide spread development of anthelmintic resistant strains of nematodes (Jackson, 1993). Therefore, several investigations were done to reduce the impact of parasitism and the extent of anthelmintic resistance from

which; nutrient supplementation (Coop and Holmes, 1996); the use of forages or plants with anthelmintic properties (Klocke and Chan, 1982 and Reese *et al.*, 1982) and biological control (Gronvold *et a.l*, 1996).

Some studies have shown that condensed tannins containing plants have a significant effect against internal parasites (Bown *et al.*, 1991, Butter *et al.*, 1998., Hoskin *et al.*, 2000 and Athanasiadou *et al.*, 2000). Tannins are the major group of secondary plant metabolites. They are water soluble polyphenolic compounds with molecular weight ranging between 500-3000 Dalton (White 1957). These compounds have been used for centuries in the traditional medicine and natural remedies of several intestinal and skin infections (Sakanaka *et al.*, 1989).

The objective of the present work was to determine the effect of tannins extract on the viability of the free larvae of *Parascaris equorum*, and to their prophylactic effect on visceral larva migrans of *Toxocara canis;Toxocara vitulorum* and *Parascaris equorum* in experimentally infected mice.

MATERIALS and METHODS

Collection and maturation of eggs:

The adult worms of *Toxocara canis; Toxocara vitulorum and Parascaris equorum* were collected from the small intestine of dogs, calves and donkeys respectively during post mortem examination. Eggs were obtained by pressing the female uteri with a blunt object (according to Stevenson and Jacobs, 1977). The collected eggs of each type were cultivated in 1% formalin solution at room temperature for three weeks to ensure the development of infective second stage larvae (Sayed, 1985). The eggs were washed from formalin by centrifugation several times with physiological saline and stored at 4°C until used (Nicholas and Stewart, 1979).

Condensed tannins extract:

The condensed tannins extract used was commercially avaliable as quebracho powder extract (Quebracho ATO;Roy Wilson Dickson) of cold soluble type.

Effect of tannins on the viability of free larvae (invitro studies):

Parascaris equorum larvae were obtained by incubation of larvated eggs with 2% sodium hydroxide and 2% sodium hypochlorite for 24 hours, then centrifuged several times with phosphate buffer saline (Annen *et al.*, 1975).Viable larvae were obtained using the magnetic

stirer (Sayed, 1985). Approximately 50 larvae were added in small petri-dishes containing either no tannins extract (control) or a different concentration of tannins (500 ;1000 and 2000μg tannins /ml tap water); incubated at 37°C and examined after 2 , 24 , 48 hours .

Experimental design for invivo studies:

120 albino mice about 40 g. weight were used . They were parasitic free and acclimatized to the experimental condition for 7 days. These animals were divided into three groups , (40 animals each) as follow :

Group I : Infected with 1000 larvated eggs of ***Toxocara canis.***

Group II : Infected with 1000 larvated eggs of ***Parascaris equorum.***

Group III :Infected with 1000 larvated eggs of ***Toxocara vitulorum.***

Infection was done using stomach tube. Each group was divided into four subgroups, (10 animals each) as follow :

Subgroup 1 : Received 500 *u*g tannins/ ml drinking tap water.

Subgroup 2 Received 1000 *u*g tannins/ml drinking tap water.

Subgroup 3 : Received 2000 *u*g tannins/ ml drinking tap water.

Subgroup 4 : Served as control, received normal tap water .

Tannins were dissolved in drinking water and given to the animals ad-libitum before the infection by 3 days and remained until the animals were sacrificed after two weeks of infection.

Pathological examination :

Fresh specimens were collected from livers , lungs, kidneys, intestines and spleen of sacrificed animals. The specimens were fixed in 10% neutral buffer formalin. These samples were dehydrated in alcohols, processed and embedded in paraffin blocks. Serial sections of 5-7 microns were prepared from each block and stained with heamatoxylin and eosin and examined microscopically (Bancroft and Stevens,1993).

RESULTS

Effect of tannins on the viability of free larvae (invitro studies):

The motility of L2 of *P. equorum* was greatly affected with tannin extract where their movement gradually decreased until the larvae become immobile after 48 hours. Most of these larvae were still alive. After washing them three times with normal saline the larvae retained their motility but the movement was sluggish (in comparison to movement of control samples). The mortality rate was 6% , 18% and 30% after 2, 24 and 48 hours, respectively on using concenteration of

2000ug tannins, while it was 20% at 1000ug tannins after 48 hours. The use of 500ug tannins had no effect.

Pathological examination (invivo studies):

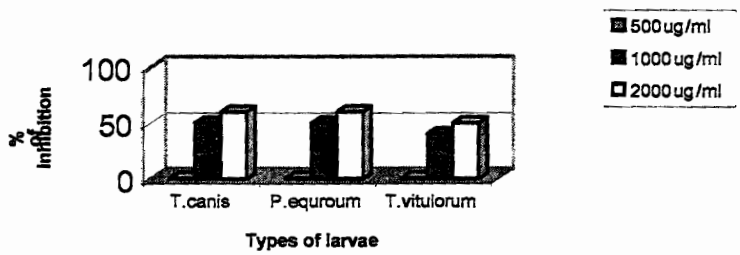
The pathological changes due to different types of larvae with different treatments of tannins concentrations were summarized in Table **(1)** and illustrated in figures **1** to **5** . These changes included:-

a) Mechanical alteration in the parenchymal tissue of the livers, lungs, kidneys and intestines due to the effect of visceral larval migration.
b) Presence of many sections of the larvae in the infected organs.
c) Exudative eosinophilic infiltration in the parenchymatous tissues
d) Chronic eosinophilic granulomatous reaction, especially in the liver and lungs .

All these changes are well expressed in the control group of *T. vitulorum* infection, which gave the severest pathological reaction followed by *T. canis and P. equorum.* The latter two types of larvae gave nearly similar pathological changes . In tannins treated subgroups, it was clear that the subgroups which received 2000 ug tannin/ ml water gave the best result in reducing the pathological changes in different investigated tissues, followed by those which received 1000 ug/ml, while the subgroups which received 500 ug/ml showed nearly similar lesions as in the control subgroups.

According to this histomorphological assessment of different investigated organs; **as showen in Digram (1)**; it was clear that:- **(A)** The percentage of inhibition on visceral larva migrans of *T. canis* and *P. equorum* was similar, reaching to 50% and 60% in 1000 and 2000ug tannins, respectively. **(B)** In case of *T. vitulorum*, the percentage of

Digram 1:Percentages of inhibation in visceral larva migrans in mice using different concenteration of tannins .



inhibition was 40% and 50% in 1000 and 2000ug tannins, respectively. (C) The use of 500ug tannins/ml had no effect on all species of larvae.

DISCUSSION

The objective of this study was to determine if tannins exhibited inhibitory effect on the viability and visceral larva migrans of some larval nematodes .The inhibitory effect was measured using both invitro assey and invivo histomorphological assessment in infected mice.

In the present invitro study it was clear that tannins had an inhibitory effect on the motility of the L_2 of *P. equorum* and increase their mortality rate. This may be due to its effect on the muscular activity of the larvae, leading to partial paralysis . This opinion agrees with Abdul *et al.* (2000) and Molan *et al.* (2000), who suggested that tannins may penetrate the cell wall of larva and affect their muscular activity and viability.

Histopathological changes in mice after invivo study showed an important response of visceral larva migrans and provided a rapid method to detect the effect on larva migration in different organs. Visceral larva migrans induced a number of lesions in liver, lung, intestine, kidney and spleen. The pathological changes are either in the form of tissue alteration or eosinophilic reaction in different visceral organs. The tissue alteration was due to mechanical damage from larval migration which lead to presence of migratory tracts, necrobiotic changes, and hemorrhages. Similar results were recorded in visceral larva migrans in Guinea pigs and mice by Mossalam and Atallah (1975) and Abd El-Ghaffer *et al.* (1999). Tissue reaction to the presence of the larvae was represented by exudative eoisnophilic infiltration (eosinophilic inflammatory response) and later on form granulomatous eosinophilic reaction. This result agrees with Clayton and Duncan (1977); Hanns (1985); Urquhart *et al.* (1987); Barker *et al.* (1993) and Hungerford (1990).

The present results show that tannins exhibited a relative inhibitory effect on the migration of infective larvae of *T. canis, P. equorum* and *T. vilulorum* in the experimentally infected mice. Abdul *et al.* (2000) and Molan *et al.* (2000) had previously reported the invitro inhibitory effect of tannins on the migration of *Trichostrongylus* and *Dictyocaulus* larvae by using a larval migration inhibition assay, while Niezen *et al.* (1995) and Athanasiadou *et al.* (2000) recorded reduction

of both worm burdens and number of their eggs in lambs and sheep by using of short term of tannins. The effective concentration of tannin extract used in the present work was 2000ug/ml which inhibited 60% of *T. canis* and *P. equorum* while in *T. vitulorum* it inhibited the larval migration in 50% of cases. This concentration was within the physiological range of free tannins in digesta recorded by Terrill *et al.* (1994). They mentioned that the physiological range of total tannin concentration in the abomasal and duodenal digesta of sheep fed condensed tannins containing diets was about 1.1% - 2.8%,.

T. canis and *P. equorum* were more susceptible to the action of tannins than *T. vilulorum.* This difference may be attributed to genetical resistance of some worms to the tannins. Hoekstra *et al.*, 1997 observed decrease in anthelmintic efficiency in some resistant nematode strains . However, the severity of pathological changes in case of *T. vitulorum* larval migration in the present work should be considered as a cause of this relative resistance.

Effect of tannins may be due to their direct anthelmintic effect or indirect physiological mediate effect. The direct effect is due to the ability of tannins to penetrate the cell wall of the larva and affect their muscular activity and viability (Abdul *et al.*, 2000). The indirect effect is by inducing physiological and biochemical changes in the gut of the host where intestinal nematodes are susceptible to these changes (Martin *et al.*, 1985). On the other hand, condensed tannins have a high affinity for both endogenous and dietary proteins, form stable complexes with it (Butler and Rogler, 1992; Mueller-Harvey and McAllan, 1992). So tannins can protect dietary protein from rumen degradation and thus increase the flow and availability of protein in the small intestine . Such an increase of absorbed protein will increase the animal resistance to the parasite by enhancement of host immune response (Abbott *et al.*, 1988 and Coop and Kyriazakis, 1999).

CONCLUSION

The present work indicated that tannins has a relative prophylactic effect against visceral larva migrans in experimentally infected mice. The best effective concentration of tannins was 2000ug/ml drinking water.

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Table 1: Histomorphological assessment of mice infected with different larvae and different conc. of tannins*.

Groups & subgroups / Pathological lesions	Group I & II (*T.canis & P.equroum*)				Group III (*T. vitulorum*)			
	Subgroup 1 (500 ug/ml.)	Subgroup 2 (1000ug/ml)	Subgroup 3 (2000ug/ml)	Subgroup 4 (control)	Subgroup 1 (500ug/ml)	Subgroup 2 (1000ug/ml)	Subgroup 3 (2000ug/ml)	Subgroup 4 (control)
1- Liver								
Presence of migratory tract containing RBCs (Fig.1a).	+++	++	+	+++	++++	+++	+	++++
Presence of larva surrounded by eosinophilic cell reaction (Fig. 1b).	++	++	+	+++	++++	+++	+	++++
Presence of larvae surrounded by eosinophilic cell reaction associated with necrosis & hemorrhage (Fig. 1c).	++	++	+	++	++++	+++	+	++++
Presence of eosinophilic cells granuloma (Fig. 1d).	+	+	+	+	++++	+++	+	++++
2- Lungs								
Migratory tract with hemorrhage (Fig. 2a)	+++	++	+	+++	++++	+++	+	++++
Perivascular hemorrhage (Fig. 2b).	+++	++	+	+++	++++	+++	+	++++
Presence of larvae associated with necrosis & hemorrhage (Fig. 2c)	++	+	+	+++	++++	+++	+	++++
Parasitic granulomatous pneumonia (Fig. 2d).	++	++	+	+++	++++	+++	+	++++
Parasitic bronchopn. (Fig.2e)	-ve	-ve	-ve	-ve	++++	+++	+	++++
3- Kidneys								
Congestion of the glomerular tufts (Fig. 3a)	++	+	+	++	++++	++	+	++++
Eosinophilic cell reaction (Fig. 3a).	+	-ve	-ve	+	++++	++	+	++++
Presence of the larvae in the renal tubules (Fig. 3b).	-ve	-ve	-ve	-ve	++++	++	+	++++
4 – Intestines								
Necrosis & sloughing of the villar epithelium (Fig. 4).	+++	++	+	+++	++++	+++	+	++++
5- Spleen:								
Hematobiotic activation (Fig. 5).	+++	++	+	++	+++	++	+	+++

*Score value. Every change is assessed using a score value from:

– ve to ++++ve -ve no change + ve mild reaction present in sporadic cases

++ ve moderate reaction present in few cases. +++ ve severe reaction present in half of cases

++++ ve very severe reaction present in most of cases.

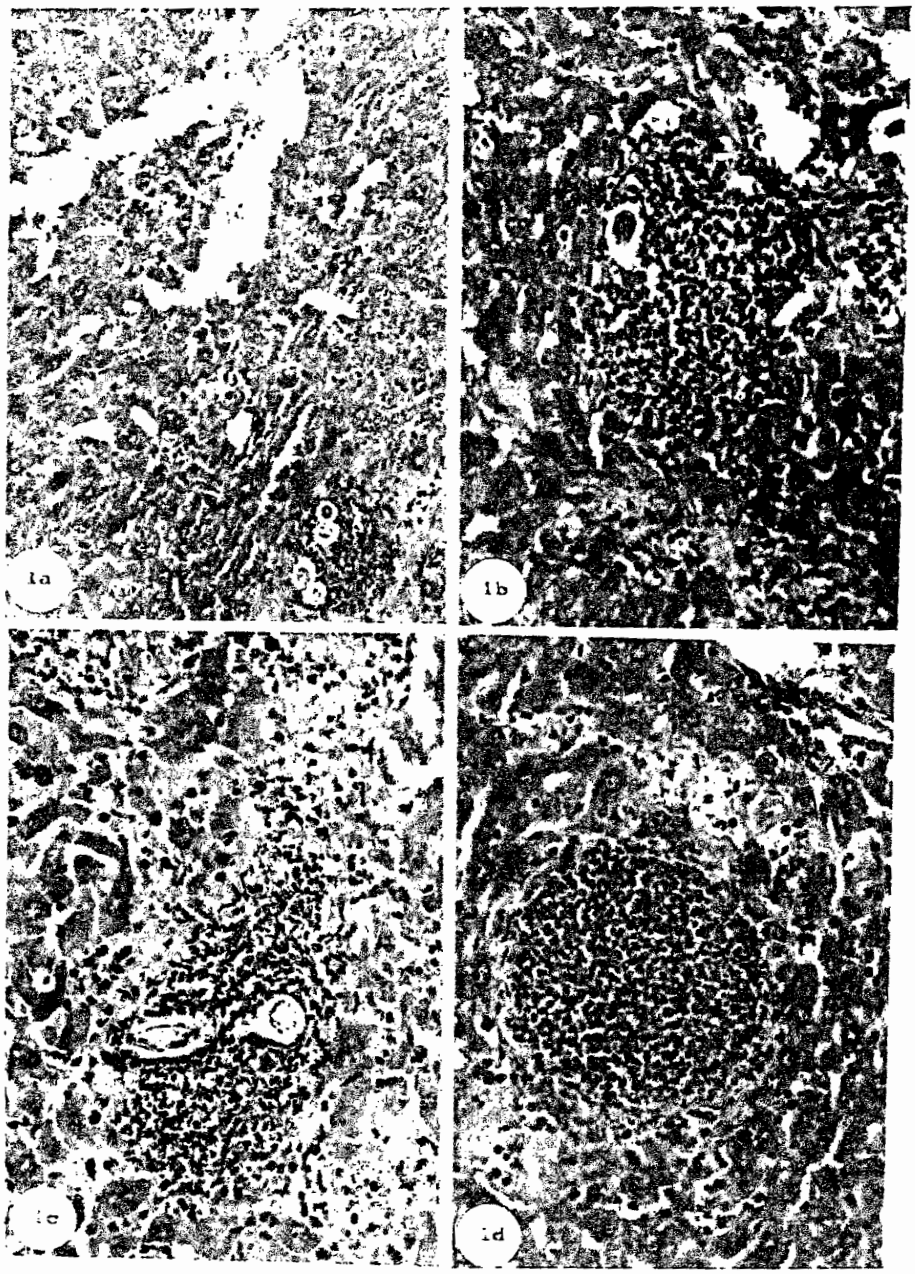

Fig. 1: Liver from mice of the control subgroups showing:

a) Migratory tract containing RBCs.

b) sections of migrating larva surrounding by eosinophilic cell reaction.

c) sections of the migrating larva surrounded by eosinophils associated with necrosis and hemorrhage.

d) nodules of eosinophil cells in the hepatic parenchyma. H&E.10 x 25.

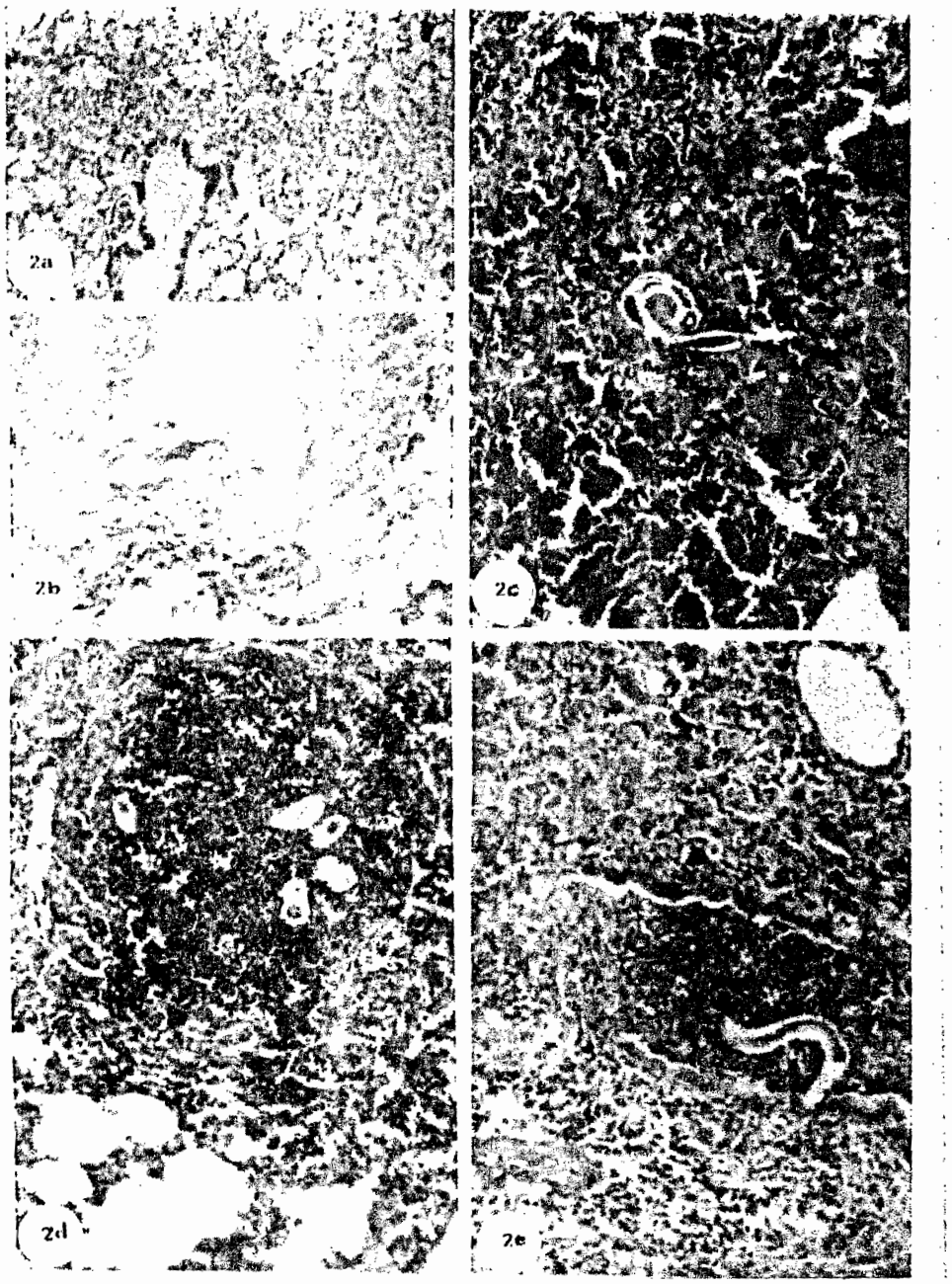

Fig . 2: Lung from mice of the control subgroups showing:

a) Migratory tract with hemorrhage.
b) Perivascular hemorrhage.
c) Sections of migrating larva associated with necrosis and hemorrhage.
d) Parasitic granulomatous pneumonia.
e) Parasitic bronchopneumonia. H&E. 10 x 25.

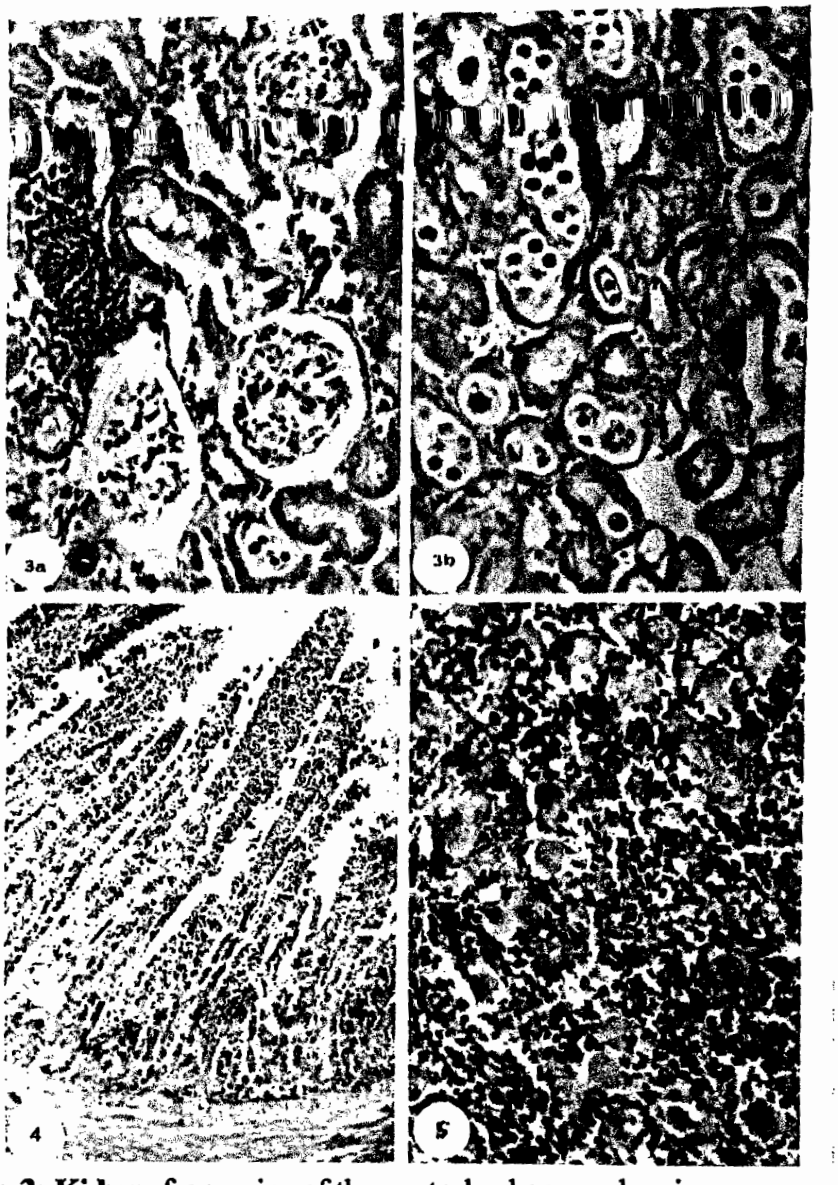

Fig. 3: Kidney from mice of the control subgroup showing:

a) Interstitial eosinophilic cell reaction with congestion of glomerular tuft.

b) Multiple sections of the migrating larva in the renal tubules . H&E.10 x 25.

Fig. 4: Intestine from mice of the control subgroups showing: Necrosis & sloughing of the villar epithelium H&E. 10 x 10.

Fig. 5: Spleen from mice of the control subgroups:- Showing heamobiotic activation with increase of heamobiotic cells mainly megakaryocytes. H&E. 10 x 25.