EFFECT OF PRUDHOE BAY CRUDE OIL ON HATCHING SUCCESS AND ASSOCIATED CHANGES IN THE PIPPING MUSCLE (*MUSCULUS COMPLEXUS*) IN EMBRYOS OF DOMESTIC CHICKEN (*GALLUS GALLUS*)

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by

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CERTIFICATION OF MASTER'S THESIS WORK

We, the undersigned, certify that

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project
has presented his/her ~~thesis~~ with the following title:

Effect of Prudhoe Bay Crude Oil on Hatching Success and Associated Changes in the Pipping Muscle (Musculus complexus) in Embryos of Domestic Chickens (Gallus gallus).
(as it appears on the title page and front cover of thesis)

that the thesis is acceptable in form and content, and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on May 26, 1992.

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ABSTRACT

Fertile White Leghorn chicken eggs were exposed to 0,1,2,4,6,8, and 16 µL of Prudhoe Bay Crude oil (PBCO) on day 9 of incubation. The effects of oil on percentage pipping,percentage hatchability, body weight gains post hatching,serum creatine kinase and pathological changes in the pipping (hatching) muscle (musculus complexus), liver, bursa of Fabricius and other organ systems were assessed during the hatching period in embryos that had survived acute toxicity and were alive on day 18 of incubation. Oil treatment greatly reduced hatchability and percentage pipping (Chi-square: $p=0.0001$). Severe edema and hemorrhage of the pipping muscle, multifocal subcapsular hepatic necrosis, lymphoid atrophy in the bursa of Fabricius with infiltration by heterophils and occasional dorso-caudal subcutaneous edema were observed in oil exposed embryos. Pipping muscle wet weight was greater in oil-exposed embryos (t test: $p =0.001$). Body weight gain post-hatching was significantly reduced in embryos exposed to 4 uL of PBCO (ANOVA: $p<0.05$). Serum creatine kinase (CK) levels were elevated only at the time of hatching in the oil-exposed embryos (t test: $p = 0.047$). Dysfunction of the pipping muscle in oil-exposed embryos may account for reduced pipping rates and hatchability.

KEY WORDS: Chicken, egg, embryo, Prudhoe bay crude oil, petroleum oil, toxicity, pipping muscle, hatchability, creatine

kinase, Experimental study.

INTRODUCTION

Embryotoxicity from transfer of crude petroleum oil from contaminated marine or fresh water to the eggs by incubating birds is of great environmental concern during oil spills. Spilled oil floats on the water and is readily absorbed by the feathers of swimming or diving birds, causing physical disruption of feathers with resultant loss of bouyancy,insulation and subsequently death (Jensen and Ekker, 1985). Sublethal external contamination of nesting birds results in transfer of oil to the shell of the eggs during laying or incubation in sufficient quantities to cause overt toxicity (King and Lefever, 1979), and teratogenicity in embryos of various avian species (Hoffman, 1979 a&b; Macko and King, 1980; Lewis and Malecki,1984; Couillard, 1989).

Petroleum oils are complex mixtures consisting mainly of aliphatic and aromatic hydrocarbon compounds (NRC, 1985). Prudhoe Bay crude oils (PBCO) are composed of approximately 41.3%(w/v) aliphatic; 35.6%(w/v) aromatic hydrocarbon; 6.8%(w/v) heterocyclic hydrocarbons and trace metals (Peakall et al., 1982; Khan et al., 1986). Among the metallic components of PBCO only molybdenum, nickel and vanadium occur in appreciable quantities (Peakall et al., 1982).

In marine or fresh water environments, spilled oil undergoes several physicochemical and biological transformations termed weathering (NRC, 1985; Greene and Trett,1989). Weathering of crude petroleum oil drastically modifies its physicochemical characteristics and results in increased concentration of polycyclic aromatic hydrocarbons (PAH) (Hoffman and Gay,1980).

Toxicity of crude petroleum oils to avian embryos has been attributed mainly to high molecular weight hydrocarbon constituents with four (4) or more benzene rings; the polycyclic aromatic hydrocarbons (Hoffman, 1979; Hoffman and Gay, 1980; Peakall et al, 1981; Ellenton, 1982; Albers and Gay, 1982). Embryotoxicity is manifested as reduced hatchability (Albers, 1980), stunted growth, malformation (Ellenton,1982), mortality (King and Lefever,1979), hepatic necrosis with mineralisation and subcutaneous edema (Couillard and Leighton, 1989;1990 a&b). The toxic effects have been shown to result from oil penetration through the eggshell into the embryonic tissues (Hoffman and Gay, 1980). However, the mechanisms by which oils cause these changes is poorly understood. Crude oil also induces mixed function oxidases in avian embryos (Lee et al.1985).

The severity of oil toxicity to avian embryos is dependent upon the dose applied on eggshell (Albers, 1980; Lewis and Malecki, 1984; Couillard, 1989), and the age of the embryos at the time of exposure (Szaro et al. 1980; Couillard, 1989). The

source and composition of crude oils involved also influence the degree of toxicity (Ellenton, 1982; Szaro and Albers, 1977; McGill and Richmond, 1979).

Escape of egg-young from the shell (hatching) at termination of incubation period is essential for life in egg laying vertebrates. The hatching process entails a series of complex and coordinated, embryological, biological and mechanical events which must occur at the proper place and time during embryonic life for successful emergence from the shell (Hamilton, 1952; Oppenheim, 1973). Prehatching activities in chickens commence around day 16 of incubation with appearance of a behavioural pattern characterized by smooth, coordinated tonic movements and a stereotyped type III activity (Oppenhiem, 1974). Four major prehatching events take place during successful hatching, namely : tucking of the head under the right wing, membrane penetration, pipping, climax and emergence (Hamilton, 1952; Oppenheim, 1970; 1973). Membrane penetration, pipping and cracking of the egg shell during climax are achieved by the egg tooth and the pipping muscle (Brook and Garrett, 1970). These two embryonic structures are usually at their maximum sizes between day 17 and 20 of incubation, just prior to prehatching activities (Fisher, 1958; Ramachamdran et al.,1969; Brooks and Garrett,1970). Therefore damage or dysfunction of the pipping muscle and egg tooth may impair pipping, cracking of eggshell and subsquently hatching of the egg-young.

Leighton (unpublished) observed that external application of PBCO on chicken eggs on day 9 of incubation impaired hatching in embryos that were alive on day 18. The aim of this study was to assess the effect of microliter quantities of PBCO on pipping and hatching success, and to test the hypothesis that failure to hatch is associated with morphological lesions in the pipping muscles of the embryos that survived acute toxic effects and were alive on day 18 of incubation.

MATERIALS AND METHODS

Two experimental studies were conducted. In both experiments, fertile White Leghorn Chicken (Gallus gallus) eggs were shipped from Keystone Hatchery, Manitoba, Canada, within 2 days of collection. On arrival, eggs were kept at 4C and tipped twice per day for 2 days. Then eggs were removed from the cold room and allowed to stand for 4 hours at room temperature before being randomly set on trays in the incubator (Humidaire,New Madison,Ohio), preset at a temperature of 37.5C and 50-55% relative humidity. The eggs were automatically turned once per hour for 18 days of incubation. From day 18 of incubation up to hatching, the turner was stopped. Trays holding eggs were rested horizontally and relative humidity was increased to 65%. On days 6 and 9 of incubation, the eggs were candled to remove infertile eggs and embryos with retarded development.

In experiment 1, on day 9 of incubation, the viable embryos were randomly divided (Sokal and Rolf, 1973) into 6 treatment groups, of 53, 48, 47, 48, 48, 48 eggs that received 0, 1, 2, 4, 8 and 16 µL of Prudhoe Bay Crude Oil (PBCO), respectively (Table 1). The control (0 µL) group had 5 embryos more than most of test groups. A prominent chorio-allantoic membrane blood vessel, near the air cell was identified in each embryo by candling. Oil was applied externally on the eggshell in a single location, overlying the prominent chorio-allantoic membrane blood vessel, using a Drummond microdispenser Pipet. The oil was allowed to spread freely on the eggshell before re-incubation.

On days 13 and 18 of incubation (i.e 4 and 9 days post treatment),the eggs were candled to check for mortality and dead embryos were removed in each group. Between day 19 and 20 of incubation five(5) embryos were randomly selected from the control group, euthanatized using carbon dioxide as soon as they pipped and fixed whole in 10% Phosphate buffered formalin to serve as control embryos for histology. Pipping and hatching of the embryos in each group was monitored at an interval of 4 hours; number pipped, number of hatched embryos,number pipped and died, and number of unpipped dead embryos were recorded. The unpipped dead embryos and those that pipped and died were removed from the eggshell on day 22 of incubation, examined grossly and fixed whole in 10% buffered formalin (Lillie and Fullmer,1976) for histological examination.The fixed portions of

liver,hearts,spleen,kidneys,lungs,pipping muscle and bursa of Fabricius were trimmed and embedded in paraffin, sectioned at 5u thick and stained with Haematoxylin and Eosin (Luna,1968) for light microscopy without knowledge of the treatment group. Von Kossa's stain was performed on hepatic sections which appeared abnormal (Lillie and Fullmer,1976).

Chicks that hatched were transfered to a brooder, provided with feed and clean water ad libitum and were weighed once every week for 3 weeks, using a Mettler PM 460 Delta range balance.

In Experiment 2, the eggs were divided into 2 groups of 60 (control) and 274 (PBCO) eggs respectively. The same procedures and conditions were used as in experiment 1 up to day 9 of incubation. On day 9, eggs in the test group were externally treated with 6uL of PBCO. On days 13 and 18 of incubation, the eggs were candled to remove and record dead embryos. On day 18, at pipping, at hatching and 5 days post hatching, ten (10) embryos from each group were randomly selected,euthanatized using carbon dioxide, weighed and blood was obtained by heart puncture (MacArthur,1950) for serum creatine kinase (CK) assay from each embryo. The CK values were analysed by modified Oliver-Rosalki method (Dart CK- NAC). Pipping muscle, gastrocnemius muscle, pectoral muscle, liver, and heart were dissected out and fixed in Karnovsky's fixative (Karnovsky, 1965) for histopathology. The

pipping muscle and heart from each embryo were bolted once (dried lightly with paper towel) and weighed before fixation.

Hatching and pipping rates were expressed as a percentage of the number treated for each group in experiment 1 and differences in treatment effects were analysed by Chi-square. Data for weight gains post hatching were analysed by One Way Analysis of Variance (ANOVA) and wet weights of pipping muscle, pipping muscle/body weight ratios and serum creatine kinase were analysed by unpaired t-test (Statworks program,Ventura Bld; Suite 250 Calabasas,CA,91302:1986). The results were considered significant at $p \leq 0.05$.

RESULTS

Experiment 1:

Two peaks of embryo mortality were observed in oil exposed groups before hatching. Acute mortality was observed 4 to 9 days post exposure, that is before day 18 of incubation. There was also chronic mortality that occurred during the hatching period, at pipping and hatching(day 19-20 of incubation). Both acute and chronic mortality increased with increasing dose of PBCO (Table 1). Hatchability and pipping rates decreased with increasing dose of PBCO,while the mortality post-pipping increased with dose, (Table 2 and Figure 1). Failure to pip was not significantly

different between embryos treated with 2.0uL and 4.0uL of oil ($p > 0.05$).

Location of the head at the narrow end of the eggshell (malposition) was observed in 2.1%, 9.1%, 9.4%, 3.9%, and 4.8% of the unpipped embryos at 0.0 µL, 2.0 µL, 4.0 µL, 8.0 µL, and 16.0 µL PBCO- dose groups, respectively (Table 3). Oil exposed embryos that failed to hatch had grossly visible, irregular pale zones at the margins of the liver which histologically corresponded to subcapsular multifocal to locally extensive areas of necrosis with mineralisation (Table 3 and Figures 2-4).

Large subcutaneous fluid filled cavities on the right dorso-caudal aspect (rump) were observed in 5.1% and 15.6% of embryos dosed with 1.0 µL and 2.0 µL of PBCO, respectively (Table 3 and Figures 5-6). Histological examination of bursa of Fabricius had markedly reduced lymphoid tissue in the plicae with indistinct lymphoid follicles, infolding of the lining epithelium and infiltration by moderate numbers of heterophils in exposed,unhatched embryos that were alive on day 18 of incubation (Table 3 and Figures 7a-c). Embryos that died within the eggshell had advanced autolysis which hampered assessment of the changes.

Pre-hatching oil exposure to 4 µL of PBCO was associated with a small but significant decrease in mean body weight gains

from hatching to 3 weeks post-hatching compared to control (Table 4).

Experiment 2:

Marked accumulation of straw colored to red tinged gelatinous fluid material (edema fluid and hemorrhage) around the pipping muscle was observed in embryos exposed to 6 µL of PBCO (Figure 8a, b). Histological examination of these revealed sparse multifocal segmental fragmentation and occasional vacuolation of muscle fibers (Figures 8c). No corresponding lesions were evident in breast and gastrocnemius muscles or heart. Mean pipping muscle wet weight was significantly higher in oil-exposed embryos than in the control group at pipping (Table 5). Mean pipping muscle wet weight to body weight ratio was significantly different between oiled groups and control on day 18, at pipping, hatching and 5 days post hatching (Table 5). Serum CK levels were elevated in oil treated embryos only at hatching (Table 6) and the range of CK value was very wide in both groups. Hepatic necrosis with mineralization observed in oil-exposed embryos in experiment 1, was also present in treated embryos in experiment 2. The lesions were still observable at the termination of the experiment at 5 days post hatching.

DISCUSSION

This study has demonstrated that mortality of chicken embryos exposed to PBCO on day 9 of incubation occurred at two different periods after exposure; acute mortality occurred before the hatching period (4-9 days post-exposure), while the second period of mortality occurred at hatching due to failure to pip and/or crack the shell and enter the climax stage after pipping. Mortality rates at both times varied with dose. Thus,there appeared to be a delayed expression of toxicity in embryos which survived acute toxic insult that resulted in failure to hatch. A dose of PBCO greater than 1.0 µL caused significant reduction in hatching success (Chi-square, $p<0.002$). This is in agreement with earlier reports about crude petroleum oil embryotoxicity (Hoffman and Gay, 1980; Lewis and Malecki, 1984). The low hatchability in oil-exposed chicken embryos, that were alive on day 18 of incubation,is attributable to failure to pip and increased mortality post pipping. Highest mortality post pipping was at 8uL of PBCO while 16 µL caused the the highest percentage unpipped dead-in-shell (Table 1).

Chicken embryos exposed to PBCO that were alive on day 18 but failed to hatch, had heavier and swollen pipping muscles with accumulation of often red tinged gelatinous material in the overlying subcutanous tissue, between the two pairs of the pipping muscle and adjacent subcutis of the neck. There were

petechial and ecchymotic hemorrhages on the pipping muscle. Histologically, these muscles showed occasional swollen muscle fibres with vacuolated sarcoplasm, wide separation of fibres by pale staining homogenous material and scant to large numbers of free extravascular red blood cells. No corresponding changes were observed in gastrocnemius or pectoral muscles. However, there were no gross and histological vascular changes to account for the hemorrhage in the pipping muscles. Thus this hemorrhage was likely by diapedesis. The increase in wet weight of pipping muscles in oil-exposed embryos was due to edema and hemorrhage. During normal hatching, the pipping muscle greatly enlarges from day 17 to 20 of incubation with mild physiological edema which cushions the muscle at pipping and when cracking the eggshell to effect hatching (Rigdon et al. 1968; Ramachandran et al. 1969; Klicka and Kaspar, 1970). Marked edema with hemorrhage, observed in oiled embryos in this experiment, suggest an exaggerated physiological process with increased vascular permeability. The failure of the affected embryos to pip or hatch suggests that there was a functional impairment of the pipping muscle as well. Necrosis in pipping muscle of chicks has been associated with failure of the otherwise normal embryos to hatch (Rigdon et al.1968). In the present study, only a very few muscle fibres were abnormal histologically.

Among the unpipped embryos a few had malpositioned heads. The normal head position of the embryo for successful hatching is

with the head at the blunt or large end of the egg (Romanoff, 1972). Therefore failure to hatch in embryos with malpositioned heads is attributable to this abnormal orientation.

Creatine kinase was elevated in oil exposed embryos at hatching. Increase in serum CK levels is commonly used as an indicator for muscle injury (Anderson et al.1976; Tripp and Schmitz,1982; Boyd, 1983). However, the minimal muscle fibre degeneration observed in these chicks at hatching may not be sufficient to account for elevated CK value. Exercise and stress have been shown to cause increases in serum CK levels in turkeys and lambs (Tripp and Schimitz, 1982). Therefore, stress of oil exposure and increased activity during hatching, together with mild pipping muscle degeneration may explain the significant increase in CK only at hatching. There was wide variation in CK values within groups.

Poor body weight gain during the first 3 weeks post-hatching was observed in the embryos exposed to 4.0 µL of PBCO. This suggests that oil has a residual toxic effect in embryos exposed to it during incubation which may impair the ability of the chicks of avian species to adapt in the natural environment post-hatching.

Severe lymphoid depletion in the bursa of Fabricius with infiltration by heterophils and infolding and hyperplasia of the

epithelial lining of the plicae was observed in oiled embryos. Atrophy of lymphoid tissues in bursa of Fabricius, thymus and spleen occur in chronic diseases, starvation, exercise and exposure toxins (Riddell, 1987). Lymphoid atrophy in the present study may have been due to a general stress response to oil or to a direct toxic efffects of PBCO.

The present study provides further evidence of the extreme toxicity of petroleum oil to the avian embryos. Data from this study are consistent with a physiological dysfunction of the pipping muscle. But the data do not support the hypothesis that failure to hatch was associated with degenerative morphological changes in the pipping muscle. The pathogenesis of failure to hatch in oil-exposed embryos still remains uncertain and,thus, further investigation is needed.

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Table 1: Effect of exposure to Prudhoe Bay crude oil on the survival of chicken embryos. Experiment 1.

	Dose of PBCO (µL)					
	0	1	2	4	8	16
No. alive on day 9[a]	53	48	47	48	48	48
No. alive on day 13[b]	52	47	38	36	36	36
No. alive on day 18[c]	52	39	33	32	26	21
% mortality between day 13 and 18[d]	0.0	18.8	29.7	33.3	45.8	56.0
No. pipped	51[e]	37	26	25	18	5
No. unpipped	1	2	7	7	8	16
No. pipped/died	0	1	1	6	17	5
No. hatched	46	36	25	19	1	0
% pipped[f]	98.1	94.9	78.8	78.1	69.2	23.8
% hatched[g]	97.9	92.3	75.8	59.3	3.8	0.0
% pipped/died	0.0	2.6	3.0	18.8	65.4	23.8
% unpipped/died	2.1	5.1	21.2	21.9	30.8	76.2

[a] = embryos alive prior to exposure to oil.
[b] = 1 embryo from control euthanized on day 13 of incubation to serve as control.
[c] = Total no. of embryos that survived acute toxic episode per group.
[d] = % total acute embryo mortality between day 13 and 18.
[e] = 5 embryos euthanized immediately after pipping to serve as control.
[f] = % pipped of the no. of embryos alive on day 18.
[g] = % hatched of the no. of embryos alive on day 18.

Oil was applied on eggshell on Day 9 of incubation

Table 2: Effect of exposure to PBCO on % hatchability, pipping, and mortality post pipping of chicken embryos. Experiment 1.

	Dose of PBCO in µL					
	0(47)[a]	1(39)	2(33)	4(32)	8(26)	16(21)
Hatchability (%)	97.9	92.3	75.8[b]	59.4[b]	3.8[b]	0.0[b]
% Unpipped/Died	2.1	2.6	21.2	21.9	30.8	76.2
% Pipped/Died	0.0	5.1	3.0	18.7	65.4	23.8

[a] = total embryos per treatment group in parentheses

[b] = significantly different from control $p \leq 0.002$. Chi square.

Table 3. Pathological changes associated with exposure to PBCO in pipped and died, and unpipped dead chicken embryos. Experiment 1.

	PBCO (µL)					
	0	1	2	4	8	16
Hepatic necrosis	0	1(3)[n]	1(5)	5(15)	7(24)	16(19)
Pipping m. edema	1	1(3)	2(5)	6(15)	10(24)	4(19)
Atrophy of bursa of Fabricius	1	1(3)	2(5)	5(15)	5(24)	3(19)
Sub-cutaneous edema	0	2(3)	0(5)	5(15)	0(24)	0(19)
Malposition	1	0(3)	3(5)	3(15)	1(24)	1(19)

[n] = number of dead embryos examined per treatment group.
Oil was applied on the eggshell on day 9 of incubation.
All embryos were alive on day 18 of incubation.

Table 4: Effects of PBCO on body weight gain of White Leghorn chicken 3 weeks post hatching. Experiment 1.

PBCO (µL)	n	wt(g)/1wk	wt(g)/3 wks	wt gained	% of control
0	46	46.6[a]±3.1	172.6±17.3	126.0	100.0
1	36	45.7±4.0	165.1±15.9	119.4	94.8
2	23	44.1±3.7	171.2±14.5	127.1	104.4
4	19	42.6±4.1	153.5±15.8	110.9[b]	88.0

Oil was applied to the eggshell on Day 9 of incubation
n = No. of successfully hatched embryos in each group
[a] = Mean body weight (gm) ± S.E.
[b] = Significant body weight gain difference from control $p \leq 0.05$. One Way Analysis of Variance and Least Significant Difference (LSD) test.
wt = weight

Table 5: Effect of PBCO on pipping muscle wet weight and pipping muscle/body wet weight ratios at various stages of chicken embryo development. Experiment 2.

Stage of embryo development	PBCO (µL)	(n)	Mean body wt. (g)	Mean pipping m. wet wt. (mg)	Mean pipping/ body wt. ratio	Hepatic necrosis (h)
day 18	0	10	29.1±0.4	0.377±0.010[a]	0.013±0.000	0
	6	10	28.2±0.7	0.432± 0.020	0.015±0.002	4
At pipping	0	10	38.4±0.6	0.561±0.040	0.015±0.001	0
	6	10	37.8±1.5	0.903±0.080[b]	0.024±0.002	4
Pipped/died	0	10	38.4±0.6	0.561±0.040	0.015±0.001	0
	6	10	39.1±1.0	1.321±0.060[b]	0.034±0.001	3
At hatching	0	10	39.6±1.0	0.503±0.030	0.013±0.001	0
	6	10	38.7±0.6	0.726±0.010	0.019±0.003	2
5 days post-hatching	0	10	57.3±2.7	0.167±0.010	0.003±0.000	0
	6	10	50.7±1.5	0.183±0.010	0.004±0.000	1

[a] = mean ± standard error
[b] = significantly different from control $p \leq 0.05$ t test.
[c] = no. of embryos with hepatic necrosis

Table 6: Effect of PBCO on serum creatine kinase (μ/l) levels. Experiment 2.

	n	PBCO (μL) 0	6
Stage of development. Day 18	10	3043 ± 710[a]	4048 ± 744
At pipping.	10	2315 ± 690	4717 ± 920
At hatching.	10	1692 ± 281	5704 ± 1862[b]
5 days post-hatching.	10	3243 ± 811	1924 ± 310

[a] = Mean ± S.E.
[b] = Elevation of CK levels significantly different between oiled embryos and control (t test: $p < 0.05$) immediately after hatching.

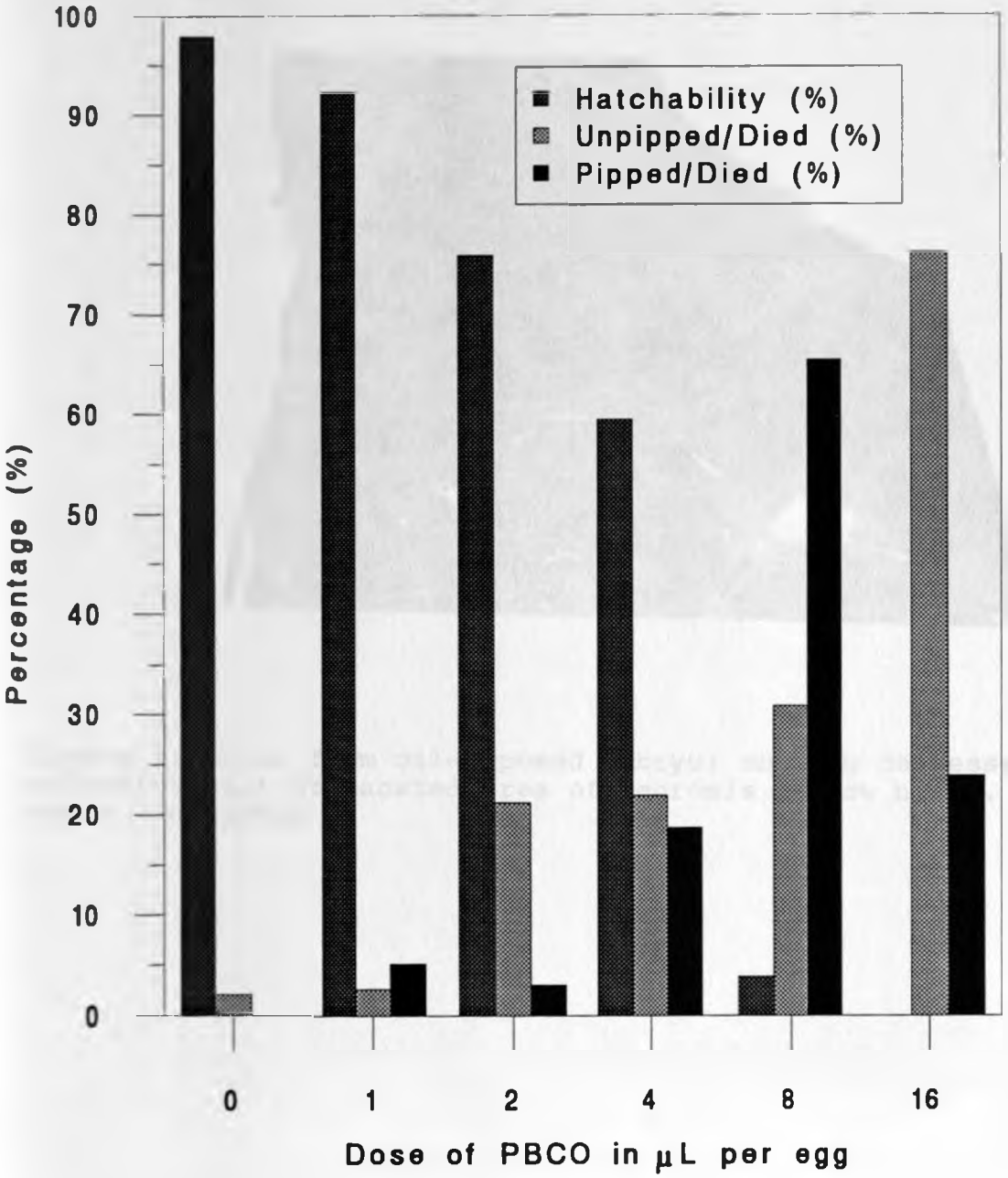


Fig. 1. Effect of PBCO on Hatchability of Chicken Egg Embryos. Exp. 1

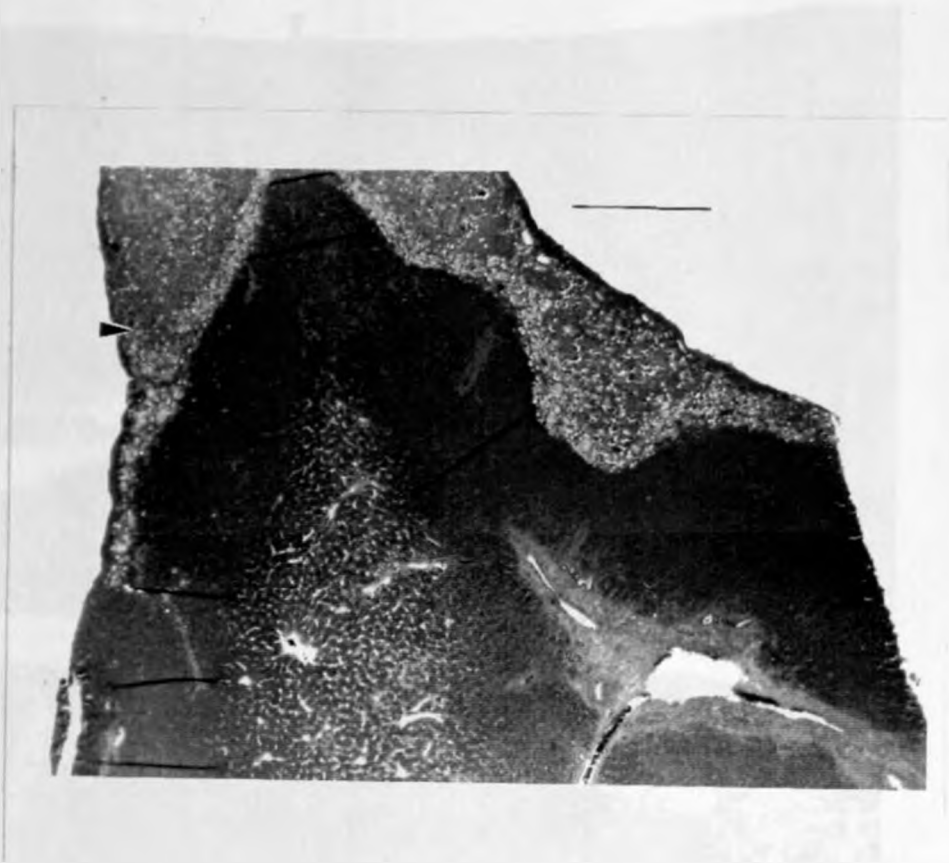
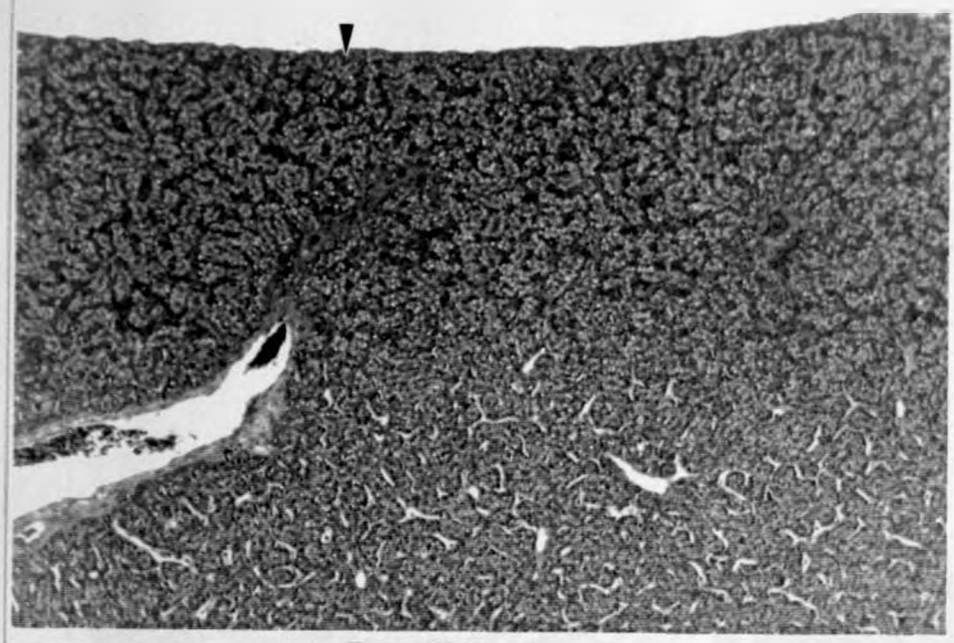


Figure 2: Liver from oil-exposed embryo; showing depressed locally extensive well dermacated area of necrosis (arrow head). H/E stain. Scale line=500μm

3a



3b

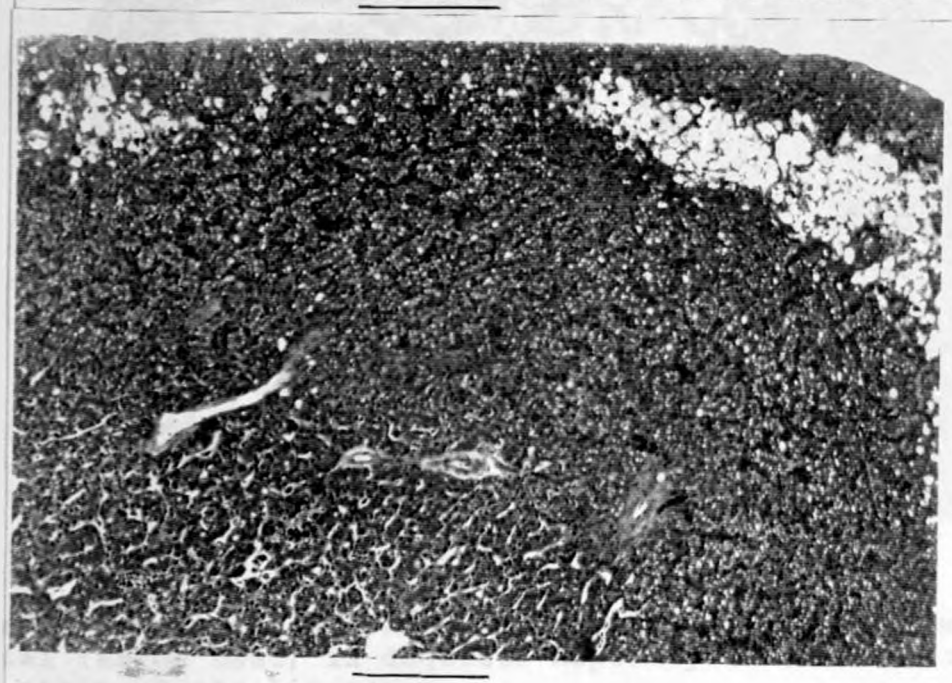


Figure 3: Chicken embryo liver sections. 3a) section from control group, note fairly smooth margin (arrow head); 3b) liver from oil-exposed embryos showing subcapsular vacuolation and loss of architectural structures of affected hapatocytes. H/E stain. Scale line=200μm

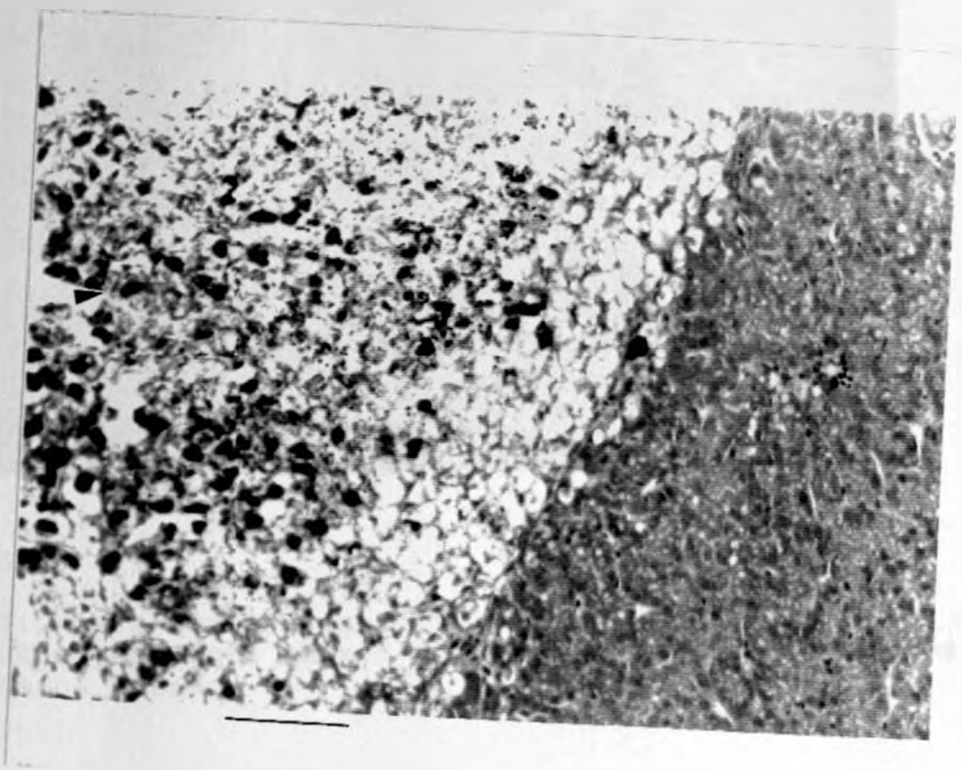
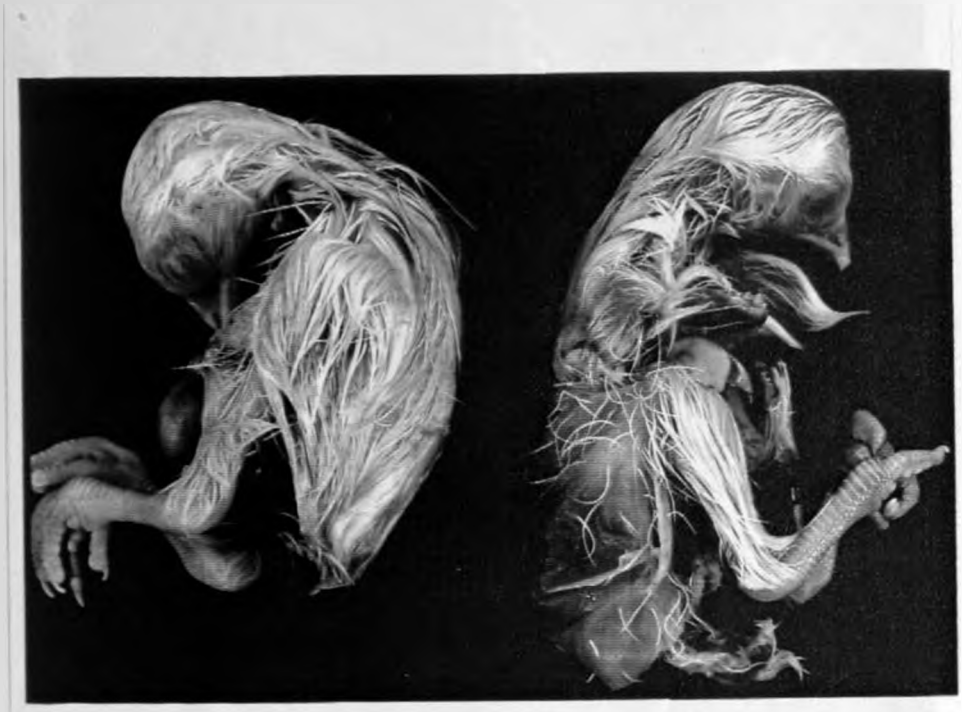


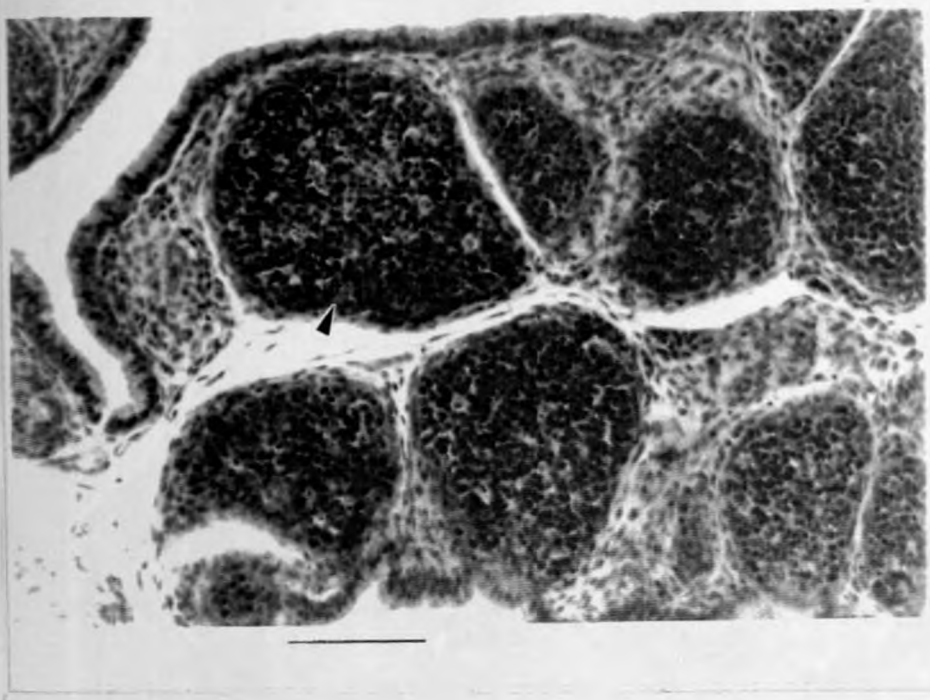
Figure 4: Liver from oil-exposed embryos; note black amorphous material (arrow head) in degerate hapatocytes (calcium minerals). Van Kossa stain. Scale line=45μm.



5a 5b

Figure 5: Whole chicken embryos: 5a) from control group. 5b) is from treatment group, note marked accummulation of fluid in the subcutaneous tissue.

6a



6b

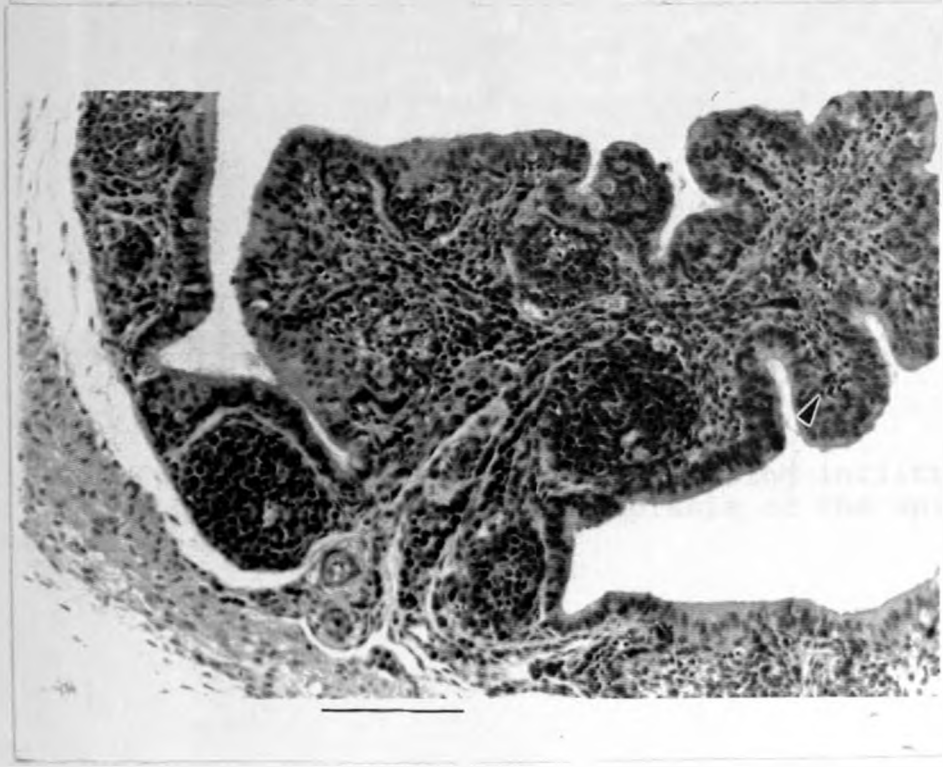


Figure 6 a & b: Bursa of fabricius. 6a) control note well formed lymphoid follicles (arrow head). 6b) oil-exposed, note depleted lymphoid tissue and infolding of epithelial lining of plicae (arrow head). Scale line=65μm.

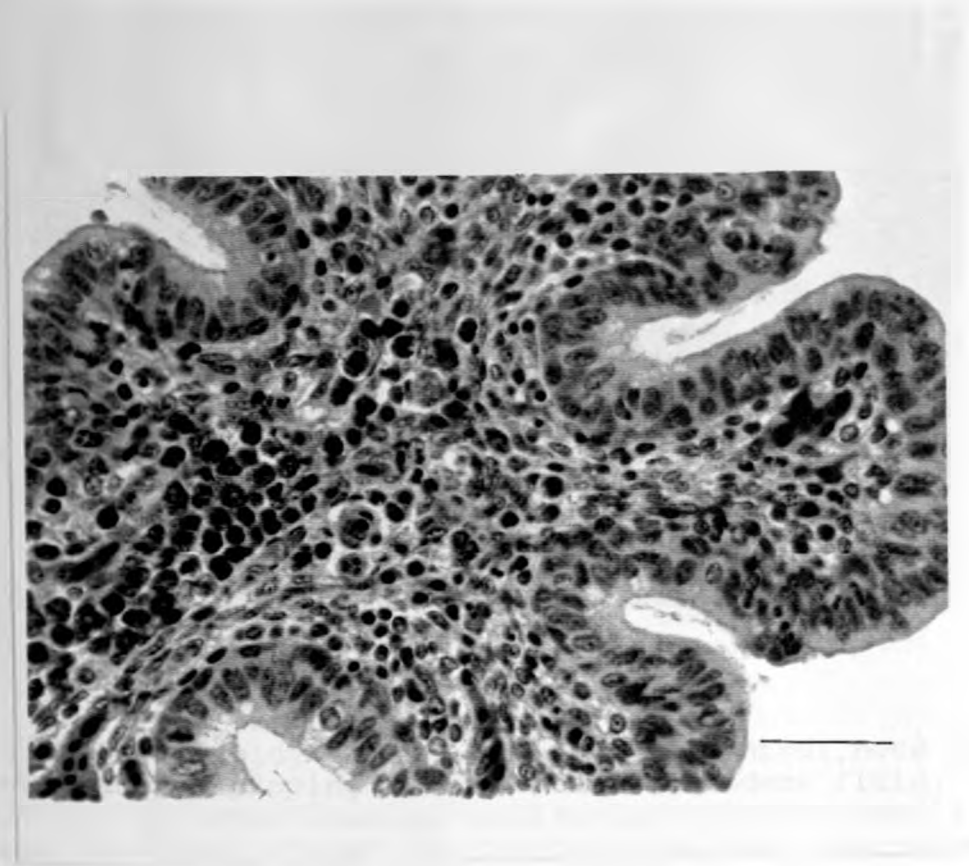


Figure 6c) is high magnification of 6b showing infiltration of interstitium by heterophils and hyperplasia of the epithelial lining of the plica. Scale line=30μm.

Figure 7 a & b. Pipping muscle; 7a(left) is control,note less prominent pairs of pipping muscle with scant edema fluid. 7b(right) from oil-exposed group showing swollen dark pipping muscle with gelatinous material between the two pairs of the pipping muscle and subcutaneous tissue. Scale in mm.

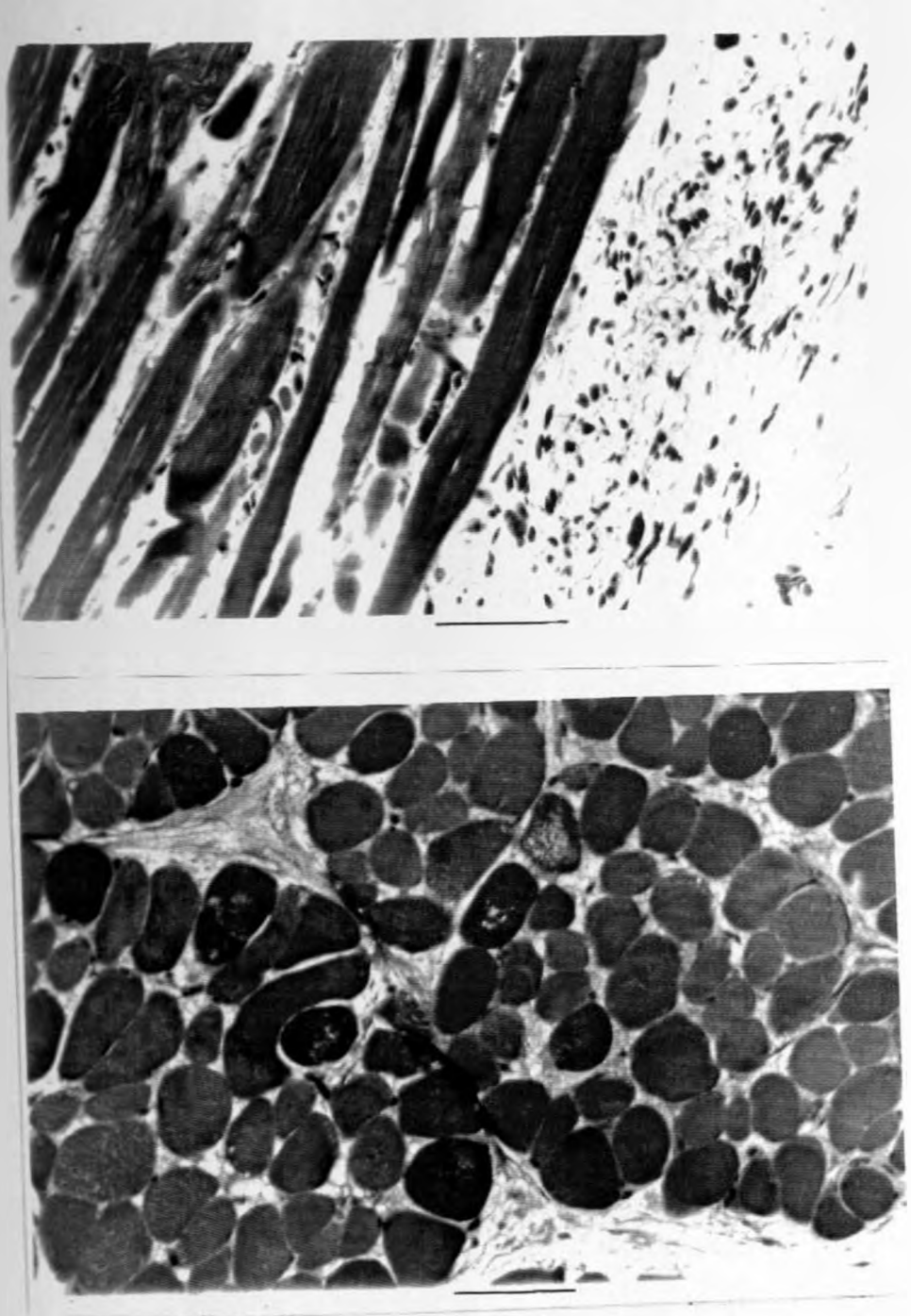


Figure 7c & d);c(above) is longitudinal histological section of 7b showing edema and hemorrhage in overlying fascia and between muscle fibres. 7d(below) showing occasional vacuolation of sarcoplasm of few muscle fibres in muscle bundles. H/E. Scale line=40μm.