5 ml sterile PBS 14 and 7 days before infection respectively. Groups 9 and 10 had 5 chickens each and they were injected i.p. with 10 ml of 10% protease peptone at 14 and 7 days before infection respectively. Group 11 had 10 chickens and were used as infected uninjected controls. All chickens were inoculated with 1 x 10^5 oocysts, sacrificed at 5 days post infection and lesions scored.

RESULTS

1. Effect of i.p. injection of irritants on lesion severity and blood leucocytes

Gross lesions seen at 5 days post infection were less severe in those chickens which received the i.p. injection of proteose peptone than in the control group (Fig. 3-1, 3-2, 3-3, 3-4, 3-5). The reduced severity was accompanied by retardation in the growth of the parasite. Second generation schizonts were fully developed in intestinal sections obtained from the uninjected control groups. On the other hand, there were only very early second generation schizonts in intestinal sections obtained from i.p. injected chickens. Total leucocyte counts were significantly higher at day 5 post infection in the control chickens when compared to those of the chickens that received the i.p. injection (Fig. 3-5). The same was observed with heterophil (Fig. 3-7) and monocyte counts while lymphocyte numbers were significantly lower in the control chickens when compared to those chickens which received the i.p. injection of proteose peptone (Fig. 3-8). Histological sections of bone marrow of chickens that received the i.p. injection of proteose peptone were full with heterophils, while those of the controls were depleted (Figs. 3-9, 3-10).

Delaying the i.p. injection of proteose peptone for 12 hours after infection did not change its effect on lesion severity. Mean lesion scores of the two groups that received the i.p. injections at time of infection and at 12 hours post infection was 1. On the other hand, all control chickens had severe hemorrhagic enteritis with a mean lesion score of 6.12 (Fig. 3-11).

Of chickens receiving other irritants, those injected i.p. with thioglycolate broth at the time of infection had the least severe gross intestinal lesions (mean lesion score 0.9). These were similar to the results obtained when proteose peptone was used for i.p. injection. Moderately severe lesions were obtained with starch (mean lesion score 2.1) and Sephadex G-75 superfine (mean lesion score 3.6) when compared to the uninjected control group where a lesion score of 6.75 was obtained (Fig. 3-12).

Intramuscular injection of proteose peptone did not change the lesion severity of E. necatrix at 5 days post infection.

2. Effect of i.p. injection of proteose peptone on oocyst production and mortality distribution.

When chickens were infected with 3×10^4 oocysts, peak oocyst production of the control group was between days 8-9, while that of the group that received the i.p. injection was between days 10-11 post infection (Fig. 3-13).

Two out of three chickens of the group that received the i.p. injection of proteose peptone were negative for oocysts at the end of day 7 post infection. The mean of daily oocyst production (Table 3-1) of group 2 (controls) was significantly higher than that of group 1

(i.p. injected) at the end of day 7 post infection. On the other hand, mean daily oocyst production of group 1 was significantly higher than the control group at the end of day 11 post infection. Total oocysts produced by group 1 was (1.9×10^7) greater than that of the control group (1.7×10^7).

At the low dose of 1×10^3 oocysts mean daily oocyst production of the control group showed two peaks, one was at the end of day 9 and the second was at the end of day 12 post infection (Fig. 3-14). On the other hand, only 1 low peak was observed at the end of day 10 post infection in the group that received the i.p. injection of proteose peptone (Fig. 3-14).

One out of four chickens which received the i.p. injection of proteose peptone was negative for oocysts at the end of day 7 post infection. Mean daily oocyst production during the period studied (days 7-15 post infection) was generally higher in the control group that in the group which received the i.p. injection of proteose peptone (Table 3-2). It was significantly higher only at the end of day 9 post infection. Total oocysts produced by the control group was 8.1×10^7 while that of the group which receeved the i.p. injection of proteose peptone was 3.7×10^7 oocysts during the period studied. A difference of 4.4×10^7 oocysts produced more by the control group. However, it was statistically significant.

Death due to E. necatrix infection was delayed by the i.p. injection of an irritant. Chickens which received the i.p. injection of proteose peptone were all alive at day 5 post infection whereas 7 out of 9 chickens died on day 5 and 1 on day 6 post infection in the uninjected control group. In the group which received the i.p. injection 4

4 chickens died at day 6 and 3 chickens died at day 7 post infection. Two chickens which were injected with proteose peptone survived the infection whereas only one survived from the control group.

3. Effect of i.p. injection of proteose peptone on the development of first and second generation schizonts

When infected birds which received an i.p. injection of proteose peptone was sacrificed at day 7 post infection, instead of at 5, lesions (mean score 4.9) comparable to those seen in the control group at day 5 post infection (mean score 6.3) were recorded. This together with the delay in oocyst production caused by an i.p. injection of irritant indicated that the life cycle was delayed by the irritant.

Mean numbers of first and second generation schizonts found in sections obtained 3 days post infection from three different areas of intestines of chickens in groups 1, 2 and 3, injected i.p. with proteose peptone at 0, 24 and 48 hours respectively, and those of the control group 4 are presented in Table 3-3. There were significantly more second generation schizonts in groups 2 ($p < 0.07$), 3 ($p < 0.001$) and 4 ($p < 0.01$) than in group 1. More second generation schizonts were detected in the group which received the i.p. injection of proteose peptone at 48 hours (group 3) than the other 3 groups. Numbers of first generation schizonts found in all groups were comparable (Table 3-3).

The first part of the life cycle of E. necatrix was studied in chickens killed at 3, 6, 12, 18 or 24 hours post infection. In light microscopic studies, using the special sporozoite stain, sporozoites were observed in villous epithelial cells by 6 hours post infection (Fig. 3-15). By 12 hours post infection sporozoites were seen in mono-

nuclear cells mostly in the villar lamina propria. The above findings were similar in the control group and in those chickens that received the i.p. injections. In the control group the sporozoites were seen in mononuclear cells between crypt epithelial cells and in crypt epithelial cells themselves 18-24 hours post infection (Fig. 3-16). On the other hand, at 18 hours post infection in the group that received the i.p. injection sporozoites in mononuclear cells were mainly in the villous lamina propria but not in the crypt. In addition, very few sporozoites in mononuclear cells were seen in the lamina propria or crypts at 24 hours post infection. There whereabouts could not be determined in this study.

4. Ultrastructural studies on the type of cell involved in the transport of sporozoites.

Electron microscopic examination of the type of cell involved in the transport of the sporozoites was done only on tissues obtained from the control group. In this group, sporozoites were detected at the tip of the villous epithelial cells by 3 hours post infection (Fig. 3-17, 3-18). Sporozoites in villous epithelial cells were near the villous basal lamina by 6 hours post infection (Figs. 3-19, 3-20). At this time post infection sporozoites were also seen in mononuclear cells. These mononuclear cells appear structurally similar to intraepithelial lymphocytes rather than macrophages (Fig. 3-21). Movement of these parasitized intraepithelial lymphocytes is indicated by constrictions and irregularities in their plasma membranes (Fig. 3-22). By 12 to 18 hours post infection the infected intraepithelial lymphocytes were seen in the lower third of the lamina propria (Fig. 3-23). Sporozoites were

also observed in intraepithelial lymphocytes around and between crypt epithelial cells by 18-24 hours post infection (Fig. 4-24) while in the intraepithelial lymphocytes (Fig. 3-25) the ultrastructural characteristics of sporozoites were unchanged. By 24 hours post infection some of the sporozoites were seen in crypt epithelial cells (Fig. 3-26). In this figure, three sporozoites can be seen. One of them is in an intra- epithelial lymphocyte while the other two are inside crypt epithelial cells. The sporozoite in the crypt epithelial cell on the top of the picture has undergone some morphological change as indicated by the enlargement of the parasitophorous vacuole and the disappearance of the micronemes (Fig. 3-27). This infected crypt cell also shows some nuclear enlargement and loss of microvilli (Fig. 3-27). The epithelial nature of these parasitized cells is also demonstrated by the presence of desmosomes between the two parasitized cells (Fig. 3-27). Normal uninfected intraepithelial lymphocytes are shown in Fig. 3-28.

All chickens injected i.p. with silica or proteose peptone or the combination showed very mild intestinal lesions with mean scores of 1.2, 1.5 and 1.2 respectively. On the other hand, chickens in the control group showed very severe lesions with a mean score of 6.33 (Fig. 3-29).

5. Effect of BCG and Propionibacterium acnes injection on lesion severity.

Chickens which received 5 mg of P. acnes vaccine as an i.p. injection showed less severe lesions (mean score = 1.62) than the control group (mean lesion score = 3.5). This means that at a dose of 5 mg/chicken P. acnes, like proteose peptone, acted as an i.p. irritant.

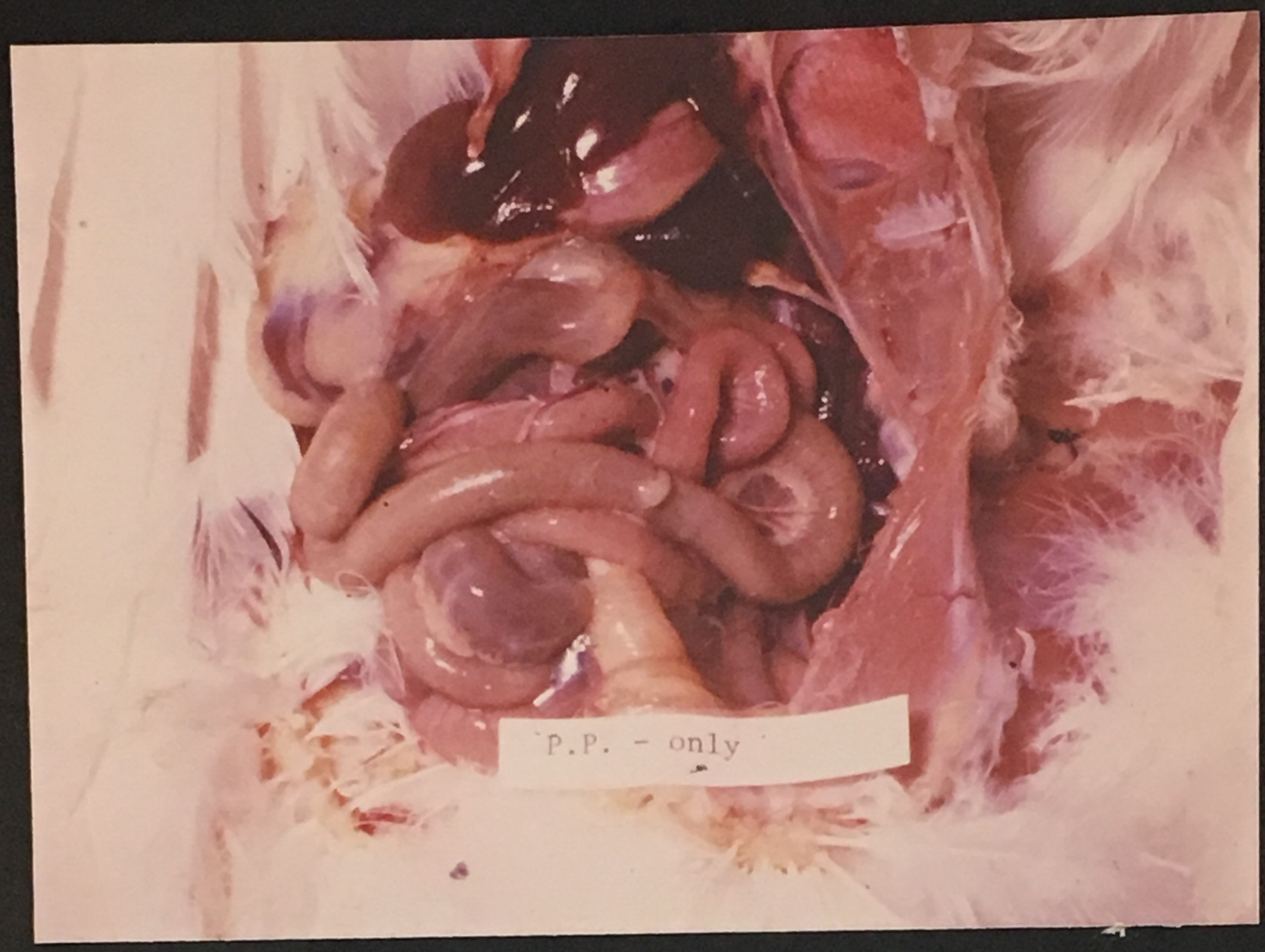
All chickens injected i.p. with 20 mg of P. acnes vaccine showed severe lesions which were comparable to the lesions seen in the control infected PBS injected group. Also lesion severity was related to the time of i.p. injection of P. acnes, the earlier the i.p. injection before infection the more severe the lesions. The results of this experiment contradicts the results obtained using 5 mg P. acnes and may indicate that the effect of P. acnes on lesion severity is dose dependent.

All chickens, injected i.v. at 14, 7 and 0 days before infection with BCG, or P. acnes showed severe lesions (Table 3-4). In the case of chickens injected i.v. with BCG, lesion severity was related to the time of injection. The earlier the i.v. injection, the more severe were the lesions. Chickens injected at 14, 7 and 0 days before infection had lesion scores of 6.6, 5.8 and 5.1 respectively. The same was true of those chickens injected i.v. and i.p. with P. acnes 14, 7 and 0 days before infection and those injected i.p. with proteose peptone 14 and 7 days before infection (Table 3-4).

Mean lesion scores of all chickens (except groups 6 and 10) in the groups which were injected i.p. or i.v. 14 days before infection were comparable to those of the control infected uninjected group (Table 3-4).

Figure 3-1. The intestine of a chicken infected with 1.5×10^5 oocysts and injected intraperitoneally with proteose peptone, (5 days post infection). Compare with Figure 3--2.

Figure 3-2. Intestinal lesion of a chicken infected with 1.5×10^5 oocysts but not injected with proteose peptone (5 days post infection).


P.P. - only

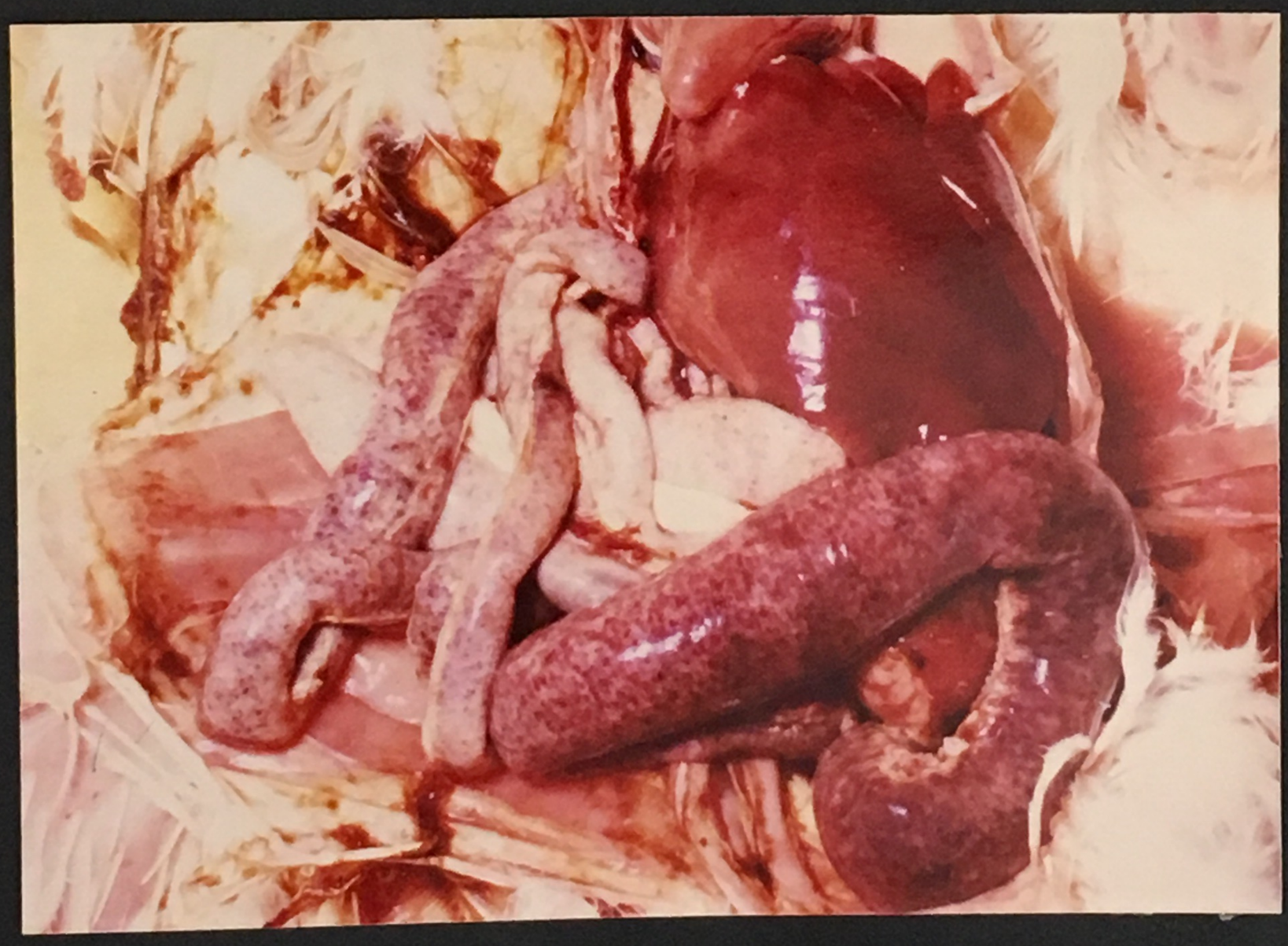
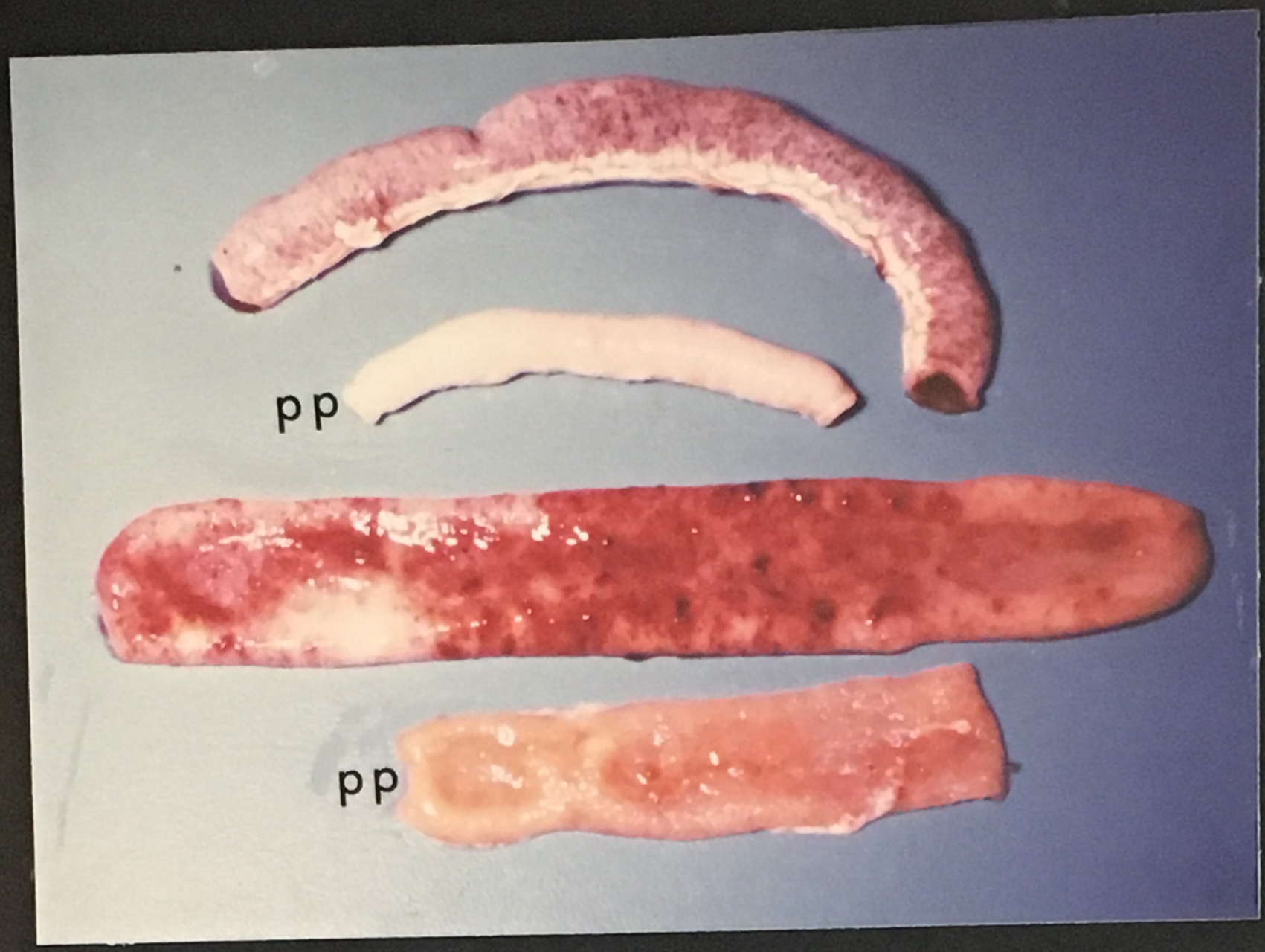
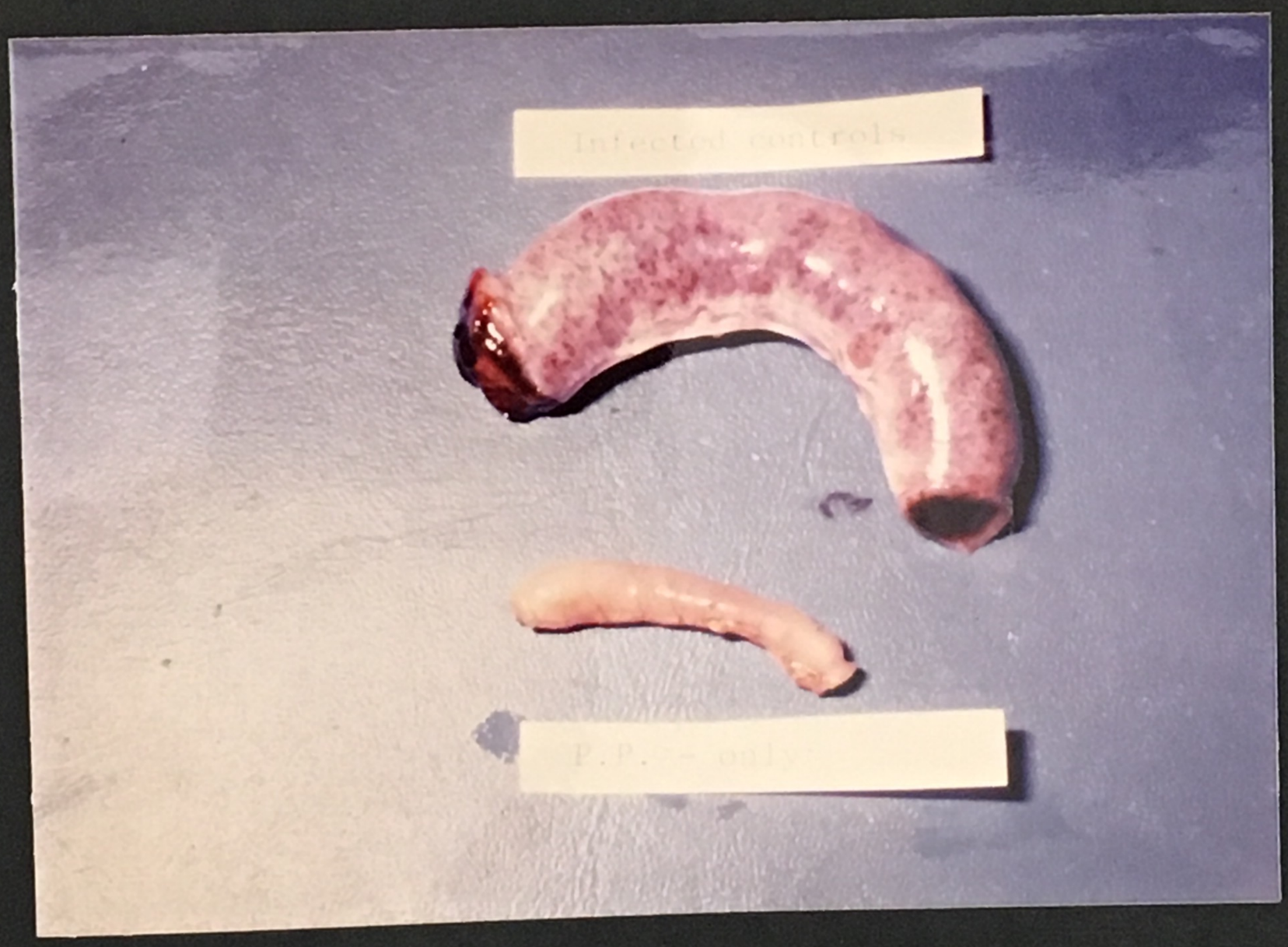


Figure 3-3. Serosal and mucosal lesions of infected chickens. Notice the difference in lesion severity between chickens injected with proteose peptone (pp) and uninjected chickens 5 days after infection.

Figure 3-4. Pieces of intestine taken from two chickens infected with the same dose of oocysts. The lower piece is from a chicken injected intraperitoneally with proteose peptone at the time of infection.


pp
pp


Infected controls
P.P. - only