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Effects of Dietary Zinc Levels on the Histological Changes Produced in White Leghorn Cockerels

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An experiment was conducted with one day-old White Leghorn male chicks to study the effects of different levels of dietary zinc on total zinc in the testes, pancreas and liver to attempt to correlate these effects with any histological changes produced. A study of the relationship between dietary zinc concentration and total contents of zinc in tissues at 30, 50 and 85 days of age suggested no significant linear trend in the testes. The corresponding trend for the liver and pancreas was significant at 1 % level at 30 and 50 days. The lack of significant linear trend at 85 days suggested that the role of zinc in an organ of high metabolic activity was impaired after long-period feeding. The histological differences were evident in the testes of all groups of chicks on the 85 th day. Immature germinal cells were seen in the chicks receiving the basal diet, while the testes of chicks receiving 50ppm zinc were packed with spermatozoa. High levels of zinc supplementation tended to produce destruction in some tubules and germinal cells. In the pancreas of the chicks receiving the basal diet, small amount of zymogen granules could be seen compared with those receiving 50ppm zinc which were packed with zymogen granules and presented the normal feature of the acini. While the pancreas of chicks receiving high levels of zinc showed an increase in the fibrous tissue and alteration of the stainability of the cells. Glycogen accumulation in the hepatic cells of the liver was highest when the levels of zinc in the diet were the lowest.

INTRODUCTION

Zinc (Zn) has been known to be an essential element for many metabolic processes in certain animals and deficiency, as well as excess, of Zn interferes with normal growth and development. There is little information available regarding the effect of Zn in the chick and limited number of experiments to date have mainly been concerned with the histological effect of Zn.

In general, dietary Zn tends to promote the growth of chicks up to about 55 ppm (Young et *al.*, 1958). The chicks are able to consume diets containing 1500 to 2000 ppm Zn without reduced growth (Roberson and Schaible, 1960).

The role of Zn in the normal function of male genital tract in the animal has been studied by Gunn and Gould (1958) and Gunn *et al.* (1961). Parizek *et al.* (1966) indicated that Zn plays an important role in spermatogenesis in rats

and most probable site of action is the primary spermatocyte. In support of this suggestion, Millar *et al. (1958)* found that Zn content was significantly reduced in Zn-deficient rats, the seminiferous tubules were atrophied and marked reduction in the number of spermatozoa occurred.

The pancreas is one of the organs in which the most rapid accumulation and turnover of retained Zn occurs (Feaster *et al., 1954).* Therefore, it is likely that this organ is most affected by Zn deficiency. Histochemical study has indicated that the granulation of p-cells in pancreatic islets was reduced in Zn-deficient Chinese hamster (Boquist and Lernmark, 1969).

The differences of response to dietary Zn in the liver were apparent in rats and calves. Rats have a highly effective mechanism of Zn homeostatic control for high dietary Zn enabling them to consume 600 to 1000 ppm dietary Zn with little or no increase of Zn in the liver (Ansari *et al.,* 1975). In contrast, with calves fed a diet supplemented with 600ppm Zn, Zn concentration in the liver was increased 4 fold after 7 days (Stake *et al.,* 1975).

In view of the lack of information on the effects of Zn on the chick, the studies reported in this paper were designed to investigate further the effects of dietary Zn supplementation on the testes, pancreas and liver in male chicks and to attempt to correlate these effects with any histological changes produced in these organs.

MATERIALS AND METHODS

One day-old White Leghorn male chicks were randomly divided into four experimental groups of 15 chicks each. One group was fed a basal diet which contained 32ppm Zn (Table 1). Three groups were fed the diets to which Zn ammonium sulphate was added at levels of 50, 250 and 1000 ppm to provide a final concentration of 82, 282 and 1032ppm Zn. The diets and distilled water" were offered *ad libitum*. Body weights were recorded at 5 days intervals. Five chicks from each group were killed on the 30 th, 50 th and 85 th day of age. The liver, pancreas and testes were removed from each chick for Zn analysis and histological examination.

The Zn content of diets and individual organs was determined by atomic absorption spectrophotometry after wet digestions of samples in the nitric perchloric acid mixture.

For histological study, the liver was fixed in Gendre's fluid and 4 μ thick sections were stained with PAS-hematoxylin and hematoxylin-eosin (HE). The pancreas was fixed in Zenker-formalin solution, the tissue was cut into 4 μ thick and stained with HE. The testes were fixed in Bouin's fluid, 4μ thick sections taken from the middle portion were stained with PAS-hematoxylin and HE.

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1) Vitamins mixture and antibiotic were added to the distilled water. Vitamins mixture (Trimix, produced by Eisai Pharm.. Co. Ltd.) was given in amount of 3 ml/day into the distilled water according to the prescription. Antibiotic (Erythrocin-PF. produced by Dainippon Pharm.. Co. Ltd.) was given in amount of 150 g/125 l.

Table 1. Composition of the basal diet.

Ingredient	(%)
Sucrose	24. 0
Corn	44.0
Fish meal	16. 0
Barley	8.0
Soy bean	7.0
Choline chloride	0.5
Sodium chloride	0. 5

The Zn content was calculated as total Zn (μg) per organ on fresh weight basis. The effect of dietary Zn *on* total Zn in organs was evaluated with regression analyses and analysis of variance (Snedecor, 1956). The standard error of the mean was given.

RESULTS

Body and organs weights of the experimental groups over treatment periods of 30, 50 and 85 days are presented in Table 2. The highest gain in body weight was obtained by the 50ppm dietary Zn but the value was statistically insignificant. High levels of Zn addition reduced the body weight from that of the control group but the reduction was not statistically significant. Also no differences were detected in the organ weights.

Total Zn contents in the liver, pancreas and testes on fresh weight basis are presented in Table 3. The Zn content in the liver and testes steadily increased with time, but in the pancreas it was not substantially higher at 85 days than at 50 days of life in the two higher dietary Zn levels. The results showed that total Zn in the liver, pancreas and testes increased in chicks receiving 50ppm Zn, a level apparently sufficient to meet requirements of growth. The largest increases were noted in the pancreas and testes on the 50 th day (100% and 59 %, respectively) over the control group, and small increases (3 %) were noted in the total Zn content in the liver.

Zn content in the liver and pancreas was increased sharply by high levels of dietary Zn. With 1000 ppm Zn supplementation at 30, 50 and 85 days the total Zn in the liver increased by 73, 34 and 38 % respectively and in the pancreas by 162 and 193 % at 30 and 50 days respectively over the control group. However, the total Zn content in the testes was not significantly affected by 1000 ppm Zn at 30 and 50 days, and it tended to be depressed by 51% at 85 days over the control group.

The results were analysed for any linear relationships between total Zn in the organs and the concentrations of Zn in the diet. No significant linear trends were found in the total Zn content in the testes at 30, 50 and 85 days of age (Table 3). The corresponding trends for the liver and pancreas were significant at 30 days (5 % and 1 % levels, respectively) and 50 days of age (1% levels in both organs, Figs. 1 and 2). But no significant linear relationships were found at 85 days.

Table 2. Effects of dietary Zn concentration on body, testes, pancreas and liver weights.

Zn added to the diet (ppm)	Body weight (g)			Testes weight (g)		
	30 days	50 days	85 days	30 days	50 days	85 days
0	314.09" ±28.80	651.50* t-43.92	932.86* ±46.37	0.08* ±0.03	0.38* ±0.12	7.20* ±3.40
50	320.84 ±31.90	691.00 ±47.70	998.00 i62.38	0.08 ±0.02	0.40 ±0.30	7.49 io. 93
250	301.32 127.60	624.44 ±69.03	922.50 ±37.68	0.09 ±0.09	0.32 10.08	6.74 il.30
1000	288.2; ±30.40	617.50 ±82.30	960.00 ±52.51	0.09 ±0.03	0.32 ±0.20	7.53 ±4.10

Zn added to the diet (ppm)	Pancreas weight (g)			Liver weight (g)		
	30 days	50 days	85 days	30 days	50 days	85 days
0	1.07* ±0.07	1.32* ±0.19	1.57* ±0.15	10.77* F1.39	14.36* ±3.80	13.47* il.90
50	0.90 ao. 09	1.24 ±0.22	1.56 to. 20	9.28 ±0.83	13.18 ±2.30	15.45 ±0.70
250	0.74 ±0.04	1.32 ±0.06	1.56 ±0.12	7.47 -10.65	14.21 ±1.00	16.40 ±2.60
1000	0.74 to. 07	1.45 ±0.22	1.89 ±0.30	6.69 io. 74	14.32 ±1.30	13.96 il.40

* Mean value ± standard error.

Histological studies of the testes at 30, 50 and 85 days old were made. Some aspects are shown in Plate I. On the 30th day, overall pictures of the testes in all groups were similar. On the 50 th day, many tubules were characterized by the presence of the primary spermatocytes after Zn supplementation (PI. I-Figs. 2 and 3), while the tubules of the chicks fed the basal diet were often lined by only spermatogonia and Sertoli's cells (Pl. I-Fig. 1). Most of the histological changes in the testes were evident on the 85th day. In the testes of the chicks receiving the basal diet, very few sperm could be seen (Pl. I-Fig. 4), whereas those receiving 50 ppm Zn were packed with sperm, as seen in Pl. I-Figs. 5 and 6. Some tubules appeared to be damaged and lined by spermatogonia and spermatocytes, the interstitial cells were reduced in number and the cytoplasm was scanty. It was apparent that the testes of the chicks receiving 50 ppm Zn carrying on normal spermatogenesis were composed of a different population of cells than those of other groups. High levels of Zn supplementation tended to produce destruction in some tubules and germinal cells, while the other tubules still remained unaffected and developed

Table 3. Effects of dietary Zn concentration on total Zn in the testes, pancreas and liver.

Zn added to the diet (ppm)	Total Zn in the testes (ppm)			Total Zn in the pancreas (ppm)			Total Zn in the liver (ppm)		
	30 days	50 days	85 days	30 days	50 days	a5 days	30 days	50 days	85 days
0	7.90* ±5.71	9.06* ±4.06	92.79* ±30.14	27.01* ±2.63	21.31" 16.38	39.49* i11.76	224.53* ±27.26	297.08* ±55.30	363.92* ±17.40
50	a.03 14.74	18.21 ±7.67	103.72[2] 13.90	35.15 i-3. a2	34.06 ±9.84	58.79 ±5.30	252.61 ±35.14	307.81 ±55.90	498.39[1] ±78.40
250	9.98 ±7.49	12.07 ±5.76	44.03[1] ±6.70	35.15 ±3.87	33.75 ±4.37	24.15 ±3.20	216.29 114.58	316.40 ±24.60	426.86 ±34.10
1000	5.03 ±2.74	9.66 ±5.643	45.18 ±22.90	70.81[1] k28.66	62.55 i-17.20	41.57 t12.90	355.62[2] ±100.28	398.84 ±50.50	503.71[2] ±47.70

* Mean value ± standard error.

[1] Significantly different ($P<0.01$) from the control group by analysis of variance.

[2] Significantly different ($P<0.05$) from the control group by analysis of variance.

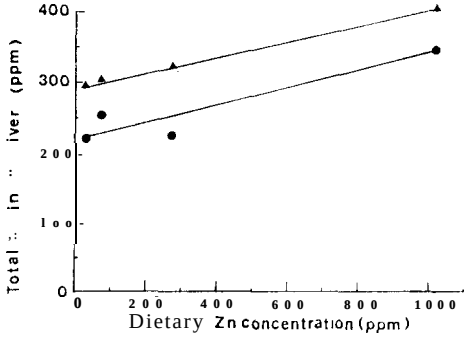

Fig. 1. Total Zn content in the liver and regression lines after 30 and 50 days of dietary Zn treatment.
●—●; 30 days (Y=0.127 X+217),
▲—▲; 50 days (Y=0.100 X+294).

toward the normal picture of maturity. The interstitial cells appeared to be destructed by the high levels of Zn in some tubules but the Sertoli's cells remained intact (Pl. I-Figs. 7 and 8).

In the pancreas, the histological appearances of the chicks of all groups were typical to the normal chicks at 30 and 50 days old, but the most obvious changes were evident on the 85 th day. The acini of the chicks fed the basal diet were almost entirely depleted of zymogen granules, but the pancreatic islets appeared unaffected (Pl. II-Fig. 9). The pancreas of the chicks receiving 50ppm Zn presented the normal feature of the acini and islets (Pl. II-Fig. 10), while the acini of the chicks receiving high levels of Zn showed in-

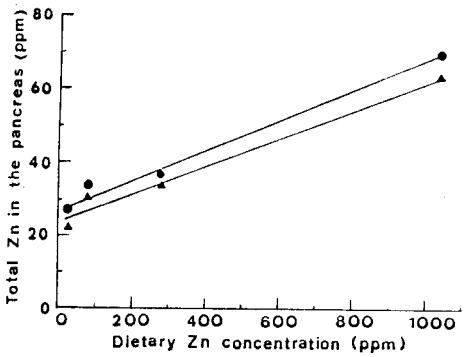

Fig. 2. Total Zn content in the pancreas and regression lines after 30 and 50 days of dietary treatment.
●—●; 30 days (Y=0.042 X+27.19),
▲—▲; 50 days (Y-0.037X-1-24.92).

creased fibrous tissue and alteration of the stainability of cells, but showed no evidence of cell degeneration (Pl. II-Fig. 11). Those acini were relatively little depleted compared to the acini of the basal control group and staining of zymogen granules persisted but varied from cell to cell.

In the liver, normal hepatic cells of the basal control chicks presented the usual feature at the end of 30 days old, while the glycogen content was increased and formed aggregated masses occupying large areas of the cytoplasm. This accumulation of glycogen persisted in a similar manner at the end of 50 and 85 days (Pl. II-Fig. 12). These glycogen masses were stained heavily by PAS reaction. The glycogen accumulation was in the form of small patches and decreased by time (Pl. II-Figs. 13 and 14). Little accumulation of glycogen was present in the chicks supplemented with high levels of Zn.

DISCUSSION

The amount of Zn needed to assure satisfactory and optimum growth in the chick and tolerance of the amount have been studied widely and intensively. The consensus of these reports was that the minimum requirement lay in the range of 30 to 35 ppm Zn; the optimum was higher than 45 to 55 ppm Zn and the tolerance was at least 1000 ppm Zn (Supplee et al., 1958; O'Dell and Savage, 1957; Johnson ***et al.,*** 1962). The dietary experiment reported in this paper is in full ageement with these findings. The present study suggests that the Zn requirement for growth of young growing chick is approximately 82 ppm Zn. Zeigler ***et al.*** (1961) claimed that a minimum dietary level of Zn was about 12ppm for the young chick when the diet contained casein. However, there is no enough evidence in the published work to substantiate this generalization of Zn requirement of the chick when diets are based on protein sources such as casein or soy bean protein.

In the present study, the decrease of the Zn level in the testes with high levels of Zn supplementation at 85 days of age suggests failure of Zn uptake by the chicken testes. Gunn ***et al.*** (1960) indicated that the uptake of ^{65}Zn in the testes of rats depend upon the presence of pituitary hormone. And, Gunn ***et*** al. (1961) found that Zn uptake in the testes was depressed by 40 % level from intact control values following hypophysectomy to the mature rat. In view of these observations, the fall in total Zn levels in the testes noted in this study using high levels of dietary Zn may represent the loss of the hormonal control necessary for spermatozoa to incorporate Zn. On the contrary, it would appear that with low levels of dietary Zn there was a high increase of Zn levels in the testes toward the end of 85 th day. This increase of Zn in the testes may be related to some morphological changes which occurred at that stage of testicular development, such as the appearance of spermatozoa. Miller and Miller (1960) have indicated that the spermatozoa was responsible for the concentration of Zn in the seminiferous tubule. In this context, it is necessary that the Zn supply must be sufficient for every new cell formation during spermatogenesis. The finding in this study shows that the testicular tissue is highly sensitive to dietary Zn. The chicks began to respond to all levels of Zn supplementation within 50 days of age. The primary spermatocytes appeared in all Zn added groups but few spermatocytes were presented in the unsupplemented chick. On the 85th day, the addition of high levels of Zn started to produce destruction in some tubules and interstitial cells, while the other tubules remained unaffected and developed toward the normal maturity. The tubules of the unsupplemented chicks were devoided of mature spermatozoa. Thus two aspects of the problem had to be considered. The first is the relation of Zn to non-spermatogenic tissue and interstitial cells. The second is the role of Zn in the spermatogenic cells. Concerning the first one, Zn was known to have an inhibitory role in the hyperplasia of the interstitial cells as occurred following the testicular injury by cadmium (Gunn and Gould, 1970). On the other hand, Elcoate ***et al.*** (1955) showed that the administration of testosterone propionate affected the distribution of Zn in the Zn deficient rats by directing more of it into the gonads. Regarding the second aspect, Millar ***et al.*** (1958) demonstrated that inadequate uptake of Zn led to degeneration of the seminiferous tubules, indicating the importance of this metal in spermatogenesis. Furthermore, the marked diminution in the germinal cells in the present study indicated that some of the spermatogonia have been destroyed when the chick maintained on dietary Zn deficiency. Considering the fact that it takes about 12 weeks for spermatogonia in the normal White Leghorn cockerels to develop into mature sperm (Kumaran and Turner, 1949), however, degrees of injury produced by Zn deficiency may slow down the maturation process.

There was a possibility that high dietary Zn suppressed the secretion of testosterone propionate as shown in the present study, and high level Zn might inhibit the secretion of follicle stimulating hormone (FSH) by anterior pituitary. In support of this suggestion, Parizek (1960) found that uptake of ^{65}Zn by the testes of immature rats was increased by a single dose of 1 to 4

IU of FSH. Unfortunately the state of the pituitary was not observed in the present study.

The observation in this study about the effect of different levels of dietary Zn on the total contents in the pancreas is in general agreement with that of other workers (Kincaid *et* al., 1975; Miller *et al.,* 1970).

The differences in the magnitude of changes in the total Zn in pancreas were apparent in comparing the present study with the other studies. These differences were probably a consequence of the duration, dietary Zn levels and species in the experiments. There was, nevertheless, agreement that dietary Zn concentration had an influence upon the total contents of Zn in the pancreas. The liner increment between total Zn in the pancreas and the dietary Zn concentration was observed with all groups on the 30 th and 50 th day of life. The lack of this increment on 85 th day suggests that the role of Zn in an organ of high metabolic activity is impaired after long-term feeding. In support of this suggestion, Miller *et al.* (1970) claimed that the pancreas must have several different types of binding sites which hold Zn with widely varying tenacity. The tendency toward a decrease in the total Zn in the pancreas with time in the high levels of dietary Zn was in harmony with the study of Miller *et al.* (1970). The histological observation in the present study indicated that Zn had a profound effect on the zymogen granules of the acinar cells. The acinar cells were known to be a major excretion pathway for Zn (Montgomery *et al., 1943).* In the present study the exocrine and endocrine portions of the pancreas demonstrated few histological changes up to 50 days of age. At 85 days old, the decrease in the zymogen granules of the acinar cells as a consequence of the unsupplemented diet might reflect the loss **of** proteolytic activity suggested by Koo and Turk (1977). After the addition of high levels of dietary Zn, the sequence of fibrosis at 85 days indicated that these levels of Zn might produce predominantly connective tissue damage.

The linear increment between the total Zn in the liver and the dietary Zn concentration with all groups was observed on the 30 th and 50 th day of age. This suggested that the additional Zn would have accumulated. The lack of increment on 85 th day fully supports the finding of Kincaid *et al. (1976)* who claimed that homeostatic control mechanism protected the chick for at least limited periods of time against increased Zn in tissues from dietary Zn levels up to 1200 ppm.

Zn has been correlated with protein synthesis and catabolism. The end products of protein catabolism are then either excreted or reutilized for a variety of metabolic processes. One of these metabolic processes is hepatic glycogenesis via glyconeogenesis.

Enzyme studies are needed to rule out the possibility of a factor controlling glycogenesis or glycolysis as a cause for glycogen accumulation. Glucose -6-phosphatase is one of the key of gluconeogenic enzymes (Weber *et al.,* 1965) and this enzyme is located in membranes of smooth surfaced endoplasmic reticulum. The absence of these membranes to move these energy store in or out of the parenchymal cell in case of Zn deficiency in rat was explained for the accumulation of glycogen (Miller *et al.,* 1974). In the present

study, the excessive accumulation of glycogen in chick receiving the basal diet and the decrease in the amount of this accumulation with increasing dietary Zn levels, add supporting evidence to the glycogen metabolism.

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Explanation of Plate I

Fig. 1. Testis from a 50 days old cock fed the basal diet. Seminiferous tubules are lined by spermatogonia and Sertoli's cells. HE stain. x400.

Fig. 2. Testis from a 50 days old cock fed the basal diet supplemented by 50ppm Zn. Seminiferous tubules show stages of mitotic division with the appearance of primary spermatocytes. HE stain. x400.

Fig. 3. Testis from a 50 days old cock fed the basal diet supplemented by 1000 ppm Zn. Seminiferous tubules show more mitotic activity than that in Fig. 2. HE stain. ×400.

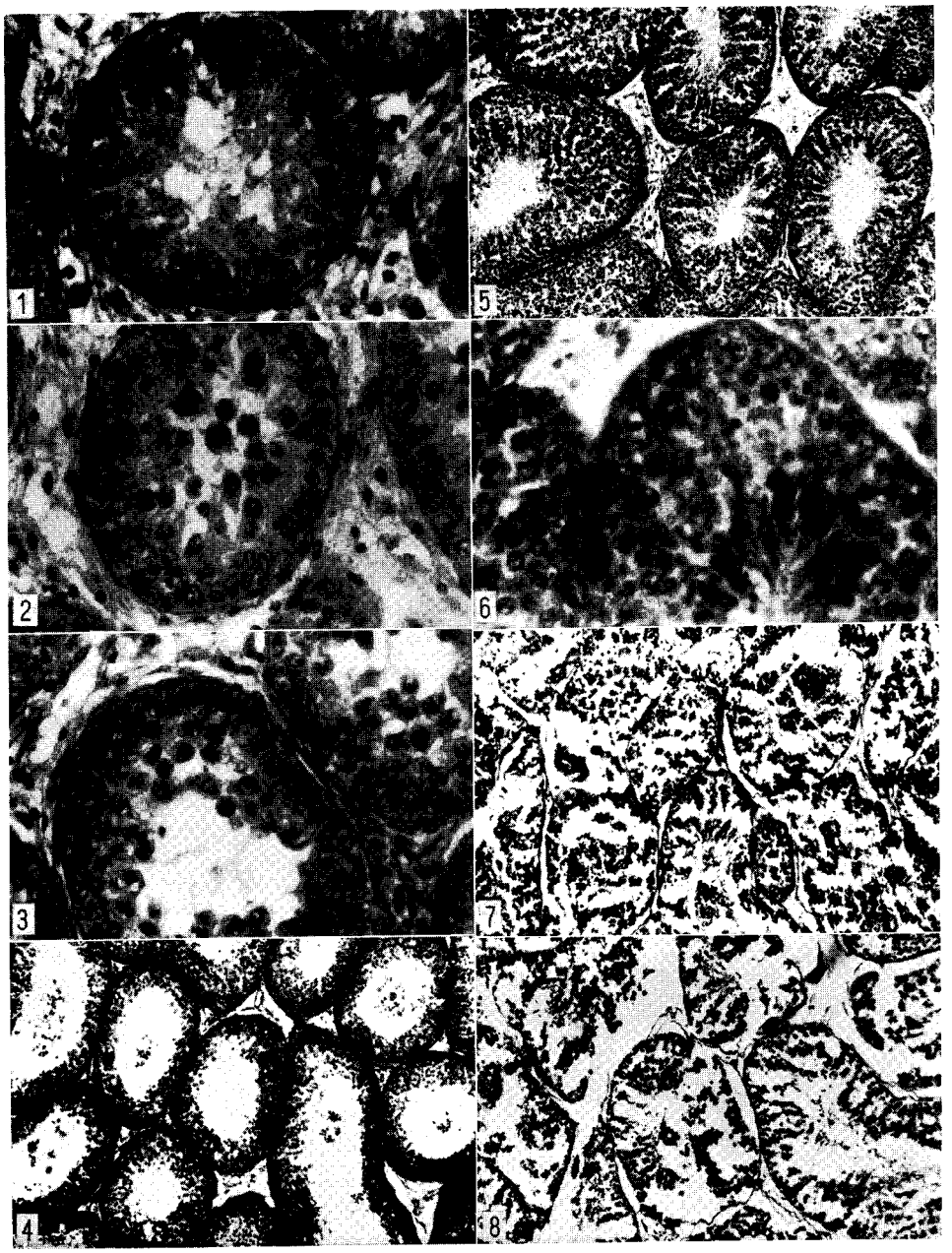
Fig. 4. Testis from a 85 days old cock fed the basal diet. Seminiferous tubules are lined by small number of maturing germinal cells, and the relative increase in the size of lumen compared with Fig. 5 is noted. HE stain. ×100.

Fig. 5. Testis from a 85 days old cock fed the basal diet supplemented by 50ppm Zn. Seminiferous tubules show various stages of spermatogenesis. HE stain. ×100.

Fig. 6. Higher magnification of testis in Fig. 5 with spermatogonia near to the basement membrane and spermatozoa projecting into the lumen. HE stain. ×400.

Fig. 7. Testis from a 85 days old cock fed the basal diet supplemented by 250ppm Zn. Destructed tubules and germinal cells are noted. HE stain. × 100.

Fig. 8. Testis from a 85 days old cock fed the basal diet supplemented by 1000 ppm Zn. Destructed tubules and germinal cells are more numerous than that in Fig. 7. HE stain. ×100.



Histological Effects of Zinc in the Cockerels

Explanation of Plate II

Fig. 9. Pancreas from a 85 days old cock fed the basal diet showing extensive depletion of zymogen granules. HE stain. ×400.

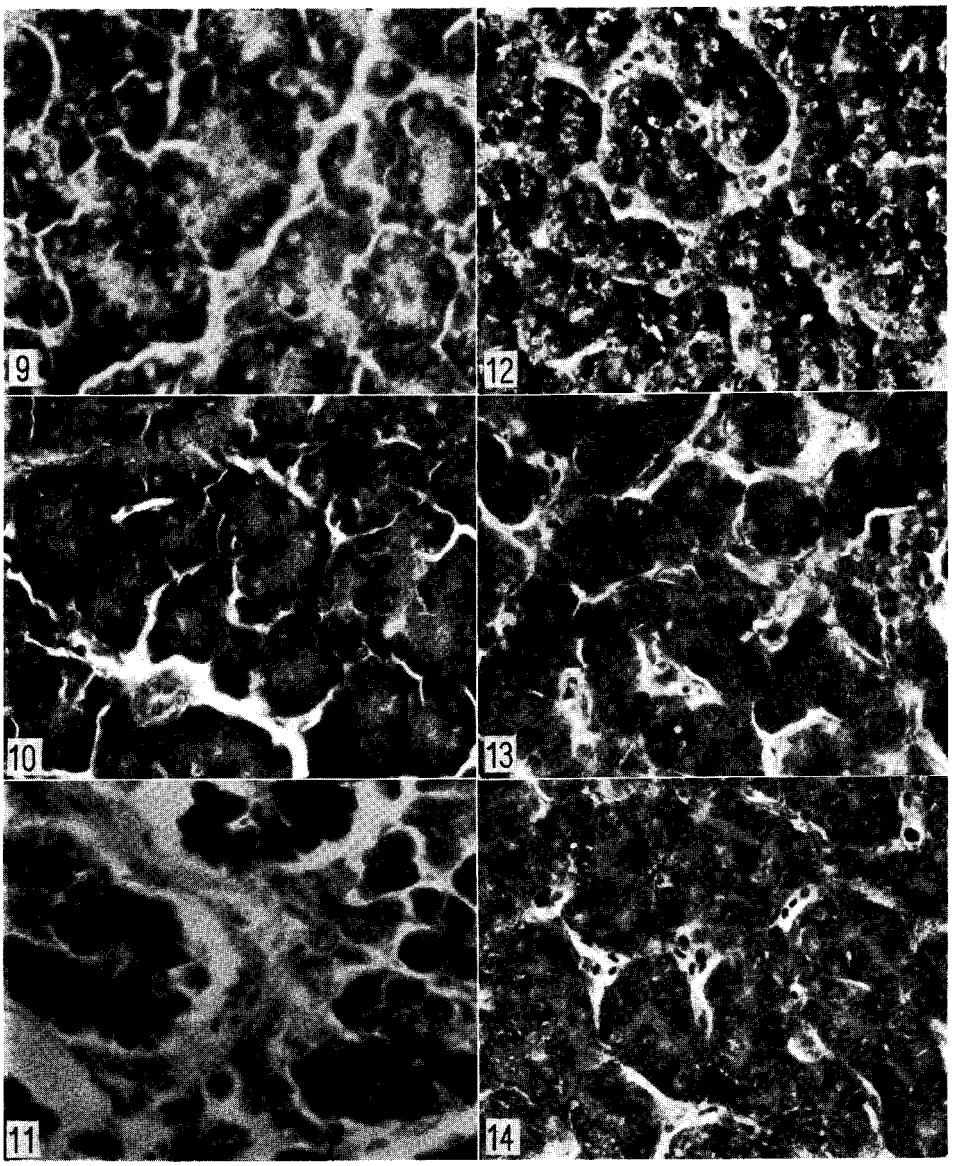
Fig. **10.** Pancreas from a 85 days old cock fed the basal diet supplemented by 50ppm Zn. The acini filled with darkly stained zymogen granules are noted. HE stain. ×400.

Fig. **11.** Pancreas from a 85 days old cock fed the basal diet supplemented by 1000 ppm Zn. Well developed fibrous tissue separating the acini are noted. HE stain. ×400.

Fig. 12. Liver from a 85 days old cock fed the basal diet showing the accumulation of small patches of glycogen. Glycogen appears black. PAS-hematoxylin stain. ×400.

Fig. 13. Liver from a 85 days old cock fed the basal diet supplemented by 50 ppm Zn. showing the distribution of glycogen. Compare the amount of glycogen with that in Fig. 12 which contained large accumulation of glycogen. PAS-hematoxylin stain. ×400.

Fig. 14. Liver from a 85 days old cock fed the basal diet supplemented by 1000 ppm Zn, showing complete glycogen depletion. PAS-hematoxylin stain. × 400.



Histological Effects of Zn in the Cockerels