SPERMATOGENESIS IN BALD EAGLES EXPERIMENTALLY FED A DIET CONTAINING DDT

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Decreasing reproductive success of Bald Eagles (*Haliaeetus leucocephalus*) along the west coast of Florida led to speculation that pesticides, particularly DDT, might be involved in causing infertility of the eagles (Broley, 1958). In England and Scotland similar decreases due to reproductive failure have been reported in Golden Eagles (*Aquila chrysaëtos*) and Peregrine Falcons (*Falco peregrinus*). Pesticide residues have been found in the eggs of these two species, and the possibility of a causative relationship has been suggested (Ratcliffe, 1963; Lockie and Ratcliffe, 1964).

As a result of Broley's observations (1958) the National Audubon Society initiated a field study to measure population trends and reproductive success of the Bald Eagle in various parts of the United States (Sprunt and Ligas, 1963).

The Bureau of Sport Fisheries and Wildlife has undertaken experimental studies in two series of tests with captive eagles to determine the approximate level of DDT in the diet that might cause death, the rate of accumulation and loss of DDT residues in various tissues, and the effects of DDT on reproductive organs. Early results have been summarized by DeWitt and Buckley (1962) and by Buckley and DeWitt (1963).

This paper reports the results of the histological examination of the testes of Bald Eagles fed a fish diet containing DDT.

METHODS

Bald Eagles were captured near Haines, Alaska, in November and December of 1961 and 1962 and maintained at the Experimental Fur Station, Petersburg, Alaska. In the first series of tests (1961–1962) the basic diet consisted, at various times, of heads of ground salmon, herring, or flounder, which was supplemented by liver meal (2 per cent by weight). At various intervals wheat germ meal, wheat germ oil, and multiple vitamins were also added. In the second test series (1962–1963), food consisted of ground salmon waste—heads, entrails, and trimmings—(99 per cent) and commercial liver meal (1 per cent), supplemented by one teaspoonful of vitamin supplement and one gram of Terramycin® per 10 pounds of food. The Terramycin® was fed for about seven days, then omitted from the diet for three or four days.

Technical grade p,p′ DDT dissolved in Wesson oil® was mixed with the diet to produce the desired concentrations. During the 1961–1962 studies DDT was fed at the following nominal concentrations: 0, 10, 160, 800, and 4000 parts per million (ppm) dry weight. These concentrations were computed on the assumption of a 70 per cent moisture content, the amount present in fish fillets. The diet of salmon heads contained less moisture than this; hence, that actual dosage was somewhat lower (see footnote, table 1). In the 1962–1963 studies all eagles were fed a diet containing approximately 10 ppm DDT for periods of 60 and 120 days. Eagles were necropsied at death or at the conclusion of the tests. Portions of selected organs were fixed in Bouin's solution or in 10 per cent formalin for later microscopic examination. Remaining tissues and organs were saved for residue analysis.

For the purposes of this report the spermatogenic cycle was divided into various histologic stages using criteria roughly similar to those used by Johnston (1956) and by Sealander and Hauser (1965).

TABLE 1
RESULTS OF HISTOLOGICAL EXAMINATION OF TESTES OF ADULT MALE BALD EAGLES FED DDT AT LOW LEVEL

Specimen number	Date killed	Treatment[a]	Time on treatment	Condition of testes	Stage[b]
3	25 Nov. 1962	None[c]	—	Normal, inactive	1
6	24 Dec. 1962	None[c]	—	Normal, inactive	1
8	2 Mar. 1963	10 ppm DDT	60 days	Normal, inactive	1–2
9	2 Mar. 1963	10 ppm DDT	60 days	Early spermat.	2
11	12 April 1963	10 ppm DDT	39 days[d]	Early spermat.	4
13	1 May 1963	10 ppm DDT	120 days	Sperm present	6
15	1 May 1963	10 ppm DDT	60 days	Sperm present	5–6
		Clean food	60 days		
16	1 May 1963	10 ppm DDT	60 days	Sperm present	5–6
		Clean food	60 days		
20	7 May 1963	10 ppm DDT	120 days	Sperm present	5–6
21	9 May 1963	10 ppm DDT	60 days	Sperm present	6–7
		Clean food	60 days		
22	12 May 1963	10 ppm DDT	60 days	Early spermat.	3–4

[a] Dietary concentration expressed on a dry weight basis, on the assumption of 70 per cent moisture content. Actual moisture content proved to be 65 per cent.
[b] Testicular stages correspond to those of Johnston (1956).
[c] Bird dead before experiment began.
[d] Bird died prematurely of pneumonia.

Stage 1. Inactive conditions; spermatogonia with a few primary spermatocytes. No evidence of cell division.

Stage 2. Primary spermatocytes in synapsis; our stage 2 includes both stage 2 and stage 3 of Johnston (1956).

Stage 3. Not seen in our limited series of eagle testes.

Stage 4. Secondary spermatocytes present.

Stage 5. Spermatids present, but no spermatozoa present.

Stage 6. Spermatozoa present in bundles with the heads of the sperm embedded in the cells lining the seminiferous tubule. Sperm occasionally seen in the lumens of some tubules. Our number 6 is equivalent to Sealander and Hauser's stages 5a and 6b (1965:160–161).

Residue analyses were made at Wisconsin Alumni Research Foundation. Tissues were dried at low temperatures (40° C), ground with sodium sulfate, extracted with petroleum ether and Skelly B in Soxhlet apparatus, cleaned by passage through a florisil column, and read by electron capture gas chromatography.

RESULTS

Since a complete evaluation of the histological changes seen in the tissues from the 1961–1962 studies had to await the analysis of the findings in the 1962–1963 trials, these latter results will be discussed first.

When Bald Eagles were fed a fish diet containing 10 ppm DDT for periods of 60 or 120 days, there was no apparent inhibitory effect upon the spermatogenic activity of the testes (table 1). DDT residues (DDT + DDD + DDE) ranged from 5 to 14 ppm dry weight. These would probably all be less than 2 ppm wet weight. Histologically the testes were at the stages of spermatogenesis one would expect in light of the known breeding season in Alaska (Imler and Kalmbach, 1955).

TABLE 2
RESULTS OF HISTOLOGICAL EXAMINATION OF BALD EAGLE TESTES IN ACUTE TOXICITY TESTS (1961–1962)

Eagle	Dosage[a]	Date died or killed	Days on test	Testicular stage
193	0	K 29 June	112[b]	6
143	160	D 18 May[c]	71	5
188	160	K 29 June	112	Destruction
74	4000	D 31 March[c]	23	1
32	4000	D 26 March[c]	18	1
156	4000	D 26 May[c]	15	Destruction

[a] Dietary concentration expressed on a dry weight basis, on the assumption of 70 per cent moisture content. Moisture content in fact assayed 42 per cent on stored frozen samples. Despite probable loss of some moisture in storage, the true dosages were evidently considerably lower than the nominal dosages shown on the table.
[b] Control. Kept in captivity 112 days.
[c] Died with typical tremors and other signs of DDT intoxication.

Two birds that died in November and December before receiving the diet containing DDT had testes that were in Stage 1. One eagle, killed on 2 March after being on DDT for 60 days, also had histologically normal testes (Stage 1–2). The testes from a second eagle killed on 2 March after 60 days on DDT were in histological Stage 2.

As shown by table 1, the testes of all the eagles (except number 22) were in histological stages compatible with the breeding season in Alaska. Histologically, the testes of number 22 were at an earlier stage than those of the other eagles, but there were no degenerative changes. Causes of this failure to develop are unknown, but cannot be reasonably considered to be associated with DDT dosage.

Results of the 1961–1962 studies are summarized in table 2. The two eagles fed DDT at 4000 ppm died after being on the diet 15 days (bird number 32) and 23 days (bird number 74) and exhibited typical DDT tremors before death. Histologically their testes were still in Stage 1, the stage to be expected at this season.

Marked degenerative changes were apparent in the testes of eagle 156, which was maintained for 15 days on a diet containing 4000 ppm DDT. A detailed discussion of these changes in eagle number 156 follows.

Testis. Lumens of seminiferous tubules were filled with sloughed cells and cellular debris which contained many degenerate cells. Developing sperm were seen in a few widely scattered tubules, but the majority of tubules were devoid of sperm and spermatids.

Epididymis. Lumen filled with degenerate cellular material, consisting of necrotic cells, nuclear fragments, cytoplasmic remnants, and sperm. Frequently the necrotic cellular elements were fused, forming irregular conglomerate masses to which sperm were frequently attached. Eosinophilic hyaline-like droplets were found in the lumen of the epididymis, lying near the degenerate cellular material.

One eagle (number 188) that survived a dietary dosage of 160 ppm DDT for 112 days showed degenerative testicular changes, while another eagle (number 143) that died after dietary dosage of 160 ppm DDT for 71 days had histologically normal testes with active spermatogenesis. No testicular material from the eagles fed DDT at the rate of 800 ppm was available for histological study.

In summary, testicular destruction occurred in two of three eagles that died or were killed at the season of the year when active spermatogenesis was to be expected.

One of these (number 156) was on 4000 ppm, a dosage that it survived only 15 days. The other (number 188) had survived 160 ppm for 112 days. The eagle (number 143) with normal testicular development, also on dosage of 160 ppm, died after 71 days. Changes in testicular tissue did not occur in the two eagles on 4000 ppm (numbers 74 and 32) that died of DDT poisoning at the time of year prior to active spermatogenesis. Thus, obvious testicular damage occurred only (but not uniformly) at dosage levels that also were generally toxic.

DISCUSSION

When these toxicological studies were initiated it became apparent that no one had studied the normal testicular cycle of the Bald Eagle. Our studies have shown that this species has a testicular cycle similar to that reported from many other birds in the Northern Hemisphere and that spermatogenesis will occur in recently caught Bald Eagles. The testes of newly captured birds in November and December were small and histologically inactive, whereas they were progressively more active, histologically, as the normal breeding season approached. Sperm were present in the seminiferous tubules of all but one male (number 22) killed in May. One bird (number 13) also had a large number of sperm in the epididymis. The peak of spermatogenic activity closely approximated the reported nesting dates. Imler and Kalmbach (1955) reported that Bald Eagles lay eggs from as early as 24 March to as late as 24 June in Alaska but that half the records are between 7 May and 14 May. Hensel and Troyer (1964) found that the egg-laying period extended from mid-April to the end of May and reached a peak in the second week of May.

The failure of reproductive success in Bald Eagles could be due to several causes, including infertility in the male or female or both, or the failure of the zygote to develop. This discussion will be restricted to the possible role a toxic chemical might have in affecting the fertility of the male.

Infertility in the male could be due to (1) the failure of spermatogenesis to occur, (2) the production of abnormal sperm, or (3) the production of sperm in insufficient amounts to allow optimum fertility (Sturkie, 1954).

Our data suggest that DDT does not interfere with spermatogenesis except at levels which are in themselves toxic to the Bald Eagle as mentioned above. Several toxic chemicals have been shown experimentally to prevent spermatogenesis in the rat (Ribelin, 1963), and certain toxic fats will prevent spermatogenesis in the domestic chicken (Allen and Lalich, 1962; Allen, 1964).

While we were unable to determine if the Bald Eagle sperm produced in the 1962–1963 study were morphologically normal and exhibited normal motility, the possibility does exist that these sperm were normal. Mallards and pheasants fed at the rate 100 ppm for varying periods had morphologically normal and motile sperm (unpublished data, L. N. Locke).

We did not obtain any information on the quantity of sperm of treated eagles, although it is possible that sperm production could be reduced by DDT treatment. It has been shown in the chicken that a minimum of 100 million spermatozoa must be inseminated to insure optimum fertility (Sturkie, 1954). Albert (1962) reported a drastic decrease in sperm production in one of two white leghorn cockerels fed rations containing 0.3 per cent (3000 ppm) DDT by weight for a period of 12 days. At this time both birds displayed minor neurological signs of DDT intoxication and were returned to a normal diet. After holding both cockerels on the normal ration for 30 days, during which time the DDT toxicity signs disappeared, and the sperm

levels returned to normal, the birds were again placed on a diet containing 0.3 per cent DDT. Both died 18 to 20 days after being returned to the diet containing DDT, and both showed a marked decrease in sperm production just prior to death. The testes from only one cockerel were examined histologically, and Albert reported that these showed degenerative changes. None of three cockerels which he fed a diet containing 0.1 per cent (1000 ppm) DDT showed any reduction in sperm production.

SUMMARY

When Bald Eagles were fed DDT in the diet at the level of 10 ppm (dry weight basis) for periods of 60 and 120 days, there was no interference with spermatogenic activity. Degenerative testicular changes were produced only by levels of DDT that produced abnormal neurological signs and usually resulted in death.

Histological examination of these testes indicates that Bald Eagles have a seasonal testicular cycle similar to that reported for many other birds of the Northern Hemisphere.

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