RAPTOR RESEARCH

Volume 6 Supplement Part J 1972

SPECIAL CONFERENCE ON CAPTIVITY BREEDING OF RAPTORS–A REPORT

Sponsored by Raptor Research Foundation, Inc.
Held at Sioux Falls, South Dakota, U.S.A., November 22-23, 1971

Part J. Reproductive Endocrinology (Panel 5)

edited by
Richard R. Olendorff
3317 Olympus Drive
Bremerton, Washington 98310

Panel Members: Erich Awender, Chairman, Timothy Lawson, James C. McIntyre, and Stanley Temple.

AWENDER. We will try to get right into things here because this is going to be a more nebulous problem and may be more divisive. I can already see things shaping up from two sides, the practical and theoretical. They do not rule each other out, but I think we will have quite a bit of discussion and not quite so much formal presentation. Mr. Temple will present the basics so that we will know what we are talking about and see in general outline what endocrinology is all about. I am not too qualified to say anything here, because I am mostly versed background-wise in human medicine and I have done very little work with birds. I will tell you what little I know later, but let's start with Mr. Temple, please.

TEMPLE. As Dr. Awender has said, when we are talking about endocrinology of breeding birds, we are talking about the control mechanism. Endocrinology is the study of hormones. Hormones are internal secretions of the body and are the mechanisms by which birds, mammals, and all vertebrates control their reproductive processes. We are dealing with the very intimate control of all of the acts that are associated with breeding. It is a very complicated picture and, unfortunately, the information for birds as a group is very scanty. Our understanding of even mammalian endocrinology, especially of the cycles of various hormones that control reproduction, is scanty; with birds we are even more at a loss. We are fortunate that the evidence collected so far indicates that most

vertebrates have the same (certainly all birds have the same) basic endocrine control of their reproductive cycles. Information that we might gain from studying the endocrinology of a pigeon or a chicken or some other bird is undoubtedly perfectly applicable to birds of prey. Basically, because they are so important to the birds, there has not been a lot of modification of these systems. It is one basic system that has worked. So what I am going to do is go through the endocrine control of a bird's reproductive system, and point out, when I finish explaining it, possible places in this system where problems imposed by captivity may be disrupting the normal function. After I have done this, I might comment a little on ways of helping the birds with reproductive endocrinology problems. I think Dr. Awender and Tim Lawson will follow up with some of the approaches that they have tried.

First of all let's start out with what it is that triggers the reproductive cycle in birds. What starts the cycle is an external stimulus. The responses to external stimuli are probably where birds have evolved all the variation that we see between species. Different species have evolved different sets of external stimuli which trigger reproduction. For many birds, photoperiod—increasing day length —is one of the most critical external stimuli triggering the reproductive cycle. For other birds, climatic factors, such as rainfall, temperature, and other things also come into play, as do important factors such as the interaction between pairs, nest-building and courtship. All of these external stimuli come from outside the individual bird. The external stimuli are received through the bird's senses. Be they visual, auditory, tactile, or whatever, the stimuli are received by the central nervous system—i.e. by the brain. They are then transferred to the region of the brain called the hypothalamus. The hypothalamus you can think of as being an integrating system. This is where all the information is synthesized, and the appropriate messages, if you will call them that, are relayed to the different portions of the brain that will ultimately trigger the production of the hormones which in turn will control the reproductive cycle. The hypothalamus secretes very little known substances called neurohormones. These hormones are secreted by nerves, a very unusual condition, and they are very, very hard to identify. These neurohormones are secreted by the hypothalamus after it has integrated all the information from the external environment. They are then relayed to the pituitary gland.

If you remember your high school biology, the common designation for the pituitary gland is the master gland of the body. Many of the important hormones that control body processes are produced in the pituitary. This is exactly the case with reproduction. The whole thing gets started in the pituitary. This is the gland that will secrete, after it has been triggered, gonadotrophic hormones. Gonadotrophic hormones, very simply, are hormones that will stimulate the gonads. With birds we are very much at a loss in identifying the gonadotrophic hormones. For mammals it is fairly well established. There are two main ones—Follicle Stimulating Hormone, commonly called FSH, and Luteinizing Hormone, commonly known as LH. These two hormones have been identified in mammals as the hormones that control the maturation of the gonads. We

have not yet been able to isolate two separate hormones from birds. In birds there may be one hormone that has both of these functions. But, suffice it to say, the anterior pituitary secretes gonadotrophic hormones which are released into the blood stream and circulated through the body to the gonads.

FSH and LH (or whatever they might be in birds) have two separate functions. FSH in the female causes development of the ovaries. It causes the ovaries to enlarge, to become mature, preparing them for ovulation. In the male, FSH stimulates the development of testes. Birds are cyclic in their gonadal development. During the winter the gonads regress; during the breeding season they enlarge. The enlargement of avian gonads is in direct response to these gonadotrophic hormones. LH, the other gonadetrophic hormone that we know exists in vertebrates, has the function in the female of causing ovulation. Once the gonad has matured, a spike or sudden spurt of LH from the anterior pituitary causes the egg to be ovulated, to leave the ovary, and pass into the oviduct where it will be developed into an egg and laid. In the male, LH does not peak quite the same way it does in the female, or we do not think it does. LH probably increases along with FSH and this is also responsible for the development of secretory products in the gonads.

The gonads not only produce gametes, sperm and eggs, but they are also important in that they secrete sex steroids, the sex hormones, as well. These play a large role in the development of secondary sexual characters, such as the comb in a male chicken. They have a tremendous role in controlling male and female behavior. The way the gonads elicit this is by secreting the sex steroids into the blood stream. We are talking about estrogens. The estrogen that is most active in birds is estradiol. In males we are talking about androgens produced by the testes. The main androgen produced by the bird's testes is testosterone. When released from the gonads into the blood stream, the sex steroids circulate and cause appropriate changes that will prime what you might think of as the accessory breeding organs. It will cause, for instance, the development of the sperm duct that will eventually carry the semen from the testes to the outside. In the female it causes the development of the oviduct, the structure which is going to be secreting the albumen and the eggshell around the egg. These structures are also cyclic, decreasing in size during the winter and increasing in size during the reproductive season. The increase is in response to steroid hormones secreted by either the testes or the ovary. Also, estradiol and testosterone control reproductive behavior. They do this once again by going back through the bloodstream to the brain, to the hypothalamus. At this point, the information on the circulating levels of these hormones causes appropriate behavior. As the testosterone levels in the blood increase, a male bird is stimulated, for instance, to defend his territory or to go through courtship display; a female responds by building nests and all the other courtship displays that are associated with the breeding cycle.

That is basically the cycle of hormones that we are talking about. It is very important that we realize that this is a cycle of hormones. All of these are interrelated; think of it as a circular thing. We are dealing with a situation known as

a negative feedback mechanism—something like a thermostatic control. Let's go back over the system and show how this negative feedback system works. Early on in the breeding season, when the external stimuli become sufficient to cause secretion of the gonadotrophic hormones, the hypothalamus is also monitoring the level of the steroid hormones from the gonads. I am going to be a little anthropomorphic here, but just for convenience suppose the hypothalamus is not detecting very much estradiol or testosterone. The gonads are small; they need the gonadotrophic hormones. The hypothalamus secretes a lot of gonadotrophic hormones to cause development of the gonads. As the gonads increase in size in response to this, they start secreting steroid hormones, either testosterone or estradiol. The blood levels increase. The hypothalamus reads this and says, "There is a high level of steroid hormones; therefore it is time to shut off or decrease the gonadotrophic level." So you can see how this is a thermostatically controlled mechanism. They balance each other out, very critically. This has important implications which I will talk about in a few minutes.

The other thing that is important about hormones and their control of reproduction in birds is that the timing of these hormones is very, very critical. The hormones have to come into play at exactly the right time to cause a synchronous series of events to occur. As I mentioned before, you have to have a gradual build-up of gonadotrophic hormones to gradually build up the gonads. Once the gonads are mature, you need a big spark, or peak, of the hormones that will cause ovulation, the release of the egg. You also have to have appropriate changes in the gonadal hormones, the steroids. These have to build up to a certain level. The intensity of the behavior that they are going to elicit is, of course, a direct function of how much of this hormone is circulated. Behavior, as you know, starts out slowly early in spring and builds up to a peak. It is probably at this peak time when reproductive behavior is peaking. We are talking about copulation and fertilization and the actual events that mean success or failure of breeding.

Now, where might this system break down in a captive bird? There are several places. The first one involves the external stimuli. When you put a bird into captivity, you are obviously depriving it of many of the external stimuli he would normally be getting in the wild. As of yet, no one has cataloged the complete array of external stimuli necessary for a Peregrine Falcon to be successfully stimulated to reproduce. I think we can safely say that photoperiod, the increasing of the day length, is very important. Also, probably, courtship, the interplay between the mates, is also a very important stimulus. Probably, having an adequate nest site is very important. Outside of these very obvious ones, we just do not know. This is where the items discussed earlier on the basic requirements for a successful breeding chamber come into play. It very well could be that some things associated with the deprivation of stimuli in captivity are blocking the whole system right at the start. Another possible place where you could get into problems is in the hypothalamus. It could be that the birds are picking up other stimuli from the captive situation that are blocking the hypothalamus from triggering production of gonadotrophic hormones. Certainly you

know that there are many things that we do with birds in captivity that are unusual in terms of stimuli. At the level of the gonads things can also go wrong. It is very possible that the gonads may not be producing enough of the steroid hormones, the sex hormones, to elicit proper behavior. When we get to the level of the gonads, we can go to an endocrinologist who is very versed in these matters and ask, "We have a bird in captivity; we have done everything we can to make conditions ideal for it, but it will not breed; what is wrong?" First thing he is going to ask is, "What were the conditions of the gonads after you gave this bird all of these sufficient stimuli—or what you thought was sufficient?" This is one place where we are sadly lacking. We have had many failures breeding Peregrines, and I would venture to say that we probably do not even have one or two instances where we knew what conditions of the gonads accompanied the failures. Were these gonads enlarged? Were they anywhere near functional? This is a critical question. If we find, for instance, that they are not enlarged—they are remaining very small and undeveloped—then we can say the problem is with the hypothalamus. If they are enlarged and we are still not getting appropriate reproduction, then we might think of looking at the sex hormones. Is there production of those to elicit the behavior that must accompany reproduction?

These are two points where we might consider helping birds out. We have two groups of hormones at our disposal, the gonadotrophic hormones and the sex hormones. I would first of all, as a physiologist, caution anyone from using these hormones in a bird for which you have not examined the gonads. Remember, this is a negative feedback system. You must know what the condition of the gonads is in your bird that is not breeding. You may well screw up the bird even more by giving it an inappropriate hormone. For instance, let's say that your bird is not doing anything. You say, "Well, I'll give the male a shot of testosterone; that ought to make him reproductively inclined." In fact, it will. If you castrate a bird and give it supplemental injections of testosterone, it will go through all of the appropriate reproductive behaviors, including mounting and copulation, but, of course, if the testes are removed, it has no sperm to contribute. So, let's say you go ahead and try injecting testosterone; let's say that was not the problem; let's say that the bird actually did not have gonads that were enlarged. What you have done then is add testosterone. It goes back to the hypothalamus, and the hypothalamus says, "Oh, here is all this testosterone," and cuts off all the gonadotrophic hormones. What you have done, if anything, is to decrease the size of your bird's gonads, because you have knocked out the gonad stimulating hormone. Let's say you do it the other way and make a mistake; you inject gonadotrophic hormones, when, in fact, the gonads are fully developed. What you are going to do, then, is harm the cycle of the testes or the ovaries; the cycle has to build up gradually. What I would caution is that before you attempt any kind of wholesale use of these hormones on the bird, you are going to have to know what the condition of the gonads is in the bird that did not breed for you.

Now, the other thing that makes manipulation with these hormones very

difficult is that for no species of birds, including chickens, do we have any information on what the normal circulating levels of these hormones are. We have ballpark estimates, but we have no information at all on what the level is, for instance, during the peak in the breeding season. We have no idea what the concentrations of sex hormones are in the blood of even a breeding chicken. As I said, these hormones are very precisely involved in this cycle. If you are going to attempt to induce breeding in a bird by injecting exogenous hormones, you are going to have to be very sure that you are going to be able to inject these hormones in the proper sequence, at the proper concentrations, at the proper time. Otherwise, you should not expect to get sure-fire results. There have been reports in some of the literature of people who have stimulated certain birds. In fact, the only successes that I know of that have been published, have been achieved with small finches. They have injected sometimes artificial gonadotrophic hormones, sometimes naturally occurring mammalian hormones. These successes are not really adequate to say that it was the injection of hormones that ultimately triggered the birds to lay eggs. The birds grew up under conditions that were definitely stimulatory to gonadal development, and it was not a very well controlled experiment. What I am trying to say is that the researchers did not show that, in fact, these hormones were the responsible agent.

One very interesting report that I should mention was carried out on Pintails. If you happen to be a waterfowl breeder, Pintails are your Peregrine Falcons. They are very difficult to breed in captivity; why, they do not know. A very well designed experiment was carried out at the Delta Waterfowl Research Station in the late 50's on this problem. The fellow who carried out this study had huge sample sizes of Pintails to work with. He could experiment; he had room to play around, which we do not have with Peregrine Falcons, obviously. He tried every trick in the book; he tried giving them increased photoperiod; he tried varying all the external stimuli he could possibly vary. He still could not get full gonadal development. Gonads would develop to a sub-optimal level, taper off and decrease. He then decided to try the hormones. He looked at the gonads. He knew that the gonads did not reach full condition, so he thought, "Well we'll try gonadotrophic hormones to see if I can beef up the hormone titers and stimulate the gonads to become fully mature." He tried a wide variety of doses on many birds. He tried FSH alone, LH alone and combinations of the two. He tried another hormone which is very difficult to get hold of. He also tried a hormone called pregnant mare's serum. The serum from a pregnant horse has a hormone in it that, as of yet, is unidentified. This hormone possesses the dual qualities of FSH and LH. It is sort of a hybrid form. You can get this very easily from the blood of a pregnant horse. He tried giving this because it's readily available. He gave it in varying doses—up to massive doses—in a complete range and, even with this, he was not able to get the gonads to come into full condition. This is probably because we have no knowledge as to how this hormone should be injected—what the cycle of it should be. He went point blank injecting, and did not get results.

Now, I am not saying that this approach is not going to work; what I am say-

ing is, if you apply the artificial exogenous hormones to a bird, you are going to have to be very careful what you are doing because you may ruin the bird. I do not want to sound pessimistic, because I know Dr. Awender and Tim Lawson have both done some experiments with exogenous hormones that indicate that perhaps there is as yet some response in birds of prey to these hormones. However, just to caution you, realize that in these experiments they were not controlled to show that it was actually the injection of the hormones that was causing the development of the gonads. I hope somebody in this room can pick up the obvious project and look at it with a common species like the Kestrel. If you get a bird that will not breed in captivity, laparotomize it, look at the gonads, and then work from there. That is the first experiment that has to be done.

AWENDER. I will be brief with my own project. I have a very small sample to work with and I am not willing to take any chances to harm the birds, kill them or operate on them; that is the main reason I shied away from injections to begin with. I have ruled out injections. Injectable stuff is very hard to come by, it is expensive, and we do not know for sure whether it would have any significance for birds. Most of the laboratory work that has been done so far involves laboratory animals, usually a rat, mouse or guinea pig, very occasionally a chicken and, once, maybe a pigeon. But the bulk of everything has been done on mammals and has been applied mostly in human medicine with variable results. I do not think many, if any, groups, as such, have applied something therapeutic to birds. It was always on the scientific level. We do not care too much if a chicken does not lay eggs; we kill it and eat it. But with falcons it is a little different. So I have ruled out shots and went to tablet .

My experimental pair, just to tell you what I was working with, are passage-tundra falcons. The tiercel is going on four; the falcon is going on nine years of age. They were ordinary passage falcons. They were trapped at the usual time at the usual place. The female was flown in falconry for about five years, the tiercel for one and a half years. From then on I put them in this project and they have been together since, with one interruption. The tiercel was gone last year for a short time. There is not too much in the line of non-injectable things to stimulate the pituitary. As I said before, I am drawing only from what I can deduce from human medicine, and I have had very little, if any, contact with veterinarians. I do not have the foggiest idea where the gonads of my falcons are. Are they big, little, or indifferent; are they even existent? The falcons were acting in a very neutral way. If the size would not have betrayed the tiercel, I would not have even known which sex they were.

So, at any rate, I thought I would start at the top, from the pituitary. I went ahead last year, in 1970, and put the two tundra falcons in their room where they were going to stay, and exposed them to light, beginning about the first of March. They had light for about a month—about 20 hours a day. It amounted to roughly 500 watts plus daylight. After the first month I put them on 24-hour daylight, figuring if a little is good, a lot might be better. This is a common

thing applied by lay people. This did not do much; there was no change in behavior at all; they were very passive to me and to each other, and stayed out of each other's way. They never perched together. Then I heard about a drug which was producing quintuplets, sextuplets and even eight babies in humans and I thought, great. The more eggs it produced in a falcon, the better. It may kill a woman, but not a falcon: they lay one at a time. This drug is called clomiphene-citrate or Clomid for short. The trade name is Clomid. It is a synthetic thing, comes in tablet form, 50 mg per tablet, and women take it for only five days. Then they are supposed to ovulate, and some of them really do. They go overboard and eventually they abort; they cannot carry them to term—not enough room. So I thought this was ideal for falcons. I figured that if a woman takes 50 mg a day for five days, a falcon needs 0.75 mg per day on a commensurate per weight basis. I could not make it; it was so little. I had to go to the pharmacy and they had trouble weighing out that little for me, but we thought we came close to 0.75 mg. I put it in a little capsule, put it in a piece of meat and gave it to the falcon for five days, figuring to do it just like for the ladies. Nothing happened; absolutely nothing happened. The falcon started her molt a little bit late. That was a pretty complete washout. In order to be good to the tiercel, too—I did not want to inject him—there is testosterone which is fairly effective by mouth if a methyl group is attached to it; it is then methyl-testosterone. In human medicine we use it in doses of 10 mg per tablet and usually one, two or three tablets will do. I gave the tiercel a human dose. It did not kill him; it did not do anything. Nothing! It may have inhibited, as we may conclude from Temple's presentation. At any rate, he had the testosterone for the same time the falcon had her drug. I stopped the experiment and that was that for 1970.

This year I thought, well, let's go one better and prime the falcon with estrogenic hormone. It did not work from the top, so I started from the bottom and gave her something by mouth. The ordinary estrogenic substance is not effective by mouth so I went to stylbestrol, diethyl stylbestrol to be precise, and that comes in my office in half mg tablets. I primed this falcon with diethyl stylbestrol, half mg per day, given by mouth. I started on May 9 and gave it to her until May 28, roughly three weeks. That did not do much at first, but towards the end of that period the falcon became just a little restless. I could not explain on what basis and I did not know if the drug had anything to do with it. She was peaceful usually, but then she sat on the highest perch and beat her wings like crazy, exercising many hours a day. I am not aware of what this meant, but otherwise she left the tiercel alone. It was the same falcon on the same photoperiod. Then, on May 29, I stopped abruptly the diethyl stylbestrol and switched over to the Clomid that I gave her the year before. Again, I gave daily doses, but I really went up high, probably too high because I figured I did not have enough time. I gave her 12½ mg; this is a quarter of a tablet. That is an enormous dose on a weight basis. She took it from May 29 to June 9 and that made a significant behavioral change. She became extremely broody, which she had never done before. She went into her brooding corner and made a

scrape there. She became extremely passive. I could touch her on the back and she did not get up. She acted like Mr. Nelson mentions several times—she acted sick—but she stayed in good physical health. She ate and I could practically feed her like a little eyass falcon; she was that tame and that quiet. I palpated her abdomen to see if she would enlarge things. This drug is supposed to induce ovulation or, at least, get the follicles to mature more. I could not palpate any increase in her abdomen. Laparotomy was out of the question.

I stopped this experiment at that time. After about a month or so she changed her behavior again and this time with a vengeance. She became extremely aggressive to me and to the tiercel. This persists to date. The tiercel has to retreat many times a day. She has crabbed with him several times. My room is 10 feet wide, 15 feet long and the highest point is about 12 feet. If she was nimble enough she could really hurt him; nothing has happened so far, but she has remained, for about a month after I stopped those drugs, extremely aggressive and downright mean to the tiercel. The tiercel received no medication this year. I have just left him alone. He behaved passively, as usual, and now he is constantly a fugitive. The female again postponed her molt even more. She started extremely late. She started about the first of September. Now, of course, she is almost finished; she molted extremely rapidly.

There is not much else I can add to this particular pair. The Clomid is something that has been used in humans successfully, as I said. In the ordinary laboratory animals, and as far as I know definitely on mice and laboratory rats, it did inhibit. It inhibited follicle maturation and ovulation. I thought, perhaps, if it works in people, it may not work in lower mammals. As you can see, with falcons nothing happened. I do not know why, but I think if somebody had more time and wanted to titrate the dosages a little more accurately, perhaps more could come of it. It has one advantage; it can be given by mouth. Most of the other hormones we are talking about here can only be injected and again we just never know where we stand with the dosage. Too much is not good; too little is not good; and whether we start on the top or on the bottom we may antagonize the other part.

I have tried other pairs, but they have not been receiving any hormone treatment. One is a pair of Peale's. I think the passage tundras needed it most. That is why I picked this pair for experimentation. Passage tundras are known to be hard to get into any kind of breeding condition, much less to lay fertile eggs. The others will probably do it naturally. I have a pair of Peale's and a pair of Prairies. The Prairie Falcons have been stimulated with light only and I think they did not do it on a natural basis because the tiercel was too young. The tiercel is a home-bred bird that Henry Kendall bred three years ago. He is one of the surviving tiercels. He has been mated with a falcon that was then three years older. Now he is three; she is six. She has laid eggs on photophasing. I turned the light on one year on December first and left it on day and night. Six weeks later, January 16 she started to lay eggs at the usual two-day interval. She laid a clutch of four. I took the eggs away and put them in an incubator; they were not fertile. I gave her two banty chicken eggs. She alone incubated them well

enough to start them growing. I could see the embryo two weeks later. The tiercel was totally passive. This year the same thing happened, except I started the light later–on March first. The eggs were laid while we were gone skiing at the end of March. When we came back, she had a clutch of four again and the tiercel helped incubate. But, again, they were not fertile. They behaved very well. The tiercel was very aggressive; the falcon was not. She was calm and broody, so I did not disturb the eggs for 32 days. Then I realized they were no good and it was too late to recycle. The moral of this story is: no matter how good it looks, candle on the ninth or tenth day.

The third pair are the Peale's and there is not much to say about those because that pair I will leave alone for natural breeding attempts, not even any light stimulation, because I think they have the potential to do it on their own. The tiercel is three; the female is six. They are eyasses. The female has raised foster babies of her own species in British Columbia. This year, just for the heck of it, I gave her a two week old Prairie Falcon. She immediately adopted it and fed it, but the tiercel did not participate in the feeding. She is a good foster mother. She is of the right age. The tiercel has been broody for the last two years even though he is young, so I hope that next year, just by leaving them alone and giving them good food, they might do it the natural way. It seems like the natural way may prevail over the injectable way or tablet drug culture way, but time will tell. We are just at the beginning.

LAWSON. I think first of all the key in the chart from Stan Temple's presentation is the very first block, the external stimuli. Unfortunately it is not always practical to create rock cliffs and Colville River in your breeding room and I think that most people will agree now, as we have talked about earlier, that the birds seem to realize where they have come from, where they were born, and they seem to always do better in a familiar environment. After they get there, the external stimuli that both initiate this process and keep it going after it is initiated are vital. For example, if you take Kestrels and put them in a breeding room and do not supply them with a nest box, you are wasting your time. They just will not do anything. The nest hole seems to be essential to success. When you take these birds out of their normal habitat, you are confusing them, completely. What we try to do with the gonadotrophic hormones, at least my rationale is, that we are trying to bypass the external stimulus needed to initiate breeding. I have been criticized, and very rightly so, by Stan Temple and John Snelling in that my experiments are not controlled so that I can say 100% that my hormones are doing the job. I am giving them hormones, increased light, warmer temperatures, and total isolation so that the limited success I claim could be any of these or all of them together. I go along with Dr. Halliwell; I do not care if I have to hang an Indian medicine bag in there to get them to lay eggs; I will do anything and this is the basis for my using these hormones. My other big misunderstanding with all breeding projects is that everybody seems to feel that these birds are not affected by human presence. Dr. Meng is living proof that I am completely wrong on this, but I am going to say it anyway.

Adrenalin, epinephrin, is proven to be directly antagonistic to follicle stimulating hormone. When you walk into the room to feed the birds and they jump around being really ill-at-ease, you are destroying everything that you have tried to build up in these birds. It has been shown that adrenalin is directly antagonistic to follicle stimulating hormones. Something else that Stan said was that if you are going to try these hormones, you must inject at the proper times and in the proper amount. I will go along with proper time, but I do not think the amounts are that critical. I do not think you are going to kill them with pregnant mare's serum. When I did this with Kestrels I gave these four-ounce birds one cc of pregnant mare's serum each day, injected intermuscularly, for five days running. That is about half of the dose you would give a 50-pound dog. So I am really whopping it to them. Dr. Awender seems to correlate that you have to increase the dosage far above what you would calculate for the weight of the bird. You have to do it at the proper time or you are defeating the bird's natural mechanism. If the gonads are beginning to develop and you give them follicle stimulating hormone or testosterone, the chances of success are nil. But I just do not believe that the amount that we are giving is that critical. I started off with Kestrels and we did perform a laparotomy on these birds to check the gonads before we started. The birds were captured about 35 miles apart on purpose to preclude their being a naturally mated pair, and we did this in mid-December. We gave the birds five injections and then left them alone, trying to initiate a response with hormones, and then let nature take its course. We let increased photoperiod and lack of disturbance take over. We have done this with Kestrels about five times, with Red-tails twice. With the Kestrels we got copulation in 11 days from capture and eggs laid in 33 days. With the Red-tails we never did see copulation, but we got fertile eggs 47 days from capture.

STODDART. When did you give them more light?

LAWSON. Immediately, 16 hours of daylight.

STODDART. You gave them injections of hormones?

LAWSON. We gave them one injection a day for five days. Before we even put them in the breeding room, we gave them the first injection.

VOICE. The tiercel as well as the falcon?

LAWSON. Both.

MARCUS. Tim, when you gave five shots did you keep them jessed up for five days and catch them?

LAWSON. No sir. These were wild birds; they were not manned. They were kept behind a one-way glass all the time with no external disturbance whatso-

ever as far as sight is concerned, just the noise that normally occurred.

MARCUS. How did you give them shots for five days?

LAWSON. Catch them at night. They never saw us.

STODDART. Do you think that causes adrenalin production, i.e. handling them at night?

LAWSON. Not nearly like going into the room would cause. It takes two minutes to do this.

HUNTER. In conversation with you before, we discussed the site of injection with Peregrines.

LAWSON. We were giving these birds 6 cc a day which is a whopping dose for the breast muscle of a Peregrine, so we went to the subcutaneous tissue behind the neck where you are going to get equal absorption. I am sure that you are going to get the same results.

SCHUBERT. You put it all in one area and it absorbed?

LAWSON. We did not have any trouble with soreness or any sign of discomfort whatsoever. In correspondence with Dr. Graham, I am sorry he is not here, he mentions that he had trouble with what he thought was a bad reaction to this foreign protein. Pregnant mare's serum is serum from a pregnant mare, just like it says, and it is a foreign protein. I think everybody is familiar with serum sickness or has heard about it. It has been shown that you can take a guinea pig and give it a shot of pregnant mare's serum, wait ten days and give it another shot and it will die in 30 seconds right before your eyes. But we have had absolutely no problems with this in our birds.

STODDART. Where were the Peregrines from that were used?

LAWSON. Don Hunter's Peregrines.

STODDART. Were they tundras or Peale's?

LAWSON. They were tundras.

HUNTER. Maybe you ought to explain what the Peregrines did.

LAWSON. I'm sorry you asked that. The first pair of birds Don sent me, the female had been in captivity I don't know how long, but she was pinioned on one side.

HUNTER. She had a bad wing.

LAWSON. The tiercel was fine. I wish I had my notes here with me, but I do not. I forget how many days it was exactly. It was around the normal time that we would expect to see some action. The female began to cluck and show amorous tendencies toward the tiercel and beg to be fed. She started to scrape and the tiercel would not run away from her. He would sit and eat within a few inches of her feet and a couple days later she died of what was diagnosed by the pathology department at Ohio State as gout. But, on autopsy, the follicles on her ovaries were about an inch in diameter, which is a lot bigger than they should have been if she was regressed.

MARCUS. Was the biggest one an inch in diameter?

LAWSON. That was the biggest one. And they were graded all the way down. Somebody asked me when that was. It was the 29th of February.

MARCUS. Have you ever done any repeats on this?

LAWSON. Just with the Kestrels. Just the five trials with Kestrels and twice with Red-tails. The second time with Red-tails was with the same pair. I gave the hormone without doing another laparotomy to see the stage of the gonads.

MARCUS. What do you mean by a laparotomy?

LAWSON. A laparotomy is just to make an incision and look at the gonads, physically, to see what size they are.

MARCUS. Is there any work similar to yours without exogenous introduction of hormones?

LAWSON. Cade and Willoughby of Cornell did this with Kestrels. We patterned our experiment after them almost totally with the exception of the hormones. They got an average of 54 days with variants of 52 and 61 days before eggs were laid by their Kestrels. That is the sole basis on which I base my decision that maybe these hormones had something to do with getting the first egg in 33 days in our experiments. I have no controlled experiments using eight hours of daylight and hormones to prove that it was my hormones that did it exclusively.

TEMPLE. Tim, were your birds maintained in a heated building?

LAWSON. The temperature in the building was about 40 degrees.

TEMPLE. Temperature definitely is shown to affect the rate of development

of gonads. The pilot experiments with American Kestrels that Cade and Willoughby did, were done in January outside in Syracuse, New York, which was very cold. This might explain the time difference.

LAWSON. Absolutely.

SNELLING. Tim, could you tell us what happened after the Red-tail's eggs were known to be fertile?

LAWSON. They were put in an incubator and the incubator went wild at night and killed all three of them. What we should have done, hindsight is always better, was just leave them alone, and let them recycle on their own, but we panicked and initiated new injections. I am sure we fouled them up.

HALLIWELL. I was unfamiliar, or did not catch exactly what you did with these Peregrines. You injected 6 cc in one massive dose, subcutaneously, behind the neck—one dose and then sat back to see what would happen.

LAWSON. Not one dose, five doses.

HALLIWELL. Six cc for five days?

LAWSON. Right.

HALLIWELL. Whereas in your Kestrels and in your Red-tails you gave one cc per day for five days. All of the injections were subcutaneous, not intermuscular?

LAWSON. No, the Kestrels' doses were intermuscular, the Red-tails' were intermuscular, and the Peregrines started out to be intermuscular for the first two days, and then we got to being afraid that maybe we were going to cause some kind of myasitis by giving this in the breast muscle, so we went to the skin behind the neck, which is voluminous. You could inject probably 10 or 15 cc back there and still have a little room. It is a much better place.

SHULTZ. If you had success with one cc in the Red-tails, why would you use a six times larger dose with Peregrines?

LAWSON. The Red-tails were given three cc for five days. We took that figure right out of the air; no basis behind it whatsoever, other than it was a massive dose for a bird of that weight. We had previous luck with a massive dose in Kestrels, so we just took three cc and then we went to six in the Peregrines after we failed to get results the second time in the Red-tails.

SHERROD. Did you use five doses of six cc each, or five doses of three cc on

the Peregrines?

LAWSON. We gave five daily doses of six cc per bird.

PLATT. In your opinion, what do you think happened to the female Peregrine? It was not gout.

LAWSON. I do not know. I found her in trouble in the morning at 10:00 and at noon she was dead.

STODDART. Would follicle size have any effect on it? Were they not particularly large follicles?

LAWSON. Oh no, that would not make her die, if that is what you mean. That was the response I was trying to get—increased follicle size.

STODDART. Was that a normal-sized mature follicle?

LAWSON. Not quite; it was growing; it had to grow half again that big before it would be ovulated.

STODDART. I thought you meant that it was overly large.

LAWSON. No.

STODDART. Now what?

LAWSON. Now what? I came in the Army in July and I have been on TDY since then and have not had enough time to get back to it, but I am going to.

STODDART. This was done on a Ph.D. at the University?

LAWSON. No, this was done on my own time while I was attending veterinary school, but not in association with any university. I used some university facilities because I begged for them, but that is the only connection.

STODDART. Is anyone continuing the work there?

LAWSON. Nobody is there now to do it, no.

MENG. Just one comment. You mentioned before the adrenalin that my Peregrines secreted when they were frightened. Actually they were not frightened. I did not subject them to fright actually; I did not go in there and scare them because I feel that is very bad; that just fouls everything up. I approached the cage from the outside, put food there in a very cautious manner and then

he started defending his territory. I cowered away and just took off, but it was not to the point of his getting frightened. He did not secrete that much adrenalin. If I went in the enclosure he would get frightened and that would probably have defeated the whole purpose. It was just like in the wild where they defend their territory a little bit, but they were not frightened to the point where he went the other way.

TEMPLE. I might add—Tim has seen the paper—the paper that proves that adrenalin will block reproduction only if given in unphysiological or massive doses. The doses were given attempting to use it as a birth control technique in mammals. So, probably, the adrenalin secretion that you would cause by getting a bird to defend is probably insignificant. I want to propose something to you to see what the general opinion is. I am doing my Ph.D. work on just this problem. I am measuring all four of these hormones, FSH, LH, estrogen and testosterone in Starlings because I can get hold of lots of Starlings around here and I have the facilities at hand to measure the amount of these hormones in the blood. How many of you—just to see whether it would at all be workable—how many of you who next spring fail in a breeding effort, would be willing to allow me to take two cc of blood from your bird and analyze it for these sex steroids? All I need is about two cc of blood and I can do an analysis. For Peregrines that is nothing; they can take that loss of blood very easily. It does not even have to be Peregrines; it can be other species. If I could measure the sex steroid levels in the blood, I could probably say whether the bird does in fact have gonads that are functioning normally.

STODDART. What about the females that are laying eggs?

TEMPLE. I would not want to touch it; that is why I said an unsuccessful attempt.

STODDART. What if the eggs are infertile?

TEMPLE. Okay, then.

STODDART. If you candle those eggs on the ninth day, let's say, and you analyze the blood that day, what are the chances of establishing good baseline levels for that point in the breeding cycle?

TEMPLE. What it would do is give us a standard and I could then compare. I could say, "Okay, this is what her sex steroid level is; let's take a sample from a bird that does not lay eggs; what is her sex steroid level?" If it is lower, then you would definitely say that her ovaries are not developing; she is not producing sufficient estrogen to supply the vesicle.

HUNTER. If you can work this technique out first, you can do it with one

drop if you want to. Could you check the phospholipid levels? There is a correlation, too, between the phospholipid, apparently, and the state of gametogenesis.

TEMPLE. Right. This is especially true of the male. Phospholipids are naturally occurring fatty types of chemicals. They are precursors for estrogen and testosterone. They are involved in the production of these hormones.

SMYLIE. What would be the requirements for getting the blood to you?

TEMPLE. Physically what you have to do is collect the blood and immediately centrifuge it to separate the plasma portion of the blood. You have to have a doctor do this who has a centrifuge. It is very easy to do. You freeze the plasma immediately.

AWENDER. I would like to give a chance to our other panel member, Dr. McIntyre, to present his case and then we will have a little more time for questions and answers. I know this topic has a lot more questions than answers, but let's get to one more panelist, please.

McINTYRE. I really do not have very much, because we have not fooled much with hormones. I am going to throw out something here that perhaps some of you know and some do not. When I first got into this business, Dr. Berthrong, a pathologist at Penrose in Colorado Springs, had done a lot of postmortems on falcons. He asked us to look at the adrenals every time we posted a bird. He thought the adrenals were much smaller in captive birds than they were in the wild. We tend, also, to believe this. What effect do small adrenals have on the stimulation of sex hormones, ACTH, corticosteroids, and so forth, I really do not know .but some of you experts may, perhaps.

TEMPLE. On this point, the one naturally occurring stress hormone, a hormone that is produced when an animal is under stress, is corticosterone produced by the cortex of the adrenal. This is known to inhibit production of gonadotropin. As its name implies, corticosterone is a steroid. Structurally it is very similar to the sex steroids. This circulates in the blood. When a bird is stressed, the titers are high, it goes to the hypothalamus. The hypothalamus is sometimes (pardon me for being anthropomorphic) is sometimes sloppy in reading the message. Many different types of steroids will be identified by the hypothalamus. It is shown that if you inject corticosterone in physiological doses into a breeding bird, you knock out the negative feedback system because the hypothalamus interprets this steroid coming from the adrenal as a sex steroid. This is another thing we are going to do at Cornell. We have Red-tails that breed perfectly normally—that are at ease, and we have others that do not. We are going to take blood samples from them to measure the corticosteroid level and see if there is a contrasting difference. This will be done next year.

EBERLY. Is there any variation at all in the various brand names of pregnant mare's serum?

LAWSON. Let's see, the one I was using is called Gonadin. I can not remember who makes it.

VOICE. Upjohn.

LAWSON. I think Donomone is another one.

AWENDER. I think Entruitrin-S might be another one.

HUNTER. If you have any more questions, you can direct them to some of the other people here for just a minute. We are going to proceed next with the artificial insemination part of the program during which there will be some demonstrations. Immediately afterward we will take a break and there will be some slides outside which you can look at, hopefully some live sperm under the microscope.

MATTINGLY. In your presentation, Dr. Awender, at a certain stage you said that laparotomy was out of the question. Might I ask you why this was?

AWENDER. Yes, I simply did not want to hurt them. I could do it easily. I cut and sew in the morning, every day in the morning; I could do it at home in the afternoon, too. But I do not like to do it without anesthesia. I know birds can stand it. I have operated on them when it was necessary. I would much rather do an open reduction and internal fixation on a broken bone of a hawk without anesthesia or very little anesthesia. They can take pain better than anesthesia, I think. I thought this was going to throw them too far off to grab them; to make a little incision there, then look, and then sew them up again. I thought it would not be the right thing for me to do with one sample, with one pair. If you have a bigger sample to work with, then it becomes statistically important. Here I know my explanation would have been she did not lay eggs because I cut her.

MATTINGLY. The question is being mumbled around back here. I think we saw the same thing when Stan asked about taking blood. I think we are a little too over-protective about our birds. Actually, do you hurt a bird when you laparotomize it?

AWENDER. I think they have some pain and, probably, it involves enough handling that if you do it with or without an anesthetic it may have far reaching effects the next day or two days later. We simply do not know. I favor experimentation with dispensable birds. Take buteos; take Kestrels if you must. I was not going to use my Peregrines. That's all.

MATTINGLY. But, we are not getting any information.

AWENDER. I admit this is quite a shortcoming. I was not about to do it. I am not that scientific.

MATTINGLY. By the way I have been working out dosages for a new very effective anesthetic called Ketelar or Ketamine.

AWENDER. Ketamine. Yes, we use it.

MATTINGLY. Very, very good. I have no worries about it. You can double the doses.

AWENDER. Yes, it splits the mind. I have not tried it, but I know people who do and it's OK. On people it works fine. You hurt them and they do not know it.

HALLIWELL. I think concerning anesthesia in these birds we have also used Ketamine and have had very good success with it in all ranges and sizes of birds from Golden Eagles all the way down to several Kestrels. We feel we have had better success using the inhalation anesthetics of which you are familiar. There are probably two on the market today: methoxy fluorane which we have not had as good a success with as we have had with the also halogenated ether called Halathane. And here, again, with Halathane we have done an extensive amount of work, although I will admit this has been done in a veterinary clinic where we are able to monitor blood pressure, heart rate, EKG, and so on. Because we have had excellent success with it, I feel reasonably competent to use it in a field situation. We have done over two dozen Kestrels, without a death. I pinned a wing on a baby owl approximately six months ago; we have also used it on the larger birds. I think very definitely that it would be worth using and I would be willing to talk to anyone who would like to undertake this sort of project. I think with either of these drugs the risk is not great at all used under judicious care by somebody who has some reasonable amount of experience.

SNELLING. With respect to laparotomy I must admit that I feel the same sort of twinges of pain when I think about laparotomizing a Peregrine. However, there has been some extensive laparotomy done on haggard Red-tails by a man who could not come today. He is a graduate student at Cornell now. And he did a similar study with haggard Red-tails injecting levels of PMS into them several years ago and he laparotomized these birds, I think, two or three times a week, completely putting them under with anesthesia every time he did it. His sample size was approximately 25 to 30 haggard Red-tails. He never experienced any difficulty. So, judging from the experience of poultry people who will tell you that a laparotomy is nothing, I really think that the risk is pretty minimal. Perhaps some of us should begin to think about this technique.

TEMPLE. I might add that when we do laparotomies on poultry or pigeons, for instance, we do not even use anesthesia. The incision that you have to make is in the very thin wall of the rib cage. It is not an area where the animal is going to experience a large amount of bleeding as long as it is done correctly. It is a very simple thing, but it takes experience.

AWENDER. I think we need a lot of help; more work needs to be done. What the Cornell people are doing is wonderful, and I would suggest that they go into it with all their might and do a lot of experimenting. I expressed here before that I was unwilling or emotionally unable (or name it what you want) to experiment with the birds in my possession, and I think a lot of small breeders may feel this way. We are too close to the birds. I think an impersonal approach is needed. One thing that was not mentioned here in endocrinology is this—we have been working on material that has been derived from veterinary sources or from human medicine. It is possible that there is a species specificity of some of these things. FSH as we know it may not work in birds and the only analogy I can draw is this: about 15 years ago or so I was dreaming; I thought in those days I would never have a Gyrfalcon in my life so I was going to make a Prairie Falcon grow bigger, and I was going to give it growth hormone. I could not get any, so the closest thing to growth hormone was a human pituitary extract closely resembling the pregnant mare's serum, a thing called Entuitrin-S. This was supposed to be contaminated with growth hormone and I thought I would utilize the contamination and I shot this Prairie. I got this eyass as small as I could; it still was about a ten day old chick, but I thought the skeletal growth was still backward enough. I gave it daily injections of Entuitrin-S and it developed into a beautiful, normal, lovely Prairie, no bigger, no smaller. And then I contacted Dr. Riddle who had done a lot of research in the early 1930's with prolactin; this hormone was not mentioned here. It is a very definite avian thing. It does not have much practical value and so we thought prolactin might be a growth stimulating factor or the growth hormone for birds. It just goes to show that what may be growth hormone for mammals may not be the same thing at all for birds, and that goes for all other hormones. Some of them are definitely showing effects; some of the others are not; I think this making extract from pituitaries of birds will become a major project. It has been suggested to me to hire some high school kids and let them take the things out of chicken hatcheries and so forth, but the little fellows cannot do it. I would obviously appreciate some help from Cornell, and I would gladly inject some stuff and watch one or two birds. But, I cannot make the stuff and I do not think most amateurs can.

HUNTER. I looked into this avian derivative of Follicle Stimulating Hormone several years ago and I ran into a brick wall.

AWENDER. Right.

TEMPLE. We do at Cornell. I might say that to get usable amounts you go through tons of chicken heads.

HUNTER. Yes, that is right. I had a serum company tell me they would make it for me, but I hate to tell you what they said it would cost.

SHULTZ. Stan Temple has mentioned that if we would send him two cc of blood from our birds that he could run some analyses, and I think this would be extremely useful and extremely helpful. From this group, I am sure that we could get enough samples to make it significant. Would you repeat this for the benefit of the people who were not listening?

TEMPLE. I think what I will do is publish a note in the next issue of *Raptor Research News* that is going to get to everyone, rather than right now.

VOICE. Do you only want falcons or do you want buteos and accipiters or what?

TEMPLE. I can assume the cost of some of these analyses, but every sample is going to cost me about $10 and about 16 hours of work to analyze it, so I would prefer to limit it to Peregrines for the time being because of the time and expense involved.

HUNTER. It certainly was my feeling during this reproductive endocrinology session and has been for quite a long time that this particular field does have some real possibilities, although I do not think that it is anything for, as Dr. Awender says, the little fellow. It needs to be done under controlled conditions and I am hoping that Dr. Lawson will find time to pursue his work a little further.