

SPECIAL EDITION
December 1985

Animal Keepers' Forum

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ANIMAL KEEPERS' FORUM, 635 Gage Blvd., Topeka, KS 66606.



Executive Editor: Alice Miser
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DECEMBER 1985
VOLUME TWELVE
NUMBER TWELVE

Animal Keepers' Forum (ISSN 0164-9531) is a monthly journal of the American Association of Zoo Keepers, Inc., 635 Gage Blvd., Topeka, KS 66606. Five dollars of each membership fee goes toward the annual publication costs of *Animal Keepers' Forum*. Second Class postage paid at Topeka, KS. Postmaster: Please send address changes to:

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METHODS OF DETERMINING ESTRUS
IN A WHITE RHINOCEROS
(*Ceratotherium simum*)

By
Kathleen Robbbaro, Animal Keeper
Pittsburgh Zoo, Pittsburgh, PA

In 1977, the Pittsburgh Zoo received a pair of 2½-year-old white rhinos. They were housed together in our old zoo building for six years. Upon the opening of our new African Savannah Exhibit in October of 1983, the two rhinos, named Deano and Kid, were moved to a more modern and more natural habitat. It was at this time that I became their keeper and considered some far-ranging plans for the pair. Deano and Kid have adapted well to their new surroundings. They are both well-behaved when being routinely handled by myself or the veterinary staff. Neither animal has had any serious illness or parasite problem. I had hoped to see signs of mating activity now that they were both eight years old. However, the male never showed any interest in the female or made any attempts at mounting her. During the summer of 1984, Dr. Wagner, our staff veterinarian, and I discussed the problem of our non-breeding rhinos and in the fall we decided to begin a lengthy work-up on her estrus cycle.

The reason behind studying and charting her cycle was because, like us, many zoos only have one pair of white rhinos. We know this is not likely to produce offspring. If we could find an alternative to housing a large number of animals in order to produce young, we could possibly have offspring in zoos like ours where only a pair is kept. After our study is more complete, we will try artificial insemination. Since there is not a great deal published about white rhino's estrus we feel our study will be useful in that respect alone.

Our zoo currently has a holding facility for two adults and possibly one immature or compatible animal. The two adults are kept separated at night to give each more room and to regulate food intake. There is a central area between the two cages that serves both as a transfer cage and an access to the outdoor exhibit. It is in this transfer cage that I could see housing for a possible third animal. In the future we may be able to expand our holding area but what we need now is a solution of sorts toward which to work. What our plan consists of is careful monitoring of the female's estrus cycle and eventually an attempt at artificial insemination during peak receptivity.

We have four methods of determining what stage of estrus our female is in. They are behavior, vaginal smears, rectal palpation, and urinalysis. The first we will discuss is behavior. This is quite dependent upon keeper observation. As a heat approaches (proestrus), the female become aggressive with the male. She initiates minor battles. These fights are more intense while the animals are indoors in close quarters. The fights occur as soon as the animals are put together in the morning. After being put outside in the exhibit the battles are less frequent. The fights consist mainly of jousting and slashing with the horns and a few head-down charges. If this behavior occurs in my absence, I have only to look at Deano's face and sides to see the superficial wounds she's dealt him. This behavior starts about 5-7 days before what we call "Full Heat".

The second method of determining estrus is by vaginal smears. These are done nearly every day to keep tabs on their accuracy in relation to the other methods. In doing a vaginal smear we first steady the animal and

roke her sides and rear legs until her tail curls up and out of the way. Sometimes this is very difficult. If she is uncooperative we may try again later or attempt to hold her tail out of the way. After the tail is up, wipe the vulva with a disinfecting solution called Virosan[®]. Next, a cotton swab is inserted three inches into the vagina, removed and rolled gently onto a clean slide to be stained after it is dry.¹ We do two separate slides. What we examine on the slides are epithelial cells. They will either be cornified or noncornified. We count 100 cells to get a ratio of how many cornified cells there are in relation to noncornified cells. The higher the number of cornified cells, the closer the animal is to being in heat. We also note white cells and debris. In some animals (dogs) this also is indicative of heat. We really do nothing more than note the presence and number of white cells. We haven't tried to correlate this into the rest of our data yet. When we count the number of cells on the two slides and the ratios are nearly the same, we take the average. If the two slides are greatly different from each other, we must redo them because one may have been improperly done and this could cause the results to be off. If the female is showing behavioral signs of heat and the epithelial cell slides don't agree with this, it may be because as the estrus cycle develops all physiological changes don't occur simultaneously. As hormone levels increase, changes happen one by one. Therefore, an animal behaving as if in heat may have a cell ratio that is nonsupportive. If it is rechecked in 2 or 3 days the cell ratio may have changed quite a bit. There can, however, be results that aren't correct due to contamination as I mentioned before or from improper technique (i.e. not getting the swab in far enough). That is why we use a three-inch insertion as our standard. When someone other than the veterinarian takes a swab we sometimes get confusing data.

These vaginal swab epithelial cells are taken from a dog, but will show the differences between a cornified cell and a noncornified cell.

6. Dog; Vaginal Smear; Early Estrus; Wright's Stain
The epithelial cells are cornified. Nuclear detail is apparent.
Leukocytes are absent.



Figure 6

Dog; Vaginal Smear; NMB Stain
The large cells are noncornified epithelial cells. Numerous neutrophils are present.

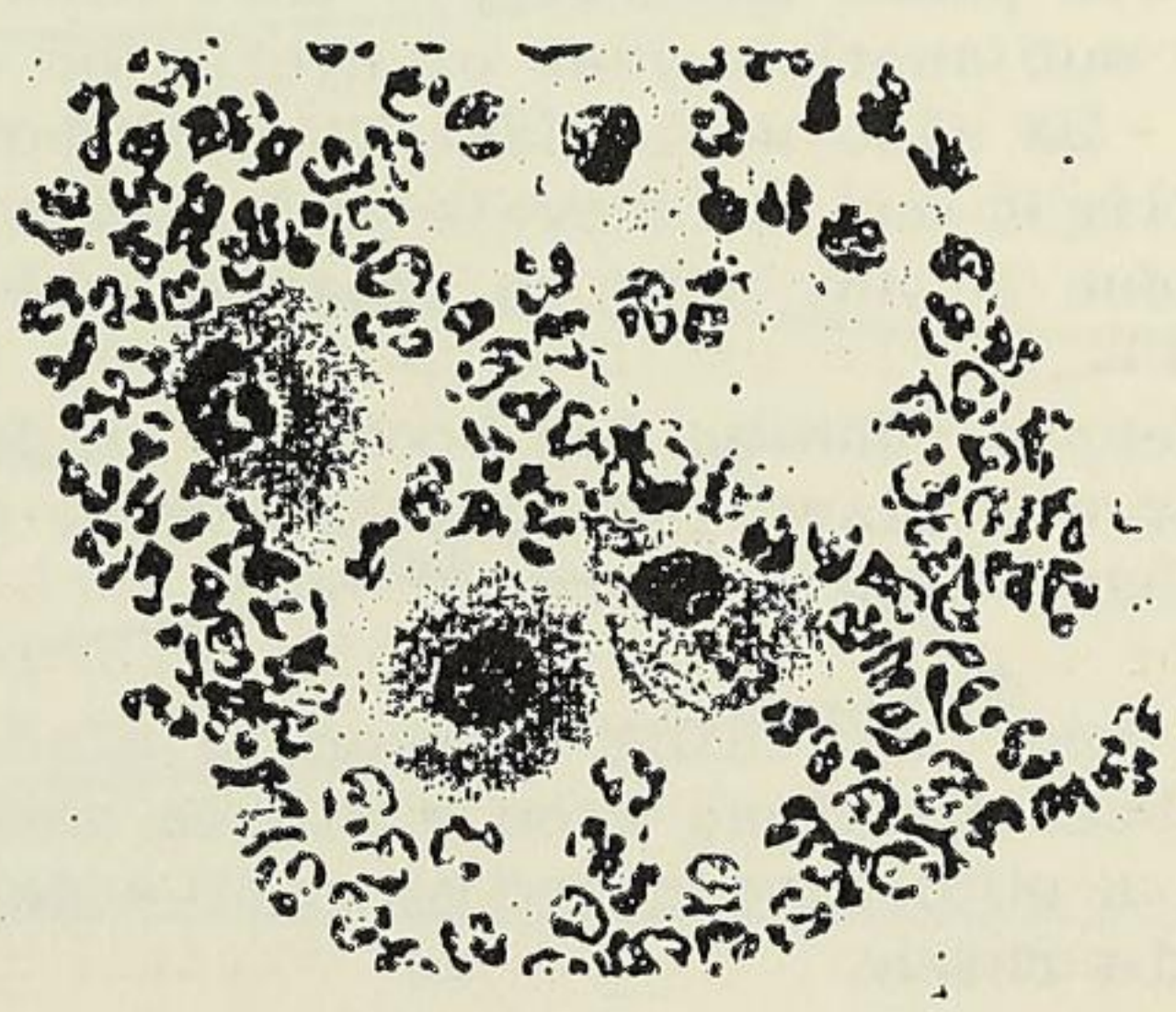


Figure 2

The third method is rectal palpation. This is done at least weekly and more often when we expect heat is nearing. Again, we get Kid to raise her tail and to steady. The veterinarian inserts his arm, covered with a glove, Virosan[®], and lubricant into the rhino's rectum and feels the shape of the uterus and fallopian tubes through the rectal walls. He then rates them by tone, length, and diameter. A tone of #1 indicates that the animal is not in heat (diestrus). A #2 could be approaching or diminishing heat (proestrus or metestrus). A #3 is full heat. During the most receptive periods the animal has a shortened and enlarged uterus, enlarged fallopian tubes, and a good tone. She will stand patiently for the exam and will slightly spread her rear legs and brace herself. We have found the rectal exam to be the most conclusive way of determining which part of the estrus cycle the animal is in.

Our final method is urinalysis. This is a very unreliable test for us at this point for two reasons. First, there are not standard hormone levels for white rhino urine established. This is currently being worked on. Second, sample collection is often infrequent. Our female tends to urinate at night, into her food, or the minute I leave the building. Urine taken from the floor is probably contaminated. We have taken steps to get a more frequent, free-catch sample by having one of our docents simply stand by all morning, cup in hand, waiting for urine. This has helped us to obtain one or two samples a week which is better than we had been doing. The samples are then frozen and when we get an adequate number they are sent to a laboratory² for hormone analysis. Once we get the results we can see how and if they agree with the other data we have collected.

None of the four methods I've mentioned can be used exclusively to determine estrus yet. There are too many variables. However, used together we can get a much better idea of what is going on in our rhino's estrus cycle.

Our findings over the past year have been interesting. I have drawn graphs comparing cell ratio with tone. It shows that the summer months produce some very confusing data. It seems that the female doesn't have a true cycle. Dr. Wagner thinks that an egg begins to form in the ovaries and instead of developing and traveling through the fallopian tubes, it is instead reabsorbed and the symptoms of approaching heat disappear (See Graph 1). The reason behind this is as yet unknown, but I understand the same thing can occur in horses during the summer in the Northern Hemisphere. From the graph you can see the slightly elevated cornified cell count and tone measurement, but the numbers never reach a sufficient level to indicate a receptive period. We call these slight increases "mini heat". The winter graph shows just the opposite (See Graph 2). There are very obvious peaks and valleys that show a cycle pattern of estrus. We want to plot out another year of cycles so we can validate what we have found so far. We also would like to collaborate with other zoos that have a similar light and dark cycle and similar temperatures to see if our findings are due in any part to geographical location.

We keep a running log book and in it is recorded the daily cell ratios, other cellular findings, behavior, urine collection, tone rating, and anything else of note.

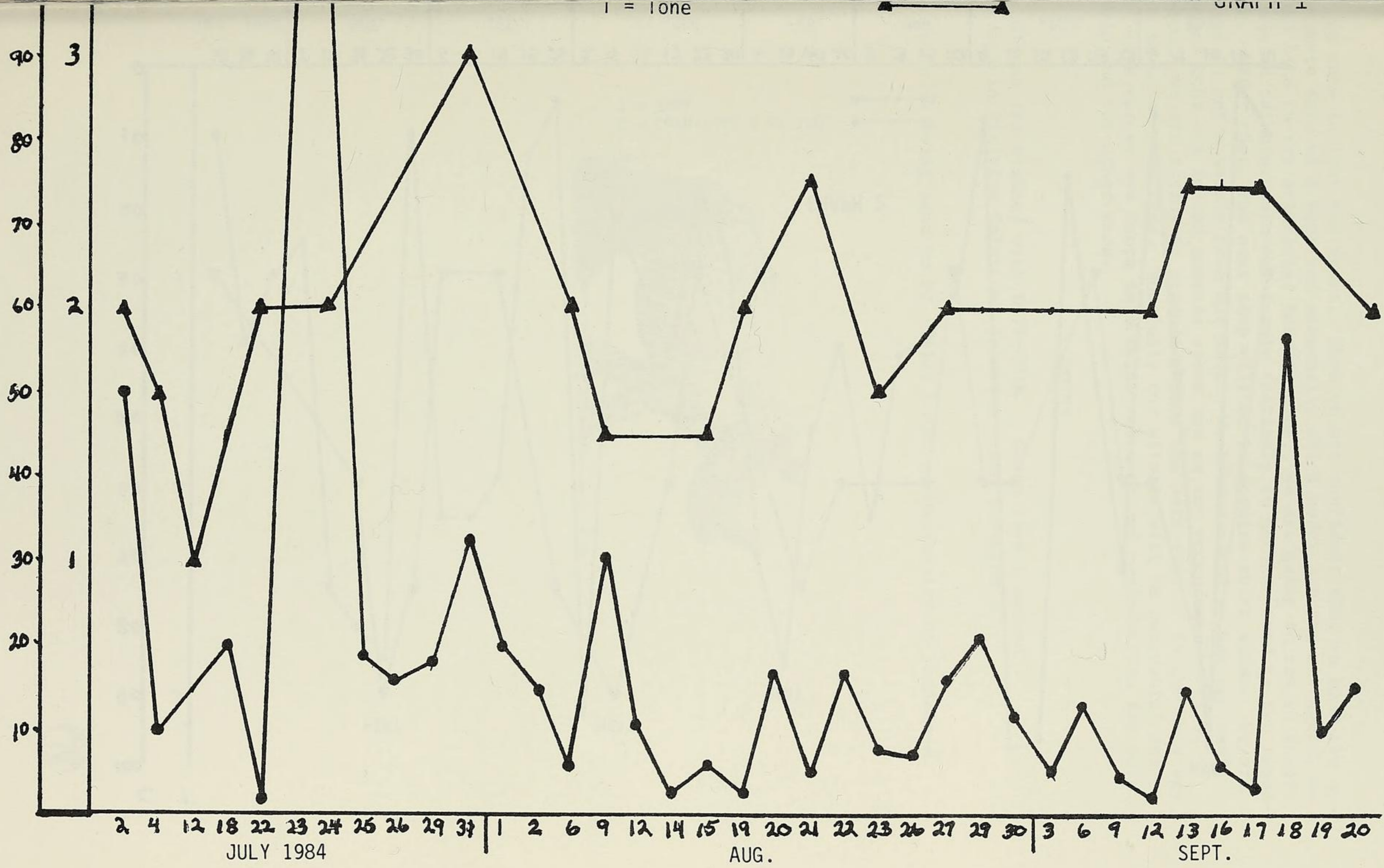
One important thing to keep in mind when acquiring this data is that this has been obtained from a single animal and should not be anything more than a guideline to go by, not a definite rule to be found with every other female rhino.

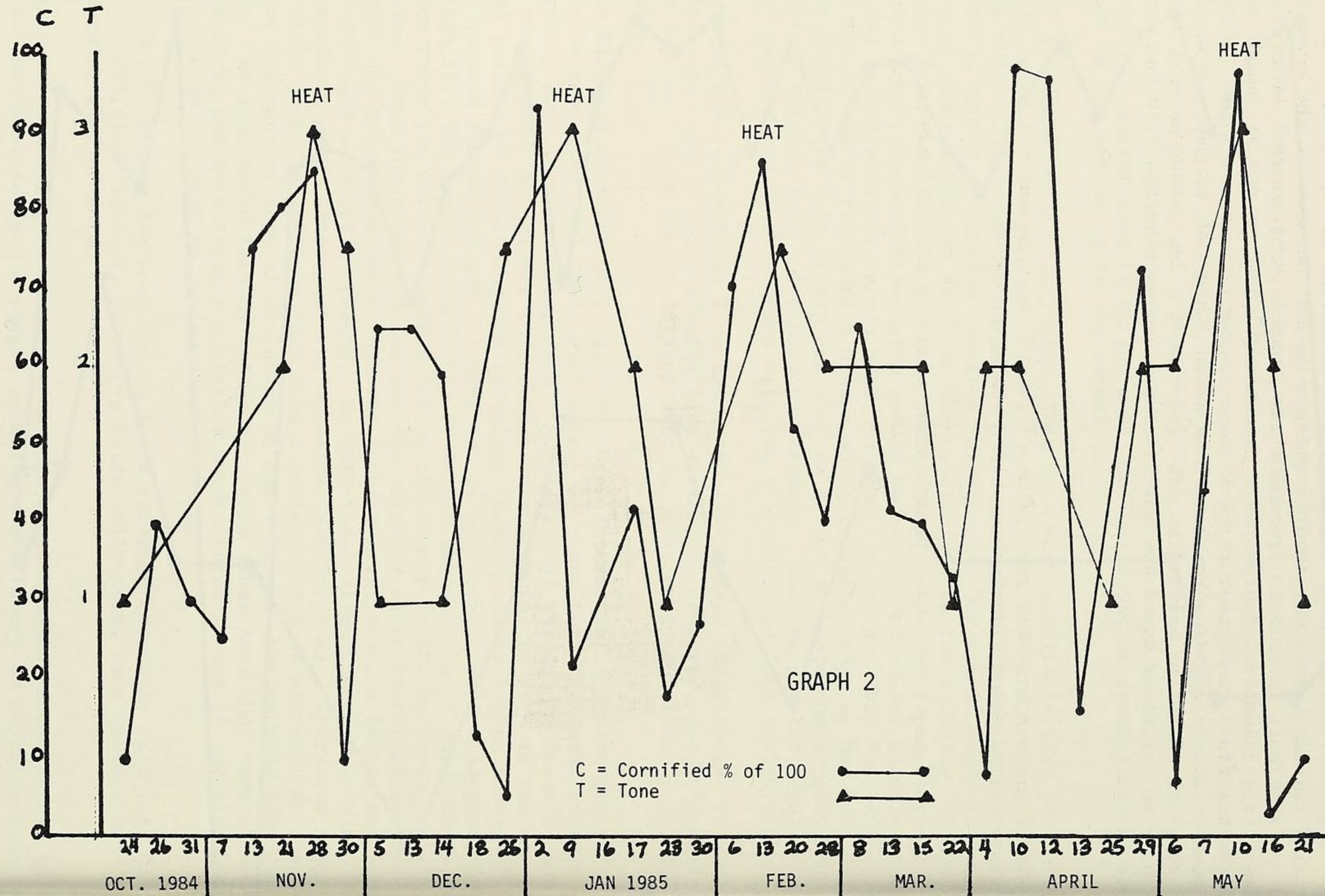
1 = lone

GRAPH 11

METHODS OF DETERMINING ESTRUS IN A WHITE RHINOCEROS, Continued

475





For us this is just the start. When we are confident that we know the approximate date of a heat and seasonally which heats are the strongest, we would like to try artificial insemination. We are going to try to insert an equine catheter into our female routinely to get her used to the sensation. After that, the next step will be to acquire rhino semen. We are trying to get a sample from our male. We have managed to get two small samples that did indeed contain sperm and we are encouraged by this. Although we are trying to produce rhinos the hard way, it is the only way open to us at this time. Hopefully our efforts will be successful and we will have opened new doors into rhino research and reproduction for ourselves and for other zoos.

NOTES

Slides are stained with DIFF-QUIK. Given five 1 second dips in Fixative Solution, Solution 1 and Solution 2.

Urine is being sent to Dr. Clint Lothrop, University of Tennessee.

