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Editors

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PREFACE

Captive breeding and, especially, the role of the world's zoological institutions in the conservation of endangered wildlife, continues to be debated. It was in this spirit of self-examination that the 5th World Conference on Breeding Endangered Species in Captivity was held in Cincinnati, Ohio on October 9-12, 1988. The conference was co-sponsored by The Fauna and Flora Preservation Society, The Cincinnati Zoo and Botanical Garden Center for Reproduction of Endangered Wildlife, and Kings Island Wild Animal Habitat.

While the presentations and discussions of previous conferences have centered primarily on specific captive breeding programs, the organizers of the Cincinnati conference attempted to develop a number of related themes. As a result, the presentations were divided into four categories - rescue and status, management and reintroduction, restoration, and recovery. The fact that 30 of the 56 papers were presented during sessions devoted to rescue and status is somewhat indicative of our current position with respect to the conservation of the world's wildlife. Perhaps by the time of the next conference, the number of presentations involving reintroduction, recovery and restoration projects will be on a par with those dealing with specific breeding programs.

The presentations by field biologists from conservation organizations, government agencies, and academia provide a special perspective and their participation underscores the fact that conservation programs involving captive propagation must be interdisciplinary to be successful.

It has been five years since the last conference was held in the Netherlands. We hope that the information presented and discussed throughout this conference will contribute in many different ways to the future success of the captive propagation of endangered wildlife. And, hopefully, this will be evaluated positively when this Conference is organized again at least four years from now.

Poster Sessions

Abstracts and/or poster text were submitted for publication.

Workshops

Workshops were organized for discussions on rhinos, bonobos, okapi, Arabian and scimitar-horned oryx, drills and exotic cats. Only the proceedings of the exotic cat workshop were submitted for publication.

Acknowledgements

The Program Committee wishes to thank all of the staff, volunteers, exhibitors and sponsors who made this Conference possible as well as Vickie Stidham who typed all of the manuscripts, and the participants and delegates who shared very many valuable thoughts and comments.

B. L. Dresser, R. W. Reece and E. J. Maruska, Editors

COMPARATIVE ASPECTS OF STEROID METABOLISM IN RHINOCEROSES: IMPLICATIONS FOR REPRODUCTIVE ASSESSMENT

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INTRODUCTION

With the possible exception of the Southern White, all species of rhinoceros are endangered in their natural habitat. Furthermore, captive populations of these species have yet to become sufficiently viable to provide a long term safeguard against continuing decline of numbers in the wild. The Black rhinoceros, for example, has experienced a 90% decline in the wild since the early 1970's, whilst the captive population, which numbers less than 180 individuals, has failed to increase over the past ten years. The Northern subspecies of White rhinoceros provides an even more extreme example, with a wild population of only 25-30, less than half this number in captivity and only a single female having bred. Ways of improving captive breeding performance of all rhinoceros species are urgently needed.

The reasons for the poor breeding record in captivity are unclear, but undoubtedly one of the main contributing factors is the lack of understanding of the reproductive biology of rhinoceros and of the factors required for successful reproduction. Virtually nothing is known about the reproductive physiology of these species and yet the application of artificial breeding techniques, such as artificial insemination and embryo transfer which may well become a necessary part of captive management of rhinoceros in the near future, is critically dependent on such knowledge and on accurate and reliable methods for assessing reproductive status. In recent years, the development of improved methods for urinary hormone analysis has been extremely valuable in providing a practical and non-invasive approach to monitoring reproductive function in exotic mammals. Assays for urinary oestrone conjugates and pregnanediol glucuronide (PdG) are now well established and have been used successfully in the detection of ovulation and pregnancy in a wide range of species (see Lasley, 1985; Hodges, 1985; Hodges and Green, 1989 for references). Application of these techniques to reproductive assessment in rhinoceroses, however, has so far met with limited success (Kasman et al, 1986; Ramsay et al, 1987).

The profile of conjugated oestrone and PdG excretion during the oestrous cycle in the Indian rhinoceros is shown in Figure 1. Here, the data derived from a study by Kasman et al. (1986), clearly show a marked increase in conjugated oestrone prior to ovulation, with elevated levels of immunoreactive PdG characterising the luteal or post-ovulatory period. Unfortunately, a similar pattern of steroid excretion cannot be demonstrated in the African rhinoceroses and methods currently available for the measurement of conjugated oestrone and PdG have failed to provide useful information on ovarian function in both the Black and the White rhinoceros. Typical data for these species (Figures 2 and 3) show that levels of both urinary metabolites are much lower than those found in the Indian (and in many cases undetectable) with little evidence of a cyclical pattern of excretion or correlation with reproductive events. The existence of species differences in steroid hormone metabolism and route of excretion and the

possibility that they may account for these observations has therefore been investigated.

RADIOLABEL INJECTION STUDY

A metabolism study was carried out in an adult female Southern White rhinoceros by administering 14C-labelled oestradiol and progesterone into the peripheral circulation. All faeces and urine excreted were collected separately over a four day period and the distribution of radioactivity determined. Both urinary (25%) and faecal (36%) routes of excretion contributed to the 61% of the administered label recovered. Of the label recovered in the urine, 92% was accounted for in the Day 2 sample, roughly half (43%) associated with metabolites in the conjugated form and half (49%) with those present as free steroids. Progestagenic (neutral) and oestrogenic (phenolic) steroids in each fraction were separated (Brown, 1955) and subjected to thin layer and high performance liquid chromatography (HPLC) in order to identify the metabolites present.

Chromatographic analysis of the neutral fraction revealed a single peak of radioactivity suggesting 20α-hydroxyprogesterone to be the only conjugated progesterone metabolite present (Figure 4). The absence of radioactivity associated with the pregnanediol standard was notable. Two peaks of radioactivity were seen in the phenolic phase and although oestrone appeared to be an abundant metabolite, the form of the original conjugate is not known. The presence of oestradiol-17α is of interest since it has not previously been demonstrated in this species and although, in this sample, appears less abundant than oestrone, its measurement may provide a better indication of ovarian function. Together these results confirm the existence of species differences in steroid metabolism and in particular provide an explanation for the failure to monitor ovarian status in African rhinoceroses by measurement of urinary PdG. It should however be remembered that our findings relate specifically to the White rhinoceros and it is not yet known whether they are applicable to the Black. The results also need to be confirmed by quantitative measurement of these metabolites during the ovarian cycle although, until the required assay validation is achieved, the uncertainty of whether such cycles are ovulatory remains a problem.

PROGESTERONE METABOLITES DURING PREGNANCY

In contrast to the lack of success in monitoring ovarian function, measurement of PdG immunoreactivity appears to be informative in indicating pregnancy in African as well as Indian rhinoceroses. Levels of immunoreactive PdG during mid to late pregnancy in the Indian and both African species of rhinoceros are shown in Figure 5. All three profiles describe increased PdG immunoreactivity associated with pregnancy and a rapid fall in levels at the end of the gestation period. Absolute values for PdG immunoreactivity however vary considerably between species with levels in the two African species being 20-100 fold lower than in the Indian. Values for the Indian rhinoceros are similar to those reported in a previous study by Kasman et al (1986). Both these and our own data (Hodges and Green, 1989) indicate a slow increase in PdG during early pregnancy with values becoming consistently higher than those in the luteal phase by approximately four months post-breeding. The limited data shown here for the African species do not indicate the onset of increased PdG excretion but Ramsay et al (1987), who reported elevated but highly variable levels of PdG

immunoreactivity during late pregnancy in six Black rhinoceroses, have suggested that it occurs as late as 6-8 months of gestation.

Although the presence of PdG has been confirmed in late pregnancy, urine of both the Indian (Hindle et al, 1988) and Black (Ramsay et al, 1987) rhinoceroses using gas chromatography/mass spectrometry (GC/MS) and HPLC analysis respectively, species differences in qualitative aspects of progesterone metabolism during pregnancy may exist. Thus, in contrast to the Indian rhinoceros in which PdG is the major progesterone metabolite, preliminary GC/MS analysis of urine from Black and White rhinoceroses suggests that metabolites other than PdG may be quantitatively more important and therefore more informative as a method of pregnancy detection. Nevertheless, data shown in Figure 5 clearly indicate that elevated levels of PdG immunoreactivity reflect the presence of a conceptus in all three species of rhinoceros and as such, its measurement provides the basis for a simple, non-invasive test for the mid-late stages of pregnancy.

SUMMARY AND CONCLUSIONS

This paper examines comparative aspects of steroid metabolism in rhinoceroses in relation to the application of urinary hormone analysis as a non-invasive method of reproductive assessment. Assays currently available for the measurement of conjugated oestrone and PdG have been successfully applied to the detection of ovulation and pregnancy in the Indian rhinoceros but, so far, have met with limited success in the African species. Results presented here provide evidence for species differences in the metabolism of oestrogen and progesterone which may account for these findings. 20α-hydroxyprogesterone and not PdG has been identified as the major metabolite of progesterone during the ovarian cycle in the White rhinoceros and possibly (by inference) also in the Black. Oestradiol-17α was detected as an abundant metabolite of oestrogen and may provide a more informative assessment of ovarian function than previously possible by the measurement of conjugated oestrone. PdG was detectable during late pregnancy in both African species of rhinoceros although other metabolites may be more abundant. Subject to the confirmation of these findings, the development of assays for the measurement of metabolites other than conjugated oestrone and PdG may provide new opportunities for monitoring reproductive function in African species of rhinoceros.

ACKNOWLEDGEMENTS

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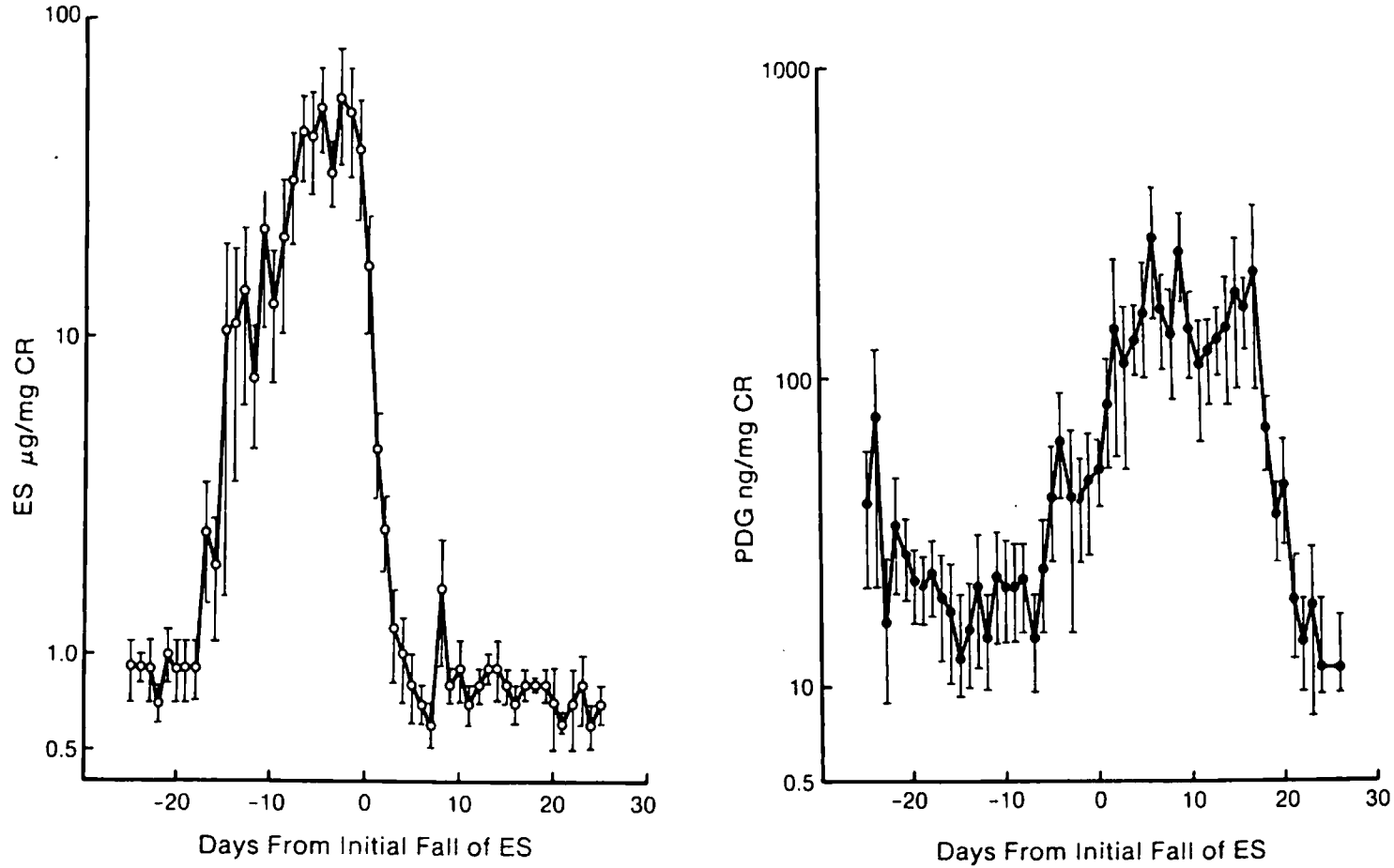

Figure 1. Profile of conjugated oestrone and immunoreactive PdG excretion during the oestrous cycle in the Indian rhinoceros. All values (mean ± SEM, taken from two successive cycles in five individuals) are aligned to the day of the fall in conjugated oestrone. Data adapted from Kasman et al (1986).

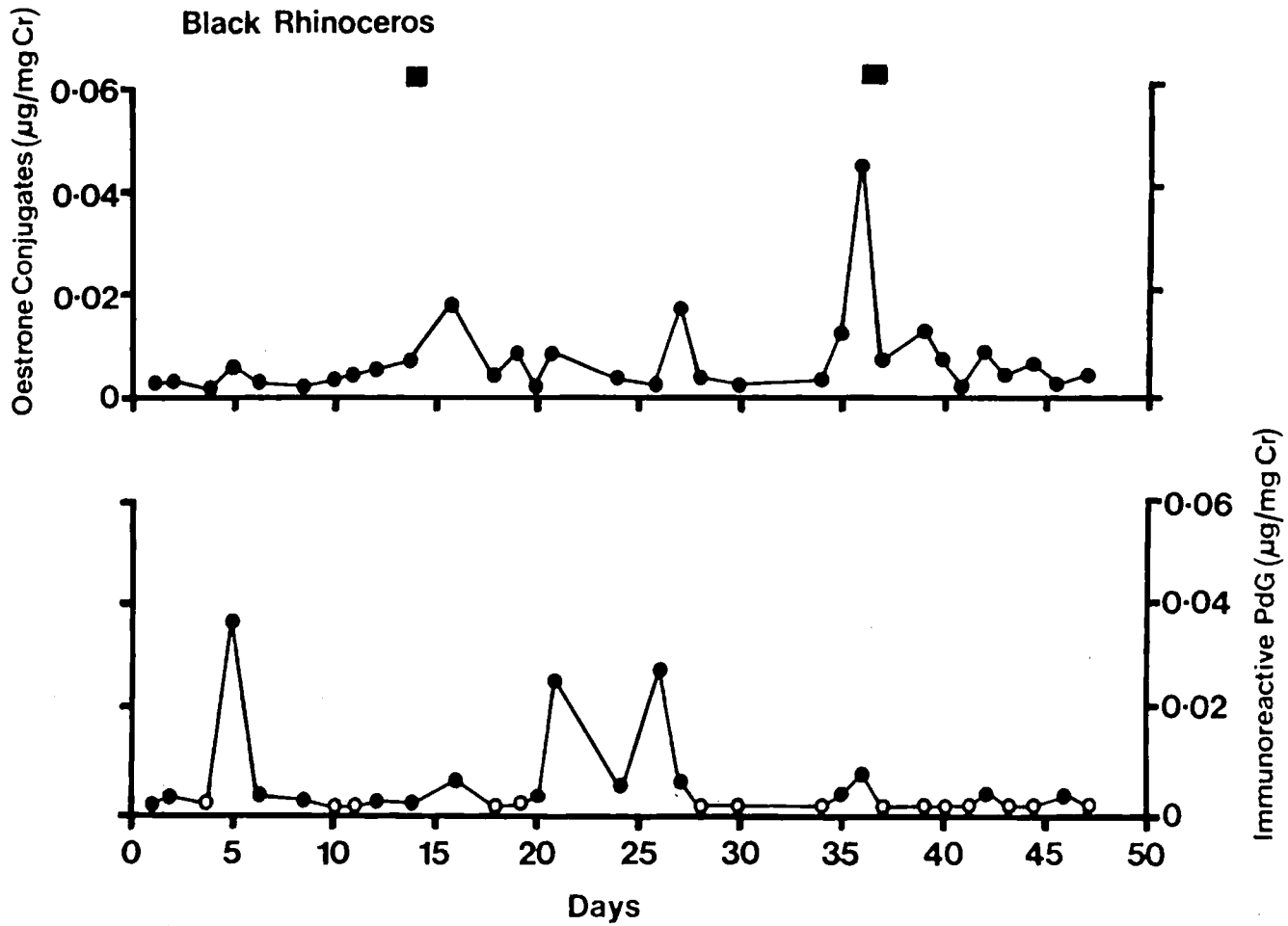

Figure 2. Profile of conjugated oestrone and immunoreactive PdG excretion during oestrous cycles in the Black rhinoceros. Oestrone conjugates were measured by RIA (Hodges et al, 1986), and immunoreactive PdG was measured by EIA (Hodges and Green, 1989). Open circles represent undetectable levels, closed squares represent periods of oestrus.

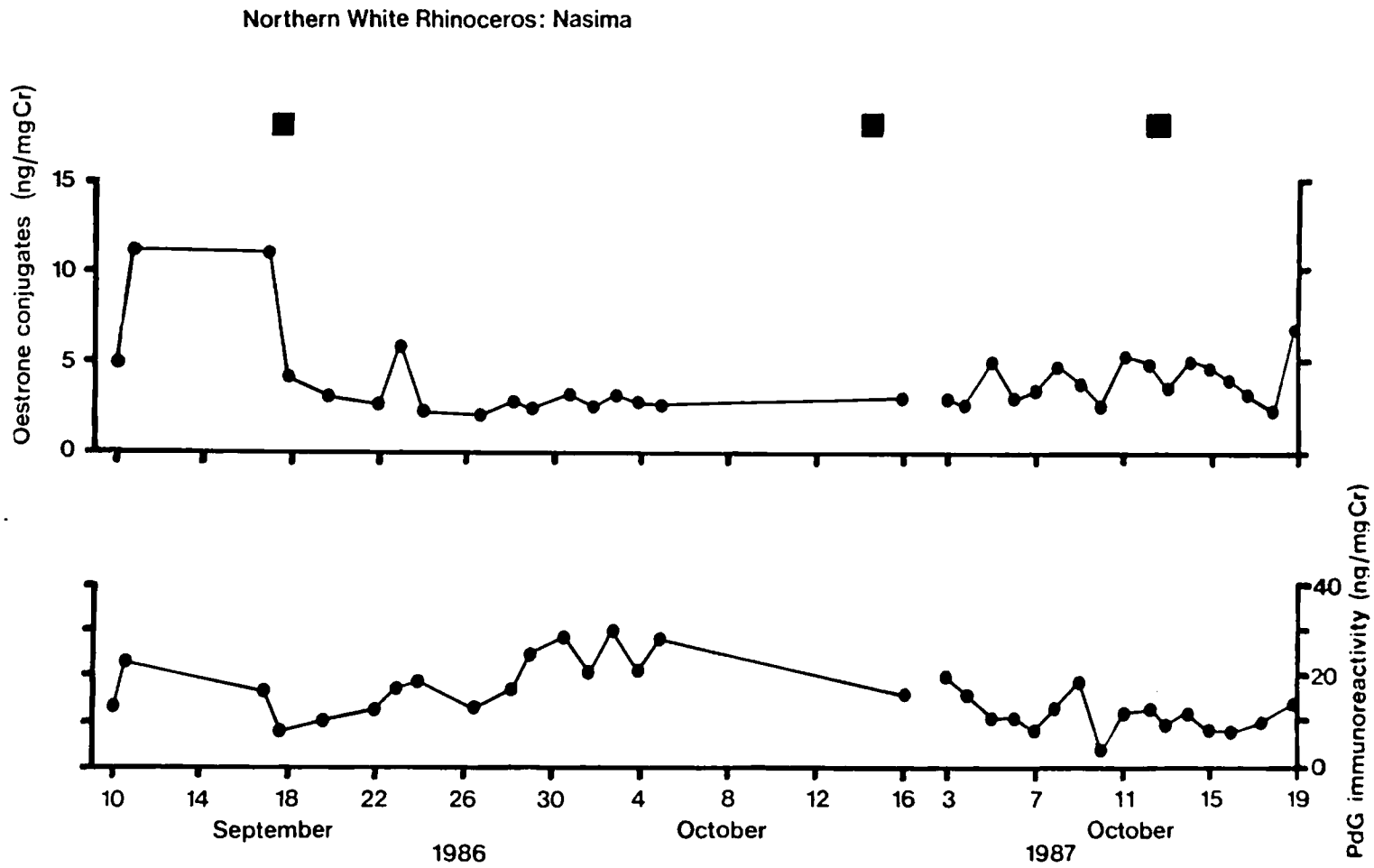

Figure 3. Profile of conjugated oestrone and immunoreactive PdG excretion during oestrous cycles in the Northern White rhinoceros (methods as in Figure 2). Open circles represent undetectable levels, closed squares represent periods of oestrus.

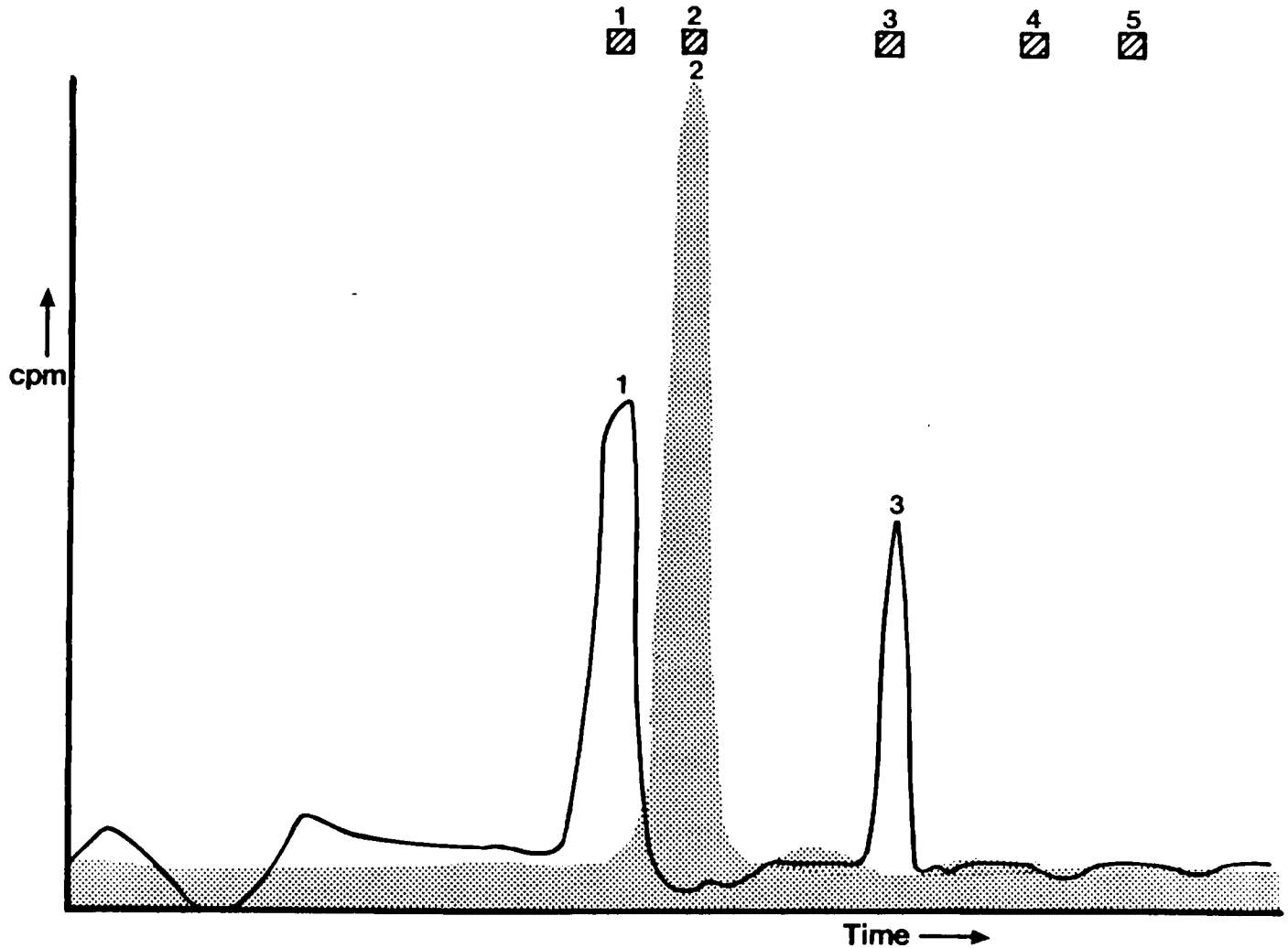

Figure 4. HPLC elution profiles of neutral (shaded) and phenolic (solid line) steroids in hydrolysed urine collected from an adult female Southern White rhinoceros after injection of 14C-labelled oestradiol and progesterone. The position of the reference standards oestrone (1), 20α-hydroxyprogesterone (2), oestradiol-17α (3), pregnanediol (4), and oestradiol-17β (5) is shown above. Neutral steroids were eluted from a C18 reverse phase column using 50% acetonitrile in water as solvent is an isocratic system; phenolic steroids were eluted from the same column using 35% acetonitrile in water in an isocratic system. Data have been combined. Adapted from Hindle and Hodges (1989).

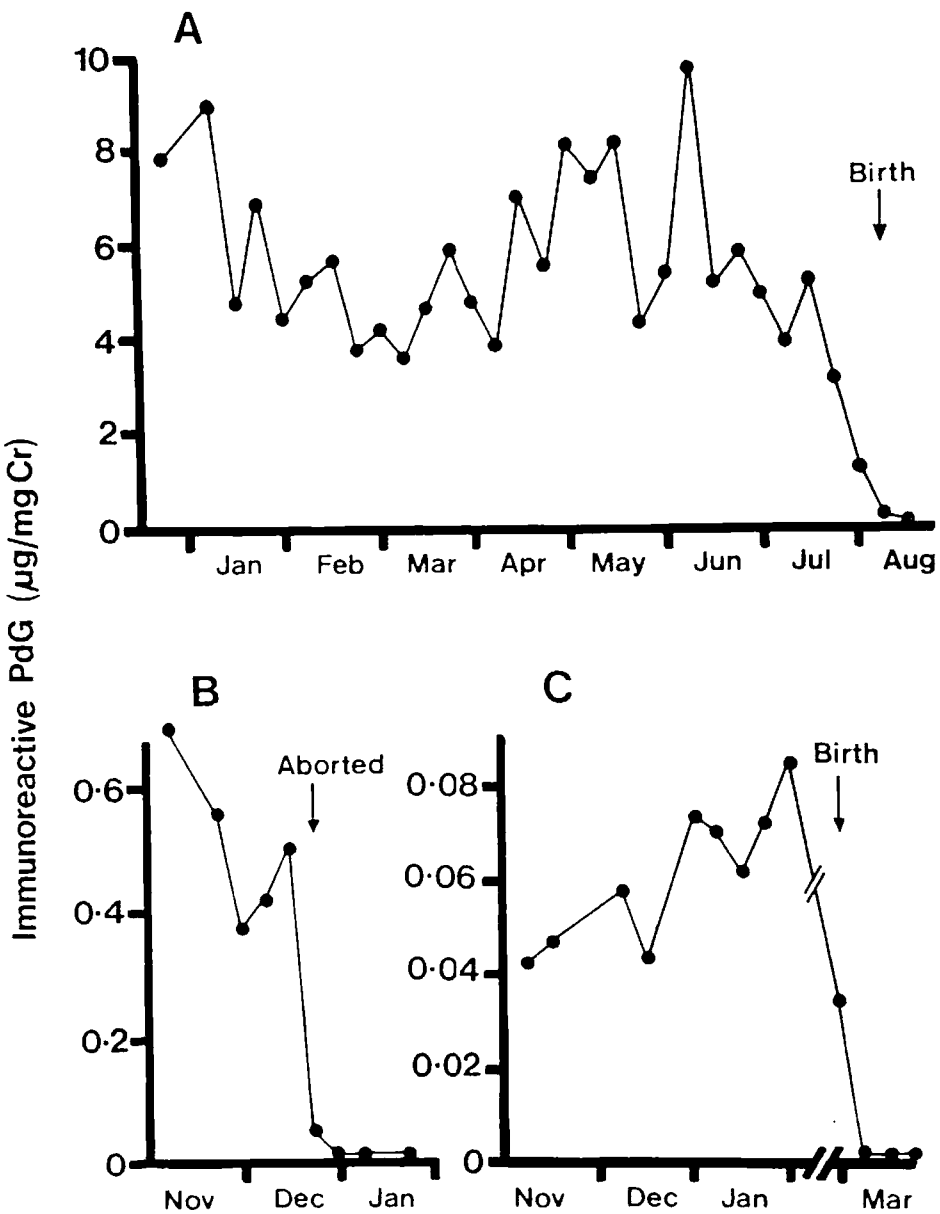

Figure 5. Levels of immunoreactive PdG during mid-late pregnancy in an Indian (A), Black (B), and White (C) rhinoceros. Pregnancies in the Indian and White rhinoceros were full term; the Black rhinoceros aborted after 228 days. Data from Hodges and Green (1989).

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