Nine rats did not show any deviations in their postoperative sexual behaviour. In seven animals, however, conspicuous changes occurred after the operation. These males tended to ejaculate after relatively few intromissions and with a short latency. The intervals of sexual inactivity following ejaculation were shortened from 5–6 min preoperatively to 1–3 min after the operation. Owing to the shortened latency periods there was an increase in the number of ejaculations achieved in a 30-min test.

The behavioural deviations appeared in the first postoperative tests and no recovery was ever seen. Exceptions to this rule were two males that did not achieve intromission and ejaculation before one or two months after the operation. When presented with the female these males approached her, followed her around the cage, and put the forepaws upon her back without making the mount with a pelvic thrust that characterizes the normal mounting responses. Frequent spontaneous penis erections accompanied by licking of the penis were displayed on these occasions. These two males also showed other abnormal features in their sexual behaviour. During the weeks following the operation they had frequent spontaneous penis erections. The erections occurred not only when these males were presented with a receptive female but also on other occasions; for instance, when being fed and weighed. The penile erections successively diminished in frequency and completely disappeared after about a month. When able to display complete copulations including ejaculation, both animals showed the same striking deviations in their sexual behaviour as described above. A demonstration of the reduction of the postejaculatory intervals and the accompanying increase in the ejaculation frequency is given in Figure 2. Histological examination of the testes did not reveal any abnormalities in any of the animals showing sexual deviations.

Comparison between the lesions in animals remaining sexually normal and in those showing sexual disturbances did not give any explanation for the behavioural differences observed. Smaller lesions within different parts of the critical region at the junction of diencephalon and mesencephalon, including complete destruction of the habenular complex, have so far not produced any sexual abnormalities[8].

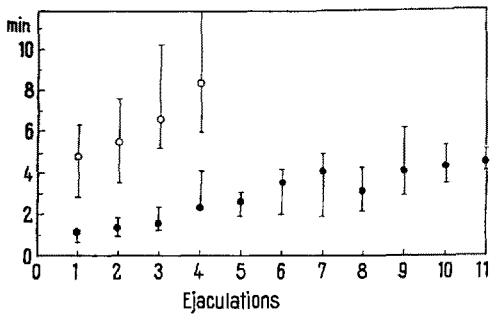

Fig. 2. The figure demonstrates the reduction of the postejaculatory intervals and the accompanying increase in ejaculation frequency in one of the operated animals observed in three 60-min tests approximately four months after the operation. Filled circles represent the median values of the postejaculatory intervals recorded during these tests. Vertical lines represent maximal and minimal values. Open circles show the corresponding performances of a group of 31 intact males.

Zusammenfassung. Umfangreiche Läsionen im Grenzgebiet zwischen Mittelhirn und Zwischenhirn bei Ratten führten zu stark erhöhter sexueller Aktivität. Die Ruheperioden nach der Ejakulation waren abnorm kurz, was die Zahl der Ejakulationen während des Versuches stark erhöhte. Auch 6 Monate nach Operation konnte kein Rückgang des Zustandes festgestellt werden.

L. Heimer and K. Larsson

Departments of Anatomy and Psychology, University of Göteborg (Sweden), March 31, 1964.

[7] J. de Groot, *The Rat Forebrain in Stereotaxic Coordinates* (Verhandelingen der Koninklijke Nederlandsche Akademie van Wetenschappen, Afd. Natuurkunde, Tweede Reeks, Del L II, 1959).

[8] *Acknowledgment.* This study was supported by Public Health Service Research Grant HD 00344-03 National Institute of Child Health and Human Development. Hormones were generously supplied by Pharmacia Inc., Uppsala.

PRO EXPERIMENTIS

Modified *in vivo* Assay for MSH

Introduction. An *in vivo* bioassay for the detection of melanocyte-stimulating hormone (MSH) has been available since 1924, when Hogben and Winton described a technique for hypophysectomy of the frog[1]. The interpretation of maximal melanin dispersal (darkening) in dermal melanocytes was aided by staging the melanocyte index (MI). This procedure was introduced in 1924[2] and altered into its present form in 1930[3] and 1952[4].

A simple modification of the assay is described here. By injection of the test material into the aortic trunk rather than into the classical location of the dorsal lymph sac, a doubling of the sensitivity is achieved.

Methods. Adult male frogs (*Rana pipiens*) weighing 30–60 g were hypophysectomized at least 3 days prior to assay. The MI in the interdigital web of the left foot was assessed as previously described[5].

For injection into the aortic trunk, the thorax of frogs anesthetized with ether was surgically exposed with 2-3 cuts of the scissors. Slight tension was placed on the right aortic trunk as a 25-gauge needle was inserted into the left aortic trunk near the base of the heart. One-tenth ml

[1] L. T. Hogben and F. R. Winton, Proc. Roy. Soc. (Biol.) *B 95*, 15 (1924).

[2] H. R. Hewer, Proc. Roy. Soc. (Biol.) *B 95*, 31 (1924).

[3] L. T. Hogben and C. Gordon, J. exp. Biol. *7*, 286 (1930).

[4] E. Thing, Acta endocr. (Kbh.) *10*, 295 (1952).

[5] G. T. Ross and W. D. Odell, Ann. N.Y. Acad. Sci. *100*, 696 (1963).

of a solution of standard Armour Melanophore Hormone in 0.01 *N* HCl-0.9% aqueous NaCl was injected into 92 frogs, although larger amounts may be used.

For injection into the dorsal lymph sac, a 22-gauge needle was introduced beneath the skin of the right leg and passed through the gluteal region in 167 frogs. One-tenth ml of solution proved to be a suitable amount, though larger quantities may be employed here also.

Standard test material response

Material	μg Producing MI of 3.0	
	Dorsal lymph sac injection	Aortic trunk injection
MSH powder[a]	0.051	0.024
ACTH[b]	0.20	0.10
β MSH[c]	0.002	0.001

[a] Armour Melanophore Hormone, Lot No. R527109, Armour Pharmaceutical Co., Chicago (Illinois USA).

[b] Armour ACTHAR, Lot No. K52004, Armour Pharmaceutical Co., Chicago (Illinois USA).

[c] Kindly supplied by Dr. A. V. SCHALLY, Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, New Orleans (Louisiana USA).

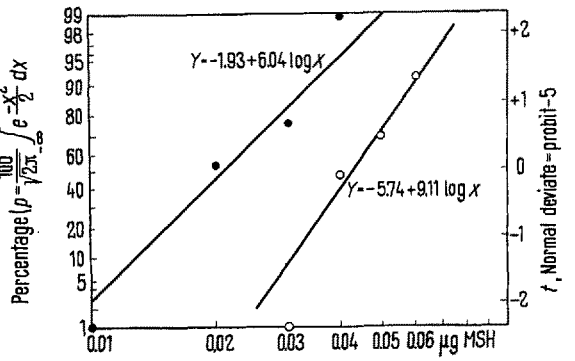

Fig. 1. Regression lines of probit analysis for MI responses of 3.0 or better. The open circles (o—o) represent responses for dorsal lymph sac injection; closed circles (•—•) responses for aortic trunk injection.

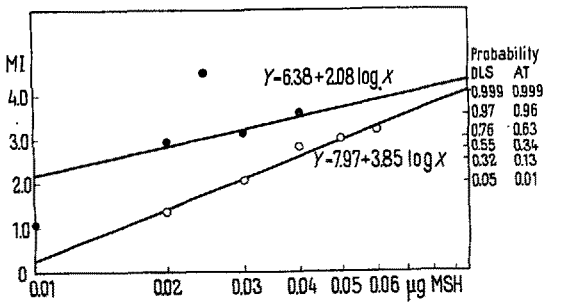

Fig. 2. Regression lines of MI responses to MSH when injected into the dorsal lymph sac (DLS) (o—o) and into the aortic trunk (AT) (•—•).

Statistical analyses of results were kindly made by Dr. M. ZELEN.

Results and Discussion. A response on the MI scale (score) was obtained sooner when the MSH powder was injected into the aortic trunk than when injected into the dorsal lymph sac. Nevertheless, the convenient 1-h response approximated the maximal MI increase and was therefore utilized in this assay.

The doses of standard test material necessary to produce an MI of 3.0[5] for each method of injection are shown in the Table. Only half the amount of test material was required to produce the same MI score when the aortic trunk route of injection was employed. Sensitivity could be further increased by reading responses at an MI of 2.75 or 2.50, rather than 3.0.

Injection by external cardiac puncture in unanesthetized frogs was attempted. In general, results were not consistent when this route was employed, although the method was more sensitive than dorsal lymph sac injection. The use of female frogs did not appear to appreciably increase the sensitivity of the bioassay.

Figure 1 summarizes the results of a probit analysis where a response was taken to be an MI of 3.0 or better. The graph estimates the probability of obtaining this response for both methods of injection. At the point where the probability of a response is 50%, the relative sensitivity was calculated to be 2.04 with 95% confidence limits of 1.70–2.38.

In addition to the probit analysis, MI responses were found to be a linear function of the log dose of standard Armour MSH over the range of doses employed. Regression equations were calculated by the method of least squares for each route of injection, and the resultant lines were drawn (Figure 2). The probability scale from Figure 1 is shown on the right-hand ordinate. Tests substantiated the visual impression of a lack of parallelism. The relative sensitivity varied from 2.7–2.1 within the MI range of 2.50–3.0.

The precision of the estimates of potency, using the 2 routes of injection, was calculated assuming a 3-point parallel-line assay design. The precision was expressed in terms of the ratio of the standard deviation of estimated potency to potency (σ k/k) or the coefficient of variation. The coefficient of variation for dorsal lymph sac injection with 3, 4 and 5 frogs at each point was 6, 5 and 4%; for aortic trunk injection it was 24, 20 and 18%. The λ-ratio (ratio of standard deviation to slope) was 0.052 and 0.21 for the dorsal lymph sac and aortic trunk routes respectively.

The dorsal lymph sac route of injection is recommended for routine measurement of MSH because of its ease of performance and precision. These virtues apply almost equally as well to the aortic trunk route of injection. However, its sensitivity, which is twice that of dorsal lymph sac injection, makes it suitable for detection of smaller amounts of MSH.

Résumé. L'épreuve *in vivo* classique pour MSH implique l'injection dans le sac lymphatique dorsal. Sa sensibilité peut être doublée par injection dans le tronc aortique de la manière décrite, tout en gardant une précision et simplicité suffisante.

A. J. KASTIN and G. T. ROSS

Endocrinology Branch, National Cancer Institute, Bethesda (Maryland USA), April 7, 1964.