

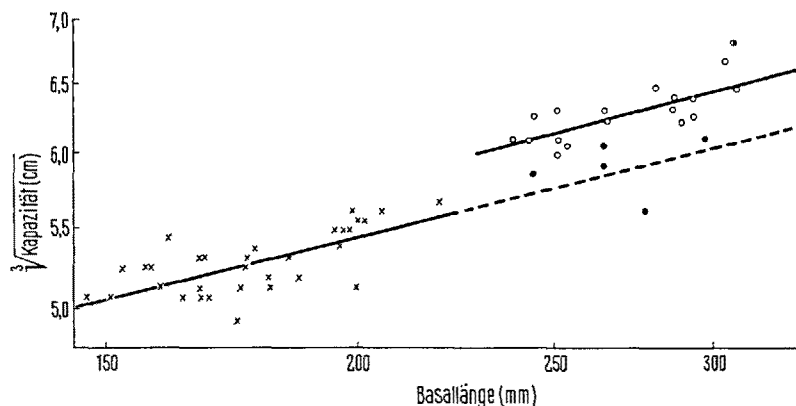


Fig. 2. Allometriediagramm  $\sqrt[3]{\text{Hirnschädelkapazität/Basallänge}}$  (doppelt-logarithmische Auftragung).  $\circ$ , *Panthera leo*, Afrika südlich der Sahara;  $\bullet$ , *P. leo persica*, Persien und Indien (2 Werte nach ANDERSON, schriftl. Mitt.<sup>5</sup>);  $\odot$ , *P. leo atrox*, Jungpleistozän Nordamerikas (Wert nach MERRIAM und STOCK<sup>11</sup>);  $\times$ , *P. pardus*, alle Teile des Verbreitungsgebietes. Allometrieachsen eingezeichnet für afrikanische Löwen und Leoparden sowie deren Verlängerung (unterbrochene Linie) in den Streubereich der asiatischen Löwen.

größe absolute Hirnvergrößerungen ... stattgefunden haben. Phylogenetische Entwicklung ist hier also dadurch gekennzeichnet, daß Allometrien «durchbrochen» werden.» Die Behauptung (RÖHRS<sup>6</sup>), derartige Transpositionsunterschiede der Allometrien zwischen verschiedenen großen, nahe verwandten Arten, wie wir sie hier etwa im Vergleich von Leoparden und afrikanischen Löwen vor uns haben, «können sicher nicht als Ausdruck einer verschiedenen phylogenetischen Ranghöhe gewertet werden», ist damit hinfällig. Die Entstehung einer sogenannten «interspezifischen» Allometrie Hirngröße/Körpergröße (Allometrieexponenten um 0,5–0,6) im Sinne von HERRE und RÖHRS<sup>6, 12, 13</sup> wird so nur als Sekundärfolge der Evolution verständlich, wenn bei körperlich größeren Arten durch Selektionsprozesse jeweils untere Cephalisationsstufen verschwinden (vgl. «intraspezifische» Allometrie zwischen Leopard und asiatischen Löwen, «interspezifische» Allometrie zwischen Leopard und afrikanischen Löwen). Als Ausdruck der Cephalisationshöhe einer Form kann daher auch nur die Integrationskonstante  $b$  der sogenannten «intraspezifischen» Allometrie Hirngröße/Körpergröße (Allometrieexponenten in der Regel um 0,23 variierend) benutzt werden, nicht aber die Integrationskonstante der im Sinne einer zwangsläufigen Folge zwischenartlicher Körpergrößenänderungen fiktiven «in-

terspezifischen» Allometrie, die HERRE und RÖHRS<sup>13</sup> als Ausdruck unterschiedlicher Organisationshöhe systematischer Einheiten vertreten.

**Summary.** Different stages of brain evolution expressed by the allometric relation of  $\sqrt[3]{\text{brain capacity}}$  and basal length of the skull are shown to be existent in the species *Panthera leo*. Whereas Asiatic lions obviously have the same level of brain size as leopards (*Panthera pardus*), African lions have higher brain capacities. A third level seems to be represented by the upper pleistocene American lion, *Panthera leo atrox*. These results permit us to reject some conceptions of HERRE and RÖHRS<sup>13</sup> concerning the quantitative expression of mammalian brain evolution.

H. HEMMER

Institut für Physiologische Zoologie,  
Gutenberg-Universität,  
Postfach 3980, D-65 Mainz (Deutschland),  
14. September 1971.

<sup>12</sup> M. RÖHRS, Z. zool. syst. Evolutionsforsch. 4, 196 (1966).

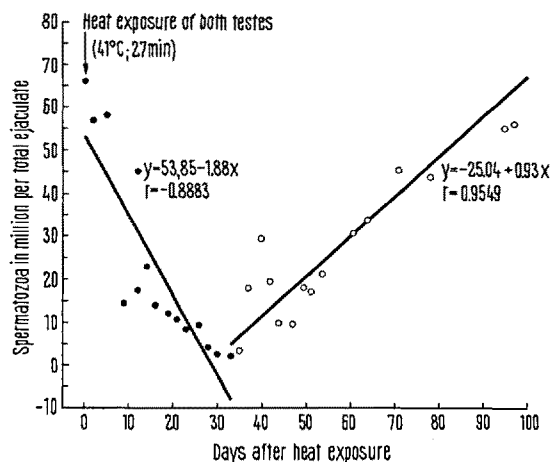
<sup>13</sup> W. HERRE und M. RÖHRS, *Die Evolution der Organismen*, 3. Aufl. (Ed. G. HEBERER; Fischer, Stuttgart 1971), vol. 2, p. 39.

## PRO EXPERIMENTIS

### A Model for Testing the Effect of Drugs on the Regeneration of Spermatogenesis in the Rat

In the rat, as in mice and guinea-pigs, the spermatozoa are trapped in a copulation plug which shows no spontaneous liquefaction. Counting or determining percent motility of the spermatozoa, and thus testing the effect of drugs on the spermatogenesis of rats continuously without taking testicular biopsies, was therefore not possible. Then 1%  $\alpha$ -chymotrypsin was found to liquefy the copulation plug of albino rats<sup>1</sup>. Spermatozoa could now easily be counted in copulation plugs collected by electroejaculation and liquefied by  $\alpha$ -chymotrypsin. To reduce individual variations of spermatozoa counts, coagulating glands and seminal vesicles of these rats were surgically removed<sup>2</sup>. Because the semen of these glandectomized rats is slightly clotted too, liquefaction by  $\alpha$ -chymotrypsin was further necessary. In the following report the effect of heat damage to the testes is demonstrated by counting spermatozoa output in regular intervals.

In 10 albino rats (Wistar strain, AF/Han<sup>3</sup>, average weight 350 g) coagulating glands and seminal vesicles were surgically removed. The animals were kept separately with water and diet ad libitum. 2 weeks later each animal was



Regression line and correlation coefficient of spermatozoa counts (mean of 10 rats) from 0 to 33 and from 33 to 97 days after heat exposure of the testes.

electro-ejaculated 4 times at 2-day-intervals and the spermatozoa counted after liquefying the copulation clot in 1%  $\alpha$ -chymotrypsin<sup>4</sup>. The scrotum containing the testes was then exposed to 41°C for 27 min in a water bath<sup>5</sup>. Until the 97th day following the exposure spermatozoa output was evaluated in 2-to 3-day-intervals in the manner described above.

Spermatozoa output before heat exposure of the testes ( $x \pm s$  in million per total ejaculate) was  $66.2 \pm 16.4$  33 days after heat exposure it was at its lowest point of  $2.2 \pm 2.1$ . On the 97th day spermatozoa output reached  $65.7 \pm 11.3$ ; the counts before heat exposure (see diagram).

Neither the heat exposure of the scrotum nor the regular electroejaculations seemed to affect the well-being of the animals. Thus it seems possible to test with this method: 1. Protective effects of drugs on heat damage to the testes. 2. Accelerating or depressing effects of drugs on the spontaneous regeneration of spermatogenesis after heat damage to the testes bei der Ratte.

**Zusammenfassung.** Methode als Modell zur Prüfung der Wirkung von Pharmaka auf die Regeneration der Spermatogenese bei der Ratte.

J. MAUSS

*Dermatologische Klinik und Poliklinik,  
Klinikum Essen der Ruhr-Universität,  
Hufelandstrasse 55, D-4300 Essen (Germany),  
6 April 1971.*

<sup>1</sup> J. MAUSS, J.-G. RAUSCH-STROOMANN, E. HAHN, R. PETRY and Z. ZAMBAL, *Andrologie* 2, 13 (1970).

<sup>2</sup> J. MAUSS, W. GERKMANN, R. PETRY and J.-G. RAUSCH-STROOMANN, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 266, 195 (1970).

<sup>3</sup> Zentralinstitut für Versuchstierzucht, Hannover (Germany).

<sup>4</sup> Firma Schuchardt, München (Germany).

<sup>5</sup> E. STEINBERGER and W. J. DIXON, *Fertil. Steril.* 10, 578 (1959).

## A Micro-Chromatographic Method for the Detection of Biologically Active Monoamines Isolated Neurons

Progress in neurochemical research is often restricted by the sensitivity and specificity of methods available for detecting and measuring small amounts of different substances. During the course of some studies concerning the uptake and conversion of certain monoamines (5-hydroxytryptamine, 5-hydroxytryptophan, adrenaline, dopamine and noradrenaline) in different neurons, it became necessary to have a simple and reliable method for separating and detecting minute quantities of monoamines in individually isolated neuron samples. It is known that microchromatography of monoamines which have been dansylated<sup>1,2</sup> allows picogram levels of 5-HT to be detected in individual snail neurons<sup>3</sup>. However, this method is not convenient for the localization of some other monoamines, e.g. dopamine, because the number of dansylated derivatives formed from the single substance makes it difficult to localize<sup>1,2</sup>.

From the reaction between certain amines and formaldehyde vapour, BELL and SOMERVILLE<sup>4</sup> described a method for the detection of as little as 30 ng of substance, using conventional paper or thin layer chromatographic techniques. The following communication describes a micro-chromatographic procedure from the original BELL and SOMERVILLE's method which permits minute amounts (5–7 ng) of dopamine, noradrenaline and 5-hydroxytryptamine to be detected.

**Materials.** 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) were supplied from Sigma adrenaline (AD) and noradrenaline (NA) from British Drug Houses and dopamine (DA) and 5-hydroxytryptamine (5-HT) from Koch-Light Laboratories; 3,4-dimethoxyphenylamine, melatonin,  $\alpha$ -methyl-m-tyrosine, bufotenine, 3-hydroxy-4-methoxy-phenylethylamine, kynurenine, 3-hydroxy-kynurenine, kynurenic acid, 3-methoxy-4-hydroxyphenylethylamine, octopamine, n,n-dimethyltryptamine, normetadrenaline, mescaline, metadrenaline and kynuramine were all kindly provided by courtesy of Dr. A. R. SOMERVILLE, Imperial Chemical Industries Ltd., Cheshire. Standard concentrations were made up in 50% acetone in 0.01 N HCl.

Chromatograms were developed on 3 × 3 cm polyamide layers (Carl Schleicher and Schüll, DC-Fertigfolie F1700 Muirpolyamide). All solvent systems were of analytical grade from May and Baker Chemical Company.

**Methods.** Standard (1–1000 ng) amounts of amine were each spotted 0.4 cm from the bottom edge of a 3 × 3 cm polyamide layer. This was achieved by sucking a known amount of amine (0.1 × 0.5  $\mu$ l) out of a 5  $\mu$ l capillary tube (Drummond Scientific Company) into the tip of an ultra thin capillary (diameter 0.5 mm) and then applying it to the chromatogram under a stereomicroscope (for details see NEUHOFF<sup>1</sup>). After drying with cool air, the chromatogram was developed in an ascending fashion in a 50 ml beaker, the bottom of which was just barely covered with either methyl acetate/isopropanol/ammonia 25% (9:7:5) or butanol/chloroform/acetic acid (4:1:1). The beaker was covered to prevent any evaporation of the solvent. When the solvent reached the upper edge of the chromatogram

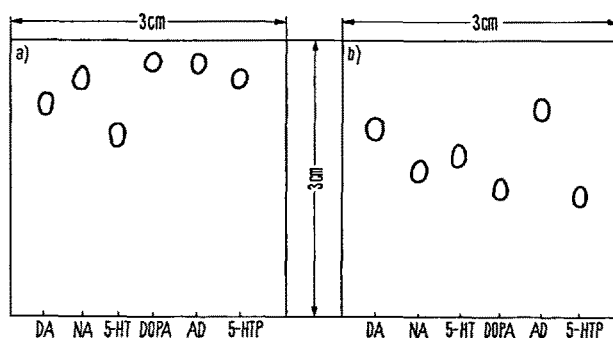


Diagram of micro-chromatograms (polyamide layers) using the solvents. a) Methyl acetate/isopropanol/ammonia and b) butanol/chloroform/acetic acid. Abbreviations as in text.

<sup>1</sup> V. NEUHOFF, *EMBO-course in Micro methods* (Max-Planck-Gesellschaft, Dokumentationstelle, Göttingen 1970a).

<sup>2</sup> V. NEUHOFF and M. WEISE, *Arzneimittel-Forsch.* 20, 368 (1970).

<sup>3</sup> G. BRIEL, V. NEUHOFF and N. N. OSBORNE, in preparation (1971).

<sup>4</sup> C. E. BELL and A. R. SOMERVILLE, *Biochem. J.* 98, 1C (1966).