

optic nerves is acute. The pons and the medulla oblongata are well developed and are well defined from one another. The median plane (Figure 4) shows a relatively narrow fore-brain and a well-developed parieto-occipital area. The corpus callosum is narrow. The rounded massa intermedia is 8 mm long. The third ventricle and the aqueduct are slit shaped and extend vertically. There is no olfactory nerve (Figure 5). The optic nerve, corresponding to the size of the eye, is very narrow. The optic structures of the Ganges dolphin (*Susu gangetica* Lebeck) are even more reduced<sup>2</sup>. The oculomotor nerve is very thin and the trochlear and the abducens are absent. The trigeminal is thinner than the acoustic nerve. The latter runs parallel to a well-developed facial nerve. The eighth is the most well-developed of the cranial nerves. The hypothalamus is quite long, the mamillary bodies are minute and the anterior commissure can hardly be seen. The fornix on the sagittal section has a diameter of 10 mm. As is the case by other Cetacea<sup>3</sup>, there is no epiphysis cerebri. There is a posterior commissure. The tuberculi optici of the

lamina quadrigemina is underdeveloped, whereas the acoustic are well-developed. The medulla oblongata has very prominent olivary bodies. The average body length of the dolphins investigated is  $184.25 \pm 16.27$  cm, the average body weight  $44.70 \pm 10.44$  kg and the average brain weight  $550 \pm 41.57$  g (Table II). The fresh cerebellar weight of No. 418 is 78.8 g which is 12.4% of the total brain weight (*S. gangetica* 6.5%, *Delphinus delphis* 16.4%). The relationship of the relative increase of the brain weight ( $y$ ) to the relative increase of the body weight ( $x$ ) is represented by the regression coefficient  $b$ . The slope of the regression line of *D. delphis* in a log-log plot is given by the significant regression coefficient  $b = 0.3290$  that is  $18^\circ 10'$  (Figure 6). A significant regression coefficient for the 4 individual values of *I. geoffrensis* could not be calculated. However it is obvious from the individual values below the regression line of *D. delphis*, that a certain relative body weight of *Inia* corresponds to a lower relative brain weight than it does for *Delphinus* (Figure 6). Therefore it may be said that *Inia* belongs to a lower grade of cephalization than *D. delphis*.

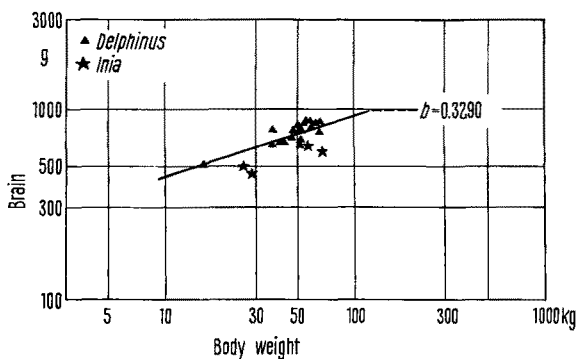


Fig. 6. Relationship between the brain weight and body weight of *D. delphis* in a log-log plot. Individual values of *I. geoffrensis* are also given.

**Zusammenfassung.** Bei einem durchschnittlichen Körpergewicht von 44,7 kg beträgt das mittlere Frischhirngewicht von *Inia geoffrensis* 550 g. Das Kleinhirngewicht macht 12,4% des Totalhirngewichtes aus. Das optische System von *Inia* ist sehr reduziert, das akustische System dagegen stark entwickelt. *I. geoffrensis* ist weniger zephalisiert als *Delphinus delphis*.

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(Switzerland), 22 April 1968.

<sup>2</sup> G. PILLERI, *Revue suisse Zool.* 73, 113 (1966).

<sup>3</sup> G. PILLERI, *J. Hirnforsch.* 8, 437 (1966).

## Use of a Combination of Metallic Compounds and Acetic Acid-Alcohol as Fixatives for Mammalian Tissue in Microspectrophotometry

It has been a fairly general procedure to fix tissues in acetic acid-alcohol (1:3) or in 10% neutral formalin for estimation of DNA by Feulgen microspectrophotometry. Acetic acid-alcohol fixation, however, appears to give a less strong Feulgen reaction, whereas formalin fixation yields better results and the nuclei are stained more intensely. The addition to the fixative of heavy metal compounds appears to vastly improve the binding of aldehyde molecules to the molecules of leucofuchsin. 3 such compounds have been tried: platinum chloride, uranyl nitrate and uranyl acetate. Presented here are DNA values of the rat kidney tissue fixed in acetic acid-alcohol fortified with each of the different metallic compounds separately and stained by the Feulgen procedure. The mixture contained in each case, 1 part of glacial acetic acid, 3 parts of absolute alcohol and 4 parts of a 1% solution of either platinum chloride, uranyl nitrate or uranyl acetate. Acetic acid-alcohol (1:3) was also used to fix part of the same tissue for purpose of comparison. Tissues fixed in the different fixatives were sectioned at

10  $\mu$ , stained simultaneously and also processed together. Measurement of the amount of DNA was carried out by a microspectrophotometer that has been described elsewhere by the author<sup>1</sup>. The measurements were made by the 2-wave-length method, the wave-lengths being 560 and 500 nm. Estimation of DNA-Feulgen was carried out in nuclei that were selected at random. However, care was taken to measure as many nuclei from the periphery as from the centre of the sections. The nuclei measured varied from 5.0–6.5  $\mu$  in diameter in all the cases. DNA values in arbitrary units were calculated according to MENDELSON<sup>2</sup>.

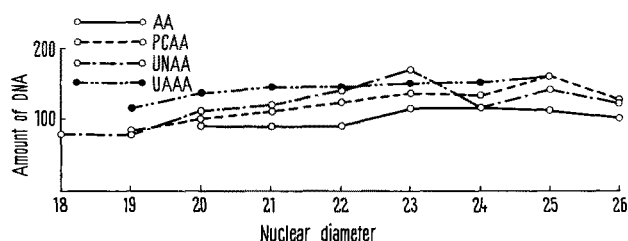
Microscopic examination of slides stained after fixing with the 4 different fixatives revealed that perfect fixation took place in all the cases. The speed of staining reaction

<sup>1</sup> M. K. DUTT, *Nucleus*, Calcutta 10, 168 (1967).

<sup>2</sup> M. L. MENDELSON, *J. biophys. biochem. Cytol.* 4, 415 (1958).

## DNA content of the rat kidney nuclei fixed in the 4 different fixatives

No. of nuclei	Fixative	DNA content	Difference between means	t-value	P
40	AA	102.45 $\pm$ 3.50 (A)	A v. B = 13.71	2.51	< 0.025
43	PCAA	116.16 $\pm$ 4.06 (B)	A v. C = 22.59	3.22	< 0.005
42	UNAA	125.04 $\pm$ 5.98 (C)	A v. D = 39.23	6.51	< 0.001
41	UAAA	141.68 $\pm$ 4.88 (D)	B v. C = 8.88	1.23	N.S.
			B v. D = 25.52	4.03	< 0.001
			C v. D = 16.64	2.15	< 0.005



The relation of the amount of DNA and nuclear diameter in tissues fixed by the different fixatives.

was also accelerated in the uranium and platinum containing fixatives as compared with that in acetic acid-alcohol preserved tissue. The mean DNA content in tissues fixed by the 4 different fixatives were statistically analysed to obtain information regarding the relative efficiency of the different fixatives for staining by the Feulgen procedure. The relevant data are presented in the Table and the Figure. From the Table it is evident that yield of DNA values is best in tissues fixed in acetic acid-alcohol containing 1% uranyl acetate (UAAA). The

DNA values, however, remain unaltered in the tissues fixed either in acetic acid-alcohol containing 1% uranyl nitrate (UNAA) or platinum chloride (PCAA). The yield is least in tissues fixed in acetic acid-alcohol (AA). These results thus indicate that the nucleoprotein complex of the cell nuclei is perhaps better precipitated in tissues fixed in acetic acid-alcohol fortified with metallic compounds such as uranyl acetate, uranyl nitrate or platinum chloride, as compared with tissue fixed in acetic acid-alcohol<sup>3</sup>.

*Zusammenfassung.* Zugabe von Platinchlorid oder Uranylverbindungen zu Eisessig-Alkohol erhöht den DNS-Wert des fixierten Gewebes beträchtlich.

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<sup>3</sup> The author wishes to express his sincere appreciation to Prof. B. R. SESHACHAR for providing necessary facilities to carry out this investigation.

## Changes in the Numbers of Deoxyribonucleic Acid Synthesizing Nuclei in the Adrenal Cortex Following Unilateral Nephrectomy in the Rat

The changes that occur in the remaining kidney following unilateral nephrectomy have been extensively studied<sup>1-3</sup> but little is known about its effect on other organs. During the course of an investigation of the changes in the numbers of deoxyribonucleic acid (DNA) synthesizing nuclei in the rat kidney in compensatory renal hyperplasia<sup>4</sup> we found that the weight of the adrenal glands was increased following unilateral nephrectomy. We decided to establish if this weight increase was accompanied by any increase in the number of DNA synthesizing nuclei in the various zones of the adrenal cortex.

*Materials and methods.* The animals used were albino male rats of an inbred strain with an initial body weight of about 200 g, which were maintained on a commercial pellet diet and tap water. Right nephrectomy was performed through a lumbar incision under ether anaesthesia with care to avoid injury to the right adrenal or its blood supply. Antibiotics were not given and post-operative infection did not occur. The rats were killed in groups of 6 or 12 animals at periods ranging from 12 h to 6 weeks after the operation. 4 groups of rats were used as controls, consisting of 2 groups of normal and 2 of sham-nephrec-

tomized animals. The 2 groups of normal rats had body weights of about 200 and 260 g. The 200 g group was used for comparison with the nephrectomized and sham-nephrectomized animals killed during the first week after operation, and the 260 g group for comparison with those killed after a longer interval. The 2 groups of sham-nephrectomized rats were killed at 2 days and 1 week after operation, which consisted of pushing the right kidney through a lumbar incision and then replacing it within the abdominal cavity.

Tritiated thymidine was used to label nuclei synthesizing DNA. It was given as an i.p. injection at a dose level of 0.7  $\mu$ Ci/g of final body weight. The labelled thymidine was injected at 10.00 and the rats killed at 14.00 to

<sup>1</sup> G. E. G. WILLIAMS, Br. J. exp. Path. 42, 386 (1961).

<sup>2</sup> H. A. JOHNSON and J. M. V. ROMAN, Am. J. Path. 49, 1 (1966).

<sup>3</sup> G. THRELFALL, D. M. TAYLOR and A. T. BUCK, Am. J. Path. 50, 1 (1967).

<sup>4</sup> H. P. R. BURY, W. A. J. CRANE and L. P. DUTTA, Br. J. Urol. 37, 201 (1965).