J. Exp. Biol. (1970), 52, 539–552
With 8 *text-figures*
Printed in Great Britain

RELATIVE GROWTH, NUCLEIC ACID CONTENT AND CELL NUMBERS OF THE BRAIN IN *OCTOPUS VULGARIS* (LAMARCK)

BY A. PACKARD* AND V. ALBERGONI†
Stazione Zoologica, Naples, Italy

(*Received* 10 *November* 1969)

INTRODUCTION

The brain of *Octopus vulgaris*, as of other cephalopods, grows throughout life. This is apparent from even a summary inspection of the animal at different stages of its life history. When it settles to the sea floor after some 6 weeks of planktonic life, the animal weighs one-fifth of a gram. Yet the brain alone weighs twice this by the time (? one year) it is subject to the many learning studies conducted at Naples; and at a maximum body size of about 10 kg. the mass of the brain may be nearly 2 g.: i.e. more than one thousand times that of the whole *Octopus* at hatching (1 mg.) (see Fig. 1). Animals of no other group except fish (Geiger, 1956) show such changes in size of the organ that controls their behaviour. This continuous growth of the central nervous system in fish and cephalopods is so unlike the situation in ourselves and other placental mammals, whose brain grows rather little after birth (at most four-fold in wet weight in man and the cat) and adds relatively few cells (< 50%, Altman, 1967), that even zoologists are unused to thinking about the problems presented by an animal whose brain cells may be increasing in number by as much as one and a half orders of magnitude.

We should like to report here the results of measurements made some years ago aimed at defining the growth rate of this organ and at providing an estimate of its cell numbers throughout the growth range. In order to avoid the enormous task of counting individual cells in the different lobes of many individual brains, their DNA content was measured and the amounts were calibrated by a method employing sperm counts. The results have led to a more general study still in progress on the distribution of nucleic acids in the central nervous system of *O. vulgaris*.

A volumetric analysis of brains from a wide variety of Mediterranean cephalopods was the subject of a monograph by Wirz (1959).

The DNA content of optic lobe and vertical fractions of the brain of *Eledone cirrhosa* has been measured by Bradford, Chain, Cory & Rose (1969). The results of the vertical lobes cannot be compared in a straightforward way with ours, however, as it is apparent from the wet weight of the tissue quoted that a large part, if not all, of the rest of the supraoesophageal lobes was contained in their slices of the vertical lobes.

* Present address: Department of Physiology, University Medical School, Edinburgh, Scotland.
† Present address: Department of General Physiology, University of Padova, Italy.

METHODS

Eighty *Octopus vulgaris* ranging from 0·3 to 8·5 kg. caught at different seasons of the year by local fisherman in the Bay of Naples were weighed shortly after capture. Their brains were dissected from the surrounding cartilage and main connectives, and each was divided grossly into two optic lobes, suboesophageal lobes and supraoesophageal lobes. (The suboesophageal-supraoesophageal division split the magnocellular lobes which are developmentally part of the 'supraoesophageal'.) In cases where the vertical lobes were measured separately they were first cut away from the top of the brain, and

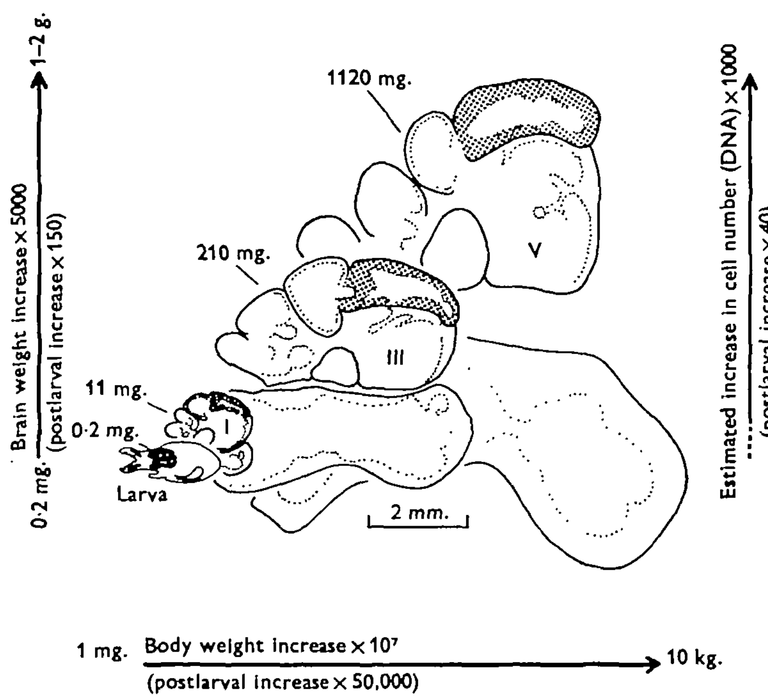

Fig. 1. Superimposed outlines of sagittal sections of brains from three of the five body-weight groups of *Octopus*, with brain wet-weights in milligrams. Approximate body weights are: I, 0·3 g, III, 40 g., V. 5 kg. The cleft running across each brain marks the line of the oesophagus; stippled areas are the vertical lobes. The optic lobes do not appear in these sections. At left, to the same scale, is a sagittal section of *Octopus* at hatching; the brain (0·2 mg.) is shown in black.

values obtained from them were later recombined with the rest of the supraoesophageal mass. The parts (Fig. 2) were weighed fresh on a high-sensitivity Mettler balance. Dry weights of the different lobes of both large and small formalin-fixed brains were 19 ± 3 % wet weight values.

Thirty seven of the brains were selected for estimation of nucleic acids. For purposes of plotting, these brains were considered as belonging to one or other of five groups (I–V) of ten-fold increase in body weight (0·1–1 g., 1–10 g., 10–100 g., 100–1000 g., 1–10 kg.). They were not selected as representing groups, however, but for their distribution at intervals throughout the growth range. The biochemical measurements were made either immediately after death or on fresh material conserved at

−20° C. DNA and RNA were estimated by the method of Scott, Fraccastoro and Taft (1956) modified so as to analyse samples varying in weight from a few milligrams to some hundreds of milligrams. This method measures the absorption of ultraviolet light principally by the purines and pyrimidines of the extracted nucleic acids. Mammalian DNA and RNA (from California Corporation for Biochemical Research)

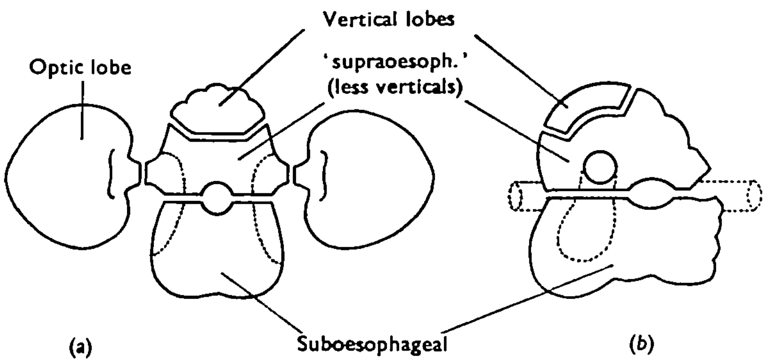

Fig. 2. Divisions of *Octopus* brain as dissected for nucleic acid analyses, (*a*) from posterior, (*b*) from the right side. Diagrammatic. The dotted tube in (*b*) is the oesophagus, the dotted lines on the brain show the extent of the magnocellular lobes (split in these dissections although morphologically belonging to the supraoesophageal lobe only).

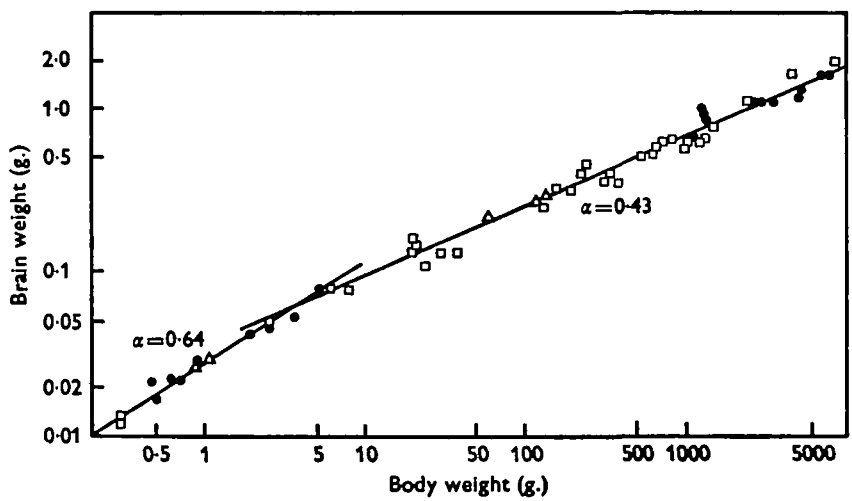

Fig. 3. Growth curve of the brain of *Octopus vulgaris*. Log-log scales. Open symbols, brains analysed for nucleic acids; closed circles, other brains from animals below 10 g. and above 1 kg. body weight; triangles, brains for which wet weights were not taken.

were used for calibration, and milligram values are therefore given as milligram equivalents of mammalian nucleic acid. In our subsequent studies we have used the diphenylamine technique for measuring DNA; they have in general confirmed the results published here.

Scott, Fraccastoro and Taft's procedure allowed us to assay the same piece of tissue first for its RNA fraction (dissolved in an NaOH-HCl mixture) and then for its DNA

Table 1. *Wet weights of brains of* Octopus vulgaris

		Newly hatched	Group I	Group II	Group III	Group IV	Group V
Body weight (g.)	Range		0·28 ~ 0·9	1·9 ~ 8·0	13 ~ 96	131 ~ 1000	1050 ~ 8500
	Average	0·001*	0·55	4·6	39·9	487	3292
Total brain (mg.)	Range		12 ~ 28	40 ~ 84	101 ~ 291	260 ~ 756	602 ~ 1950
	Average	0·2*	20	65	171	485	1150
Brain as % of body weight	Range		5·1 ~ 2·9 %	2·1 ~ 1·0 %	0·88 ~ 0·30	0·24 ~ 0·06 %	0·07 ~ 0·02 %
	Average	20 %	3·8 %	1·6 %	0·51 %	0·13 %	0·04 %
No. of animals			7	8	14	28	20
Mean age estimate in days† (Days post-settling)†		0	> 50 (20)	> 70 (40)	> 90 (60)	6 months–1 year	1–2 years

* From five grouped specimens.

† Mean age estimates for groups I–III extrapolated from data for the Japanese commercial octopus '*Octopus vulgare* Cuvier' Itami *et al.* 1963. This is probably *Octopus vulgaris* Lamarck (see Sasaki, 1929; 'Dibranchiate cephalopods of Japanese waters').

Table 2. *Wet weights of optic lobes of* Octopus vulgaris

		Group I	Group II	Group III	Group IV	Group V
Body weight (g.)	Range	0·28 ~ 0·90	1·9 ~ 8·0	13 ~ 96	124 ~ 1000	1050 ~ 8500
	Average	0·56	4·6	43·3	469	3435
Optic lobes (mg.)	Range	7·3 ~ 20	28 ~ 55	71 ~ 172	158 ~ 420	330 ~ 850
	Average	12·6	43·6	110	270	564
Optic lobes as % total brain wt.	Range	63 ~ 72 %	72 ~ 60 %	70 ~ 55 %	64 ~ 53 %	58 ~ 44 %
	Average	67 %	68 %	66 %	58 %	52 %
No. of animals		4	8	17	31	21

(dissolved in 1·6 N perchloric acid). Some of the RNA findings are published in Tables 5 and 6.

Sperm heads were counted on 5 μl. aliquots of sperm suspended in distilled water placed under 24 × 24 mm. coverslips; the suspensions were assayed for nucleic acids by the method used for brain homogenates. Histological counts were made on photographs of Feulgen-stained serial sections of four *Octopus* brains, one of 0·3 g. and the others of 12 g., 22 g. and 288 g. body weight. Calculations for the whole brain were made after applying the Abercrombie correction (Abercrombie, 1946).

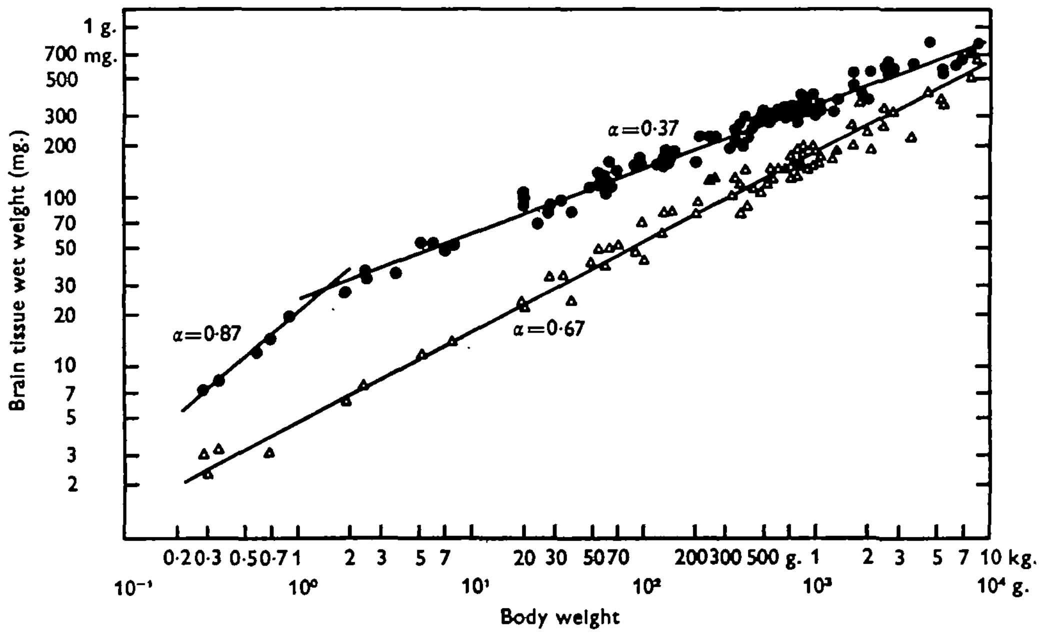

Fig. 4. Growth in wet weight of the suboesophageal divisions (△) and two optic lobes (●) of *Octopus* brain. Log– og scales.

RESULTS

Brain growth (*wet weight*)

Tables 1–4 present the wet-weight values and their averages for both the whole brain and its main divisions. Percentages are for the whole brain relative to body weight and for the suboesophageal, supraoesophageal and optic lobes relative to the total brain. Table 1 includes the brain at hatching. Even though the body weight at this stage contains a substantial contribution of yolk, the brain is one-fifth of the body. Nor is this all the central nervous tissue present, for each of the eight arms has a stout nerve cord. On a conservative estimate these, together with the brain and eyes, must account for one-quarter of the living tissue of the larva. By contrast, our largest brain contributes only one five-thousandth part (0·02%) of body weight; 40% of this is suboesophageal (lower motor) tissue and almost equals the two optic lobes in size, while at a body size of 1 g., optic lobes are together nearly three times as large as the suboesophageal lobes.

Table 3. *Wet weights of supraoesophageal division, including vertical lobes (but excluding optic lobes) of brains of* Octopus vulgaris

		Group I	Group II	Group III	Group IV	Group V
Body weight (g.)	Range	0·28 ~ 0·72	1·9 ~ 8·0	13 ~ 96	131 ~ 1000	1050 ~ 8500
	Average	0·45	4·9	37	512	3130
Supraoesophageal lobes (mg.)	Range	1·0 ~ 3·3	6 ~ 13	12 ~ 47	39 ~ 131	99 ~ 384
	Average	2	9·5	25	83	185
Supraoesophageal lobes as % total brain	Range	9 ~ 15 %	13 ~ 16 %	10 ~ 17 %	12 ~ 21 %	13 ~ 23 %
	Average	12 %	15 %	15 %	16 %	17 %
No. of animals		3	4	11	23	18

Table 4. *Wet weights of suboesophageal division of brain of* Octopus vulgaris

		Group I	Group II	Group III	Group IV	Group V
Body weight (g.)	Range	0·28 ~ 0·72	1·9 ~ 8·0	13 ~ 96	131 ~ 1000	1050 ~ 8500
	Average	0·45	4·9	38	512	3130
Suboesophageal lobes (mg.)	Range	3·2 ~ 3·4	6·4 ~ 14·0	21 ~ 72	64 ~ 203	168 ~ 690
	Average	3·3	10	30·5	147	313
Suboesophageal as % of total brain	Range	28 ~ 15 %	15 ~ 17 %	15 ~ 27 %	22 ~ 30 %	25 ~ 40 %
	Average	23 %	16 %	20 %	26 %	30 %
No. of animals		3	4	10	23	18

Table 5. *Total amounts of brain nucleic acids (mammalian equivalents) in* Octopus vulgaris *at different body-weight stages (groups of* 10*-fold increase in body weight)*

Group	No. of animals	Body weight (g.)		DNA (mg.)		RNA (mg.)		DNA diploid equivalent × 10⁶	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
I	10	0·3	0·28–0·35	0·11	0·10–0·12	0·078	0·078	11	10–12
II	2	5·3	2·5–8·0	0·51	0·32–0·70	0·38	0·32–0·44	49	31–68
III	4	22	20–28	1·01	0·97–1·10	0·58	0·54–0·68	98	94–107
IV	14	490	131–1000	2·01	1·46–2·67	1·80	1·11–2·87	196	142–259
V	7	3010	1050–8500	3·32	2·43–4·40	3·60	2·73–4·46	322	236–427

Fig. 3 is the growth curve of the total brain plotted on double logarithmic co-ordinates. The average rate of increase in weight throughout the life span (the average slope of the log–log curve) is given by α in the allometric equation $y = bx^{\alpha}$ (where y is brain weight and x is body weight). The rate changes during ontogeny, the value of α being 0·64 during the first stages of post-larval life, flattening to 0·43 before a body size of 10 g. is reached. This is chiefly due to change in the growth rate of the optic lobes; the relative growth rate of the optic lobes is particularly high up to a body weight of 1 gram (α = 0·87 based on seven animals), flattening thereafter to 0·37

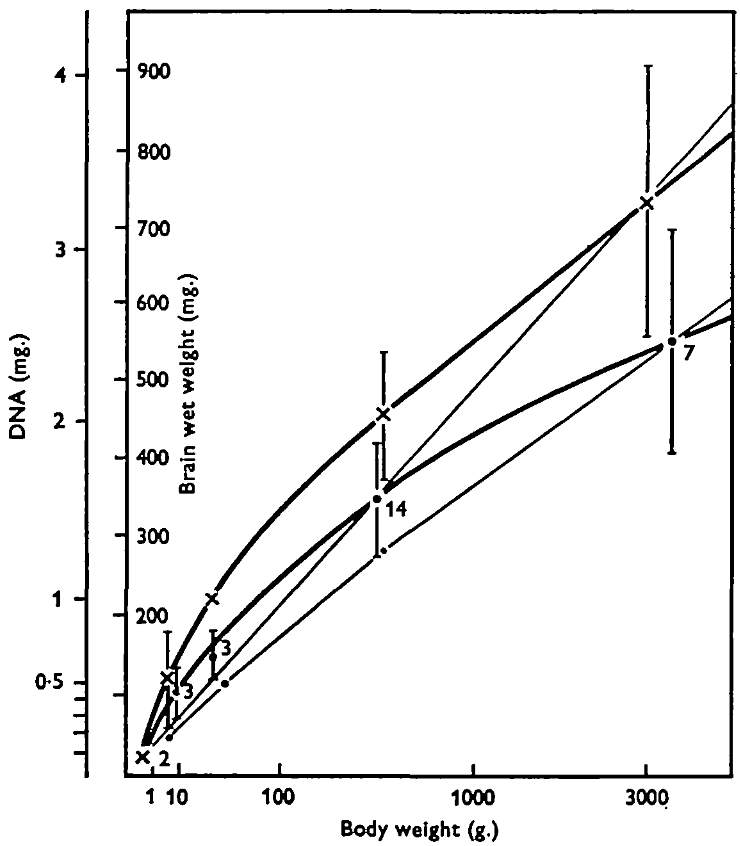

Fig. 5. Curves of DNA increase and of wet weight (straight lines) of the total brain (crosses) and the optic lobes (closed circles) of *Octopus*. Plots are grouped values for each body-weight group (see text). Numbers of samples are indicated alongside, and standard deviations by a line through the plots.

(Fig. 4). Alterations in the value of α in the supraoesophageal lobes are slighter and occur much later, while there appears to be no change in the relative growth rate of the suboesphageal lobes (Fig. 4).

In Figs. 5 and 6 (see next section) in order to facilitate comparison between DNA increase and wet-weight increase of the same part, body weight values on the abscissa have been scaled according to the power value of α for the parts of the brain concerned; thus wet-weight curves preserve a straight line.

DNA increase

The increase in DNA content of the various parts of the brain during growth is given in Figs. 5 and 6. The grouped values (totals only) that served as a basis for these figures are given in Table 5; representative values for individual brains are given in Table 6. The distribution, by weight, of the specimens analysed may be seen in Fig. 1 (open plots).

Table 6. *Representative results of DNA and RNA measurements (mammalian equivalents) on five brains of individual* Octopus vulgaris *designated by letters of the alphabet with body weight in parentheses. The last column gives the number of diploid equivalents (twice the sperm cell equivalent) for the DNA measured*

	mg. wet weight	mg. DNA	mg. RNA	mg. (DNA)/g. (wet weight)	Diploid equivalent $\times 10^6$
ZEY, ZEZ (0·32 g.)*					
Total brain	12·8	0·101	0·078	7·8	10·0
Two optic lobes	7·8	0·086	0·051	11	8·3
Supraoesoph. lobes†	1·3	0·006	0·010	4·7	0·6
Suboesoph. lobes	3·1	0·009	0·017	2·9	0·9
ZCZ (1·5 g.)					
Total brain	50·0	0·32	0·44	6·4	31·0
Two optic lobes	36·0	0·27	0·33	7·5	26·0
Supraoesoph. lobes†	5·2	0·036	0·047	5·8	3·5
(Vertical lobes)	(2·0)	(0·021)	(0·013)	(10)	(2·0)
Suboesoph. lobes	7·7	0·011	0·061	1·4	1·0
ZDA (28 g.)					
Total brain	128·0	1·10	0·68	6·6	107·0
Two optic lobes	82·0	0·83	0·27	8·2	81·0
Supraoesoph. lobes†	12·1	0·15	0·19	5·2	14·5
(Vertical lobes)	(2·5)	(0·074)	(0·027)	(30)	(7·2)
Suboesoph. lobes	34·0	0·125	0·22	1·7	12·0
ZNN (380 g.)					
Total brain	351	1·90	1·40	5·4	185·0
Two optic lobes	210	1·51	0·81	7·2	146·0
Supraoesoph. lobes†	58·5	0·30	0·21	5·1	29·0
(Vertical lobes)	(11·5)	(0·200)	(0·045)	(17)	(19·5)
Suboesoph. lobes	82	0·096	0·39	1·2	9·3
ZDP (4·450 g.)					
Total brain	1598	3·73	4·34	2·3	364·0
Two optic lobes	880	2·98	2·22	3·4	289·0
Supraoesoph. lobes†	289	0·49	0·65	1·7	48·0
(Vertical lobes)	(22)	(0·24)	(0·07)	(11)	(23·0)
Suboesoph. lobes	431	0·26	1·13	0·6	25·0

* Mean results from the pooled brains of two animals of 0·35 and 0·28 g. body weight.
† Including vertical lobes.

The figures show that although DNA amounts continue to increase throughout life, the rate of increase drops relative to the rate of increase in wet weight (DNA curves and wet-weight curves cross).

In Fig. 7 the average rate of increase of DNA in the different lobes is shown as a percentage function (compound interest) of growth in body weight. In simple terms this figure shows, for instance, that every time the body weight doubles during early ontogeny there is an average 40% increase in the DNA content of the supraoesophageal lobes and a 29% increase in the DNA content of the suboesophageal lobes. By the end of ontogeny the averages have dropped to 13% and 10%.

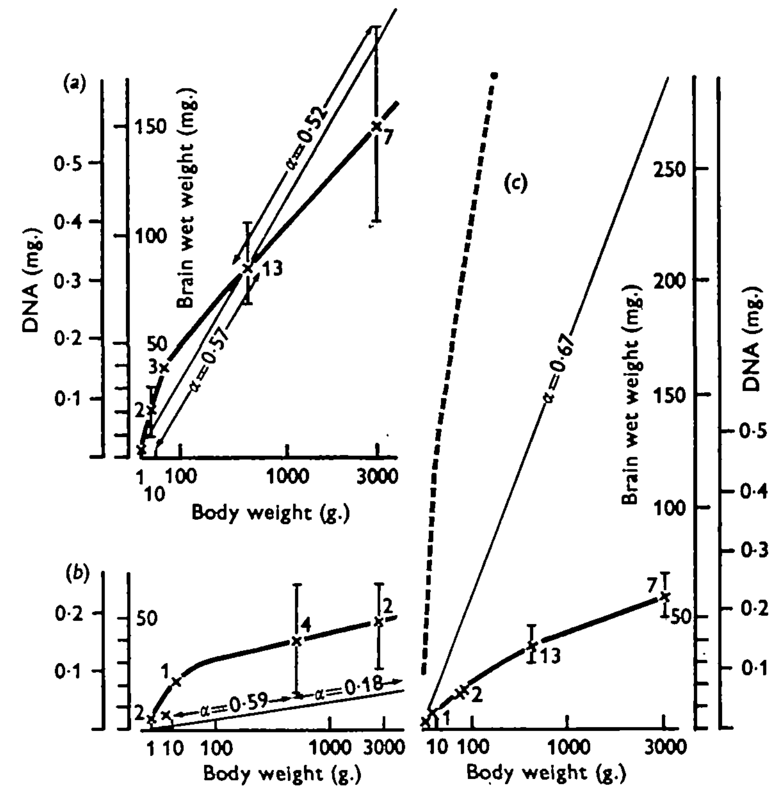

Fig. 6. DNA increase in (*a*) supraoesophageal division, (*b*) vertical lobes, (*c*) suboesophageal division of *Octopus* brain. Conventions as in Fig. 5. The hatched line in (*c*) is the first part of the optic lobes curve.

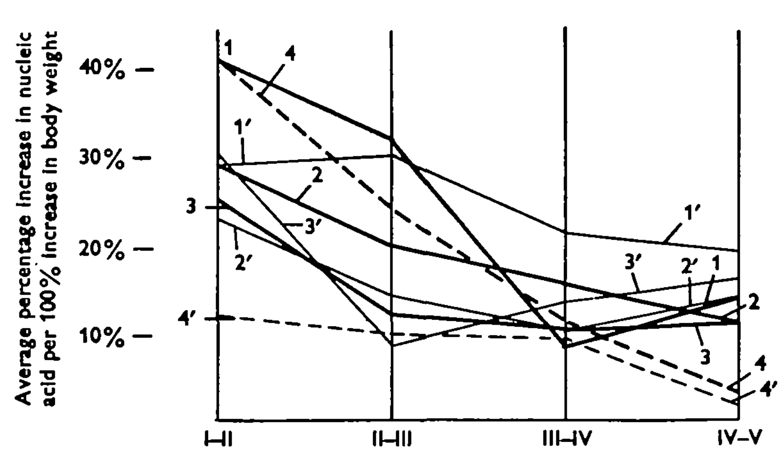

Fig. 7. Rates of increase of nucleic acids per 100% increase in body weight. Rates are given as between successive body-weight groups. Thick lines DNA, thin lines RNA. 1, 1′ suboesophageal division, 2, 2′ supraoesophageal division, 3, 3′ optic lobes, 4, 4′ vertical lobes.

Table 7. *RNA/DNA ratios of grouped brains*

	Body-weight group				
	I	II	III	IV	V
No. in sample	8–12	2–3	4–11	4–17	2–7
Vertical lobes	0·47	0·28*	0·26	0·53	0·30
Supraoesophageal lobes including vertical lobes	0·72	0·68	0·73	0·87	0·86
Two optic lobes	0·62	0·68	0·60	0·65	0·80
'Suboesophageal' lobes	1·7	2·4	1·7	3·5	4·7
Total brain	0·73	0·74	0·57	0·88	1·1

* By diphenylamine technique from pooled results of four brains, 1966.

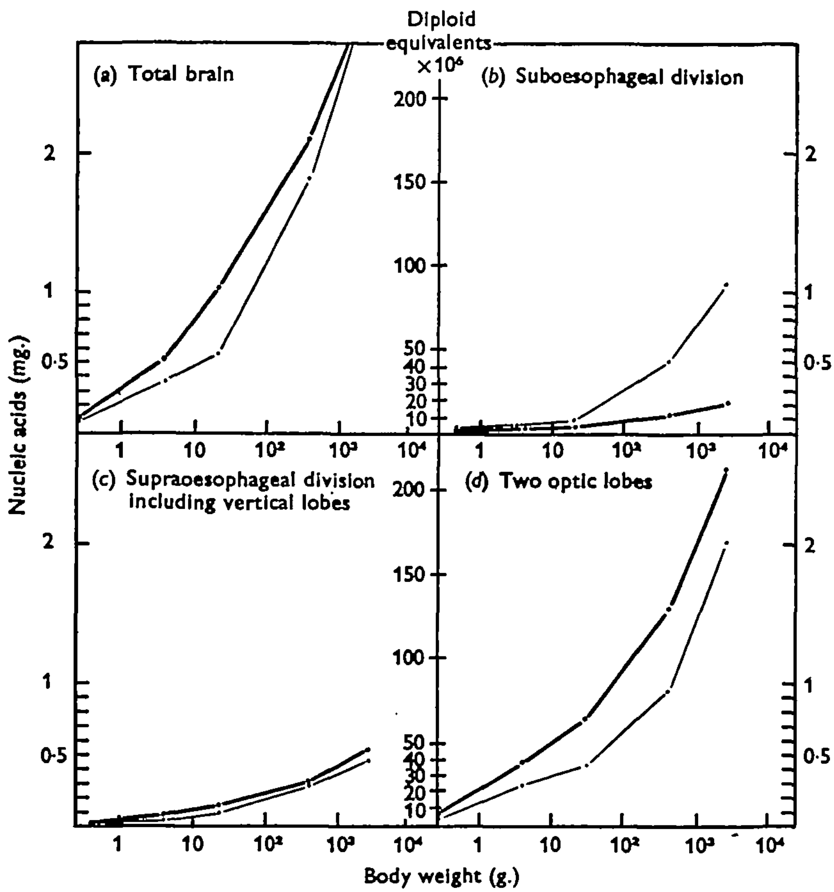

Fig. 8. Nucleic acids (DNA thick lines and RNA thin lines), and the diploid equivalents estimated from DNA in the main divisions of the brain of *Octopus*.

RNA

During most of the life history there is rather less RNA in the brain than DNA. Only in the largest animals does the amount equal or exceed that of DNA, due almost entirely to increase in the suboesophageal division. In the other main divisions the curves of RNA increase (Fig. 8) follow the same general course as the DNA curves. There is, however, considerable individual variation in the ratio of the two nucleic acids from one brain to another. Overall ratios for the different body-weight groups are shown in Table 7. In the vertical lobes, in which there is rather little cytoplasm surrounding the nuclei, the RNA/DNA ratio is particularly low.

Cell numbers (the diploid equivalent)

The right-hand columns of Tables 5 and 6 provide an estimate of total cell numbers —the diploid cell equivalent—for whole brains and their various parts that the given quantity of DNA represents. These estimates are based on the finding that 1 mg. equivalent of DNA is yielded by $1{\cdot}94 \times 10^8$ sperms ($5{\cdot}2 \times 10^{-9}$ mg. DNA/sperm). Thus, assuming all DNA to be in the chromosomes, 1 mg. equivalent of DNA in *Octopus vulgaris* represents $9{\cdot}7 \times 10^8$ diploid cells ($1{\cdot}03 \times 10^{-8}$ mg. DNA/diploid nucleus).

As our method of counting sperms was not the most accurate available, the haploid and diploid figures are provisional.

Table 8. *Nuclear counts. Comparison between histological counts and 'diploid equivalents' calculated from sperm-calibrated DNA analyses. Indications in parentheses refer to the body weightgroup or to the specimen(s) and body weight*

Octopus and body weight	Brain division	Histological count $\times 10^6$	Diploid equivalent $\times 10^6$	Ratio
Cajal-stained nuclei (neurones only)				
400–600 g. (IV)	Vertical lobes	25*	14·5 (Group IV average)	1:0·6
	Supraoesophageal lobes (less vertical lobes)	12*	16 (Group IV average)	1:1·3
	Two optic lobes	130*	154 (Group IV average)	1:2
	'Suboesophageal lobes'	2·8*	14 (Group IV average)	5 1·
Feulgen-stained nuclei (all nuclei)				
0·3 g. (I)	Vertical lobes	1† (upper limit 2)	0·51 (ZGH-P‡, 0·3 g.)	1:0·5
ZFL 12 g. (III)	Suboesophageal lobes	1·26§ } 2·1	3·7 (ZFF-G‖, 20 g.)	1:3
FAL 22 g. (III)	Suboesophageal lobes	2·9§ } 2·1		1:1·3
FAG 228 g. (IV)	Vertical lobes	11§	14·5 (Group IV average)	1:1·3
	Suboesophageal lobes	5·6§	13 (ZFA, 218 g.)	1:2·4

* From Young (1963).
† Specially counted by Prof. J. Z. Young.
§ Counted by C. Tagliacozzo.
‡ Eight specimens of average body weight 0·3 g.
‖ Three specimens of 20 g. body weight each.

The quantity of DNA (4·4 mg.) found in the largest brain is equivalent to a diploid figure of 43×10^7 nuclei, and that in the smallest brain (12 mg.) to a figure of $9{\cdot}7 \times 10^6$.

A detailed count of cell nuclei in a single brain of *O. vulgaris* has been undertaken by Young (1963). The body weight of the animal is not given, but judging by its size the brain probably belonged to an animal weighing between 400 and 600 g. Cells were stained by the Cajal method, and Young's values are for neuronal nuclei—including amacrines and 'granular' cells—but not glia, blood vessels and membranes (personal communication). They are compared in Table 8 with those obtained from DNA determinations on animals of the 100–1000 g. body weight group (IV). The table also gives histological counts of nuclei in the suboesophageal and in the vertical lobes. The right-hand column of this table shows the ratio between numbers of nuclei counted histologically and the average diploid equivalent of the same part in the relevant size group. It will be noticed that the ratios are as high as 1:5 for the suboesophageal lobes and low (close to a 1:1 correspondence) for the lobes containing few large cells (see Young, 1963). Since the large cells of the suboesophageal areas have many more glial elements attached to them than do small neurons (Gray, 1969)—while the very small cells of the vertical lobes are in direct contact one with another, with few glia interspersed—the additional nuclei indicated by our biochemical assays are probably due to the glial contribution.

DISCUSSION AND CONCLUSIONS

The relative growth of the octopus brain is rather modest compared with that of squids and cuttlefish (A. Packard, unpublished, and Wirz, 1959). For instance, its size in our largest (8·5 kg.) specimen is no larger than that possessed by *Sepia officinalis* at 1 kg. body weight. It is also considerably smaller than the brain of the dogfish (*Mustelus canis*) whose growth was measured by Kellicott (1908); in a newly hatched dogfish of 70–80 g. the brain weighs 0·8 g. and attains 6 g. in a 7 kg. animal. On the other hand, the octopus brain is larger, body weight for body weight, than that of most teleosts studied by Geiger (1956). While the slope of the allometric curve is similar, only the most advanced of the teleost species (the cyprinid, *Barbus fluviatilis*) have absolute brain weights similar to those of *Octopus vulgaris*. After converting the *Octopus* figure to dry weights, it is apparent that most of the other teleosts, of comparable body size range but belonging to more primitive families (such as the pike, *Esox lucius*), have absolute brain-weight values along the allometric curve as low as or lower than one third the values of *Octopus vulgaris*. Unfortunately we have no data on cell numbers during growth in the brains of these or other fish species.

The differential growth of the suboesophageal lobes which continues in later stages at a higher rate than the other divisions is presumably related to the increased amount of muscle in large bodies and the greater distance which the neurones have to travel (requiring larger cell bodies), but the substantial increase, also into late stages, of the optic lobes is puzzling. They contain not only higher motor centres, but also a cortical region of deep retina that goes on expanding with growth of the eyes. The eye growth curve throughout the life span together with receptor counts in early stages has been published elsewhere (Packard, 1969) and an estimate of the increasing number of receptors playing onto the optic lobes was given in Packard (1966, Fig. 3); however, although the size of the optic lobe is clearly related to size of the eyes—in fact octopuses with one eye abnormally small also have a small optic lobe—we have no functional explanation for the sustained growth of the eye and of its central connexions, growth that continues well beyond the time when behaviour can be expected to have matured.

During the large accumulation of cells that takes place in *Octopus* with age there may well be changes in the glia/neurone index in favour of glia, but the accumulation cannot be entirely due to glial cells even in the lower motor centres. Motor structures (e.g. the chromatophore organs) increase in number throughout life and the histological counts on adult brains show many motor neurones that were not present in the young animal. Certainly, the lower centres of the octopus brain are in this respect very different from the insect ventral nerve cord. Edwards (1967) found that in post-embryonic growth of the terminal ganglion of the house cricket, *Acheta*, cell increase was entirely due to glial contribution, the number of neurones remaining constant.

Before we had read Gray's paper we were inclined to think that the excess of DNA in the suboesophageal regions of the brain (containing large nuclei and large cells) might be due either to polyploidy or to a substantial component of cytoplasmic DNA. However, neither seems to be the case. The latest counts (unpublished) on nuclei in brain homogenates give a rather close correspondence between the amounts of DNA per nucleus in the different lobes. Also, although we have not made direct measurements on single nuclei, intensity of staining with the Feulgen reaction decreases as the size of nucleus increases. In the large (15–25 μ) class of nucleus found in the sub-oesophageal part of the brain, Feulgen pink is hardly discernible at all; this is in marked contrast with the large, polyploid, nuclei of gastropod ganglia which react with the same intensity as small nuclei when sections are stained by the Feulgen method.

In the vertical lobe, whose cells are uniformly small and closely packed together with very little cytoplasm surrounding each nucleus, histological counts and DNA estimates of nuclear content correspond sufficiently closely to allow one to assume not only that these cells are diploid but that the curve of DNA increase (Fig. 5*b*) is the curve of total neurone increase in the vertical lobes. Unfortunately it is established on readings of only eight specimens and it is not easy to ensure that all of the vertical lobe was dissected away in every case. In the other lobes the DNA curves, based on readings of many more samples, also indicate trends in neuronal increase even though the histological counts did not reveal a one-to-one correspondence between the diploid 'equivalent' figure and cell numbers.

The changes in connectivity that accompany growth of the brain have not been investigated, but connexions can not remain unaffected by the increase in cell number (Packard, 1966). Changes will presumably be of two kinds: (i) increase in the total of connexions, producing parallel circuits and resulting in increased capacity, and (ii) alteration in the number of connexions per cell producing greater discreteness or greater diffuseness. Both will have behavioural implications, and a whole field of study relating stages of cerebral development to performance is waiting to be explored. We have some general evidence from body-pattern repertoires (Packard & Sanders, 1969) that there is a shift from limited fixed-pattern type of behaviour in the earliest stages, when the number of brain cells is of the order of 10 million, to the more differentiated and less predictable behaviour characteristic of later growth in *Octopus* when the number is of the order of 100 million.

It would be interesting to know whether the very large increase during the life history is the result of an expansion in the numbers of all neuronal elements equally, or predominantly in one class of neurones only, especially in lobes such as the optic with a mixed population of nerve cells and an extensive growth range. We have no evidence, however, which would enable us to answer this question, just as we are still gnorant of the source of new neurones in the growing brain.

SUMMARY

1. The brain of *Octopus vulgaris* grows allometrically with respect to body weight from 12 mg. at the beginning of post-larval life to 1·95 g. at a body weight of 8·5 kg.

2. The two optic lobes contribute 64% to the wet weight total at the beginning of the growth range and 44% at the end.

3. The 150-fold increase in brain weight is accompanied by a 40-fold increase in total DNA content, 80–85% of which is in the optic lobes. DNA concentrations of the order of 20 mg./g. wet weight are found in the vertical lobes.

4. An average of 1 mg. DNA is yielded by $1 \cdot 9 \times 10^8$ *Octopus* sperms and this figure is used to calibrate the nucleic acid content of the optic lobes, and of the suboesophageal and supraoesophageal divisions of the brain in terms of their 'diploid equivalent'.

5. The ratio between the 'diploid equivalent' and the numbers of neurones counted histologically is highest for the suboesophageal division and lowest for the vertical lobes and probably indicates a high glia/neurone index in brain areas with many large cells and low index in those with a homogeneous population of small cells. The brain cells of *O. vulgaris* do not appear to be polyploid.

6. The RNA/DNA ratio is less than 1·0 in the optic and supraoesophageal lobes, higher than 1·0 in the suboesophageal lobes. The ratio increases during growth, especially in the suboesophageal division.

We wish to thank Prof. J. Z. Young for vertical lobe counts and Mrs C. Tagliacozzo for her patient assistance, also Prof. F. Ghiretti and Dr P. Tardent for their advice in the early part of this work which was supported by a grant from the National Research Council of Italy to one of us (V.A.).

REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–47.

ALTMAN, J. (1967). Postnatal growth and differentiation of the mammalian brain, with implications for a morphological theory of memory. In *The Neurosciences, a Study Program*, pp. 723–43. Eds. Quarton, G. C., Melnechuk, T. and Schmitt, F. O. Rockefeller University Press.

BRADFORD, H. F., CHAIN, E. B., CORY, H. T. & ROSE, S. P. R. (1969). Glucose and amino acid metabolism in some invertebrate nervous systems. *J. Neurochem.* **16**, 969–78.

EDWARDS, J. S. (1967). The development of the insect nervous system. I. Analysis of postembryonic growth in the terminal ganglion of *Acheta domesticus*. *J. Morphol.* **123**, 191–8.

GEIGER, W. (1956). Quantitative Untersuchungen über das Gehirn der Knockenfische, mit besonderer Berücksichtigung seines relativen Wachstums. I. *Acta anat.* **26**, 121–63.

GRAY, E. G. (1969). Electron microscopy of the glio-vascular organisation of the brain of *Octopus*. *Phil. Trans.* B **255**, 13–22.

ITAMI, K., IZAWA, Y., MAEDA, S. & NAKAI, K. (1963). Notes on the laboratory culture of the octopus larvae. *Bull. Jap. Soc. sci. Fish* **29**, 514–20.

KELLICOTT, W. E. (1908). The growth of the brain and viscera in the smooth dogfish (*Mustelus canis*, Mitchill). *Am. J. Anat.* **8**, 319–53.

PACKARD, A. (1966). Operational convergence between cephalopods and fish: an exercise in functional anatomy. *Archo. zool. ital.* **51**, 523–42.

PACKARD, A. (1969). Visual acuity and eye growth in *Octopus vulgaris* Lamarck. *Monit. zool. ital.* (N.S.) **3**, 19–32.

PACKARD, A. & SANDERS, G. (1969). What the octopus shows to the world. *Endeavour* **28**, 92–99.

SCOTT, J. F., FRACCASTORO, A. P. & TAFT, E. B. (1956). Studies on histochemistry. I. Determination of nucleic acids in microgram amounts of tissue. *J. Histochem. Cytochem.* **4**, 1–10.

WIRZ, K. (1959). Étude biométrique du système nerveux des céphalopodes. *Bull. biol. France Belg.* **93**, 78–117.

YOUNG, J. Z (1963). The number and sizes of nerve cells in *Octopus*. *Proc. zool. Soc. Lond.* **140**, 229–54.