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STUDIES IN NEURO-ENDOCRINOLOGY:

STUDY 1: THE EFFECT OF PRE-PUBERTAL GONADECTOMY ON THE GROWTH RATE OF CATS.

STUDY 2: A COMPARATIVE STUDY OF SELECTED ASPECTS OF THE POSSUM (TRICHOSURUS VULPECULA KERR) BRAIN.

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Physiology at Massey University.

Anthony Kettle
1983.

ABSTRACT

Gonadectomy is the commonest surgical procedure carried out in the cat, mainly for fertility control. However, the effect of gonadectomy on body weight in the cat has received little study although this subject is well researched in other species, such as the rat. Part I of the present study involved gonadectomy at 20 weeks. The cats were housed in outdoor colony cages and given food ad libidum, adjusted to leave daily residues. Body weight was measured weekly and transformed to $\log_{10}$. For statistical analysis, differences in body weight of each cat were tested by one-way analysis of variance and serial covariance using the previous week's body weight as the covariate. Differences between groups were investigated with 't' tests and growth rates were studied by regression. Up to 32 weeks of age there was no statistically significant difference between the growth rates of entire versus the castrates in either sex. However, when extended to 55 weeks of age prepubertal gonadectomy in the female cat caused significantly increased growth. This was not observed for the male cat.

Little information is available on the anatomy of the brain of the Australasian possum (Trichosurus vulpecula). Part II of the present study aims at presenting a simple description of the possum hypothalamus viewed in three planes of section and concentrating on some of the fibre tracts which are clearly visible. The main findings were that the mammillothalamic tract appears in a similar position to that as seen in other mammals such as the rat, cat, and sheep, while the fornix appears much steeper in its descent into the anterior hypothalumus. In addition, there is described a fibre tract emanating from the optic chiasma and passing to the caudal part of the paraventricular nucleus. This tract has not been described in other mammals, such as the rat, cat, and sheep.

PREFACE

In 1977 Ms E Sommerville and Dr M Tarttelin of Massey University conducted a preliminary study on the effect of pre-pubertal gonadectomy on the growth rate of cats. The results of this preliminary study were viewed with caution due to a suspected nutritional inadequacy in the diet. The present study was therefore undertaken using an experimental canned cat diet produced by J Wattie Canneries Ltd., which had an increased protein level. The results of this study were compared to the former investigation.

Part II of this thesis arose from a personal interest in the New Zealand possum (Trichosurus vulpecula) and was carried out during the later part of the year when the daily work involvement for the cat growth study was reduced.

The two studies are quite unrelated except in so far as it was hoped to be able to identify a definitive ventro-medial hypothalamus (VMH) in the hypothalamus of the possum. The VMH region in cats has been implicated in the control of growth, thus providing a tentative link between the two studies.

The title 'possum' for the New Zealand species of Trichosurus vulpecula and 'oppossum' for the American, Didelphis virginiana was adopted in line with a growing convention amongst investigators in the field, although there is still considerable controversy over the use of these titles as commented on in a recent edition of Possum Post (Possum Post No. 2:2 December 1980).

ACKNOWLEDGEMENTS

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Mr M.J. Birtles kindly provided expert technical assistance with the possum histology and put the equipment of his laboratory at my disposal, while Mrs U Darell, Mr E. Ormsby, Ms E Sommerville, Miss S Merritt, and Miss C Collins, helped me with the management of the cat colony in the Small Animal Production Unit of Massey University.

The possums were kindly trapped by Mr and Mrs R. McCammon and Mrs E. Bristol.

I also acknowledge the staff of J. Wattie Canneries Ltd., in particular Mrs J Newell and Mr B. Trass for their help in compounding the CX9 canned cat diet and in supplying the entire diet free of charge.

I would like to thank Mr T. Law for the photography and Wordcom for typing of the manuscript.

Anthony Kettle
1983

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PART I

THE EFFECT OF PRE-PUBERTAL GONADECTOMY ON THE GROWTH RATE OF CATS.

CAT STUDY INTRODUCTION

The control of growth, and in particular, the endocrine control of growth, has been the topic of a great deal of research for a number of years. It is now generally agreed that there is a polypeptide hormone, growth hormone, secreted from acidophilic cells in the anterior pituitary (Merimee, 1979) which is capable of producing somatic growth either directly, or indirectly through somatomedin. In the absence of growth hormone, somatic growth ceases (Nalbandov, 1963). Currently, it is thought that growth hormone release is mediated via any of three neutral centres - the ventromedial nucleus, the arcuate nucleus, and the limbic system (Merimee, 1979), although there are a number of stimuli whose locus of action remains unclear (Sorentino et al., 1972; Merimee, 1979); these include stress (Greenwood and Landon, 1966), estrogens (Frantz and Rabkin, 1965), exercise (Schalch, 1969), and protein depletion (Pimstone et al, 1966).

Whole-body growth is merely an amplification of cellular development. Thus, growth may be divided into two components: - an increase in cell numbers (hyperplasia), or an increase in cell size (hypertrophy). There is now considerable evidence (Rosenfield, 1979), that these two components may be mediated via cyclic nucleotides (cAMP and cGMP), which may be important regulators of growth (Burger et al., 1972; McMahon, 1974). Therefore, factors that can act directly on cellular nucleotide levels may have additional growth inducing or inhibiting properties (for example, prostaglandins, Samuelsson et al., 1978). Thus growth is the integrated result of many hormonal, neuronal, and metabolic factors.

Many studies have been performed to investigate the influence of sex and gonadectomy on the growth and development of several species of animals including sheep (Everitt and Jury, 1966); cattle (Arthur, 1959; Maclean, 1969); pigs, (Walstra and Kroeske, 1968); turkeys (Smith and Smyth, 1963), and rats (Kakolewski et al., 1968; Tarttelin and Gorski, 1973; Clarke and Tarttelin, 1978). However it would appear that very few similar studies have been carried out in the cat or bitch.

For the bitch, correspondence on the consequence of gonadectomy has appeared in the Veterinary Record from time to time (Warren, 1965; Smythe, 1968; Pengelly, 1968; Rhodes, 1968; Lane, 1968), While Joshua (1965) has published a paper on the spaying of bitches. This wealth of

anecdotal information is, however, seriously lacking in experimental evidence which would enable a comparison between the hormonal control of growth in the bitch and the other species studied.

For the cat, there is some evidence for a sex difference on growth rates (Latimer and Ibsen, 1932; Payne et al., 1966; Scott P. 1972; Rosenstein and Berman, 1973) but no evidence for the influence of gonadectomy on the growth rates, although work on behavioural aspects (Hart and Barrett, 1973) and the effect of prepubertal castration and urethral and periurethral tissues (Herron, 1971; Herron, 1972) has been undertaken. The lack of evidence for the influence of gonadectomy on the growth rates is a surprising find for an operation which, according to Burke (1977), is performed on 50 to 60 percent of all male cats and which, for the female, is the 'most frequently performed veterinary surgery'.

In an attempt to ascertain the role, if any, of the gonadal steroids in physiological growth the present investigation of prepubertal gonadectomy in cats was carried out and forms Part II of an earlier study by Sommerville and Tarttelin (unpublished observations 1977-78). Growth changes following postpubertal gonadectomy, as a sequal to the present study, is currently being investigated.

Palsson and Verges (1952) found that differences in growth rate between sexes in sheep were apparent only when the nutritional regime on which the lambs were reared was adequate for growth. On an inadequate plane of nutrition, there was virtually no difference between the growth rates of males and females. A search of the available literature has revealed several experiments on the nutritional requirements of cats (Dickinson and Scott, 1956; Payne et al., 1966; Schneck and Cumberland, 1968; Edney, 1972; Kronfeld, 1976) and these diets were evaluated with the standard diet used in the present investigation.

This study was discussed as part of a paper read at the Physiological Society of London meeting in Cambridge (UK) in June 1981 (see Appendix 4).

CAT STUDY MATERIALS AND METHODS SECTION

Eight female cats were mated to the same male and housed in individual cages (see Appendix 1) one week prior to the expected date of parturition. The cats were kept in these cages with their litters until weaning at 8 weeks. At weaning, the kittens were sorted out such that entire males were kept with females assigned to gonadectomy and entire females with males assigned to gonadectomy. The assignment of kittens to treatment groups was purely random, but housing was by selection to ensure that entires of opposite sexes were not housed together.

Table 1 Summary of cat birth data

Female Cat	No. in litter	Survived	Males	Females
1	2	2	2	-
2	5	5	1	4
3	2	2	2	-
4	5	2	2	-
5	4	4	3	1
6	5	4	2	2
7	3	3	3	-
8	5	2	1	1
Total	31	24	16	8

From these eight females 24 kittens were raised (see Table 1). The eight litters were born over a twelve week period and as each litter reached the 8 week mark, the kittens were weaned and wormed with Piprazine tablets (see Appendix 2). All kittens were innoculated at 12 weeks with Vaxitas FVR-CP (see Appendix 2).

In spite of vaccination there were sporadic bouts of rhinitis, characterised by occasional sneezing, oculo-nasal discharge, and coughing in some instances. In the majority of cases, treatment was unnecessary, but in persistent cases when weight loss occurred treatment with the antiboitic Tylan (see Appendix 2) was given. Occular infections and the occasional conjunctivitus were treated with Soframycin eye ointment (see Appendix 2).

At 20 weeks of age all kittens underwent surgery as per Table 2. Twenty weeks was chosen for the time of gonadectomy, as a preliminary study (Sommerville and Tarttelin 1977, unpublished data) had carried out gonadectomy at this time, twenty weeks is 3 months post-weaning and allows sufficient time to study the growth patterns at this age and 20 weeks is well before puberty.

Table 2 Summary of kitten treatment groups.

Litter No.	Males		Females	
	Gonad.	Sham.	Gonad.	Sham.
1	1	1	-	-
2	1	-	2	2
3	-	2	-	-
4	1	1	-	-
5	1	2	-	1
6	1	1	2	-
7	2	1	-	-
8	1	-	1	-
Total	8	8	5	3

The panhysterectomy of the kittens involved a left flank incision with the animal in a lateral recumbent position, anaesthesia being induced and maintained on a 4% fluothane-oxygen combination. Both ovaries and uterii were removed through the same incision while the sham surgery involved only the initial skin incision, and subsequent suturing with fine nylon thread. Prophylactic treatment by subcutaneous injection of Propen LA (see Appendix 2) was given, and the skin wound was dressed by a spray of Nobectane (see Appendix 2). In only one instance did a minor infection occur, and this was treated with Penbritten tablets (see Appendix 2) for one week.

Castration of the male kitten was by a single incision through the scrotum to the tunica albuginea. The testis was removed complete with its tunic in each case, steady traction ensuring a high severance and retraction of the vascular and spermatic cords. No suturing was needed. The sham surgery involved only the plucking of fur from the scrotum whilst under anaesthetic.

The kittens were fed entirely on a specially formulated canned cat diet (see Appendix 3). As the kittens were not housed individually but rather in groups of 3 or 4, an accurate measure of food uptake could not be made. However, the daily consumption of this food was estimated to range from just under half a 425 gram tin per cat to an entire can per cat. All feeding was carried out within a two hour period centred on 3.00 p.m. Each cage received a sufficient quantity of food estimated to leave a small residue at the end of each day.

Newborn kittens weighing less than 100 grammes were weighed on a small Avery balance whose scale was marked in 1 gramme divisions, and which could weigh to 1 kilogramme. This gave a more accurate reading of the weight of the younger kitten and helped to reduce the error in the smaller readings.

Older kittens were weighed Monday and Thursday (approximately 3.00pm) in a tared plastic cage on a larger Avery platform balance capable of weighing to 10 kilogrammes. The cats were weighed to the nearest gramme. All weighings were carried out by the same person to minimise weighing errors.

BIOMETRICAL CONSIDERATIONS

Body weights (BWt) of the cats were measured twice weekly but were reduced to one weekly mean in each case. Body weights measured over a period of time show heterogeneity of variance and highly signiciant correlation of mean and standard deviation as can be seen from Fig 1a, in which BWt is plotted against standard deviation. A simple logarithmic transformation of BWt removed this correlation with variance (Fig. 1b). A similar result was reported with rat BWt analysis (Clark & Tarttelin, 1978). Data analysis was by a series of one-way analyses of variance (ANOVAR) on the transformed BWt data of the four treatment groups. Although no treatment was instigated until 20 weeks of age, cats were assigned to treatment groups at weaning and were analysed in those groups from week one. Where the ANOVAR revealed a significant difference, further tests were carried out to identify the groups contributing to the significant difference by using "t" test. The error mean square derived from the ANOVAR was weighted according to the number of each treatment group and used in place of the usual pooled error variance in the computation of the value of "t". There were 4 treatment groups giving a total of 3 degrees of freedom available for test; the degrees of freedom were allocated as follows:

1. Female entire versus female spayed
2. Female entire versus male entire
3. Male entire versus male castrate.

Further analysis was by an analysis of covariance (ANCOVAR) using the body weight at each week as the covariate for the following week's BWt. The ANCOVAR tested differences between treatment groups independent of the previous week's BWt. Where a significant "F" ratio resulted then the groups contributing to the difference were identified using a series of "t" tests (with 3 degrees of freedom according to the comparisons above)on the adjusted means using the appropriate weighted error mean square derived from the ANCOVAR computation.

RESULTS

BODY WEIGHT

The untransformed body weight data measured twice weekly from all the cats is plotted in Figure 2. The log transformed data expressed as weekly means for the 4 treatment groups is plotted in Fig. 3. Data taken from an earlier study (Sommerville & Tarttelin, 1977, unpublished observations) and plotted in a similar manner is given in Fig. 4 for comparison.

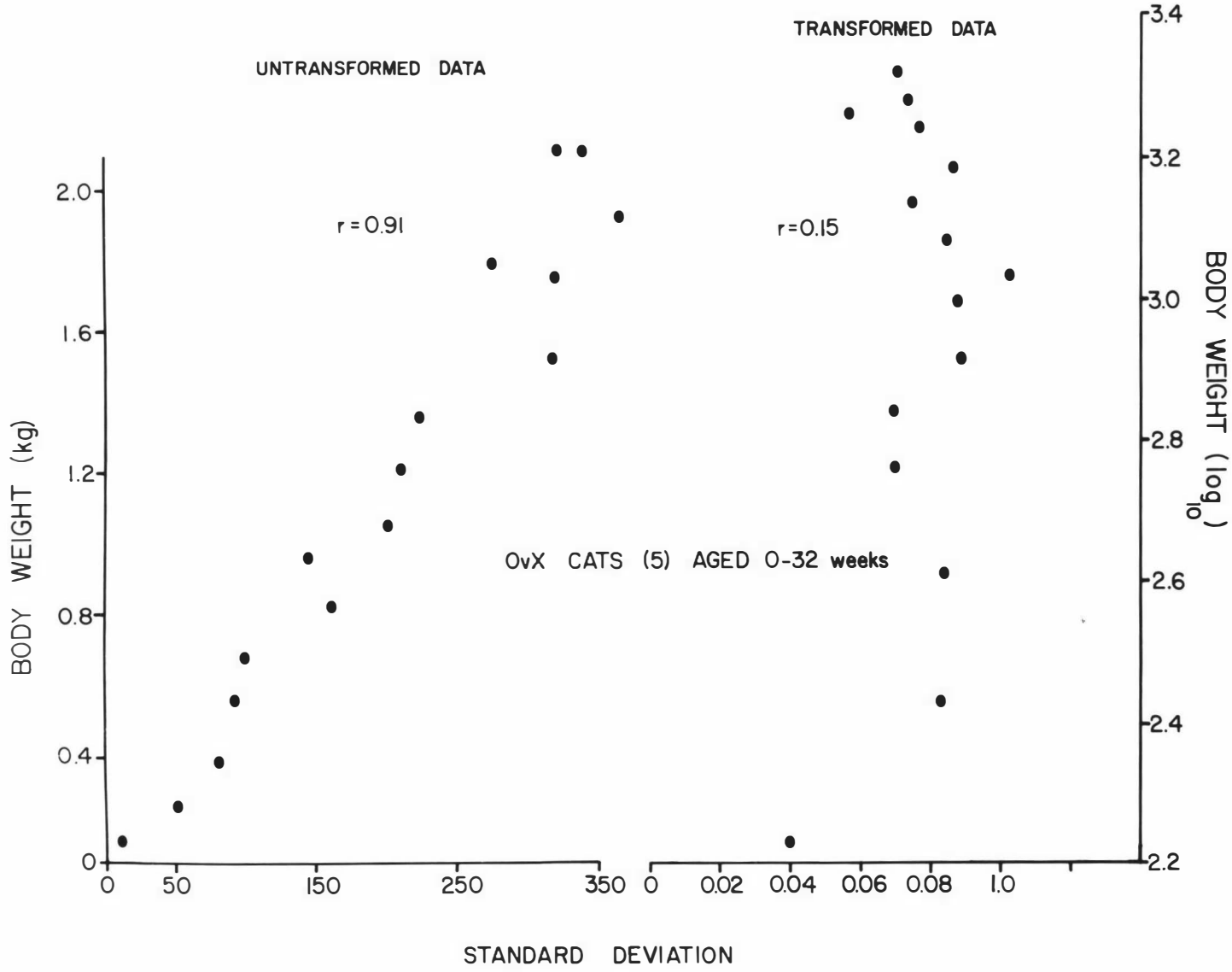
A summary of the transformed means and the results of the ANOVAR and ANCOVAR is given in Table 3. It can be seen from Table 3 that the ANOVAR shows significant differences from week 7 onwards. Further examination of data using the "t" as discussed above shows that the predominant contribution to the significant difference was the entire male versus the entire female comparison which was significant from week 8 onwards. A few weeks (weeks 8, 9 & 10) showed a difference between the groups allocated entire males and castrated males but at that time both groups were entire and the difference was due to random selection of a heavier cat in the one group. Generally there were no differences in the group designated female spayed and entire female nor the entire male and castrated male.

The critical period for examination was after week 20 following surgery. The data analysis shows that there was no affect of surgery, neither spaying nor castration, on BWt up to week 32 when the present study terminated.

The ANCOVAR showed significant differences only from week 7 to week 9. The "t" tests on the adjusted means showed that only the entire male-entire female comparison was significantly different. What the ANCOVAR shows is that from week 7 to week 9 there is a significant divergence of BWt between the male and female cats. After week 9 there is no further separation of BWts but the ANOVAR proves that the male cats remain heavier than the female cats.

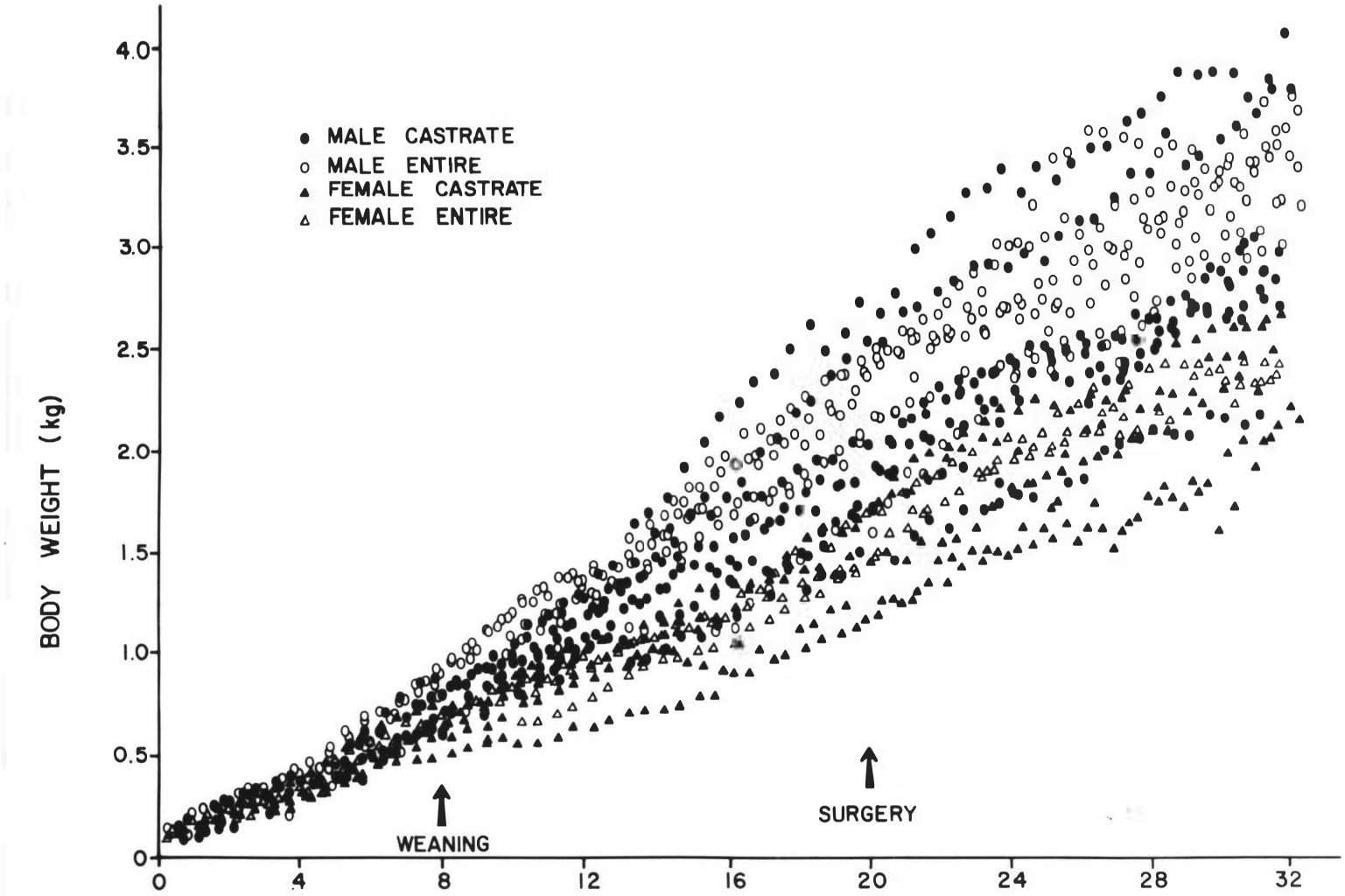
Legend Figure 1a and 1b.

The Graph on the left relates body weight (g) of one group of cats (female, OvX at week 20; representative to all groups) with standard deviation showing a high degree of correlation. The other graph shows no relationship between mean and standard deviation when the body weight is expressed as a logarithmic transformation.


UNTRANSFORMED DATA
TRANSFORMED DATA
r = 0.91
r = 0.15
OvX CATS (5) AGED 0-32 weeks
BODY WEIGHT (kg)
BODY WEIGHT (log10)
STANDARD DEVIATION
0
0.4
0.8
1.2
1.6
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
0
50
150
250
350
0
0.02
0.04
0.06
0.08
1.0

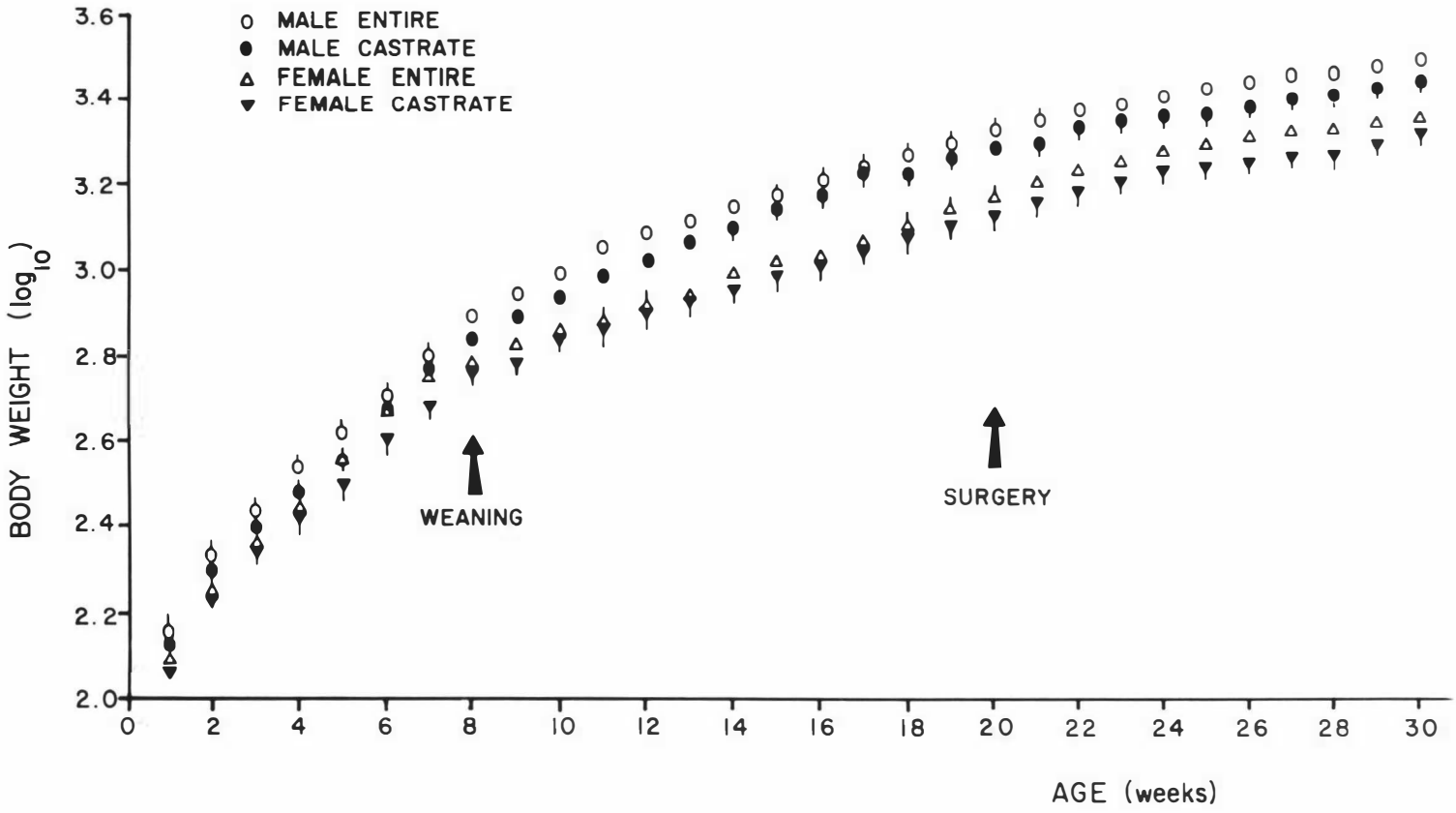
Legend Figure 2

Scattergram of all measured body weight data in the untransformed form (BWt in g) for all treatment groups during the 32 week study period.


MALE CASTRATE
MALE ENTIRE
FEMALE CASTRATE
FEMALE ENTIRE
BODY WEIGHT (kg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
4
8
12
16
20
24
28
32
WEANING
SURGERY

Legend Figure 3

Graphs relating body weight in logarithmic transformation of all treatment groups during the 32 week study period. Arrows indicate the time of weaning and time of surgery; entire cats had "sham" surgery at 20 weeks.


MALE ENTIRE
MALE CASTRATE
FEMALE ENTIRE
FEMALE CASTRATE
BODY WEIGHT (log10)
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
WEANING
SURGERY
AGE (weeks)

Legend Figure 4

Graphs relating body weight in logarithmic transformation of all treatment groups during the 32 week study period of the preliminary experiment carried out in 1977 (Sommerville & Tarttelin, unpublished data). Arrows indicate the time of weaning and surgery; entire cats and "sham" surgery at 20 weeks.

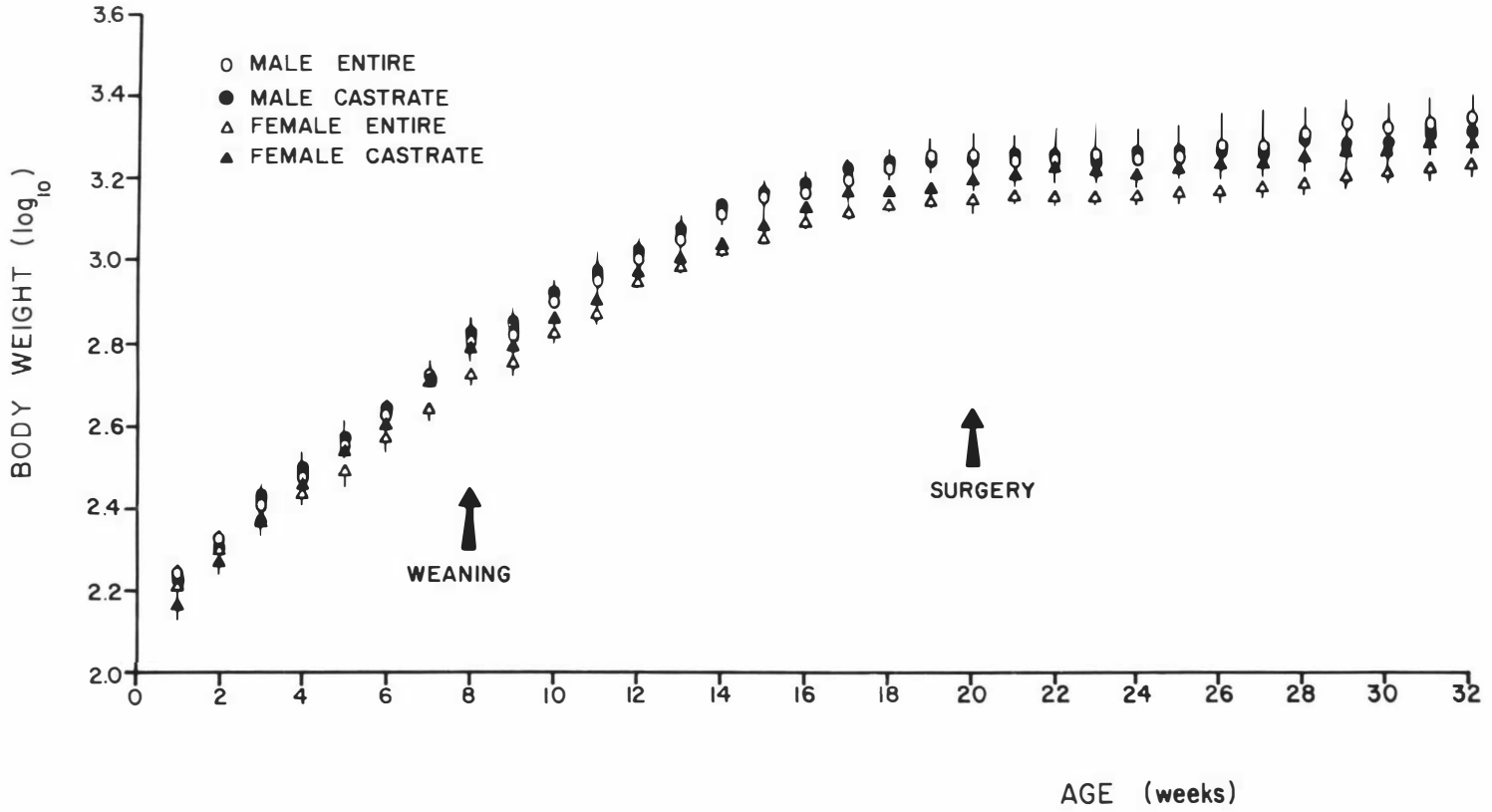

o MALE ENTIRE
● MALE CASTRATE
△ FEMALE ENTIRE
▲ FEMALE CASTRATE
BODY WEIGHT (log_{10})
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
WEANING
SURGERY
AGE (weeks)

Table 3: Body weight expressed as logarithms of BWt in grammes (±sem)

	Females		Males					
Weeks	entire	spayed	entire	castrated	Error mean square	"F"	Level of Sig.	Covariance	Sig.
1	2.10 ±.037	2.07 ±.017	2.16 ±.041	2.14 ±.041	0.009	0.904	NS	-	
2	2.26 ±.039	2.24 ±.018	2.35 ±.036	2.30 ±.046	0.010	1.276	NS	1.410	NS
3	2.37 ±.026	2.36 ±.027	2.46 ±.029	2.40 ±.035	0.006	1.857	NS	0.734	NS
4	2.45 ±.019	2.43 ±.037	2.54 ±.034	2.49 ±.029	0.006	1.991	NS	0.995	NS
5	2.58 ±.029	2.51 ±.037	2.62 ±.03	2.57 ±.025	0.005	2.109	NS	1.937	NS
6	2.68 ±.023	2.61 ±.037	2.71 ±.032	2.66 ±.025	0.005	1.636	NS	0.133	NS
7	2.76 ±.013	2.69 ±.033	2.81 ±.025	2.76 ±.022	0.004	3.184	< 0.05	4.133	< 0.05
8	2.80 ±.011	2.76 ±.031	2.90 ±.021	2.84 ±.019	0.003	5.871	< 0.01	4.536	< 0.05
9	2.84 ±.018	2,79 ±.029	2.95 ±.022	2.89 ±.015	0.002	9.169	< 0.01	2.929	< 0.1
10	2.87 ±.024	2.84 ±.031	3.02 ±.026	2.94 ±.016	0.003	10.206	< 0.01	0.888	NS
11	2.88 ±.032	2.88 ±.042	3.06 ±.02	2.99 ±.018	0.004	9.893	< 0.01	1.015	NS
12	2.93 ±.035	2.91 ±.04	3.10 ±.017	3.03 ±.019	0.003	10.560	< 0.01	0.542	NS
13	2.96 ±.023	2.94 ±.038	3.12 ±.013	3.07 ±.019	0.003	12.550	< 0.01	1.982	NS
14	3.00 ±.020	2.97 ±.034	3.15 ±.02	3.11 ±.027	0.004	9.079	< 0.01	0.281	NS
15	3.02 ±.017	3.00 ±.039	3.18 ±.024	3.15 ±.028	0.005	8.238	< 0.01	1.673	NS
16	3.04 ±.017	3.02 ±.041	3.21 ±.03	3.19 ±.036	0.007	6.92	< 0.01	0.060	NS
17	3.08 ±.021	3.05 ±.035	3.24 ±.031	3.21 ±.031	0.006	7.278	< 0.01	0.084	NS
18	3.11 ±.024	3.08 ±.038	3.27 ±.027	3.23 ±.033	0.006	6.908	< 0.01	0.298	NS
19	3.14 ±.024	3.11 ±.035	3.30 ±.025	3.26 ±.033	0.006	7.025	< 0.01	0.106	NS
20	3.17 ±.025	3.14 ±.033	3.32 ±.026	3.28 ±.032	0.005	7.053	< 0.01	0.850	NS
21	3.20 ±.017	3.16 ±.037	3.36 ±.024	3.30 ±.031	0.005	7.316	< 0.01	0.431	NS
22	3.24 ±.015	3.19 ±.039	3.38 ±.019	3.34 ±.031	0.006	7.918	< 0.01	1.776	NS
23	3.29 ±.020	3.22 ±.035	3.4 ±.018	3.36 ±.032	0.005	6.973	< 0.01	0.134	NS
24	3.28 ±.006	3.24 ±.034	3.42 ±.017	3.37 ±.033	0.005	6.708	< 0.01	1.287	NS
25	3.30 ±.007	3.25 ±.031	3.44 ±.016	3.38 ±.032	0.004	7.629	< 0.01	0.933	NS
26	3.32 ±.012	3.26 ±.025	3.45 ±.019	3.40 ±.029	0.004	9.336	< 0.01	1.275	NS
27	3.34 ±.01	3.28 ±.029	3.46 ±.021	3.41 ±.028	0.004	8.668	< 0.01	0.015	NS
28	3.34 ±.017	3.28 ±.033	3.47 ±.019	3.42 ±.028	0.004	9.125	< 0.01	0.065	NS
29	3.35 ±.017	3.31 ±.027	3.49 ±.014	3.44 ±.029	0.003	9.449	< 0.01	0.724	NS
30	3.37 ±.013	3.33 ±.032	3.50 ±.012	3.45 ±.028	0.003	9.489	< 0.01	0.121	NS
31	3.37 ±.009	3.34 ±.034	3.51 ±.01	3.47 ±.028	0.003	9.383	< 0.01	0.265	NS
32	3.38 ±.006	3.37 ±.025	3.53 ±.012	3.48 ±.031	0.003	8.437	< 0.01	0.151	NS

Table 4(a) "t" Test Results on Nonadjusted means during periods when ANOVAR showed a significant difference.

Comparisons:
1. entire females versus spayed females
2. entire female versus entire male
3. entire male versus castrated male

Comparison	1		2		3	
Week	"t"	P	"t"	P	"t"	P
7	1.515	NS	1.145	NS	1.527	NS
8	0.999	NS	2.645	< 0.05	2.116	NS
9	1.224	NS	3.564	< 0.01	2.596	< 0.025
10	0.749	NS	3.968	< 0.005	2.822	< 0.02
11	0.000	NS	4.124	< 0.005	2.138	NS
12	0.499	NS	4.497	< 0.005	2.469	< 0.05
13	0.499	NS	4.233	< 0.005	1.763	NS
14	0.649	NS	3.436	< 0.01	1.222	NS
15	0.387	NS	3.279	< 0.02	0.819	NS
16	0.327	NS	2.944	< 0.02	0.461	NS
17	0.530	NS	2.993	< 0.02	0.748	NS
18	0.530	NS	2.993	< 0.02	0.997	NS
19	0.530	NS	2.993	< 0.02	0.997	NS
20	0.580	NS	3.074	< 0.02	1.093	NS
		---------- time of surgery ----------				
21	0.774	NS	3.279	< 0.02	1.639	NS
22	0.968	NS	2.869	< 0.025	1.093	NS
23	1.355	NS	2.254	< 0.05	1.093	NS
24	0.774	NS	2.869	< 0.025	1.366	NS
25	1.082	NS	3.207	< 0.02	1.833	NS
26	1.299	NS	2.978	< 0.02	1.527	NS
27	1.299	NS	2.749	< 0.05	1.528	NS
28	1.299	NS	2.978	< 0.02	1.527	NS
29	0.999	NS	3.704	< 0.01	1.763	NS
30	0.999	NS	3.439	< 0.01	1.763	NS
31	0.749	NS	3.704	< 0.01	1.411	NS
32	0.250	NS	3.969	< 0.01	1.764	NS

** time of surgery at 20 weeks.

Table 4(b) "t" Test Results on adjusted means during periods when ANCOVAR showed a significant difference.

Comparison	1		2		3	
Week	"t"	P	"t"	P	"t"	P
7	0.862	NS	3.007	< 0.03	2.466	< 0.05
8	1.025	NS	3.562	< 0.01	1.748	NS
9	0.601	NS	4.093	< 0.005	1.030	NS

The results of the present study are similar to the earlier study illustrated in Fig. 4, when the cats were fed a less satisfactory diet and the same pattern of significant differences between the entire males and females was only in evidence between weeks 12 and 20. Of great interest is that ANCOVAR showed a significant difference at week 7 which is confirmed in the present study that a divergence in BWt between males and females is seen starting at week 7 but which does not persist for long.

DIET

The analysis of the the canned diet, as supplied by J. Wattie Canneries Ltd., is given in Appendix 3. It will be seen that the diet was supplied in three batches. Whilst having the total annual requirement in one batch might seem appropriate, we were advised that the keeping quality of the canned diet might not be satisfactory as it was an experimental diet and long-term storage tests had not been carried out. Therefore the diet was supplied in three batches. Table 5 gives the pooled data from the three analyses.

Table 5: Analysis of pooled data from the three analyses of the canned cat diet

	% moisture	% protein	% fat	% ash	% Carbo-hydrate	Energy KCal/Kg	% salt	$\%Ca^{++}$	$\%P^{+++}$
X	71.12	11.39	12.67	2.46	2.36	1687.06	0.54	0.51	0.54
SD	0.621	0.915	0.33	0.24	1.10	23.917	0.10	0.09	0.04
SEM	0.150	0.222	0.08	0.06	0.27	5.80	0.03	0.03	0.02

An ANOVAR was carried out on all constituents in the three batches (see Appendix 3). Batch 2 showed a significantly lower protein content, although the actual level was only 0.98% lower than the value obtained for batch 1 (the carbohydrate content was also significantly lower, but the carbohydrate content is obtained by difference so would be affected by a significant change in another constituent). J. Wattie Canneries Ltd. suggested that this lower value probably is reflected more by sampling errors than actual significant overall lowering of protein in the entire batch. We could not detect any changes in the growth levels during the change over between batch 1 and 2. Cats were born over a period of 2-3months and so at the time of the first batch changeover cats were

ranging in age between 8 and 21 weeks which would reduce the effect, if any, of a change in dietary component. An important measurement is the energy value of the diet which was not different in any of the diets. The other important point is that the actual level of protein is not as important as the nutritional value, in terms of amino acid content, of the protein fed; the smooth growth curves suggested that the second batch was not deficient in this regard.

DISCUSSION AND CONCLUSIONS

The canned cat diet provided an adequate plane of nutrition for the growing cats and this conclusion is derived from two observations. Firstly the levels of the constituent elements in the diet agreed with values obtained from the literature and secondly, the growth rates observed in the growing cats compared very favourably with the rates of growth from other published studies.

While there would appear to be no minimum require for cats for carbohydrate in their diet, levels of protein and fat are critical (Kronfeld, 1976). Payne, Seamer, and Short (1966) favour a protein level of 36% of the total calories provided by the diet, but do not mention fat or carbohydrate levels. Edney (1972) cites a report by Anderson, 1972 which recommends a protein calorie intake in excess of 29% of the total calories, Schneck (1968) cites Gershoff, 1962, who recommends 30-40% protein, and 25-30% fat; Dickinson and Scott, (1956), who did much of the pioneering work in cat growth and nutrition, maintained that the protein fraction should exceed 30% of the dry weight of the diet. Kronfeld (1976) provides one of the more recent surveys of the cat nutritional requirements and he suggests a range of 33-45% protein and 25-30% fat. The protein level of the canned cat diet used in this study was approximately 44% of the dry weight (See Table 6). Relatively less information is available as to the nutritional requirements in terms of vitamins and minerals, however, in the absence of any clinical signs of vitamin or mineral insufficiency then it can be assumed that the diet used was adequate in this regard also.

The growth rates of the cats produced satisfactory growth curves with no deflection at weaning and later body weights which were in line with other published studies (Dickinson and Scott, 1956; Payne, Seamer, and Short, 1966; and Berman and Rosenstein, 1973). At 24 weeks of age, the mean weights of both the male and female cats in the study had attained a body weight in excess of the average of the mean body weights of cats in other studies of a comparable age (see Table 7).

Up until 32 weeks of age or 12 weeks post-operatively there was no statistically significant difference between the growth rates of the entire versus the castrates in either sex. This contrasts with similar investigations in other species which have shown an almost immediate alteration in the rate of growth of the castrate.

Table 6

	Protein	Fat
Present Study	39%	44%
Payne et al	36%	-
Kronfeld	33-45%	25-30%
Edney	29%	-
Schneck	30-40%	25-30%
Dickinson & Scott	30%	-

Recommended levels of protein and fat in a cat diet expressed as percentage, of the dry weight compared to levels in the present study.

Table 7 Mean BWt (g) of entire cats at 24 weeks of age

	Male Cats	Female Cats
Present Study	2630	1905
Dickinson & Scott	2419	2115
Payne et al	2900	2200
Berhman & Rosenstein	1800	1400
Average	2437	1905

The literature provides abundant data on the effect of gonadectomy on male farm animals, but little information is available for the effect of gonadectomy on the body weight of female farm animals as these are seldom ovariectomised. In most cases gonadectomy of the male results in a decrease in the rate of weight gain; steers are lighter and slower growing than bulls (Maclean, 1969; Arthur, 1959), and wethers are lighter than rams (Everitt and Jury, 1966). Walstra and Kroeske (1968) point out, however, that the effect of castration on the rate of growth of pigs is not clear as investigators have found for both barrows and boars. Castration of poultry generally slows down weight gain (Kakolweski et al., 1968). Smith and Smyth (1963) noted, for example, that the body weight of castrated turkeys was significantly less than that of the intact controls.

Rats are one of the few animals for which the effect of gonadectomy has been determined for both sexes. In line with the males of other species, the castrated male rat loses body weight compared to the entire, while the ovariectomised female rat gains weight compared to the entire female rat (Kakolewski et al., 1968; Tarttelin and Gorski, 1973; Clark and Tarttelin, 1978).

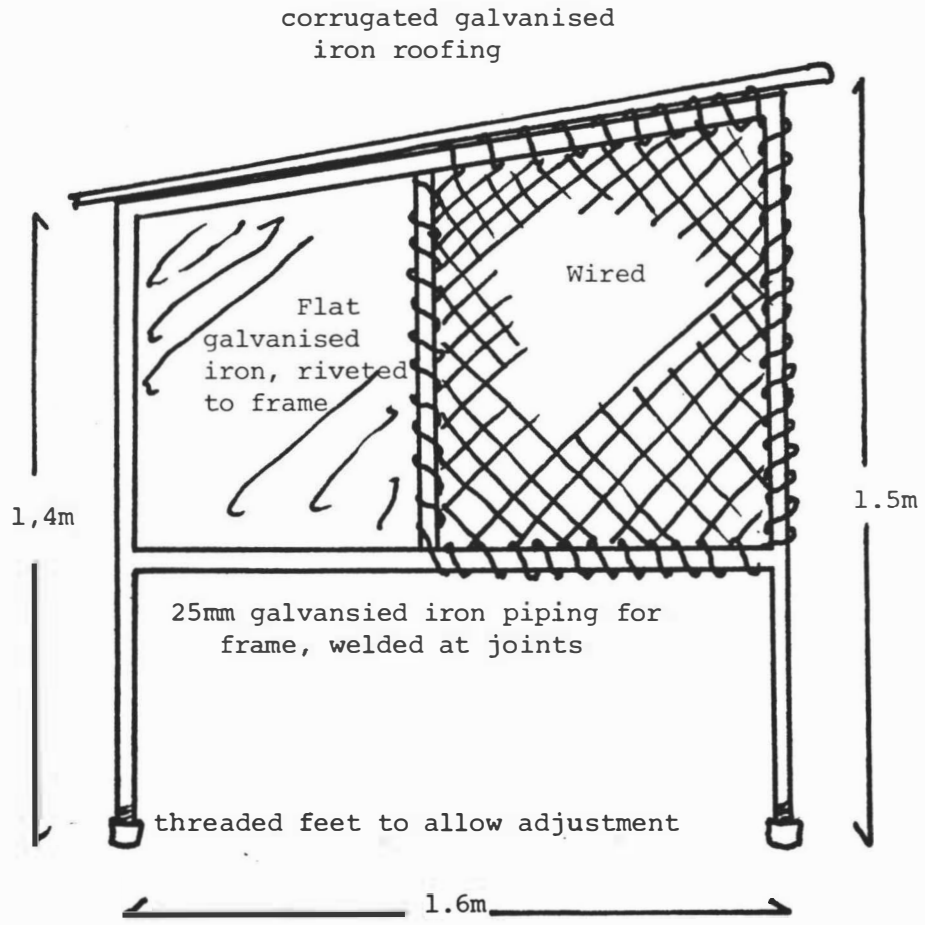
The present study supports previous investigations into cat growth, and demonstrates that the male cat body weight is significantly heavier than that of the female cat of a corresponding age. Significant differences in body weight at 32 weeks of age between entires and castrates were not shown, however further study showed that the prepubertally castrated females were significantly heavier when analysed up to 55 weeks. A significant increase in body weight in prepubertally castrated males was not seen up to 55 weeks.

APPENDIX 1 The cat pens

Inside each cat pen was a sheet of hardboard, covered in a marine varnish to improve wear and which was raised approximately 5 cm at the rear to ensure that any liquids spilt drained away. This hardboard base covered three quarters of the available floor space in each pen. Also inside the pen was a plastic nesting box, feed bowl, water bottle, and a dirt box. These pens were cleaned out as part of the daily maintenance programme, dirt boxes were disinfected and feed bowls cleaned with detergent.

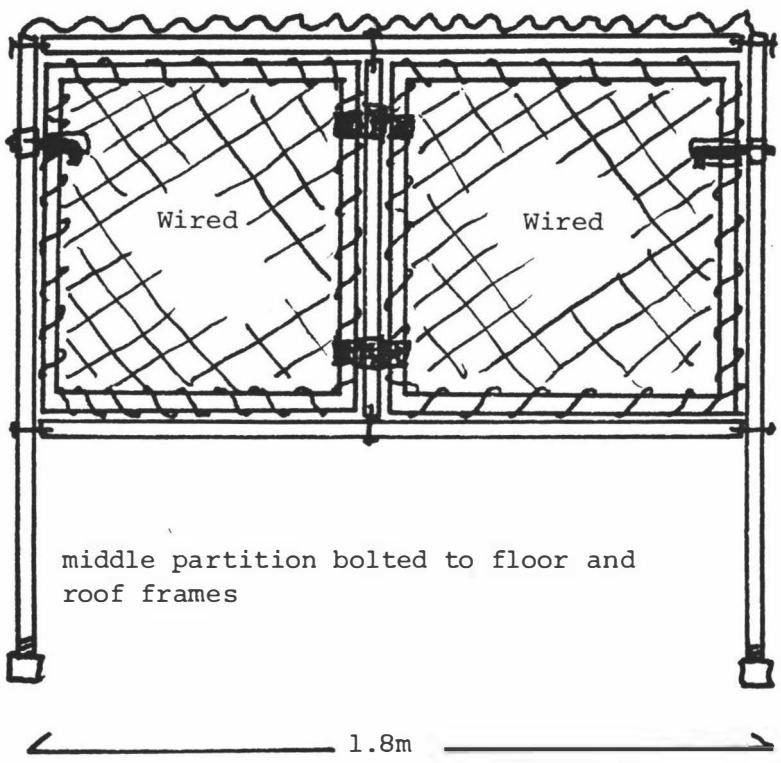
APPENDIX 1a

Details of the cat cages. (Not to scale)



SIDE ELEVATION

APPENDIX 1b



FRONTAL ELEVATION

APPENDIX 2 Descriptions of pharmacological agents employed.

VAXITAS FVR-CP (ICI Tasman Limited, Upper Hutt, New Zealand) Vaxitas FVR-CP is an oil adjuvanted, inactivated vaccine for the prevention of Feline Viral Rhinotracheitis (FVR). The innoculation programme consisted of two 1 millilitre dosages given intramuscularly at an interval of four weeks, the first dose being at 12 weeks of age. The vaccine is supplied as a single dose in a disposable syringe, which was stored in a refrigerator at 4 degress C until required. The rationale behind the booster programme is a standard procedure where the initial vaccination may be countered by residual maternal antibodies, and the follow-up vaccination is to allow the young kitten to form its own protective antibodies following the antigenic challenge of the vaccine.

TYLAN (Elanco Products (NZ), and Company. A division of Lilly Industries (NZ) Limited, Auckland, New Zealand). Tylan tablets containing 200 milligrammes of tylosin base activity with starch powder and magnesium stearate were used. Tylosin is a broad spectrum antibiotic which is particularly effective against many pleuro-pneumonia-like organisms, and for this reason is employed to counter minor respiratory ailments. A 200 milligramme tablet was broken down to an appropriate dosage and given orally twice daily over a 4 day period. The dosage ranged from 40 to 60 milligramme per kilogramme body weight daily. This dosage is greater than is recommended for treatment by the manufacturers but still within the margin of safety for tylosine, and was used because of technical problems in fractioning the tablet into any smaller portions.

PROPEN LA. (Glaxo Laboratories (NZ) Limited, Palmerston North, New Zealand). Propen LA is an aqueous suspension of Procaine benzyl penicillin and benethamine penicillin for intramuscular injection. Each millilitre contains procaine penicillin 150,000 units and benethamine pencillin 150,000 units. This preparation is reported to give high initial levels of pencillin in the blood and to maintain effective levels for four or more days following a single intramuscular injection. A subcutaneous route was chosen here for its slower rate of absorption, and a low dosage of 0.5 millilitre was given as this was prophylactic treatment.

SOFRADEX (Roussel Pharmaceuticals Pty., Limited, New South Wales, Australia). Sofradex eye ointment contains soframycin (as the framycetin sulphate) 5 milligramme per gramme, as the active antibiotic. For

conjunctival infections treatment consisted of an ectopic application of the ointment over the cornea over a 2 or 3 day period, or until symptoms ceased.

PENBRITTEN (Beecham Research Laboratories)
Penbritten tablets consist of 125 milligramme ampicillin which is a semisynthetic penicillin, whose action is through inhibition of the biosynthesis of cell wall mucopeptides in sensitive organisms. It was used here because of its wide spectrum and bactericidal action. Treatment consisted of 1 tablet per day for 4 days.

PIPRAZINE ADIPATE, (Burroughs Wellcome)
Piprazine tablets containing 500 milligramme piperazine as the adipate salt were used in the treatment of roundworm which may have been transfered in utero. Treatment consisted of a single dose of half a tablet. Tapeworm was not treated as there was no history of tapeworm within the unit, which tend to be carried by other ectoparasites such as fleas.

NOBECUTANE (BDH Pharmaceuticals Limited, London, England).
Nobecutane consists of an acrylic resin dissolved in acetic esters with inert propellant gases. When the Nobecutane Spray is applied to the wound the solvent evaporates quickly leaving a transparent, adherent elastic film. This film is impermeable to bacteria yet allows for normal skin transpiration and thus provides additional protection for the wound.

APPENDIX 3 ANALYSIS OF CX9NP AS SUPPLIED BY J. WATTIE CANNERIES LTD

Batch	% moisture	% protein	% fat	% ash	% Carbo-hydrate	Energy KCal/Kg	% salt	%Ca^{++}	%P^{+++}
	70.7	11.9	13.1	2.8	1.5	1710	0.52	0.54	0.56
	70.7	11.7	13.0	2.6	2.0	1710	0.55	0.49	0.56
	70.7	11.5	12.6	2.8	2.4	1670	0.49	0.52	0.56
1	70.9	11.4	12.6	2.6	2.5	1690	0.55		
	72.3	11.3	13.4	2.3	0.7	1680	0.55		
	72.9	11.4	12.5	2.1	1.1	1620	0.54		
X	71.366	11.533	12.866	2.533	1.7	1680	0.533	0.516	0.65
Sem	.398	0.091	0.145	0.114	0.295	13.66	0.009	0.014	0.00
	70.9	10.2	13.1	2.2	3.6	1730	0.72	0.4	
	71.1	9.9	12.2	2.3	4.5	1760	0.8	0.53	
	71.1	10.9	12.7	2.3	3.0	1700	0.65	0.37	
2	71.2	11.0	12.8	2.3	2.7	1700	0.6	0.42	
	71.2	10.9	12.6	2.4	2.9	1680	0.46	0.52	
	71.0	10.4	12.4	2.4	3.8	1680	0.45	0.48	
X	71.083	10.55	12.633	2.316	3.416	1693.3	0.613	0.453	
Sem	0.047	0.183	0.128	0.03	0.277	8.819	0.057	0.027	
	70.6	13.1	12.3	2.8	1.2	1680	0.5	0.71	0.46
	70.3	13.2	12.4	2.9	1.2	1690	0.51	0.58	0.54
3	71.0	11.1	12.6	2.4	2.9	1700			
	71.2	11.2	12.3	2.3	3.0	1670			
	71.2	12.6	12.8	2.3	1.1	1700			
X	70.86	12.24	12.48	2.54	1.88	1688	0.505	0.645	0.5
Sem	0.177	0.456	0.096	0.128	0.437	5.83	0.005	0.065	0.04
ANOVAR									
"F"	0.909	10.25**	2.24	1.74	8.37**	0.44	1.38	insufficient data for analysis	

** Sigificant P 0.01

APPENDIX 4

(From the Proceedings of the Physiological Society, 18-20 June 1981 Journal of Physiology, 319, 55-56P)

The effect on body weight of prepubertal or postpubertal gonadectomy in the cat (Felis domestica)

BY A. KETTLE, E.M. SOMMERVILLE AND M.F. TARTTELIN, *Department of Physiology Massey University, Palmerston North, New Zealand*

Gonadectomy (GX) is the commonest surgical procedure carried out in the cat, mainly for fertility control. However, the effect of GX on body weight has received little study although this subject is well researched in other species such as the rat. A recent study of the effects of age at GX on body weight in the rat (Clark & Tarttelin, 1978) reported that the increase seen in body weight in the female rat was unaffected by different ages at GX.

The present study involved gonadectomy at 20 weeks (prepubertal GX, GXPre) and at 55 weeks of age (postpubertal GX, GXPost); all surgical procedures were carried out under halothane anaesthesia. The GXPre study used kittens bred from eight queens. After 32 weeks of study further cats from a previous mating were added to increase group numbers and allow study of a postpubertal surgical group. The cats were kept in outdoor colony cages and given food ad lib. adjusted to leave daily residues. Various commercial canned diets were tested and modified until a satisfactory growth died (CX9) was produced; a crude analysis was (%); water, 71; protein, 11; fat, 13; ash 2; CH0, 2; kcal kg '. 1695. Body weight was measured weekly and transformed to $\log_{10}$. For statistical analysis differences in body weight at each week were tested by one-way analysis of variance and serial covariance using previous week's body weight as the covariate. Differences between groups were investigated with "t" tests using the weighted error mean square. Growth rates were studied by regression. A priori comparisons were: males vs females, entire females vs. GXPre females and entire males vs GXPre males. After 55 weeks, entire cats were compared with GXPost groups. A total of twenty-four cats were studied in the GXPre section and thirty-three cats in the GXPost part.

Significant differences were seen between body weight of males and females from week 7 onwards; serial covariance showed these differences were initiated over a 3-week period (weeks 7-9). From week 20-32 there were no differences between GX cats and controls, however, when a regression analysis was carried out over a period from 20-55 weeks significant differences were seen between GX females and controls. Analysis of data from 55 weeks onwards using one-way analysis of variance and covariance proved differences between entires only. However, regression analysis proved significant increases in growth rates in both GX Post males and females compared to entire controls.

We conclude that prepubertal or postpubertal GX in the female cat causes significantly increased growth. We could only show increased growth in the post-pubertal GX male cat. These findings resemble data from female rats but the findings regarding growth changes in male cats contrasts with that of male rats: castrated male rats show depressed body weights.

We acknowledge J. Wattie Canneries for supplies of CX9 cat diet.

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PART II

A COMPARATIVE STUDY OF SELECTED ASPECTS OF

THE BRAIN OF THE POSSUM (TRICHOSURUS VULPECULA)

POSSUM STUDY INTRODUCTION

De Blainville, in 1816, was the first to recognise the marsupials as a distinct taxonomic group based on their mode of reproduction, prior to this time they had been allocated to various groups depending on their life forms. Linneaus for example, in his Systema Naturae, classified the Virginian opossum (Didelphis) along with the pig, armadillo, hedgehog, and shrew in the Bestiae because of their common possession of sharp teeth.

In 1834 de Blainville revised his earlier classification recognising the monotremes as a distinct group, while retaining the condition of the reproductive tract as the basis for their separation. Thus the monotremes were termed the Ornithodelphia because their oviducts are partially united in the vaginal region and all other mammals were included in the Monodelphia (Tyndale-Biscoe, 1973).

These three groups of living mammals are still recognised today although they have not retained de Blainville's nomenclature (see Table 1). Affinities of the Ornithodelphia with reptiles, such as the laying of eggs, led T.H. Huxley to suggest that they and the marsupials represented earlier serial stages in the development of the viviparous mammals and hence the terms Proto-, Meta-, and Eu-theria.

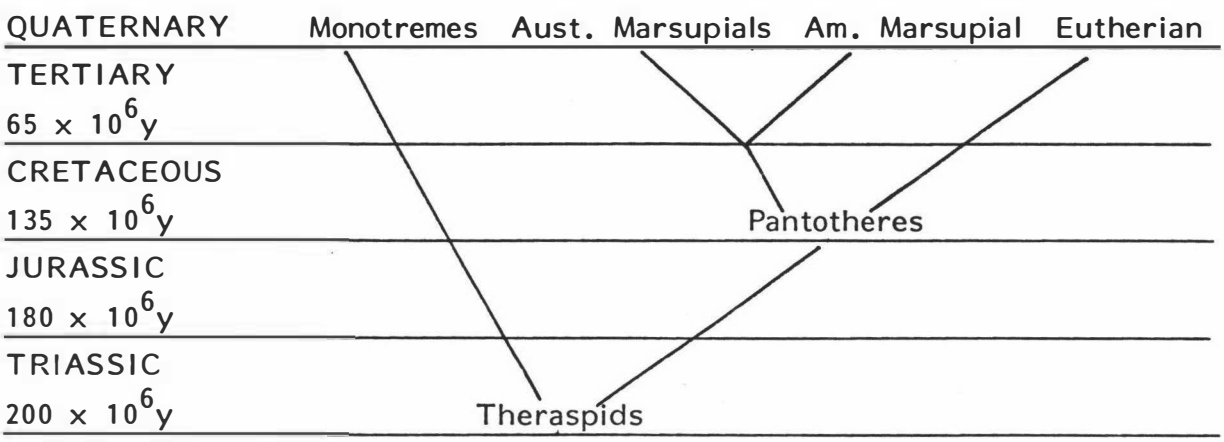
Table 1

de Blainville	Bonaparte	Huxley	Illiger, Owen
Ornithodelphia	MONOTREMATA	Prototheria	
Didelphia	Ditremata	Metatheria	MARSUPIALIA
Monodelphia		EUTHERIA	Placentalia

Comparison of nomenclature with those in common use in capital letters - Taken from Tyndale-Biscoe 1973.

These ideas of Huxley of a serial stage development of the viviparous mammals had a profound influence on earlier research and led to a surge of interest in the marsupials and monotremes. Researchers felt that by studying these animals they would gain some insight into the origin of mammals. Fossil evidence, however, indicates that the ancestors of the viviparous mammals separated early from the prototherian lineage paralleling prototherian development, and not two successive derivations from early mammals (Parker, 1967).

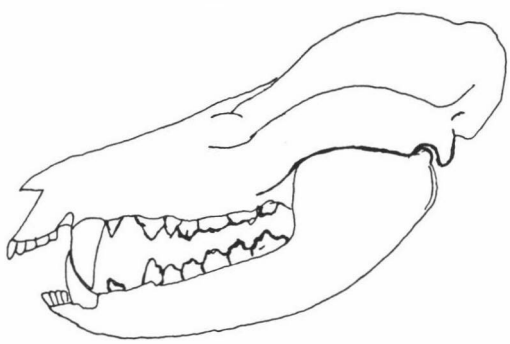
Figure 1 Mammalian relationships following Simpson (1959). Adapted from Waring, Moir, and Tyndale-Biscoe (1966).



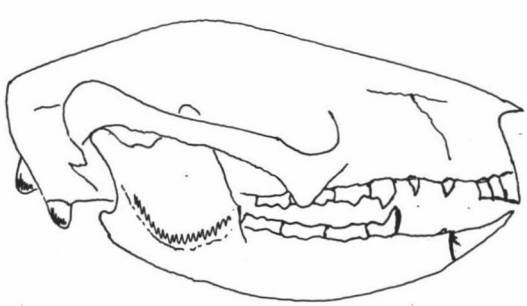
It would appear from Figure 1 that the marsupials divided in the early Cretaceous period into two groups - the Australian and American marsupials, although the fossil evidence for the means of separation of these two groups is patchy and uncertain. A complete lineage of the American marsupials is reasonably clear commencing from the early Cretaceous period but the derivation of the Australian marsupials from this Cretaceous stock is far from clear as no fossil remains of early marsupials have been found in Europe or Asia. Nor is there any fossil evidence for a Southern route via Antarctica although fossil remains of placentals of the

corresponding era have been found along both proposed routes. What is certain is that two separate marsupial groups have arisen in both the Australian and American continents.

Two sub-orders of Marsupialia - the Polyprotodonta and the Diprotodonta are found within these two groups of marsupials. All the living South American species and the carnivorous Australian marsupials are termed Polyprotodont because of their long snouts bearing a large battery of sharp pointed teeth; each ramus contains four molars, three premolars, one prominent canine, four or five incisors in the upper jaw and three in the lower jaw (see Figure 2). The Diprotodonts on the other hand are herbivorous Australian marsupials whose jaws have fewer premolars, with perhaps a canine in some cases and with three incisors in the upper jaw, and one large procumbent incisor in each dentary (see Figure 2) (Tyndale-Biscoe, 1973).



POLYPROTODONT — Virginian Opossum (Didelphis virginiana)



DIPROTODONT — Australian Bush-Tailed Possum (Trichosurus vulpecula)

Figure 2 A comparison of the dentary features of polyprotodont and diprotodont marsupials.

It would appear therefore that during the course of evolution several groups of mammals have evolved along genetically distinct lines, and yet many of these animals share similar adaptive features; ruminant digestion, counter current exchange kidneys, embryonic diapause and euthermy to name but a few. Thus the evolution and development of a species may have relatively few options and be dependent upon environmental demands (Heath and Jones, 1971; Tyndale-Biscoe, 1973) and so we see in mammals a similar set of adaptive features which have evolved independently. This is especially evident in the brain structure of marsupials.

The marsupial brain does not possess a corpus callosum, the hemispheres being connected via other commissural pathways. In the Didelphis virginiana and other polyprotodonts, Ebner (1971) has shown that the anterior commissure is used to link the hemispheres as in reptiles and monotremes. While Heath and Jones (1971) have shown that the two hemospheres of the diprotodonts (of which Trichosurus vulpecula is an example) are linked via a unique neocortical commissure, the fasiculus aberrans, in addition to the anterior commissure. In spite of this unique commissure the commissural connections of the diprotodonts are functionally equivalent to other species studied (Heath and Jones, 1971). Furthermore, Heath and Jones (1971) maintain that there is evidence for the development of certain cortical areas, particularly the visual areas, in Trichosurus vulpecula which may be markedly in advance of that of other areas and systems.

While a great deal of knowledge on the structure and functioning of the opossum brain, Didelphis virginiana, for example: rhinencephalon (Adey, Sunderland & Dunlop, 1957; Ebner, 1971), pyramidal tract (Bautista & Matzke, 1956), neocortex (Ebner, 1967), basal ganglia (Martin & Biggert, 1969), and hypothalamus (Petajan, Morrison & Akert, 1962; Roberts, Steinberg & Means, 1967; Roberts, Bergquist & Robinson, 1969; and Royce, 1971) has been accumulated over the years, comparatively little work has been done on its near cousin Trichosurus vulpecula. The New Zealand Forest Service has studied the ecology of the possum in New Zealand in some detail (Kean, 1959; Kean, 1961) and other papers on brain structure have been published (Goldby, 1939; Goldby, 1941; Packer, 1941; and Hayhow, 1967) but only one paper appears to have been published on the hypothalamus (Warner, 1969). Therefore it was decided to investigate the microanatomy of the hypothalamus of Trichosurus vulpecula, to compare it with the polyprotodont, Didelphia virginiana, and to investigate a stereotaxic approach as an investigative technique.

In brief, these aims required the successful completion of the following studies:

1. Assessment of anaesthetic suitable for satisfactory analgesia for the duration of the stereotaxic surgery.

2. Preparation of suitable material to practice histological sectioning and select appropriate stains.

3. Selection of appropriate histological technique to accurately age the possums in the study.

4. Investigation of the stereotaxic instrument for locating internal neural structures within the possum brain.

5. Microanatomical study of a selection of fibre stained sections in 3 stereotaxic planes (Anterior-posterior, sagittal, horizontal).

POSSUM STUDY - MATERIALS AND METHODS

For this study 22 possums were live-trapped in wire cages, in the Manawatu region and subsequently housed in 30 x 30 x 60 centimetre wire cages for up to 2 - 3 days until they could be utilised. The possums were maintained on a combination of apples and pelleted rodent diet with tap water ad libidum. This diet was acceptable for the majority of the animals as they remained in good condition, however, one or two would not accept the rat pellets and lost condition.

The possum was transferred from its holding cage to a long narrow wooden box, with a plunger at one end and a removable shutter at the other. By means of the plunger, the animal was persuaded to move through the open shutter and into a circular roll of plastic metting fixed at one end by a metal dowel and covered by sacking. A second metal dowel placed behind the possum effectively secured the animal. The possum could then be weighed and handled with safety.

ANASTHAESIA

To investigate the optimum dosage of anaesthetic a series of trials were held using pentobarbitone sodium ("Nembutal" - Abbot's) intraperitoneally in dosages ranging from 20-60 mg/kg body weight, to obtain the required duration of anaesthesia. The anaesthetic was injected intrahepatically in some possums and the suitability of this site compared to the I/P site.

SURGERY

Once anaesthetised, the possum was placed in a Trent-Well's stereotaxic instrument. The head was securely fixed by ear-bars located in the external auditory canal, eye-bars hooking over the infra-orbital ridge, and bars behind the incisor teeth.

The hair from the top of the head was then clipped and a midline incision, approximately 2 centimetres in length centred on the rostral base of the ears, exposed the skull. The periosteum was scraped off and a 2 millimetre burr in a dentist's drill was used to penetrate the skull either side of midline at the level of the rostral base of the ears. There are no suitable skull landmarks in the possum for locating neuronal groups in the

diencephalon and previous acute studies had demonstrated that the rostral base of the ears was a reliable starting point for locating structures within the diencephalon with the aid of the stereotaxic instrument. The electrodes used in the study were made from stainless steel, 2 inch long, No. 3 gauge (0.5mm O/D) gauge insect pins which were soldered into 22 gauge hypodermic needles. In the electrode implantation study, the electrode was lowered to the base of the brain and then cut off as close to the surface of the skull as possible. The incision was sutured in the normal manner, and the possum allowed to recover.

The following day the posum was re-anaesthetised and a midline incision over the trachea was made, the two carotid arteries and one jugular were then dissected out. Physiological saline (0.9%) was infused through one carotid artery while the other was occluded, the blood being allowed to drain out through the severed jugular vein. When all the blood from the brain had been washed out by the saline, a solution of 10% formal-saline was infused (pressure head was 1200mm H_2O) through the cannula to fix the brain. Once fixed, the head was removed and the brain dissected out and kept in 10% formal-saline until required for further histological processing.

HISTOLOGY

Blocks of brain tissue were cut from the whole brain using a Paragon disposable brain knife, and these blocks were then embedded in paraffin wax (see Appendix 1 for embedding procedure). Excess wax was trimmed off and the block was fixed to a wooden base which was used to hold the block in the Leitz base-sledge microtome. Sections of tissue were taken at 15 and 10 microns, floated in water at 60°C, then transferred onto albuminised slides. Once dried the sections were stained using a combination of solochrome cyanin and cresyl fast violet stains for myelin and cell bodies respectively (see Appendix 3 for procedure).

AGE DETERMINATION OF THE POSSUM

Accurate age determination of animals used in many scientific studies is often essential, and in the present study was needed for the correlation of the stereotaxic data.

The ages of possums have been determined by a variety of methods, such as body length, weight, pouch development and dental development as reviewed by Petrides (1949). However, more recently, dental cementum layers in molariform teeth have been used for age determination. Eleanor Kingsmill (1962) was the first to report the existance of layers in the dentine and cementum of incisor teeth sections from the possum Trichosurus vulpecula although she concluded that these layers were not related to age. Subsequently, however, Pekelharing (1970) demonstrated that layers in the cementum of molariform teeth of the mandibular were annual.

Recently, Clout (1977), and Beauchamp (1978) have published methods for examining mandibular molariform teeth of the possum. The method used in this study was largely that of Clout (1977) with some modifications from Beauchamp (1978).

After a period of rigorous boiling to remove the flesh, the mandibles of each possum were separated and the third molar (M3) extracted from the left mandible by carefully nibbling away the bone from around the roots of the tooth with bone forceps. The teeth were labelled ready for histological processing, and the mandibles preserved in case there was some difficulty in counting the layers in the selected tooth and additional teeth were needed for clarification. Normally only M3 was required.

The teeth were decalcified by immersion in 'RDO', a commercially available rapid decalcifying agent*, for three hours. The decalcified teeth were rinsed in water and stored in 70% alcohol prior to embedding.

The teeth were embedded in paraffin (see Appendix 1 for procedure) and subsequently sectioned at 10 microns on a rotary microtome. A series of sections was taken through the region of the cementum cushion between the roots of each molar. These sections were floated onto albuminised microscope slides as in the preceeding section, and then stained with Mayer's Haemalum (see Appendix 4 for procedure).

Some teeth needed a period of further softening to render them soft enough for sectioning and this was carried out while in the block by placing the block, cutting-face downwards in Mollifex. It was found that

* Dupage Kinetic Labs. Inc. Illinois.

the tissue tended to expand under Mollifex so that the decision to soften had to be made before the region of the cementum cushion was reached, otherwise the optimum region for counting the layers tended to disappear with the first sweep of the microtome blade.

The mounted sections were examined under the microscope, and in most cases, broad lightly stained bands alternating with narrow, darkly stained bands, as described by Clout (1977) could be seen in the dental cementum. These bands represent annual layers with one complete light band and one dark band being formed each year - the dark band during winter (Clout, 1977).

It was assumed that the majority of the animals used in the study were born in the autumn (Kean, 1959; Clout, 1977). Thus an animal with a single, dark, narrow band went into the 1+ category, with three, dark, narrow bands the 3+ category, and so on. The estimated error in this system is six months, provided the layers in the dental cementum have been accurately counted.

RESULTS AND DISCUSSIONS OF ANAESTHESIA

Intraperitoneal administration of pentabarbitone sodium ("Nembutal" - Abbot's) of dosages up to 40 mg/kg body weight produced a light surgical anaesthesia after approximately 12 minutes, with eyelid and conjunctival reflexes still evident. Higher intraperitoneal administration, 50-60 mg/kg, resulted in surgical anasthesia and the absence of these after 12 minutes. However, the intraperitoneal route did not produce consistant results and it is thought the inconsistancy resulted from absorption of the barbiturate by an intraperitoneal fatty depot, leading to a slower absorption of the drug into the central nervous system.

Intrahepatic administration of 60 mg/kg pentabarbitone sodium produced more consistant results with surgical anaesthesia within 10-12 minutes. However, the obviously painful site can further excite the already nervous animal, and, as the level of excitability of the central nervous system is an important predeterminant in the effectiveness of a barbiturate anaesthetic, this can make the possum more difficult to anaesthetise.

The primary aim of this section was not an anaesthetic evaluation as such but rather to bring about a stage of surgical anaesthesia whereby the experimental work could begin. Further trials are therefore necessary to evaluate a quick and reliable method for anaesthetising the possum. These trials would include a wider range of both anaesthetic and route.

RESULTS AND DISCUSSION OF AGE ANALYSIS

From the analysis of the possum ages, as estimated through the histological examination of dentine layers in the molar teeth (see Table 2) it can be seen that the age of the possum is related to its weight. However, this is not an absolute relationship and thus weight can not be used as an accurate indicator of age in the possum (Kingsmill, 1962).

In the present study, because the ages of the possums were variable (less than 1 - 9 years) and numbers relatively few, the influence of age of stereotaxic variability was not considered a feasible study.

Table 2 Estimates of age of possums used in study.

Possum Number	Age Estimate	Weight (kg)
10	3+	2.6
11	1+	1.7
13	2+	2.1
14	1+	1.4
15	7+	2.8
16	4+	2.9
17	6+	2.6
18	4+	2.4
19	0+	1.6
20	9+	3.2
21	2+	1.6

RESULTS AND DISCUSSION OF AN INVESTIGATION INTO THE USE OF THE STEREOTAXIC INSTRUMENT FOR LOCATING INTERNAL NEURAL STRUCTURES WITHIN THE POSSUM BRAIN

The Measurement System for Analysis of Possum Histology

In order to measure accurately the distance between internal neural structures it was necessary to amplify their separation and thus reduce the error in the measurement. A screen was set up onto which a calibration vernier was projected via a projecting light microscope. the divisions of the vernier were marked onto the screen. The mounted sections were then placed onto the microscope stage and rotated until the desired structures were in line with the marked vernier scale. The measurement was then read directly off the scale.

In the selection of sections to be measured two criteria were obeserved. In the first instance, internal neural structures had to be selected with a clearly defined border, and secondly, the sections had to be in approximately the same relative position for accuracy of measurement. These two criteria are essential considerations for while it may be assumed that the electrode tract would be uniform in diameter and travel in a clearly defined plane by virtue of its stereotaxic implantation, the same does not apply in general to neural structures within the brain: internal neural structures tend to be non-uniform in shape and dispersed within the brain.

Anterior-Posterior Measurement - with reference to Supraoptic Nucleus (SON)

The distance between the posterior border of the SON and the posterior border of the chronically implanted electrode tract in the anterior-posterior plane (A/P) was measured via the optical system described above (see Figure 3). The measured distance was corrected for shrinkage and then related to stereotaxic co-ordinates in the A/P plane only.

For example, SON to electrode tract distance - 4.5mm

Applying correction for shrinkage (0.8166 from Table 3) - $\frac{4.5}{0.8166} = 5.5\text{mm}$

Hence, if the A/P stereotaxic co-ordinates were 13mm for the electrode then the stereotaxic co-ordinate of the SON is 13 + 5.5 = 18.5mm.

Key to appreviations used in the Figures to accompany the measurements (Figs 3,4 & 5)

MT	Mammillothalamic tract
OT	Optic tract
SON	Supraoptic nucleus
3V	Third ventricle

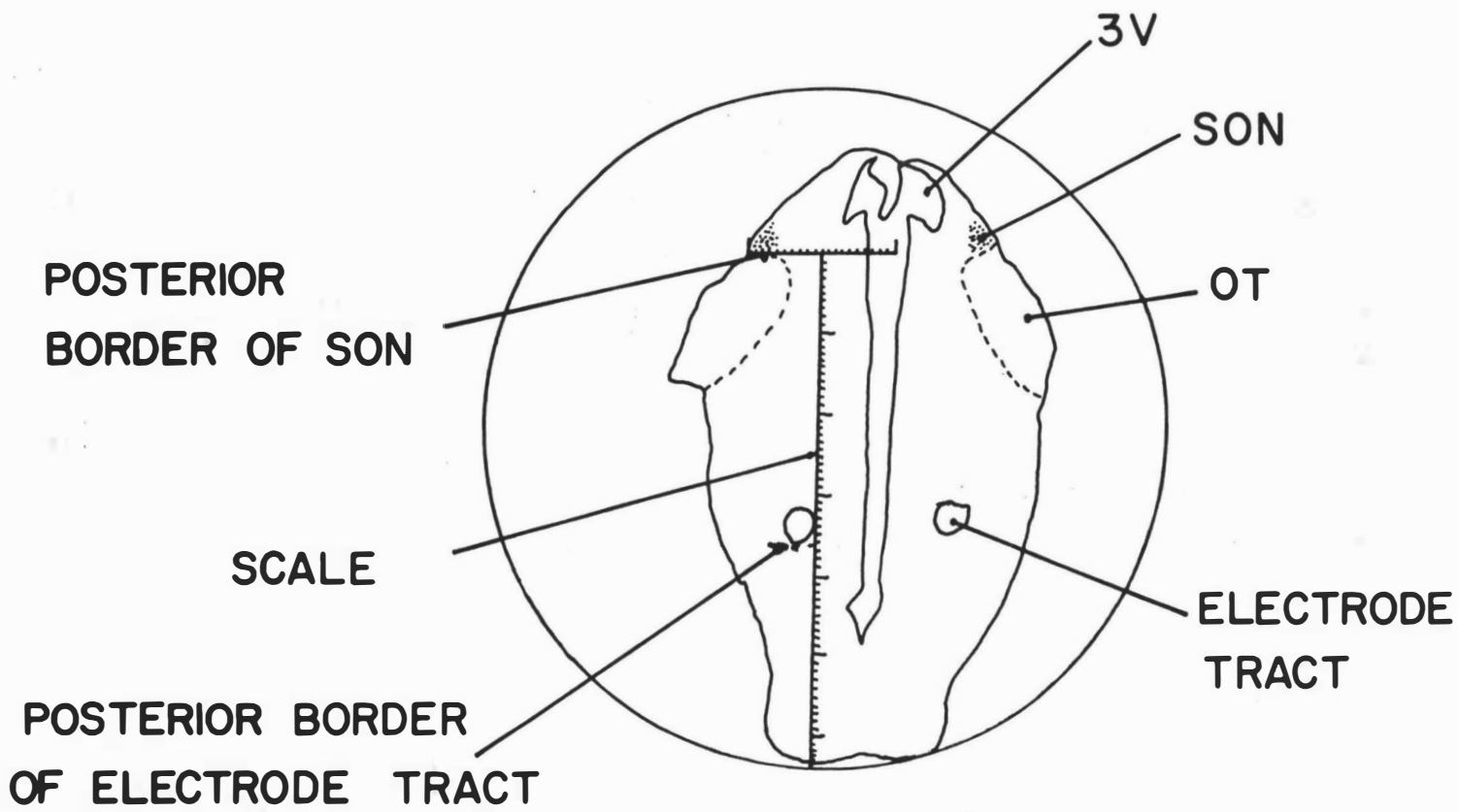

3V
SON
OT
POSTERIOR
BORDER OF SON
SCALE
ELECTRODE
TRACT
POSTERIOR BORDER
OF ELECTRODE TRACT

FIG. 3

Table 3 Shrinkage Estimates for Possum Sections

Possum No.	Wet Length (mm)	Embedded Length (mm)	Final Length (mm)	Shrinkage % Change from wet length	Shrinkage Final %
13	8.9	7.9	7.7	86.52	13.48
14	10.2	9.2	8.8	86.27	13.73
15	11.3	10.0	9.7	85.94	14.06
16	8.6	7.3*	6.8	79.07	20.93
17	11.0	9.2	8.5	77.27	22.73
18	10.6	9.1	8.8	83.02	16.98
19	11.1	9.3	8.8	79.28	20.27
20	11.8	9.2	8.8	74.58	25.42
21	10.0	8.3	8.3	83.00	17.00
			$\bar{X}$	81.66	18.34
			SD	4.31	4.31
			SEM	1.44	1.44

* Not fixed internally

In tissue processor pm 5.8.80 - out am 6.8.80

Wet Length - Fixed length (in Formal-saline)

Embedded Length - Length of block after paraffin embedding

Final Length - length of section on slide

Shrinkage % change from wet length - ratio of final length to wet length as a percentage

Shrinkage Final % - actual shrinkage from wet length to final length expressed as a percentage

$\bar{X}$ - mean

SD - Standard Deviation

SEM - Standard Error of the Mean

Anterior-Posterior Measurement - with reference to Mammillothalamic Tract (MTT)

A similar procedure was carried out to determine the distance from the electrode implantation site to the MTT using the myelin stained sections that clearly depicted the heavily myelinated tracts. The anterior border was taken for both the electrode tract and the MTT (see Figure 4).

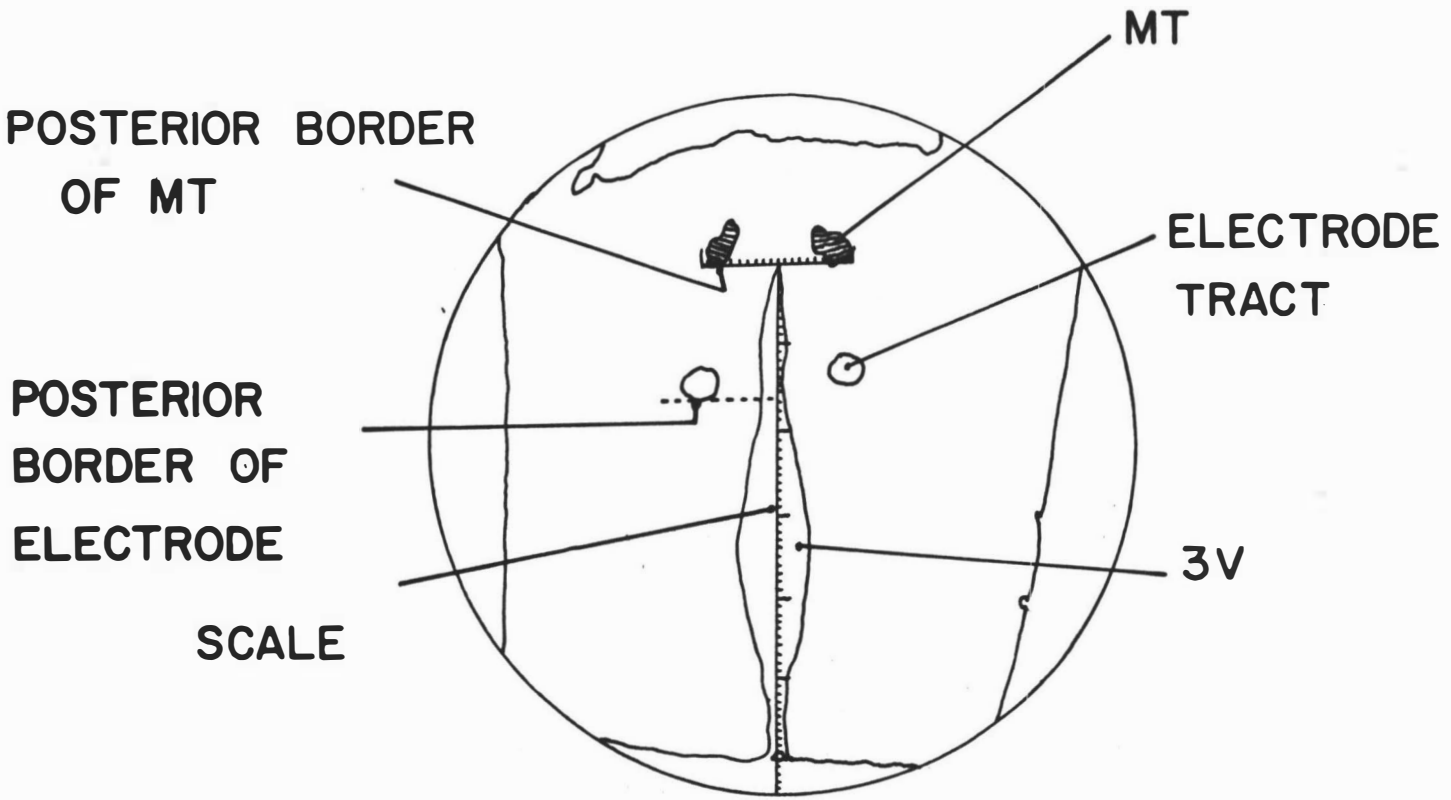

MT
POSTERIOR BORDER
OF MT
ELECTRODE
TRACT
POSTERIOR
BORDER OF
ELECTRODE
3V
SCALE

FIG. 4

For example, MTT to electrode tract distance - 1.8mm

Applying correction for shrinkage - $\frac{1.8}{0.8166}$ =2.2mm
(0.8166 from Table 2)

Hence if the A/P stereotaxic co-ordinates for the electrode tract were 13mm A/P of stereotaxic zero then stereotaxic co-ordinates of the MTT are 13 - 2.2 = 10.8mm, taking into account the relative positions of the electrode tract and the MTT.

LATERAL MEASUREMENTS

The lateral distance between the MTT border most proximal to the epithelial wall of the III ventricle and a corresponding position on the electrode tract was measured (see Fig 5) and correction factors applied as in the above examples. In cases where the sections were tilted on the slide these were aligned by eye. In this manner it was possible to assign lateral stereotaxic co-ordinates as with the A/P co-ordinates for each of the possum brains.

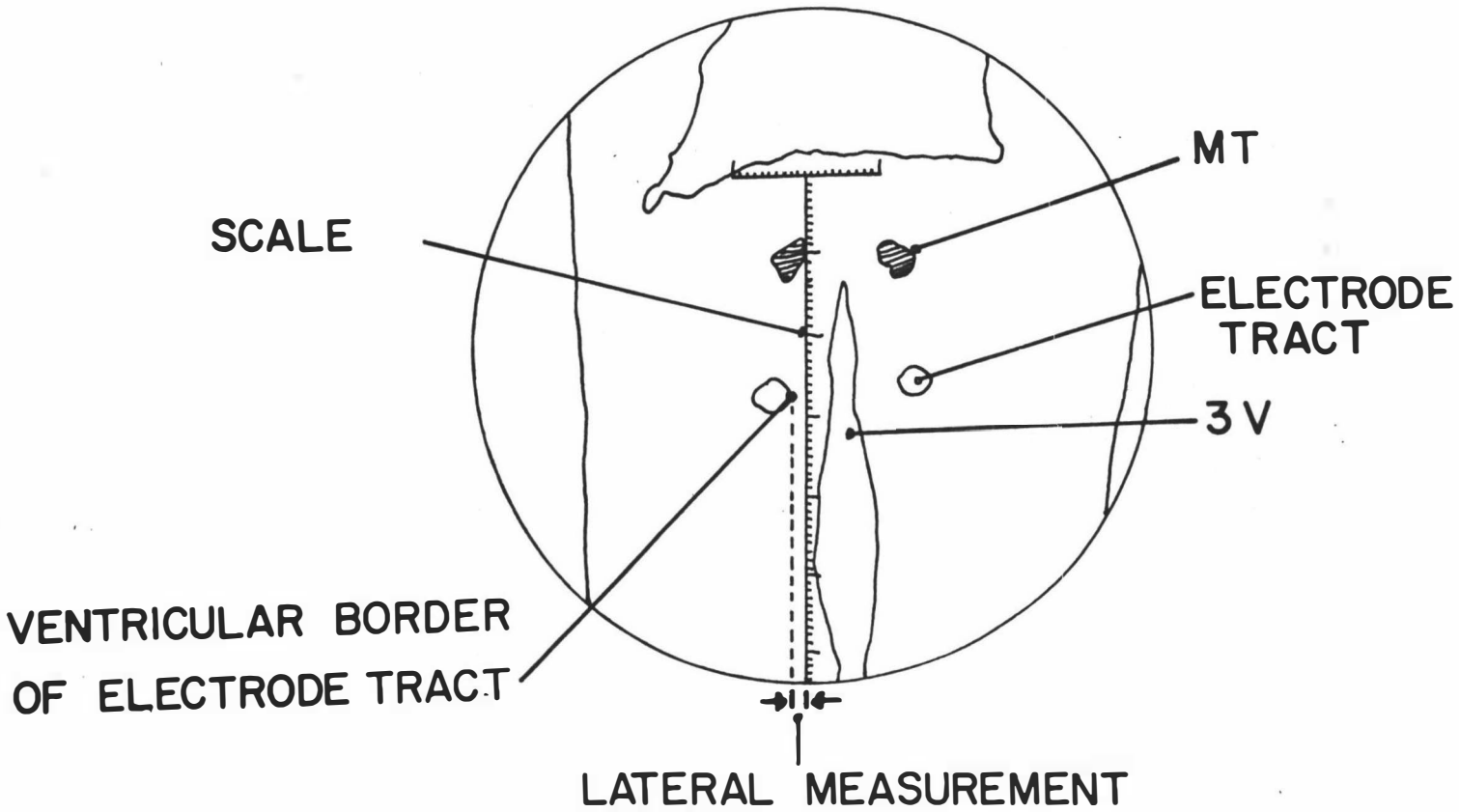

MT
SCALE
ELECTRODE
TRACT
3 V
VENTRICULAR BORDER
OF ELECTRODE TRACT
LATERAL MEASUREMENT

FIG. 5

MEASUREMENT OF STRUCTURES WITHIN THE POSSUM BRAIN

The mammillothalamic tract (MTT) and the supraoptic nucleus (SON) were chosen for investigation in this study because they were readily identifiable, had clearly defined borders in the anterior-posterior plane (A/P), and appeared to occupy the same relative position in the selected possum brains. However, lateral measurements were determined for the MTT only, as the lateral border of the SON was too diffuse to measure accurately.

The collection of data from these measurement studies is summarised in Tables 4 and 5 and the stereotaxic co-ordinates of selected structures are compared in Table 6. It can be seen that all of the collected data falls within the 95% confidence limits which, for statistical analysis implies a high degree of agreement. A 95% confidence limit may, however, not be acceptable for stereotaxic purposes.

Table 4 Anterior-Posterior Measurements

Possum No.	A/P co-ord	SON	Corrected SON	co-ord	MTT	Corrected MTT	co-ord
13	13	+3.4	+4.2	17.2	-1.6	-2.0	11.0
14	10.4	+3.5	+4.3	14.7	-1.3	-1.6	8.8
15	15	+3.6	+4.4	19.4	-1.7	-2.1	12.9
16	18.2	+2.9	+3.6	21.8	-	-	-
17	17.9	+1.5	+1.8	19.7	-	-	-
18	19.7	-2.2	-2.7	17.0	-6.5	-8.0	11.7
19	15.2	-0.4	-0.5	14.7	-4.2	-5.1	10.1
20	13.5	+4.9	+6.0	19.5	-0.7	-0.9	12.6
21	14.1	+1.9	+2.3	16.4	-2.4	-2.9	11.2

Table 5 Lateral Measurements

13	13	1.5	+0.5	+0.6		2.1
14	10.4	0.4	+0.5	+0.6		1.0
15	15	1.2	0.0	0.0		1.2
18	19.7	0.5	+0.5	+0.6		1.1
19	15.2	0.7	-0.2	-0.2		0.5
20	13.5	0.3	+0.2	+0.2		0.5
21	14.1	0.4	+0.2	+0.2		0.6
Electrode measured on LHS					$\bar{X}$	1.0
+ measurement of MTT w.r.t. midline					SD	0.566
- measurement medial of MTT w.r.t. midline					SEM	0.21

95% Confidence Limits ± 1.4 (0.0 - 2.4)

Table 6 Stereotaxic Co-ordinates of Selected Structures

Possum No.	External		Internal	
	I/A Line	Basisphenoid Bone	SON	MTT
13	13.0	21.9	17.2	11.0
14	10.4	20.0	14.7	8.8
15	15.0	24.4	19.4	12.9
16	18.2	23.9	21.8	-
17	17.9	23.9	19.7	-
18	19.7	23.0	17.0	11.7
19	15.2	20.2	14.7	10.1
20	13.5	24.5	19.5	12.6
21	14.1	22.5	16.4	11.2
$\bar{X}$	15.2	22.7	17.8	11.2
SD	2.937	1.707	2.43	1.42
SEM	0.98	0.57	0.81	0.54
95%CL	±6.8	±3.9	±5.6	±3.5
Range	8.4-22.0	18.8-26.6	12.2-23.4	7.7-14.7

Confidence Limits (CL) are obtained by multiplying SEM by the "t" $_{0.05}$.

N.B. i) all the above data falls within the 95% confidence limits.

ii) Inter-aural (I/A) line being a hypothetical line drawn between the rostral base of the pinnae while the basisphenoid bone measurement relates to the anterior tip of the basisphenoid bone covering the optic canal. The basisphenoid bone measurement was taken after the removal of the brain.

Consider, for example, two conditions for use of the stereotaxic apparatus: firstly, it may be important to strike the centre of the structure concerned, or secondly, a partial strike may be sufficient, particularly where lesioning work is being considered.

For a direct hit by stereotaxic co-ordinates on the SON from the results obtained in this study, the A.P stereotaxic co-ordinate would be 17.8mm anterior of stereotaxic zero. This value of 17.8mm is obtained from the mean of the results on the A/P co-ordinates of the SON (see Table 6) and represents the value which is most likely to be accurate on any randomly selected possum brain. No possum in the sample population chosen had 17.8mm as the A/P stereotaxic co-ordinates for the SON. The closest was possum number 13 at 17.2mm and therefore the probability is 0 (0/9) for making a direct hit on the SON using absolute stereotaxic co-ordinates. Following the same line of reasoning for the measurement on the MTT the chances are slightly better with one possum having as its A/P co-ordinates 11.2mm, the same as that of the mean (see Table 6). Here the probability is 0.14 (1/7).

The electrode diameter was 0.4mm so that a partial strike on any neural structure could be accomplished 0.2mm either side of the mean stereotaxic value for that structure. For the SON, this extends the A/P range to 17.6-18.0mm, but once again, no brain has its SON lying within this range, whereas for the MTT the chances are actually improved as two MTT are found within the range 11.0-11.4mm A/P. Thus the chances of just touching the MTT with the electrode are increased to 0.29 (2/9).

The probability of striking the MTT based on the lateral measurements is slightly better (the SON had diffuse lateral borders and was not able to be defined accurately in the lateral dimension). Within the 95% confidence limits imposed for a direct hit there is a one in seven chance, or 0.14 (1/7), while the odds for a partial hit are at 0.43 (3/7).

In any stereotaxic investigation, lateral, A/P, and vertical measurements cannot be viewed in isolation but must be taken collectively. The vertical measurements are not available for this preliminary investigation as the electrode tract was needed to go to the base of the brain for reference purposes. However the lateral and A/P measurements can be assessed for a direct hit and a partial hit on the MTT. As probability results are multiplicative and not additive in this case it can be seen that the probability of a direct hit on the MTT is 0.0196 (0.14 A/P x 0.14 lateral) while the probability of a partial hit is 0.123 (0.286 A/P x 0.429 lateral).

There are three basic methods for use of the stereotaxic apparatus, the first being the method described above based on absolute stereotaxic co-ordinates, the second method is based on a reference external skull landmark, and the third method is based on X-ray evidence. An evaluation of a stereotaxic method based on X-ray evidence was not attempted, however it was hoped that an inter-aural line based on the intersection of the rostral base of the pinnae and the mid-saggital plane might prove a reliable external skull landmark. When the SON and MTT are referenced to this external skull landmark (see Table 6) it can be seen that this method is not as reliable as absolute stereotaxic co-ordinates. It is surprising that an external landmark is not as reliable as absolute stereotaxic co-ordinates because external skull landmarks are frequently used in stereotaxic investigations. In rat stereotaxic techniques, for example, using rats of the same age and sex, the intersection of the frontal and parietal bone suture lines (known as Bregma) has been used for locating neural groups in the hypothalamus, however, it is possible that much of the variance in this study is attributable to the age variation in the subjects. The measurements on the basisphenoid bone obviously cannot be used for comparison in this study as it was a post mortem investigation prior to which the animal was removed from the stereotaxic instrument and the skull frozen after the removal of the brain. The errors involved in thawing and resetting the skull in the stereotaxic instrument are unknown but assumed to be small.

Table 7 SON and MTT Referenced to External Landmarks (inter-Aural Line)

Possum No.	SON	MTT
13	4.2	2.0
14	4.3	1.6
15	4.4	2.1
16	3.6	-
17	1.8	-
18	-2.7	8.0
19	-0.5	5.1
20	6.0	0.9
21	2.3	2.9
$\bar{X}$	2.6	3.2
SD	2.917	2.49
SEM	0.97	0.94

SUMMARY

An optical system was set up to measure accurately the distance between internal neural structures. The system involved projecting the section onto a screen upon which a calibration vernier had been projected.

Two neural structures, the SON and the MTT, were selected because they were readily identifiable, had clearly defined borders and occupied the same relative position in the selected possum brains. Lateral measurements were not made for the SON.

From the results of this study the MTT has a mean A/P co-ordinate of 11.2 ± 3.5, and a mean lateral co-ordinate of 1.0 ± 1.4, while the SON has a mean A/P co-ordinate of 17.8 ± 5.6. The ± figures representing the 95% confidence limits.

The probability of a direct on the MTT in any electrode implantation study is 0.0196 while the probability of a partial strike is 0.123.

Clearly stereotaxic co-ordinates are not sufficiently accurate (less than 0.02 chance of a direct strike) to enable localisaton of electrodes into brainstem structures. Neither was referencing neural structures to an external skull landmark.

RESULTS AND DISCUSSION OF THE MICROANATOMICAL STUDY OF A SELECTION OF FIBRE STATE SECTIONS IN THREE STEREOTAXIC PLANES - ANTERIOR-POSTERIOR, HORIZONTAL, AND SAGITTAL

Table 8

Separation (microns) between each plate, corrected to wet brain measurement (using the calculated value of 18% shrinkage, see Table 2). The first plate in each plane of section is taken as the reference starting position. *NB in the transverse sections two blocks were used.

Plate reference		Separation (m)
Transverse	P1	0
	P2	146
	P3	610
	P4	878
	P5	1318
	P6	1952
	P7	2403
	P8*	0
	P9	354
	P10	695
	P11	1183
Horizontal	P12	0
	P13	146
	P14	683
	P15	1025
	P16	1354
	P17	1635
Sagittal	P18	0
	P19	146
	P20	207
	P21	512
	P22	781
	P23	903
	P24	1025

Key to abbreviations used in the following Plates

AC	Anterior commissure
BP	Basis pedunculi
CP	Chorioid plexus
FT	Fibre tract
F	Fornix
IC	Internal capsule
Inf Rec	Infundibular recess
Lat V	Lateral ventricle
MB	Mammillary Body
M Rec	Mammillary recess
MT	Mammillothalamic tract
OC	Optic chiasma
OT	Optic tract
PV	Paraventricular nucleus
SON	Supraoptic nucleus
SO Rec	Supraoptic recess
3V	Third ventricle

Results Plates 1 - 11 photographs of sections cut in the frontal planes

Plate 1: Frontal section in the preoptic area. The plane of section was inclined slightly about a vertical axis with the right hand side structures more posterior than the left hand side.

This section shows the separation of the optic tract from the optic chiasma. There appears to be a collection of fibres extending posteriorly from the optic chiasma (see also subsequent plates) seen here between the separating optic tracts.

The anterior commissure is large and the fornix bears the same relationship to the anterior commissure as in the cat (Bleier, 196/1) and the rat (Albe-Fessard et al., 1966).

Although the stains used in the present study do not markedly distinguish groups of neurons, the magnocellular supraoptic nucleus is clearly visible.

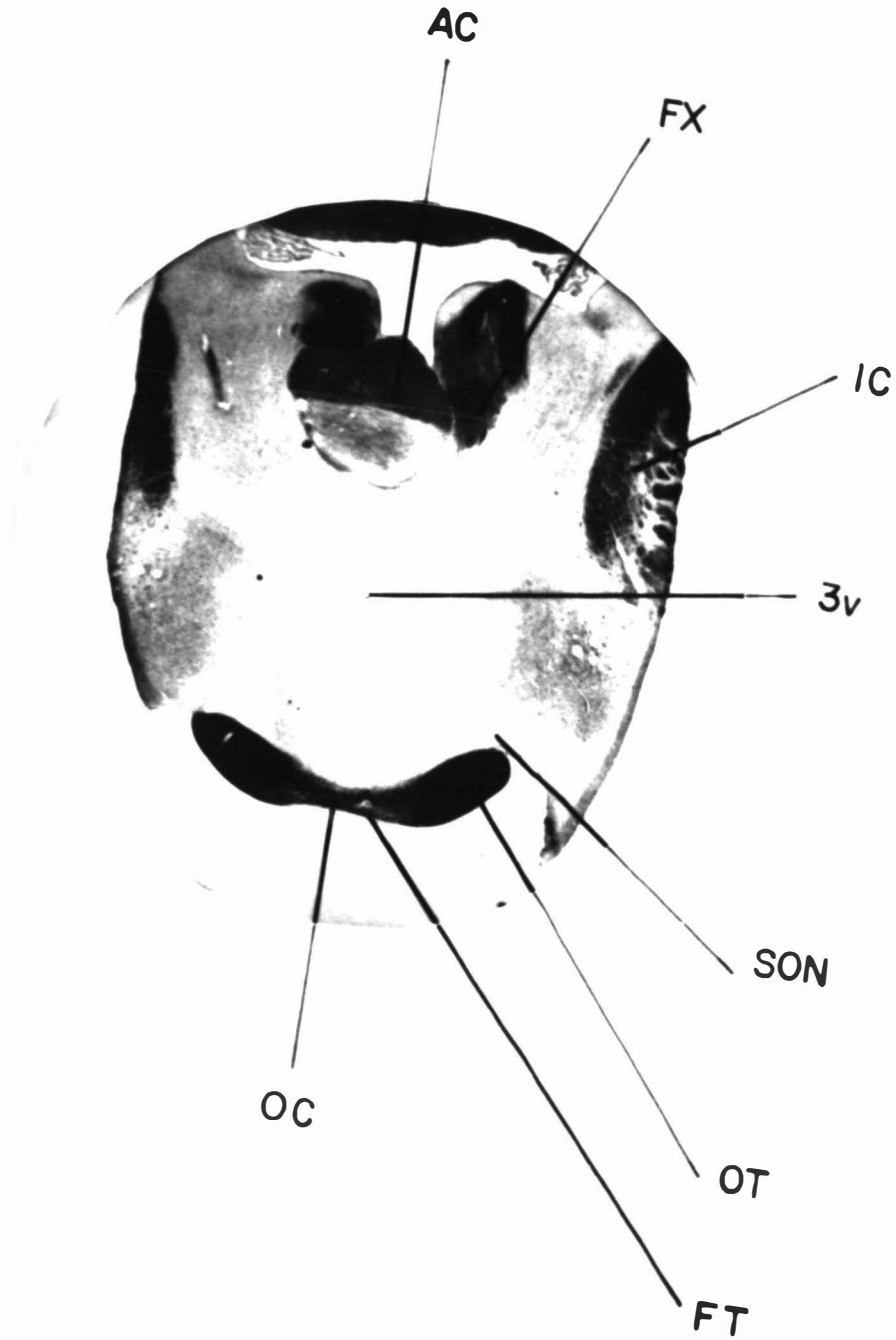

AC
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Plate 2: This section sees the end of the anterior commissure and the interconnection of the lateral ventricle (with its chorioid plexus) with the third ventricle is seen. The fornix is beginning its descent into the hypothalamus and the optic tracts are widely separating with the large system of tracts, which we think are coming from the optic chiasma, being clearly visible.

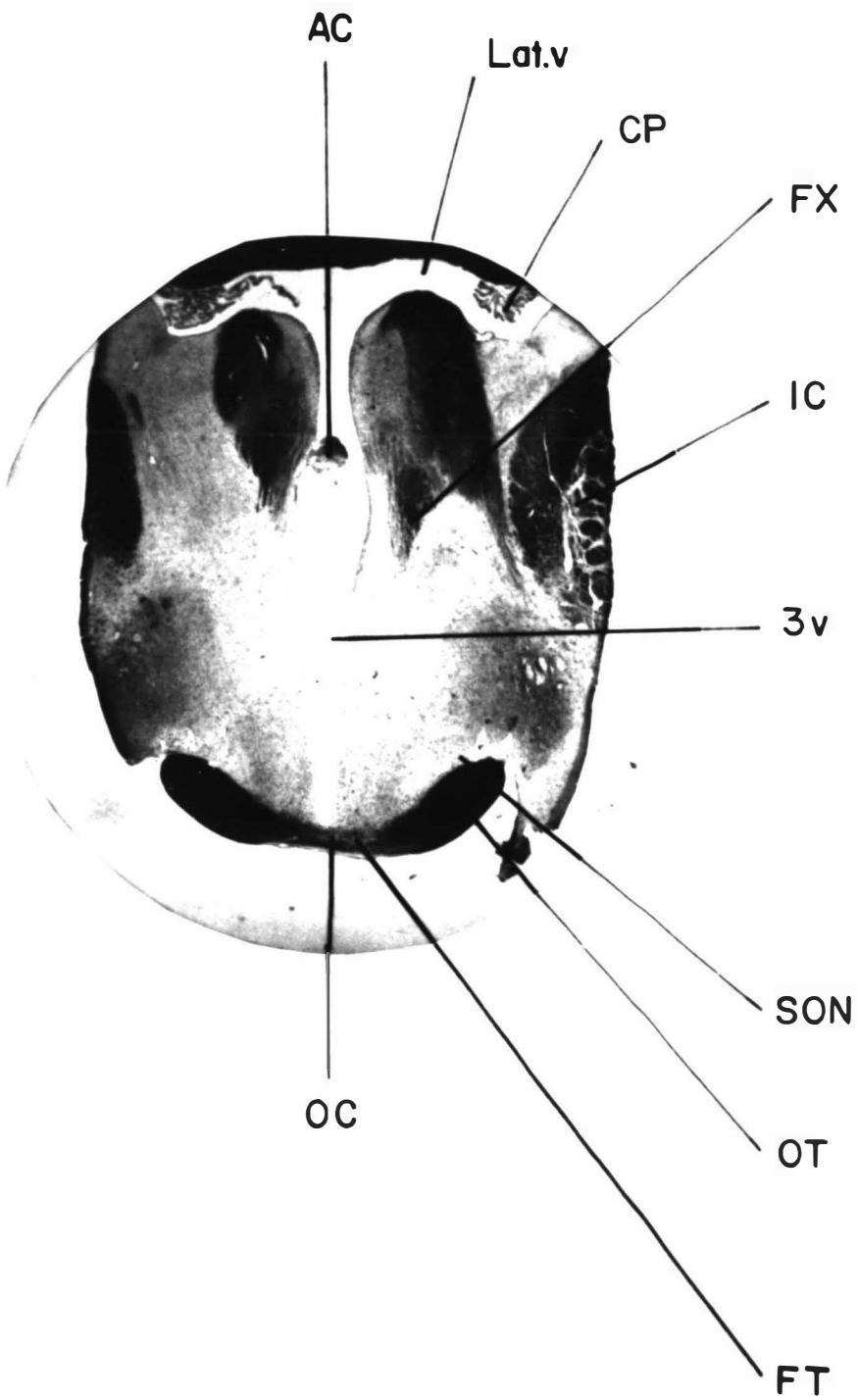
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Plate 3: The most distinguishing feature is the fornix showing a steep descent into the anterior hypothalamus; this feature is not seen in the hypothalami of the cat (Bleier, 1961) the rat (Albe-Fessard, 1966) nor the sheep (Tarttelin, 1976), where the curve of the fornix descending into the hypothalamus is much more gradual and the fornix in frontal section is usually more circular in cross-section. The fibre tract from the optic chiasma is separating from the optic tracts and consolidating in the midline. A secondary fibre tract appears a short distance above this fibre tract, curving underneath the ventral part of the third ventricle.

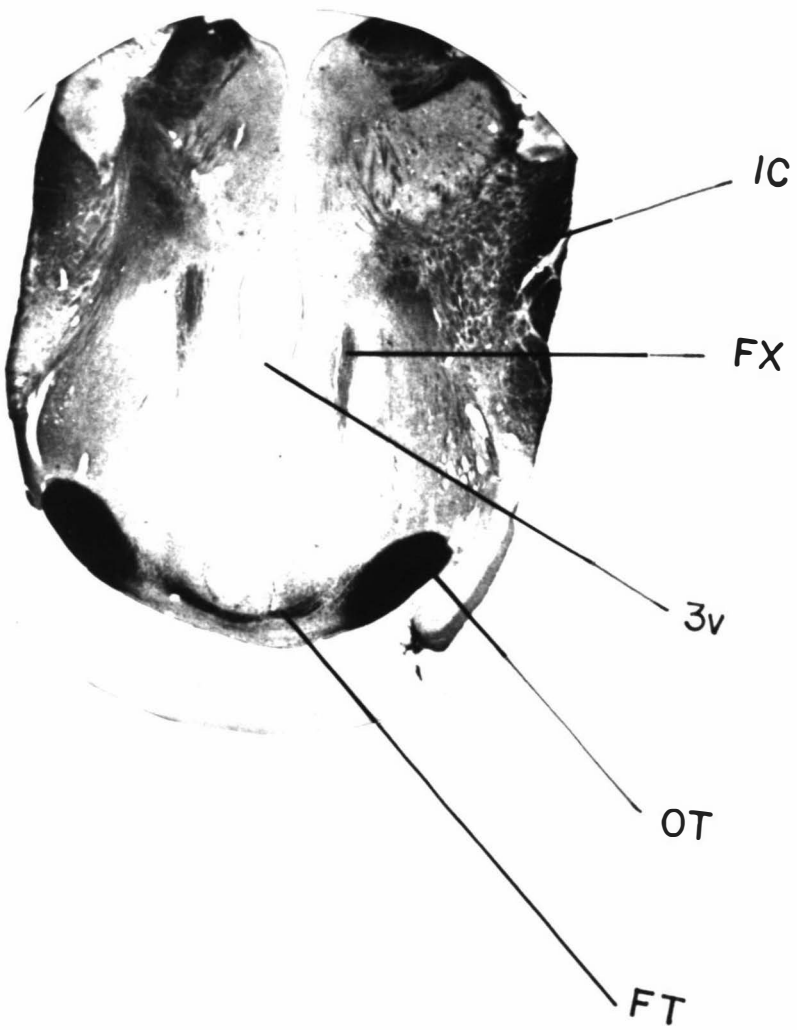
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Plate 4: This section is cut through the posterior part of the anterior hypothalamus immediately rostral to the median eminence. A small group of neurons which is through to be the paraventricular nucleus (the second magnocellular nucleus) is visible. The ventral fibre tract which appears to originate from the optic chiasma is seen curving around the ventral part of the third ventricle. This section has cut the fornix in the middle of its steep descent into the hypothalamus.

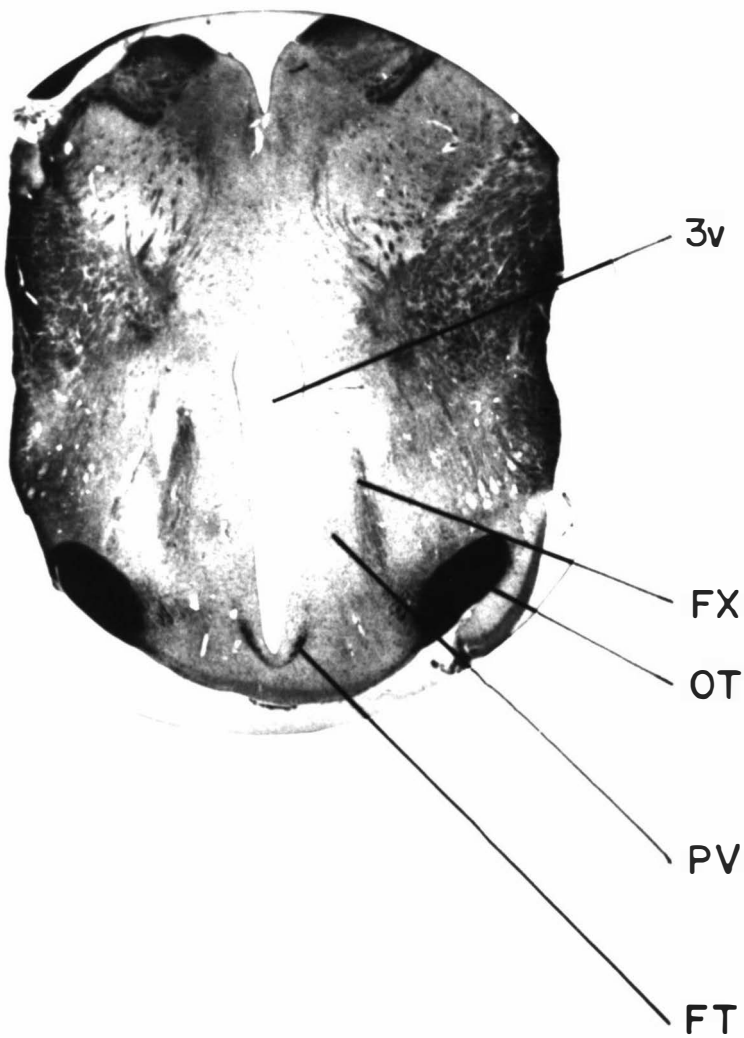
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Plate 5: In this section the fornix is cut in circular cross-section where it has descended into the ventral part of the hypothalamus. The fibre tract seen in previous Plates has moved dorsally and appears to be at a level but slightly posterior to the paraventricular nucleus seen in the previous Plate (P. 4). The infundibular recess is seen so Plate 5 is cut at the level of the median eminence.

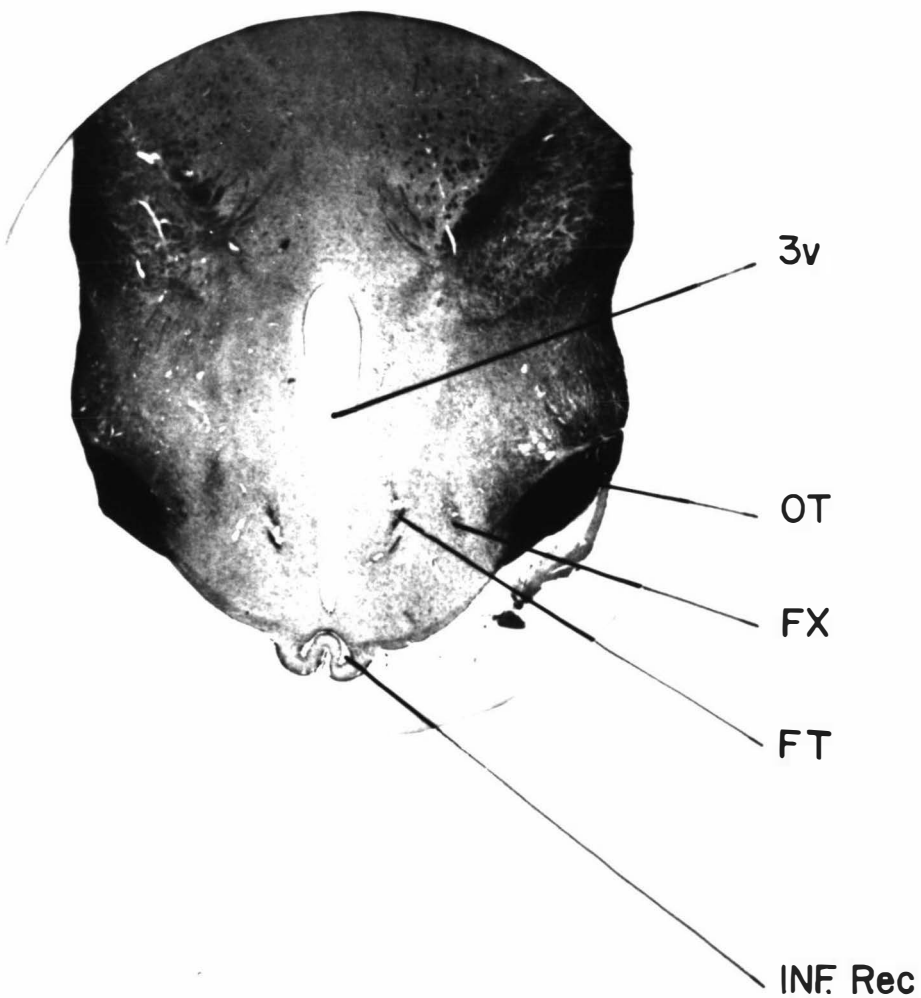
3v
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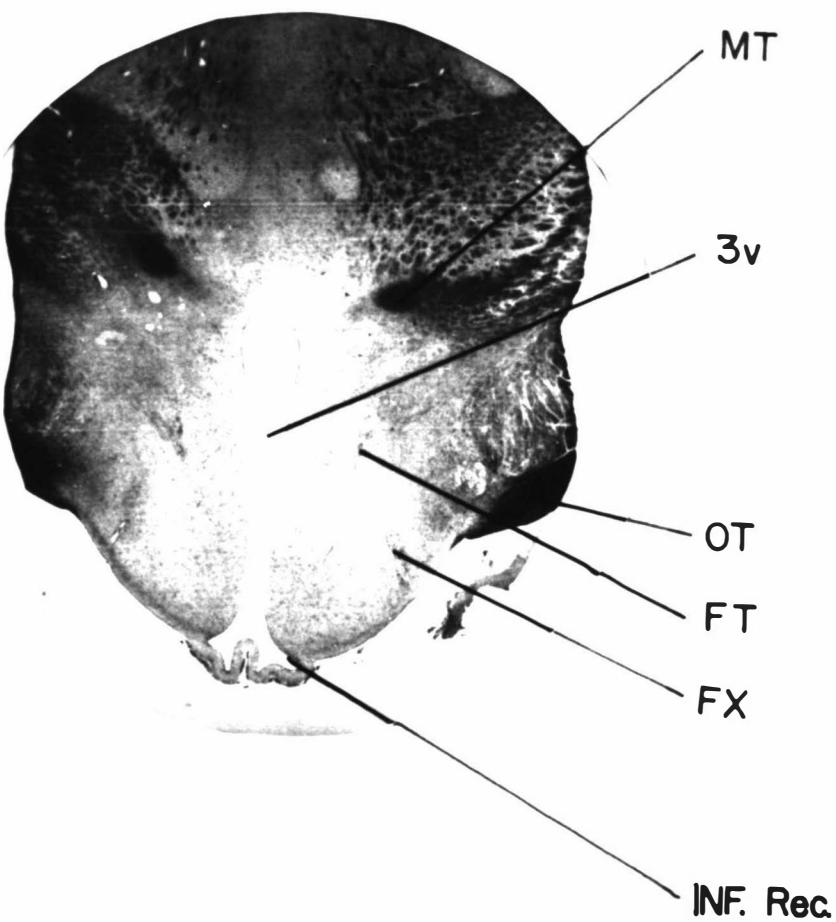
Plate 6: The fornix is faint, but still visible. A much more densely stained tract, the mammillothalamic tract is seen in the dorsal part of the section. This tract originates in the mammillary body and rises steeply (see sagittal plates) through the posterior hypothalamus into the thalamus. A part of the previously described fibre tract is still visible in the dorsal part of the hypothalamus, on a level with the optic tracts.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INF. Rec.

Plate 7: The premammillary area is beginning so we see the end of the median eminence area. The fornix has reached the base of the hypothalamus and is merging with strongly staining fibres in this area. No further evidence of the fibre tract seen in previous Plates can be seen.

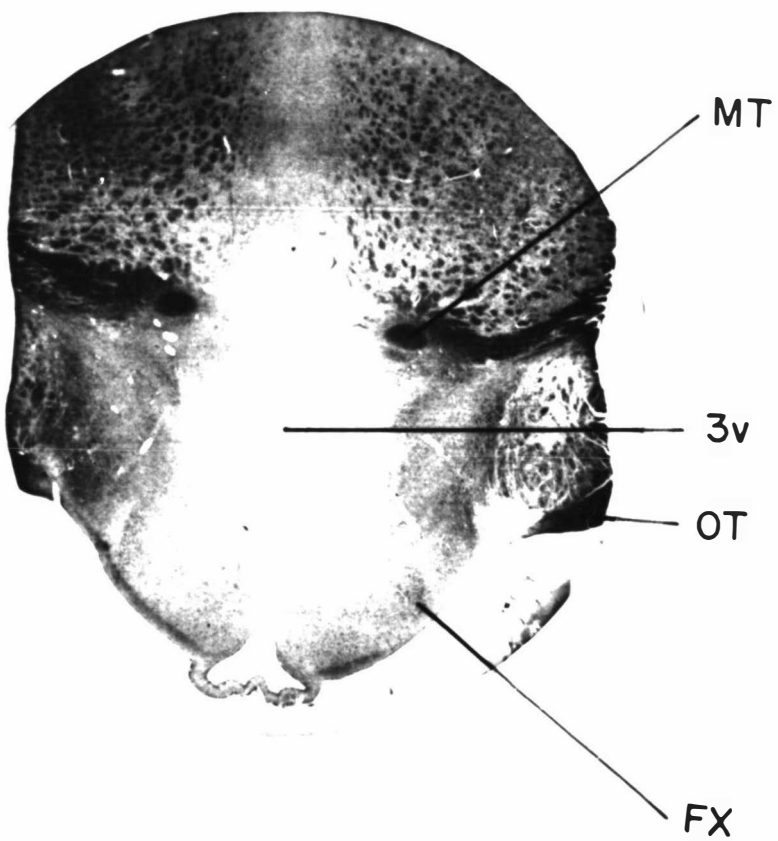
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Plate 8: The fornix is still visible but its associated ventrobasal fibres are more visible than in the previous Plate. The mammillothalamic tract is lower in the posterior hypothalamus. The basis pendunculi, consisting of the descending motor tracts which have traversed the internal capsule are visible.

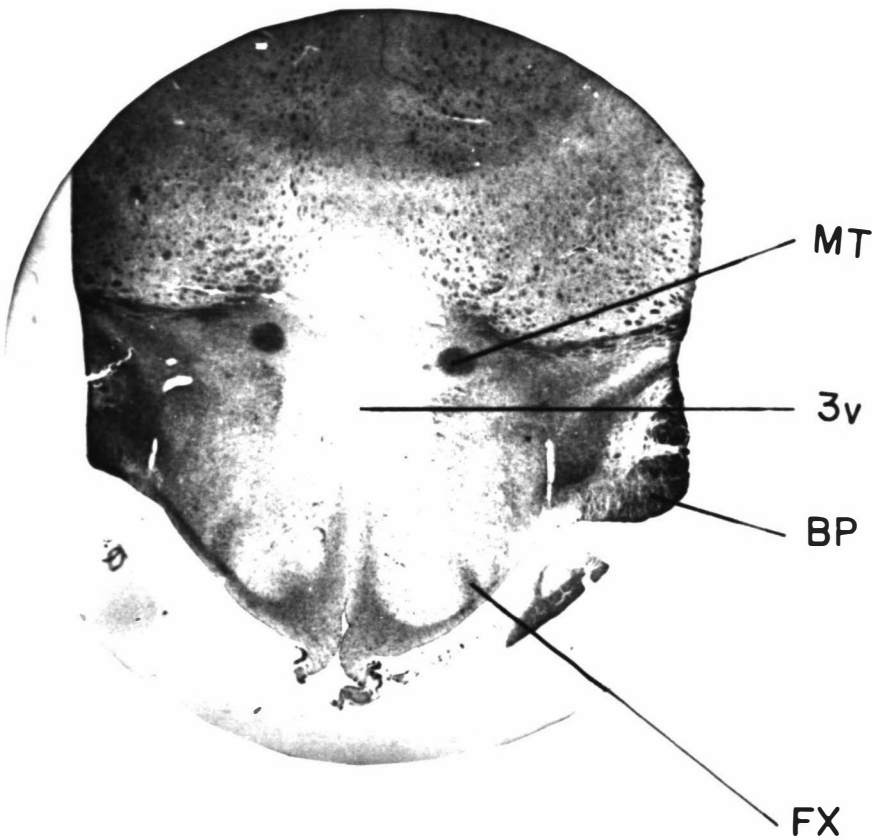
MT
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Plate 9: Similar to Plate 8. The fornix has almost disappeared.

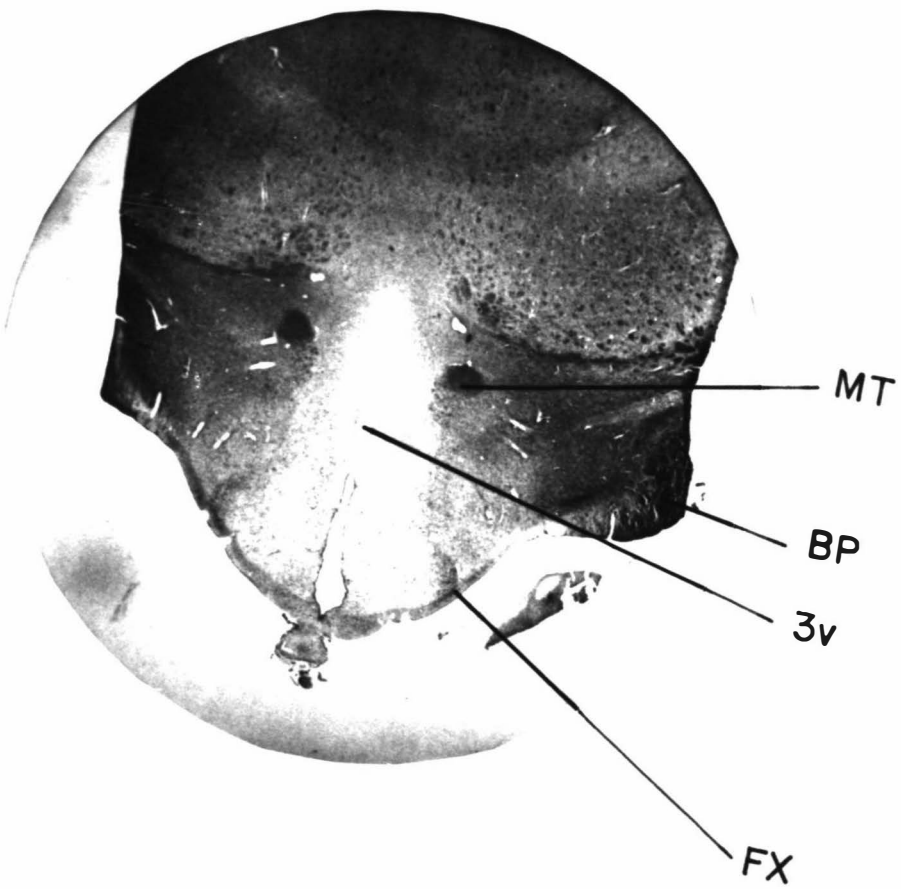
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Plate 10: The shape of the mammillary body is being formed and its mammillary recess of the third ventricle is clearly seen. The massive fibre system of the mammillothalamic tract is seen splitting into its various origins.

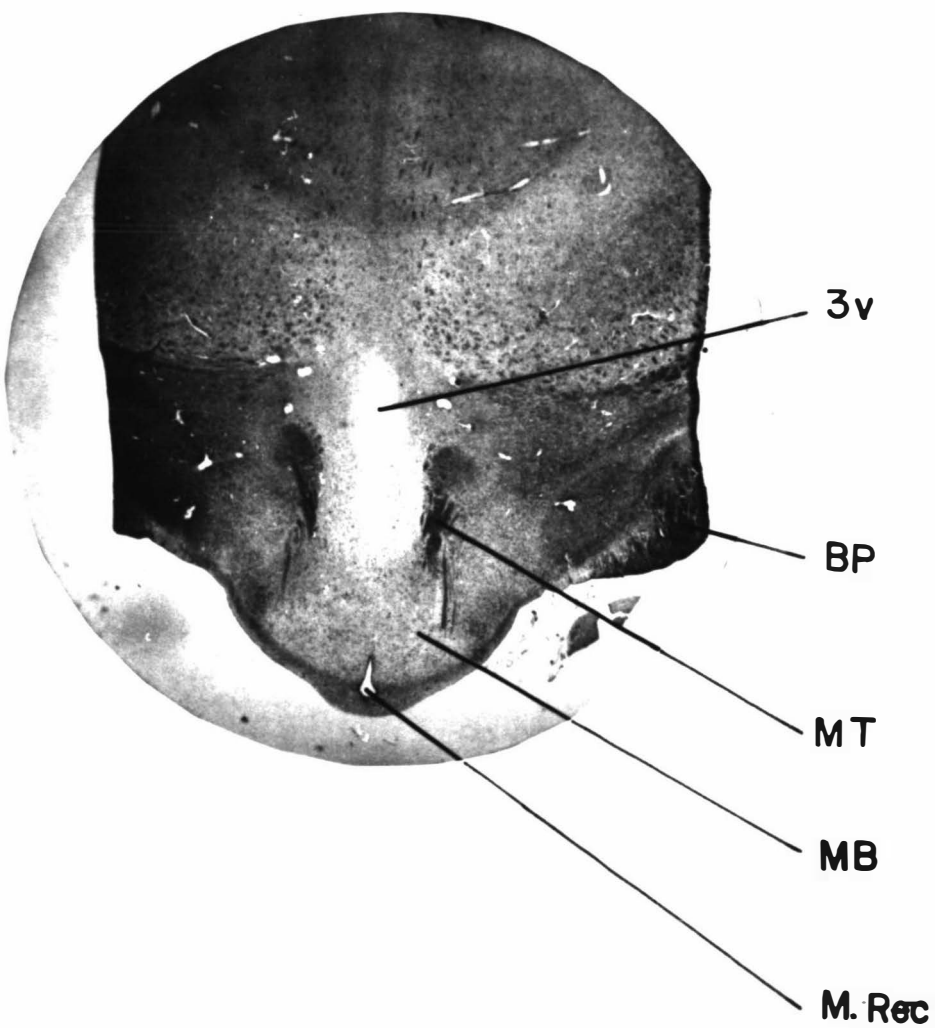
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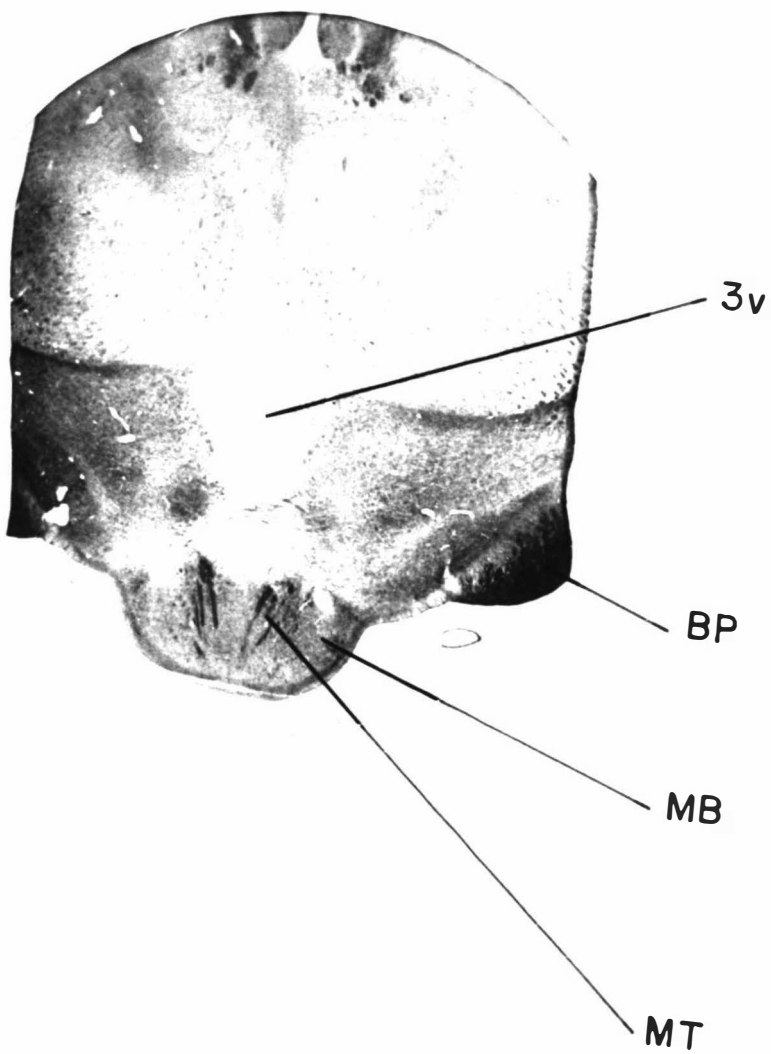
Plate 11: This is the last Plate in this series and represents the most caudal part of the hypothalamus which is dominated by the mammillary body. The mammillothalamic tract is seen as its originations. The third ventricle is seen as a small cavity immediately rostral to the commencement of the interventricular foramen.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Plates 12 - 17 Photographs cut in the horizontal plane

Plate 12: This is the most basal of the horizontal sections and shows the heavily myelinated fibres of the optic tract and the optic chiasma. The third ventricle is clearly defined (as it is throughout the horizontal series). The numerous heavily staining fibre systems of the mammillary body make this structure particularly evident. A fibre system, described in the Plates of the frontal series, is seen at its originations with the optic chiasma. There is a slight asymmetry in the angle of section of the horizontal series.

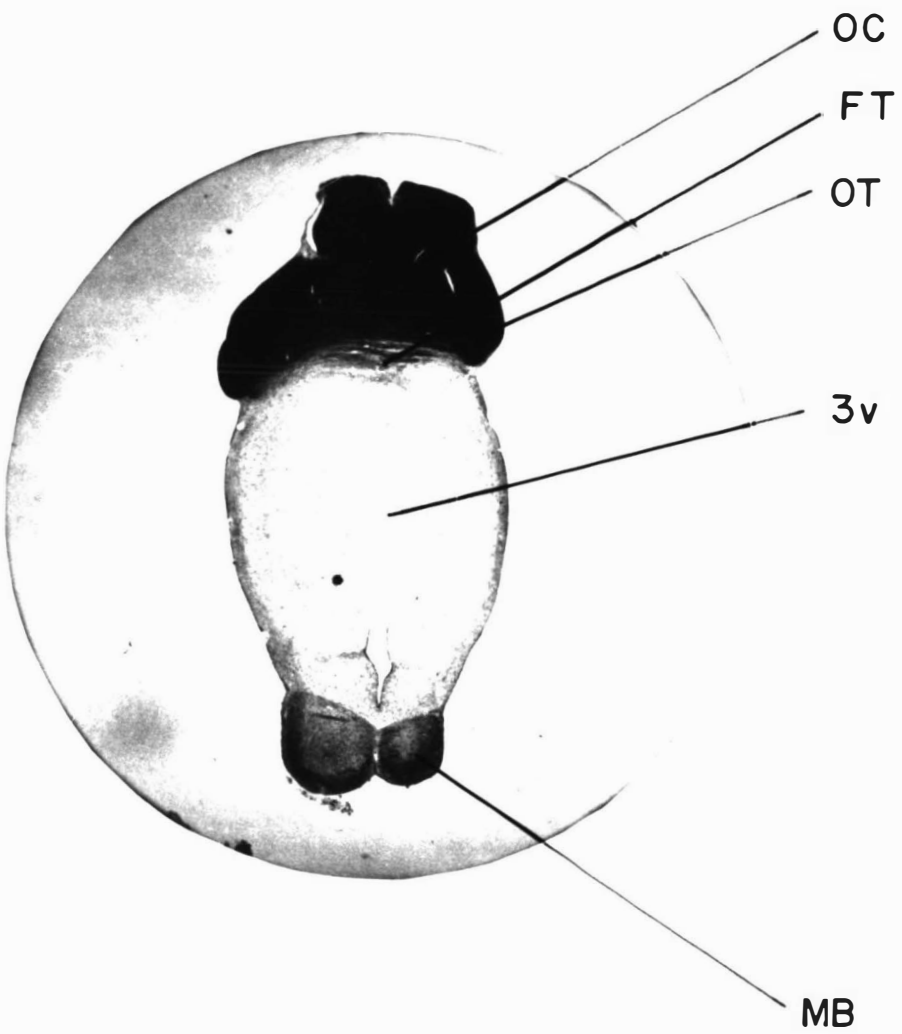
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Plate 13: Most of the features described in the previous plate are still obvious particularly the fibre system leaving the optic chiasma. In addition, the fornix is seen faintly on one side (because of the asymmetry of section).

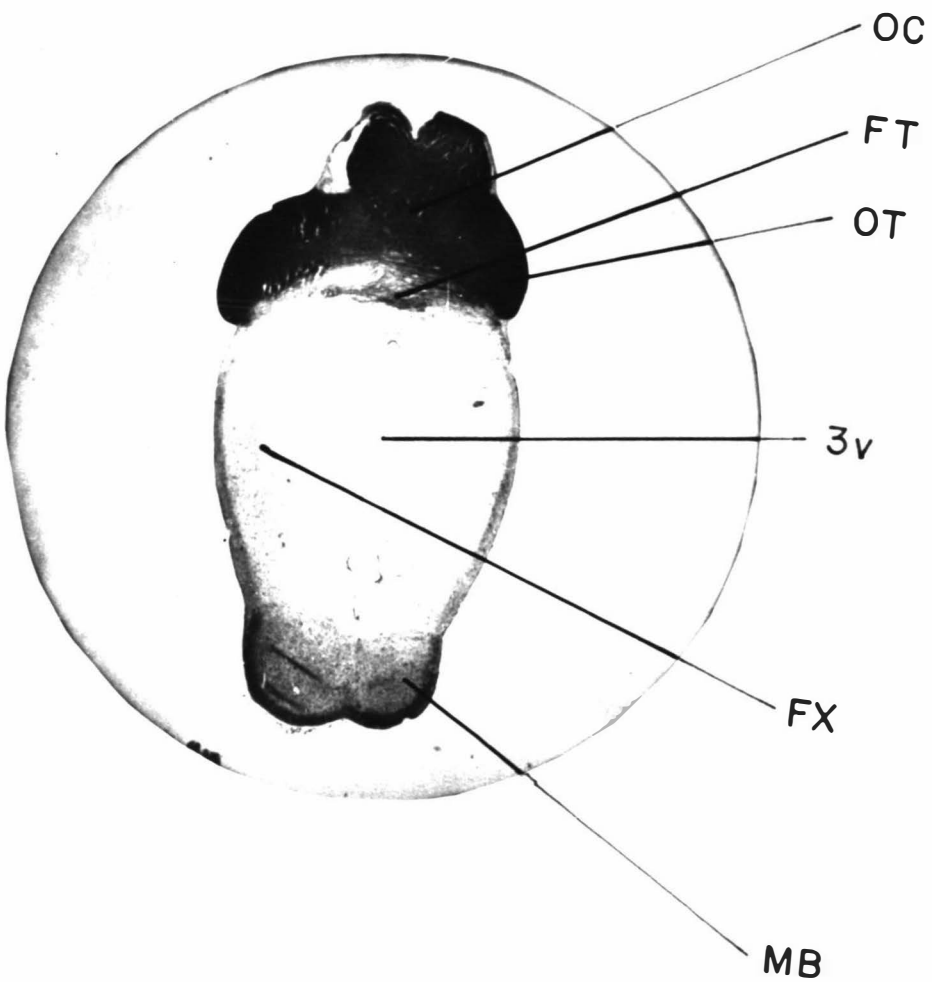
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PLATE 14: The fornix is seen on the left side clearly in this section and shows the extent of its traverse of the hypothalamus in a horizontal plane after the steep descent in the rostral hypothalamus. The originations of the various fibre systems constituting the mammillothalamic tract are seen. The fibre system originating from the optic chiasma is seen clearly stained in this section.

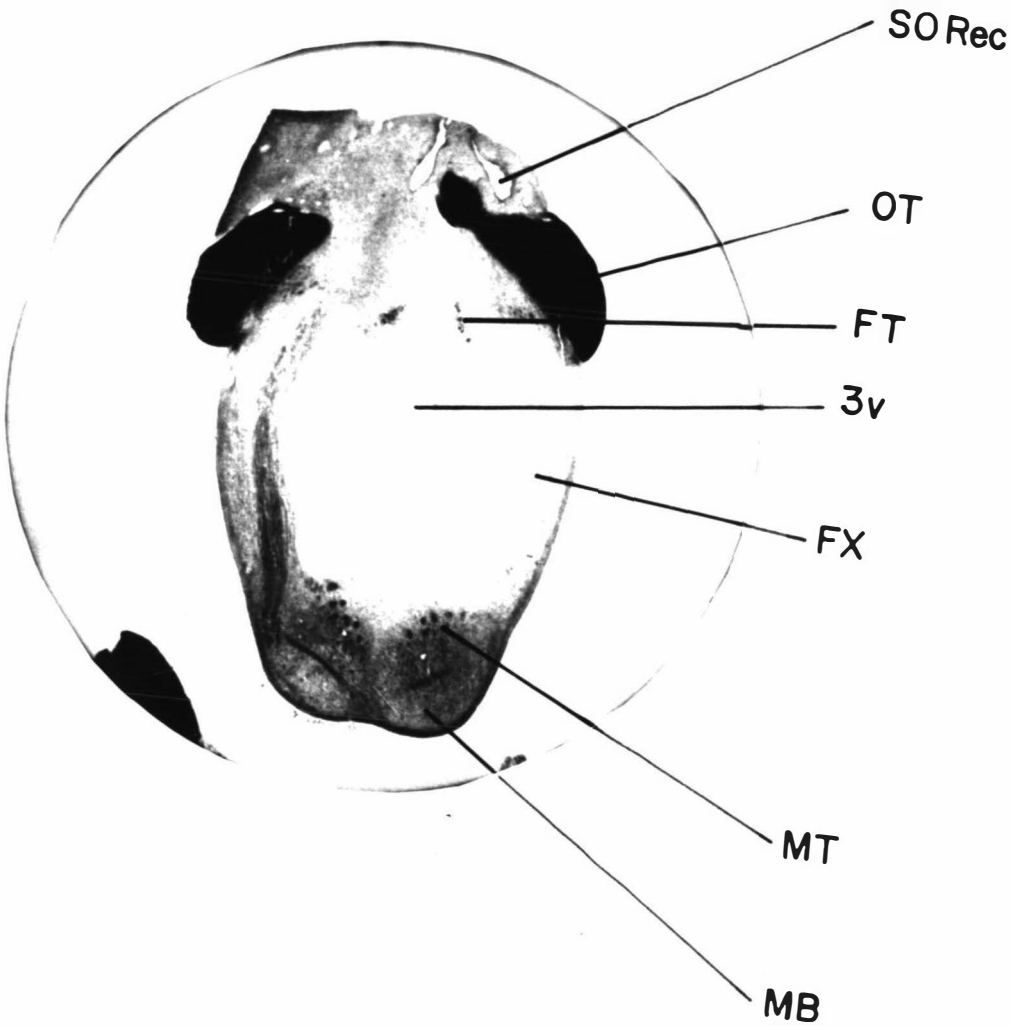
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PLATE 15: The fornix on both sides is now apparent. The fibre tract from the optic chiasma is much smaller in crossection at this level, but still clearly visible.

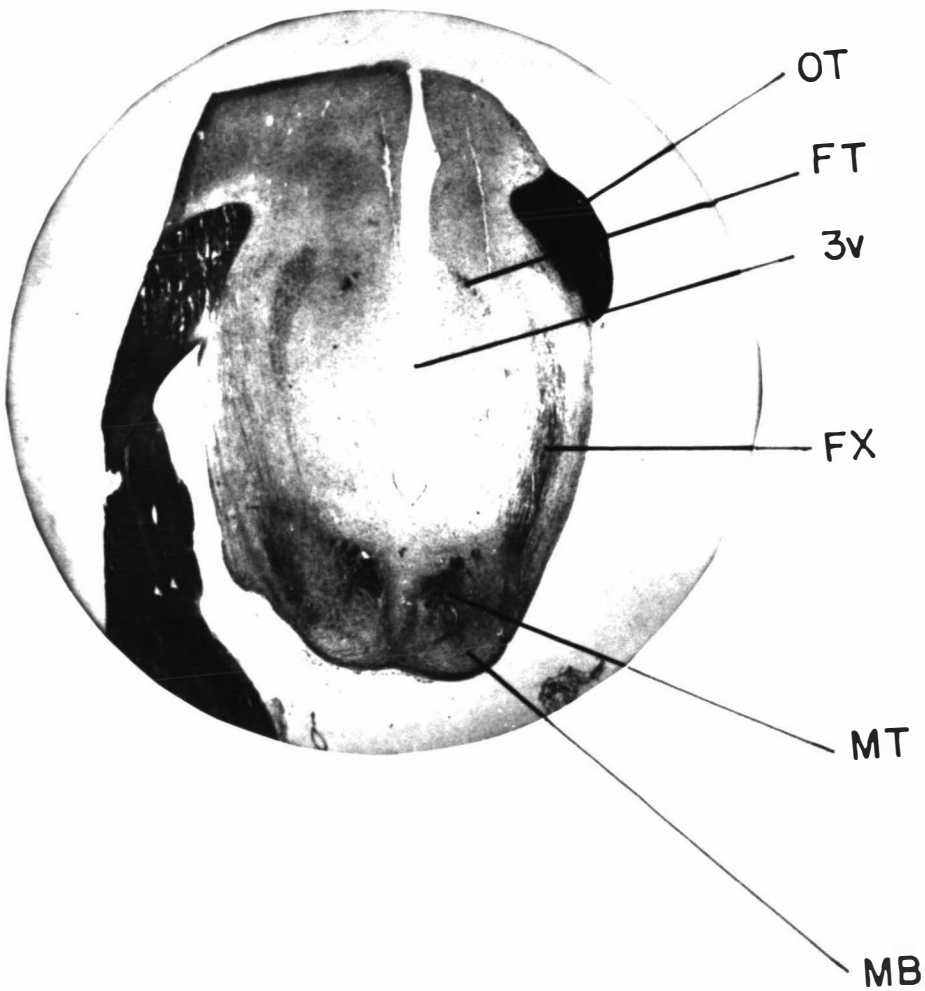
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PLATE 16: This section shows the ascending component of the mammillothalamic tract, the descending limb of the fornix on the right hand side and the position of the steeply descending limb of the fornix on the left. The fibre tract from the optic chiasma is still visible, but less clear.

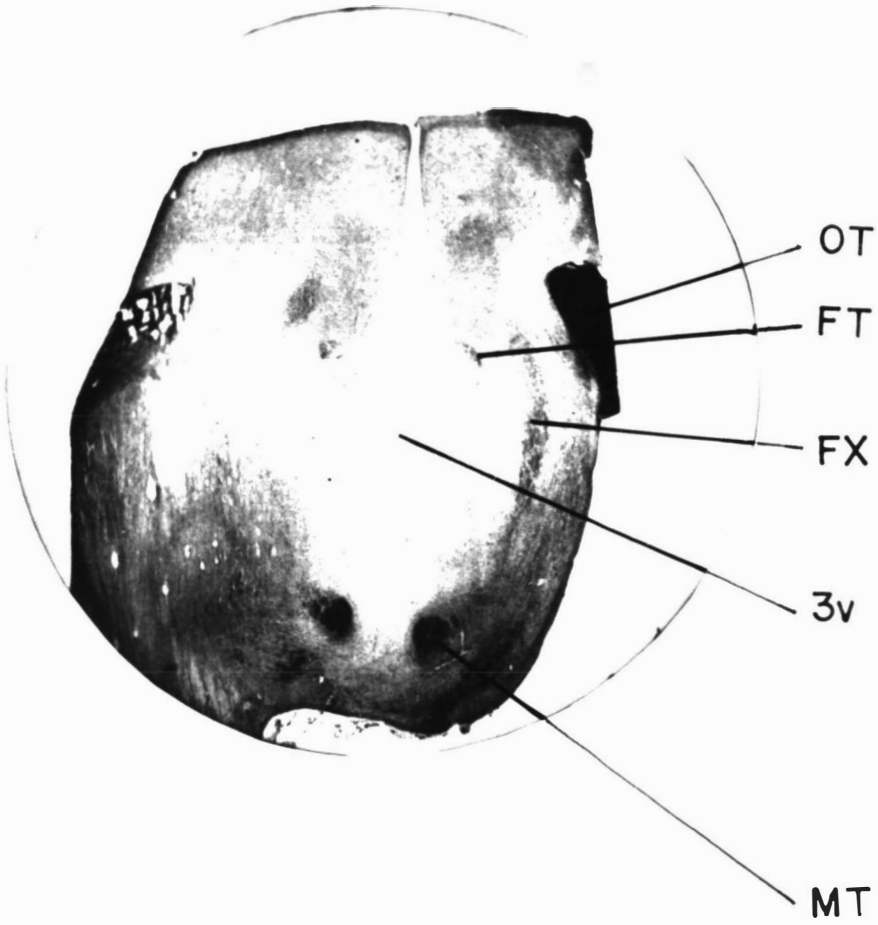
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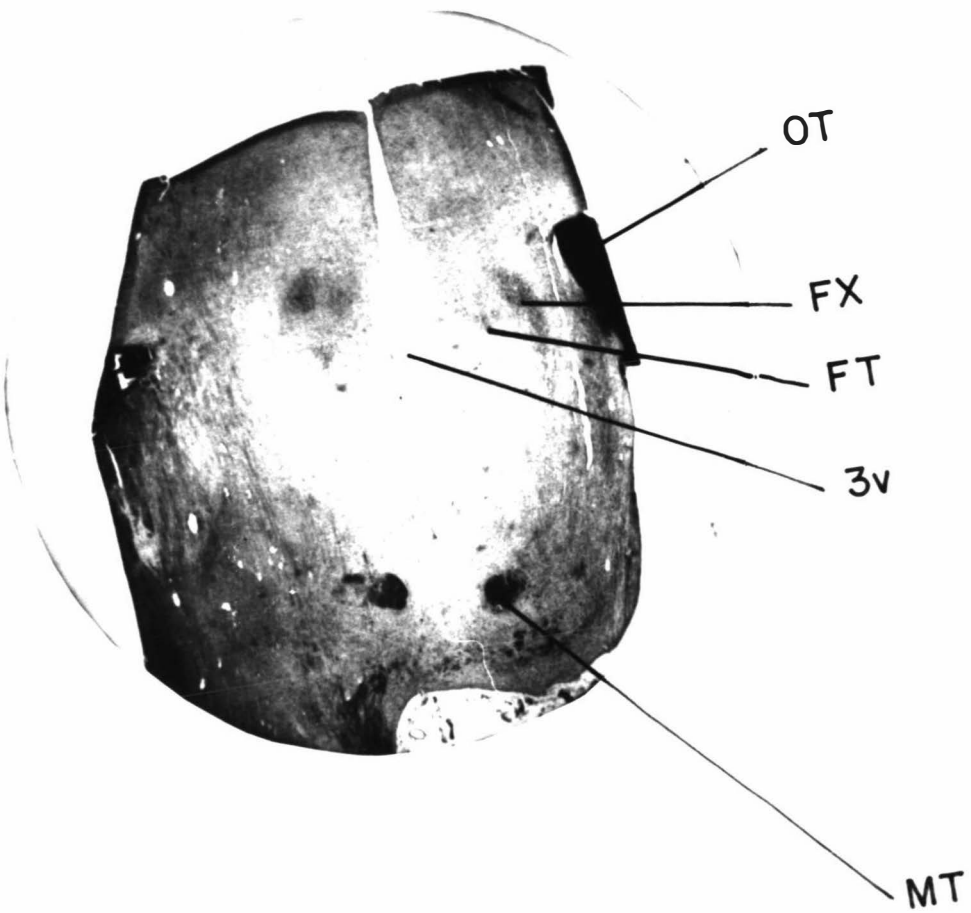
PLATE 17: This is the last section in the horizontal series and shows the descending limbs of the fornix in their steeply descending position in the rostral hypothalamus and the steeply ascending part of the mammillothalamic tract in the caudal hypothalamus. The fibre system from the optic chiasma has almost disappeared and there is no information in these sections regarding its terminations.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Plates 18 - 25 Photographs of sections cut in the saggital plane

PLATE 18: This is the most medial of the sagittal sections and shows the infundibular and the supraoptic recesses of the third ventricle. A fibre tract which appears to originate from the optic chiasma is seen in this section (and the next four Plates). This fibre tract would appear to be curved since it is seen in two parts in this section (see Plates 4 and 5).

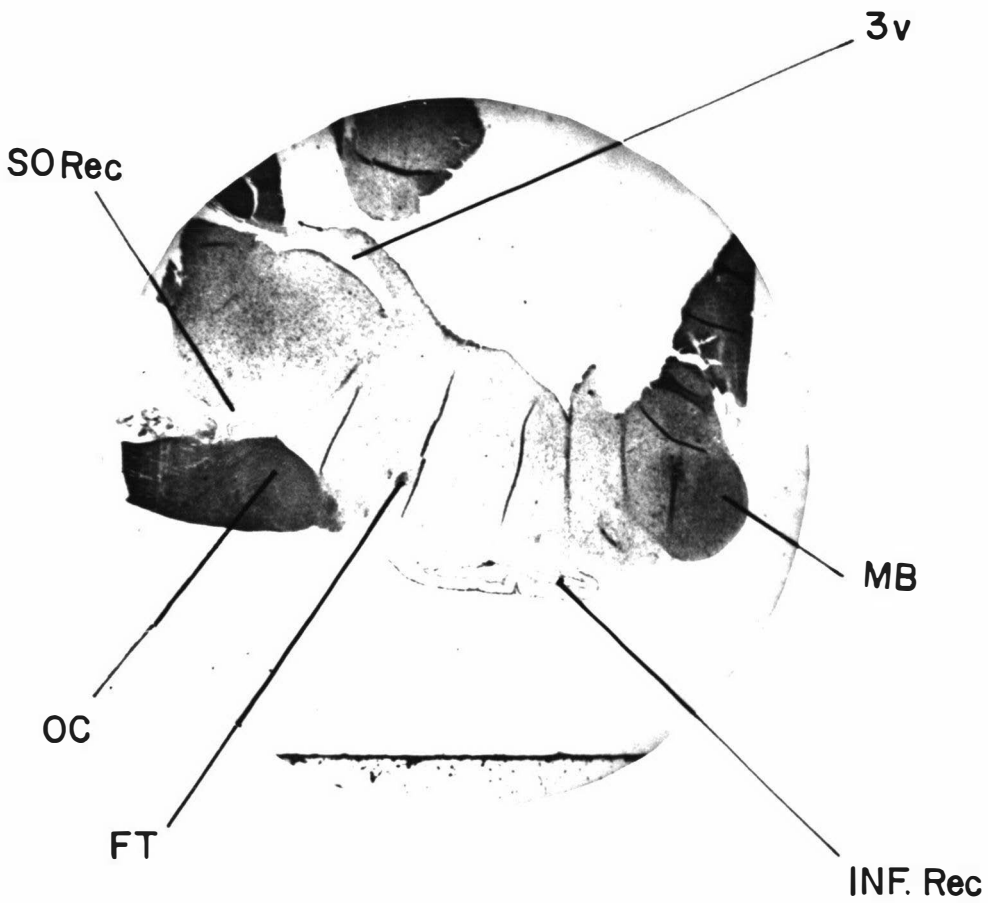
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PLATE 19: The third ventricular recesses are less evident. The origin of the mammillothalamic tract is strongly stained. The fibre tract from the optic chiasma is clearly shown.

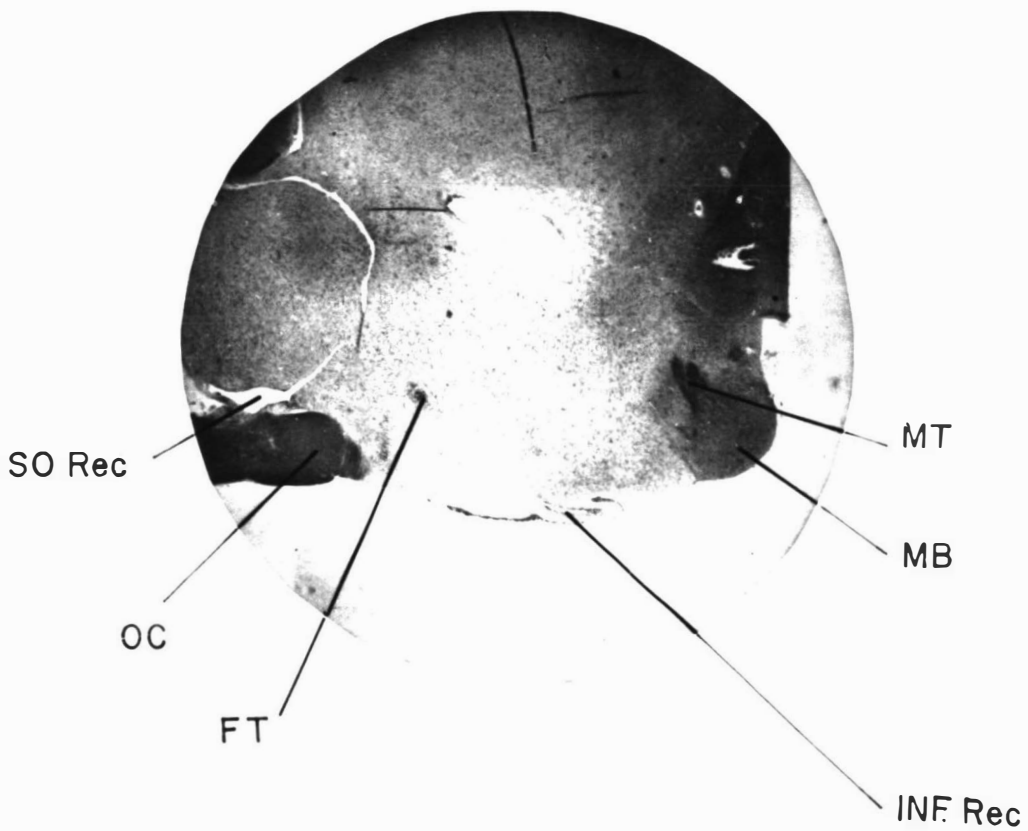
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PLATE 20: The features visible in this Plate are as described in the previous Plate. The two distinctive parts of the fibre tract from the optic chiasma are clearly seen.

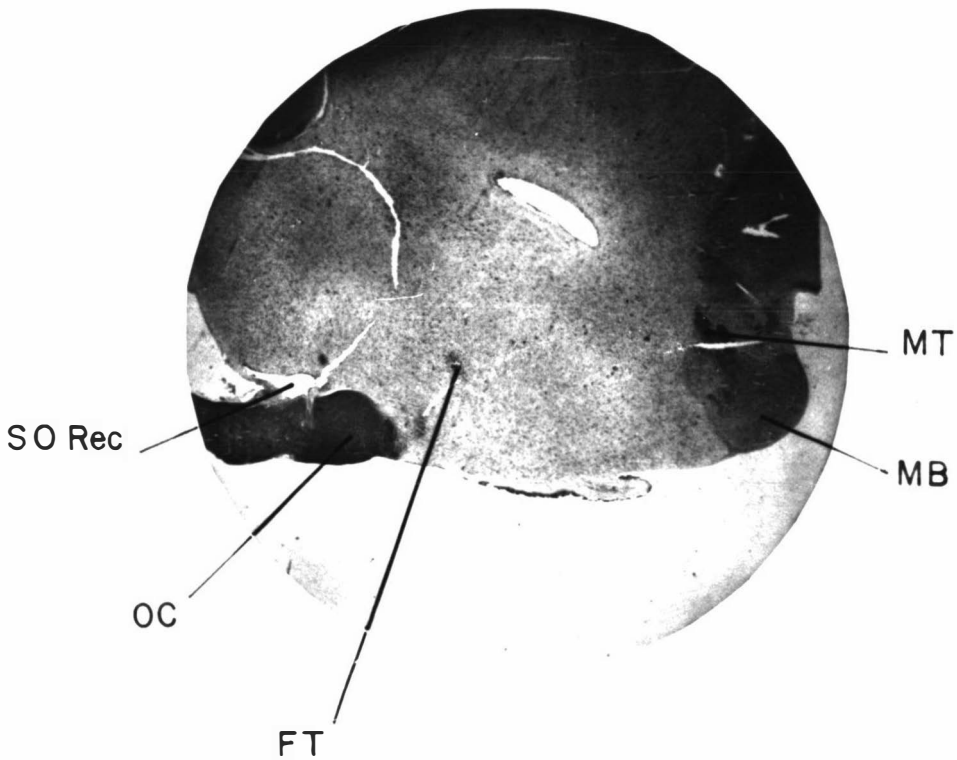
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PLATE 21: The fibre tract from the optic chiasma is seen at a higher level in the dorsal part of the hypothalamus, clearly defined since there are no other myelinated tracts in its vicinity. The first sight of the descending limb of the fornix is seen and a rather diffuse sight of the ascending component of the mammillothalamic tract.

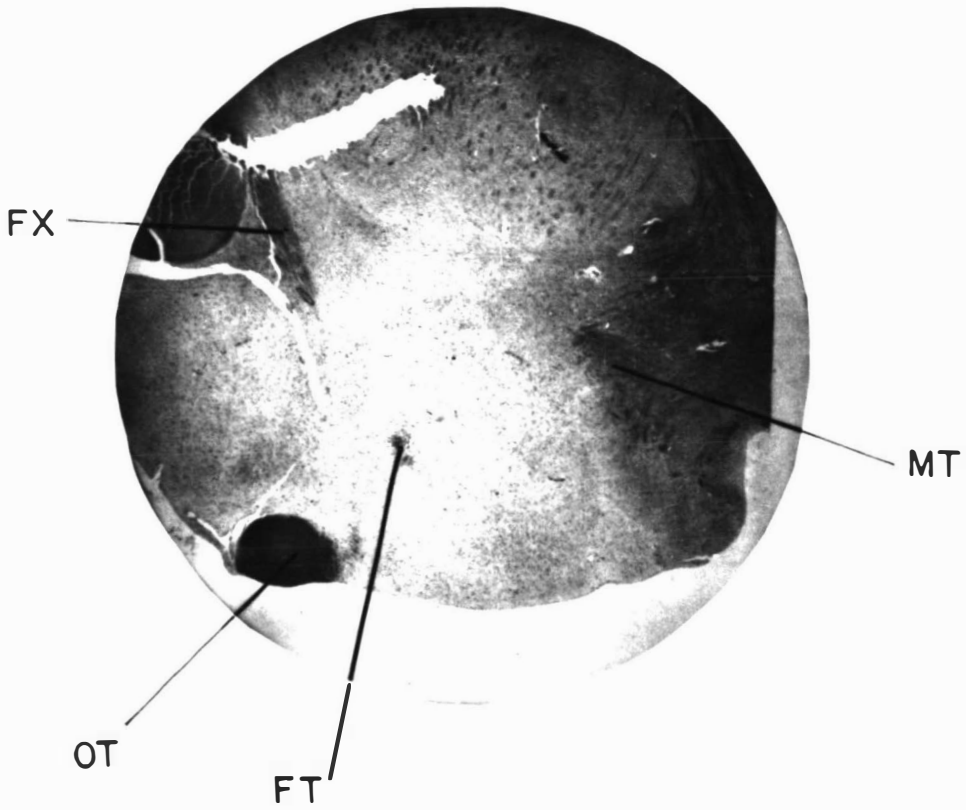
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PLATE 22: The fornix is seen as its descending component at the rostrodorsal level of the hypothalamus and also at its destination in the mammillary body. The ascending component of the mammillothalamic tract is clearly defined. The position of the paraventricular nucleus is seen. High power examination of this section reveals the magnocellular details of this nucleus and also fibres which appear to be the fibre tract referred to throughout the descriptions of the plates presented so far. This section in the sagittal plane shows the continuation of the fibre tract with the paraventricular nucleus because the tract appears to go towards the posterior border of the paraventricular nucleus.

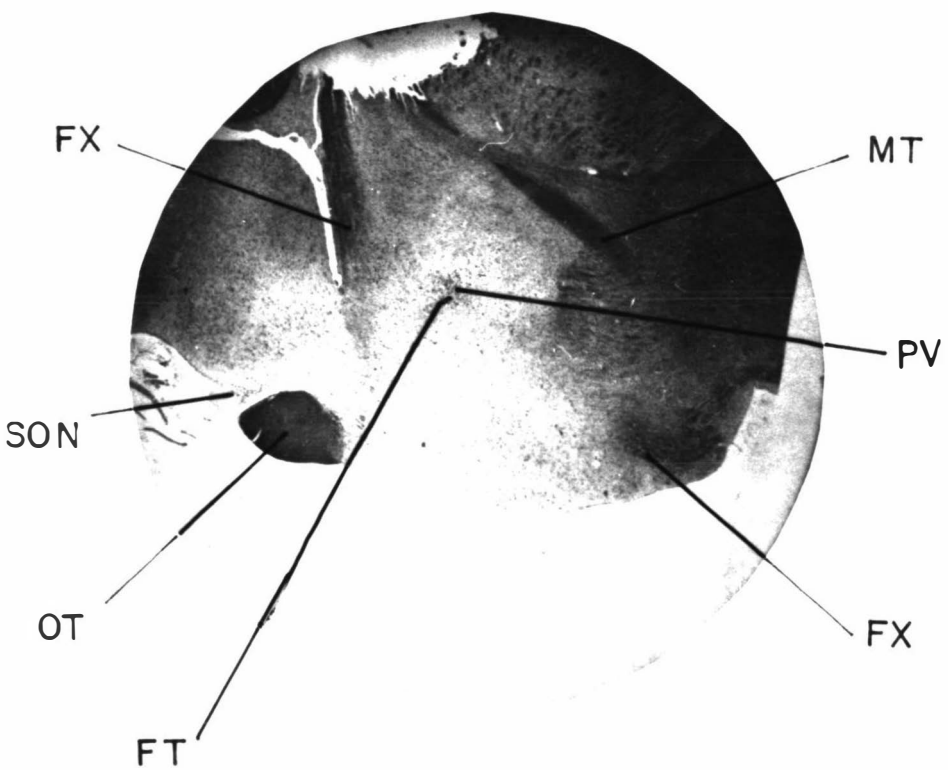
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PLATE 23: The cells of the paraventricular nucleus can still be determined under high power but the ascending (?) fibre tract is no longer clearly defined although a system of myelinated fibres connected with the dorsoposterior part of the hypothalamus appears to be continuous with this region.

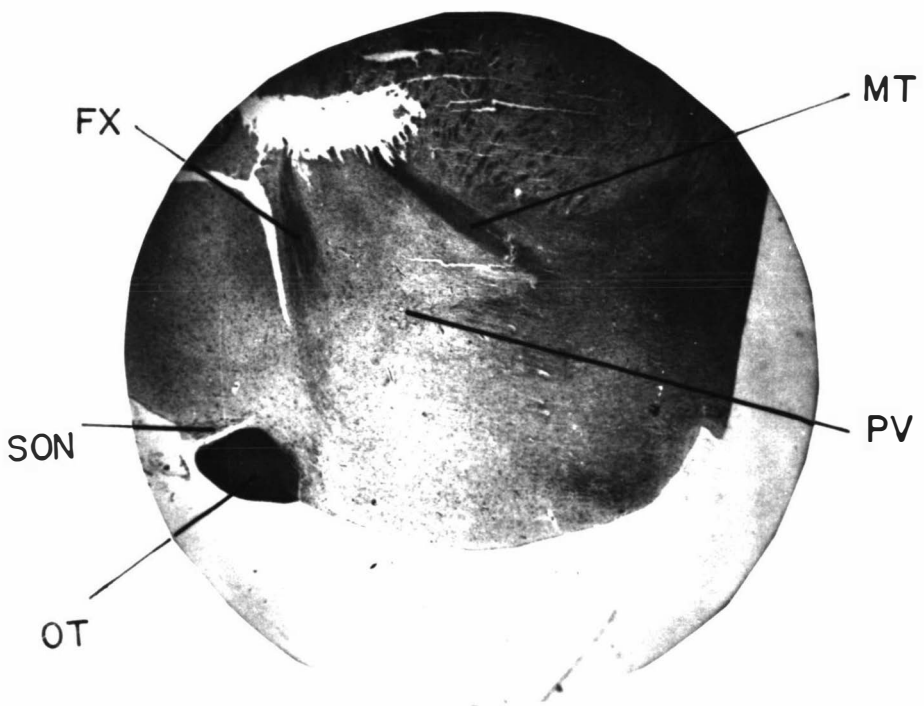
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PLATE 24: The fibre system which appeared to be continuous with the region of the paraventricular nucleus discussed in the previous Plate is no longer clearly defined. The fornix can be seen in its two components the anterior descending component and the ventrobasal destination in the mammillary body.

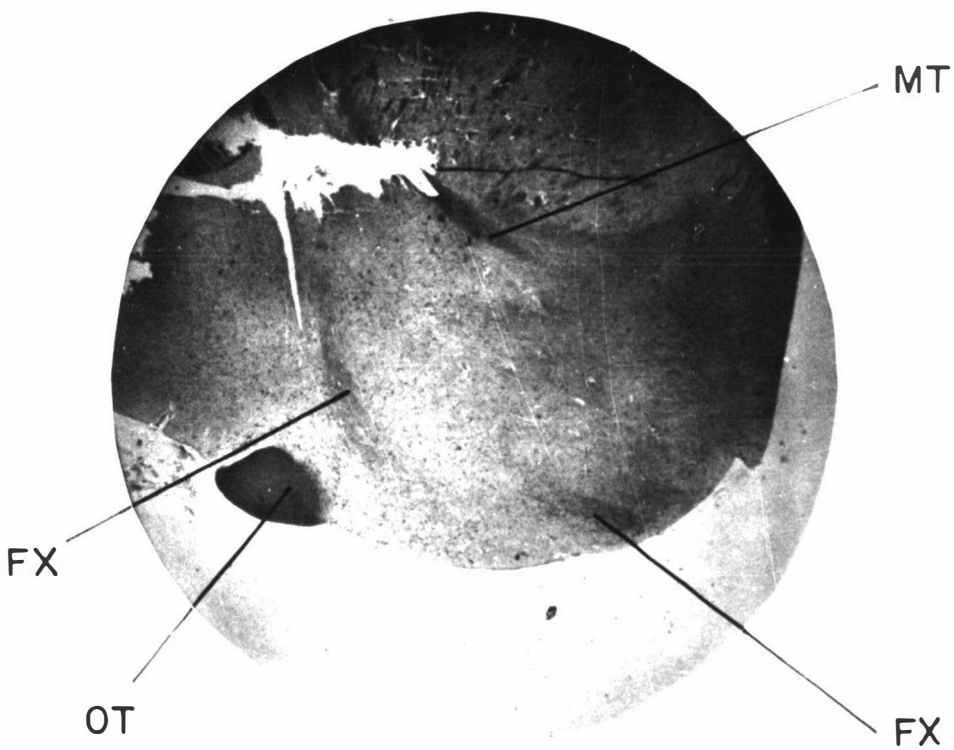
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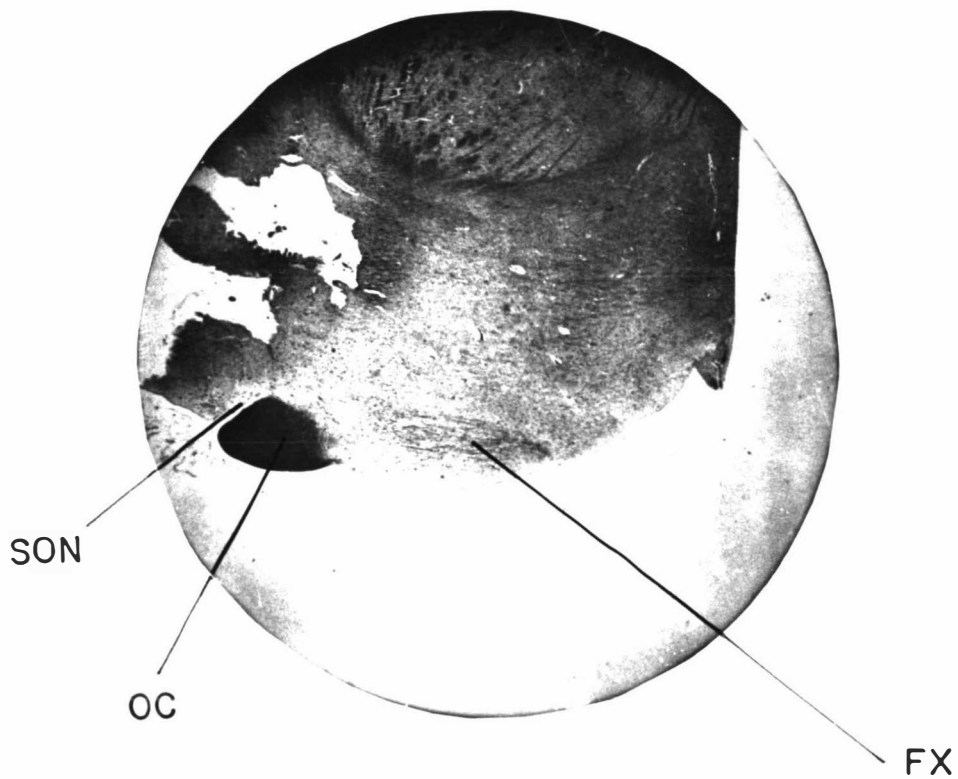
PLATE 25: This is the last Plate in this series and shows the component of the fornix in the ventrobasal part of the hypothalamus.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DISCUSSION

Little information is available on the anatomy of the Australasian possum (Trichosurus vulpecula) brain. A study by Warner (1969) on the hypothalamus of the developing possum brian fails to adequately demonstrate distinctive groups of neurons and merely quotes the standard structures seen more clearly in such brains as the rat and the cat. In addition, Warner's study does not employ a fibre stain thus making a specific study of fibre tracts difficult. The present investigators are not aware of any other published research on fibre tracts within the possum hypothalamus.

There is an abundance of published material on the anatomy of the American opposum (Didelphis virginiana) (Oswaldo-Crus and Rocha Miranda 1967, Salino et al 1971) but even these references fail to convincingly portray distinctive nuclear groups within the hypothalamus. One concludes that the possum and the American opossum maybe do not have the clearly distinguishable magnocellular and parvocelluar nuclear groupings seen in other mammals. The studies on the American opossum also do not adequately describe fibre tracts.

The present study aims at presenting a simple description of the possum hypothalamus viewed in three planes of section and concentrating on some of the fibre tracts which are clearly visible. The main findings were that the mammillothalamic tract appears in a similar position as seen in other mammals such as the rat, cat and sheep. The fornix appears much steeper in its descent into the anterior hypothalamus and runs parallel to the base of the hypothalamus throughout the tuberal region. We have described a tract which does not appear to have been described before which according to our studies appears to branch away from the optic chiasma and cup around the base of the third ventricular before dividing into two lateral parts which pass up to the caudal part of the paraventricular nucleus. We did not have the opportunity to study this area further and cannot be certain if the fibre tract connects with the paraventricular nucleus or not. Also we are not certain whether this fibre tract then branches posterior and laterally to link up with another fibre system (see sagittal plates).

Clearly this interesting departure from the pattern which is present in many other mammals needs further research. It is possible that this fibre tract represents the "elusive" accessory optic tract which has been so controversial in the study of the mammalian hypothalamus for so many years.

APPENDIX 1

Embedding Process for Possum Brain Material

70% Alcohol for 2 hours
95% Alcohol for 1 hour
Absolute Alcohol for 1 hour
Absolute Alcohol for 1 hour
Absolute Alchohol for 1 hour
Absolute Alcohol for 2 hours
Chloroform for 1 hour
Xylene for 1 hour
Xylene for 1 hour
Wax (i) for 2 hours
Wax (ii) for 2 hours

Total time 15 hours

APPENDIX 2

BRAIN HISTOLOGY

1. Cool off slides from oven.
2. Xylene (i) - 5 minutes
3. Xylene (ii) - 5 minutes
4. Absolute alcohol - gently agitate for approximately 1 minute
5. 70% alcohol - gently agitate for approximately 1 minute
6. Sit in slow running tap water for approximately 1 minute.
   If cloudy change to clean 4) and 5) and repeat same.
7. Place in Solochrome Cyanin solution for 10 minutes
   (see Appendix 3 for staining solution)
8. Wash well in running tap water until sections turn blue.
9. Differentiate in 2% $FeCl_3$ until grey matter appears almost colourless.
10. Place in water again for approximately 2 minutes (until turn blue).
11. Into freshly made Cresyl Fast Violet solution at 60°C for 6 minutes.
    (See Appendix 3 for staining solution)
12. Differentiate and dehydrate in absolute alcohol (i) - briefly
13. Absolute alcohol (ii) - briefly
14. Clear in Xylene (i)
15. Xylene (ii)
16. Cover slip with DPX.

Birtles modification of Page (1965).

APPENDIX 3

STAINING SOLUTIONS FOR HISTOLOGY

SOLOCHROME CYANIN

Place 0.2 gram solochrome cyanin R.S. (C.I. 43820) in a 250 millilitre flask and add 0.5 millilitre concentrated sulphuric acid. Effervescence occurs and a thick solution of creamy consistency is formed. Stir well to incorporate all the dye. Add 90 millilitre distilled water and 10 millilitre of 4% iron alum. Mix and filter. This solution keeps well.

CRESYL FAST VIOLET SOLUTION

To make c.400 millitre solution:-

Stock solution	-	42 millilitre
Distilled water	-	378 millilitre
10% acetic acid	-	3.5 millilitre

Heat to 60°C.

This solution will keep for 2 days in the oven at 60°C.

CRESYL FAST VIOLET STOCK SOLUTION

1% aqueous cresyl fast violet.

APPENDIX 4

PROCEDURE FOR STAINING POSSUM TEETH SECTION

1. Dewax in Xylene (i) for 5 minutes
2. Xylene (ii) for 5 minutes
3. Hydrate in absolute alcohol - gently for approximately 1 minute
4. 70% alcohol - gently agitate for approximately 1 minute
5. Wash in slowly running tap water for approximately 1 minute
6. Stain in Mayer's Haemalum for 20 minutes
7. Rise in tap water
8. Blue in Scott's tapwater until sections turn blue (2 minutes)
9. Rinse in tapwater
10. Dehydrate in alcohol
11. Clear in Xylene
12. Mount sections in DPX.

Sections cut on a Spencer AO 820 rotary microtome at 10 microns.

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