

SC-18862 (ASPARTAME): A SUPPLEMENTAL STUDY OF RAT BRAINS FROM
TWO TUMORIGENICITY STUDIES (P-T Nos. 838H71 and 892H72)

Pathology-Toxicology
Project No. 1227

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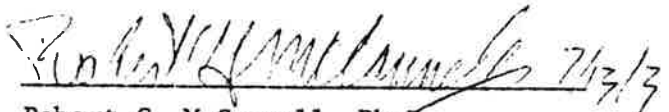
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A Brief Introduction To The Attached Report
On Brain Neoplasms In The Rat

In an initial postmortem assessment of tissues from rats administered Aspartame for 104 consecutive weeks (P-T Project No. 838H71), two coronal sections of brain were examined microscopically from each rat in the high dose and concurrent control groups. Additionally, histopathologic examination was performed on any rat exhibiting gross evidence of a brain lesion. Five brain neoplasms were identified and reported in the 440 control and treated rats examined in this initial assessment, an incidence of 1.1%. All such brain neoplasms were in the treated groups, however, and it was suggested that a more comprehensive microscopic surveillance of brain tissue (all major neuroanatomical areas) from all treated and control rats on the study would provide more reliable data on the actual incidence of such tumors.

Reference to current experimental neurooncology literature and procedures regarding the rat indicated that microscopic examination of eight carefully selected coronal (whole brain) tissue specimens per rat would constitute an acceptable comprehensive examination of brain tissue, and should reliably reveal both "macro" and "micro" tumors. Such a procedure was adopted. H&E stained sections were examined independently by two highly reputable comparative pathologists.

To enable comparison of data, the same tissue specimen selection and examination procedure was employed in a second study of Aspartame in the rat (Lifetime study: P-T No. 892H72). The data generated by this unusually comprehensive examination are most interesting and useful, and are presented and discussed in the attached document. As previously known by some comparative pathologists, and suspected by many others, the traditionally accepted incidence of intracranial neoplasms in untreated rats would be markedly different (increased), had such a comprehensive examination procedure been performed routinely on both non-survivors and survivor rats in the past.


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SC-18862 (aspartame). A Supplemental Evaluation Of Rat Brains
From Two Tumorigenicity Studies

Supplemental histopathologic evaluation of intracranial tissues from two SC-18862 (aspartame) tumorigenicity studies in the rat was performed to determine the presence or absence of neoplasms. All neoplasms were examined by both consulting pathologists, with concurrence on diagnostic terminology. This report presents both the results and their interpretation, from:

- 1) SC-18862: An oral tumorigenicity study of 104 weeks duration in the rat; P-T No. 838H71.
- 2) SC-18862: An oral tumorigenicity study involving prenatal, neonatal, and 104 week postnatal administration (Lifetime) to the rat; P-T No. 892H72.

Both sexes of Charles River Caesarean-derived rats were employed in equal numbers in control and treated groups in each study. Microscopic evaluation of eight coronal sections representing all major neuroanatomic areas of the brain was performed from each control or treated rat, survivor or non-survivor, in each study. Data are presented independently from each study, and are interpreted both independently and jointly. A full report of study P-T No. 838H71 has been submitted as Master File Entry No. 33 and 34 and the full report of study P-T No. 892H72 has been submitted as Master File Entry No. 70.

P-T No. 838H71

This 104 week study employed a total of 120 control and 320 treated rats. Compound was administered via the diet starting promptly after weaning (28 days age), and was continued uninterrupted for 104 weeks. The study was then terminated.

As a result of this in-depth evaluation of 440 animals, 12 neoplasms involving the brain were discovered in rats from treated Groups 2, 3, 4, and 5. None were detected in control rats. The nature and distribution of the tumors within the various experimental groups is presented in Table 1 on the following page.

TABLE 1

SC-18862: 104 WEEK ORAL TOXICITY STUDY IN THE RAT
P-T No. 838H71
(HLI 700-233)

Survival Data at Termination					
Group No.	Test Level g/kg/day	Percent Survival \pm S.E.		Mean Survival Time - Days	
		males	females	males	females
1	0 (Control)	38.4 \pm 6.3	46.7 \pm 6.5	569	657
2	1	45.0 \pm 7.9	57.5 \pm 7.9	629	663
3	2	52.5 \pm 7.9	50.0 \pm 8.0	636	640
4	4	57.5 \pm 7.9	35.0 \pm 7.6 ^{S-}	666	613
5	8	52.5 \pm 7.9	25.0 \pm 6.9 ^{S-}	651	423

S.E. = Standard Error.

S- = Significantly lower than control at $p < 0.05$.

Distribution of Intracranial Neoplasms			
Animal No.	Sex	Survival Time Days*	Diagnosis

GROUP NO. 1 - 0 G/KG (Control); 60 ♂, 60 ♀

None

GROUP NO. 2 - 1 G/KG; 40 ♂, 40 ♀

83745	Male	728	Astrocytoma
83750	Male	728	Astrocytoma with ependymal components
83766	Female	483	Astrocytoma with ependymal components
83769	Female	728	Astrocytoma

GROUP NO. 3 - 2 G/KG; 40 ♂, 40 ♀

83837	Male	539	Astrocytoma with ependymal components
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GROUP NO. 4 - 4 G/KG; 40 ♂, 40 ♀

83888	Male	420	Oligodendroglioma
83892	Male	343	Ependymoma
83895	Male	700	Ependymoma
83919	Male	728	Astrocytoma with ependymal components
83934	Female	595	Ependymoma

GROUP NO. 5 - 8 G/KG; 40 ♂, 40 ♀

84010	Female	91	Sarcoma (Meningeal)
84019	Female	469	Glioma - unclassified

* Week of Survival X 7.

These results suggest an apparent treatment related, but not dosage or sex related occurrence of intracranial neoplasms. The overall incidence of such neoplasms in this two year study was 2.7%. In controls the incidence was zero; in treated rats it was 3.7%.

Treated group mean survival rates per sex were comparable to concurrent control values except in the very high dose female group (No. 5). It is unlikely that the statistically significant reduction observed in the latter group exerted a meaningful effect on tumor incidence, since tumors in the lower dose groups occurred more often in males than in females and were not observed in group No. 5 males. Thus, enhanced survival of females in this group would not be likely to increase the tumor incidence.

P-T No. 892H72

This second study was designed to evaluate the tumorigenic potential of SC-18862 in the rat exposed to the compound or its constituent moieties transplacentally throughout gestation, and via maternal milk and the dry diet mixture during lactation; treatment was then continued for the ensuing 104 weeks postweaning. Thus, in addition to being treated with compound throughout their lifespan, these treated rats were presumably exposed to compound from conception onward, and parental gametes were provided by rats pretreated with compound for 60 days prior to mating.

Litters were routinely culled to 10 pups (five males, five females as possible), and 28 such litters distributed by litter into two treated groups of 40 pups/sex and a third control group containing 60 pups/sex. Thus, a total of 280 pups was employed.

As a result of this evaluation, 9 neoplasms involving the brain were discovered in the 280 animals on this study. Their nature and distribution within the control and test groups is presented in Table 2 below.

TABLE 2

SC-18862: LIFE-TIME TOXICITY STUDY IN THE RAT
P-T No. 892H72
(HLI 700-240)

Survival Data at Termination					
Group No.	Test Level g/kg/day	Percent Survival S.E.		Mean Survival Time - Days	
		males	females	males	females
1	0 (Control)	41.7±6.4	48.4±6.5	643	648
2	2	50.0±8.0	45.0±7.9	654	632
3	4	57.5±7.9	52.5±7.9	650	661

S.E. = Standard Error.

No statistically significant differences in survival.

Distribution of Intracranial Neoplasms			
Animal No.	Sex	Survival Time Days*	Diagnosis
GROUP NO. 1 - 0 G/KG (Control); 60 , 60			
90818	Male	728	Astrocytoma
90819	Male	728	Astrocytoma
90838	Male	714	Astrocytoma
90876	Female	595	Astrocytoma
GROUP NO. 2 - 2 G/KG; 40 , 40			
90943	Male	378	Ependymoma
90967	Male	630	Astrocytoma
90969	Female	728	Astrocytoma
GROUP NO. 3 - 4 G/KG; 40 , 40			
91016	Male	728	Astrocytoma
91061	Female	595	Meningeoma

* Week of survival X 7.

These results indicate a random occurrence of intracranial neoplasms, unrelated to treatment, dosage or sex. The overall occurrence of such neoplasms in this lifetime toxicity study was 3.2%. In controls the incidence was 3.3%; in treated rats it was 3.1%.


DISCUSSION AND CONCLUSIONS


A short relevant treatise discussing the problem of selecting diagnostic terminology for nervous system tumors, and also exploring use of the terms benign and malignant, is appended for examination by the interested reader.


In comparing these two studies (104 week and Lifetime), there does not appear to be consistent evidence of an intracranial tumorigenic effect. While there was an apparent relationship of tumor occurrence to treatment in the 104 week toxicity study, there was no relationship of treatment to tumor incidence in the lifetime toxicity study, a study which included prenatal as well as postnatal administration of the test material. One might expect a more consistent and significant increase in tumor incidence with an unequivocal carcinogen. The overall incidence of intracranial neoplasms was actually lower in the two year toxicity study (2.7%), in which there was an apparently increased incidence in the treated groups, than in the lifetime toxicity study (3.2%) in which there was a random, non-treatment related distribution. A comparable incidence of intracranial neoplasms (2.1%) has previously been encountered in control (untreated) Charles River rats receiving comprehensive evaluation of intracranial tissues.¹

1. Ulland, B. M., Presentation at Society of Toxicology Meeting, March 1973; Abstracts of Papers; Acad. Press. (To be published in detail in the near future.)

In conclusion, the results of these two studies do not provide consistent evidence of an intracranial tumorigenic effect. They do provide evidence of the study-to-study variability in the incidence of such neoplasms in the untreated rat.


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NOMENCLATURE AND CLASSIFICATION OF TUMORS OF THE NERVOUS SYSTEM, WITH A DISCUSSION OF BENIGNANCY VERSUS MALIGNANCY

The following is a brief explanation of the basis of our attitudes on the great problems of classification and nomenclature of neoplastic diseases which affect the nervous system. Much of it is taken from Innes and Saunders (Comparative Neuropathology; 1962; Academic Press).

As a start to nomenclature and classification, attention must be given to the normal histogenesis of the cells of the central nervous system, as it applies to man and the lower animals. This is best demonstrated by the diagram on page 1A, followed by a table of classification on page 1B. Note the disturbing complexity of synonyms.

As far as classification of brain tumors is concerned, some pathologists (medical, veterinary and experimental) take a stance either with those who favor the finer splitting as originally done by Bailey and Cushing (1926), or with others who prefer a simpler classification [Willis (1967), Russell and Rubinstein (1971), and Winston Evans (1968)]. In general, a greater tendency among medical neuropathologists has been towards the latter approach, for cogent reasons given in the various texts mentioned. Zimmerman et al. (1956) stated succinctly that these include: (1) the danger of making the classification too complex; (2) recognition that beliefs on the rigidity of the embryologic cellular antecedents to tumors have rested on shaky ground; (3) appreciation that many tumors show a mixed bag of cells, defying concepts

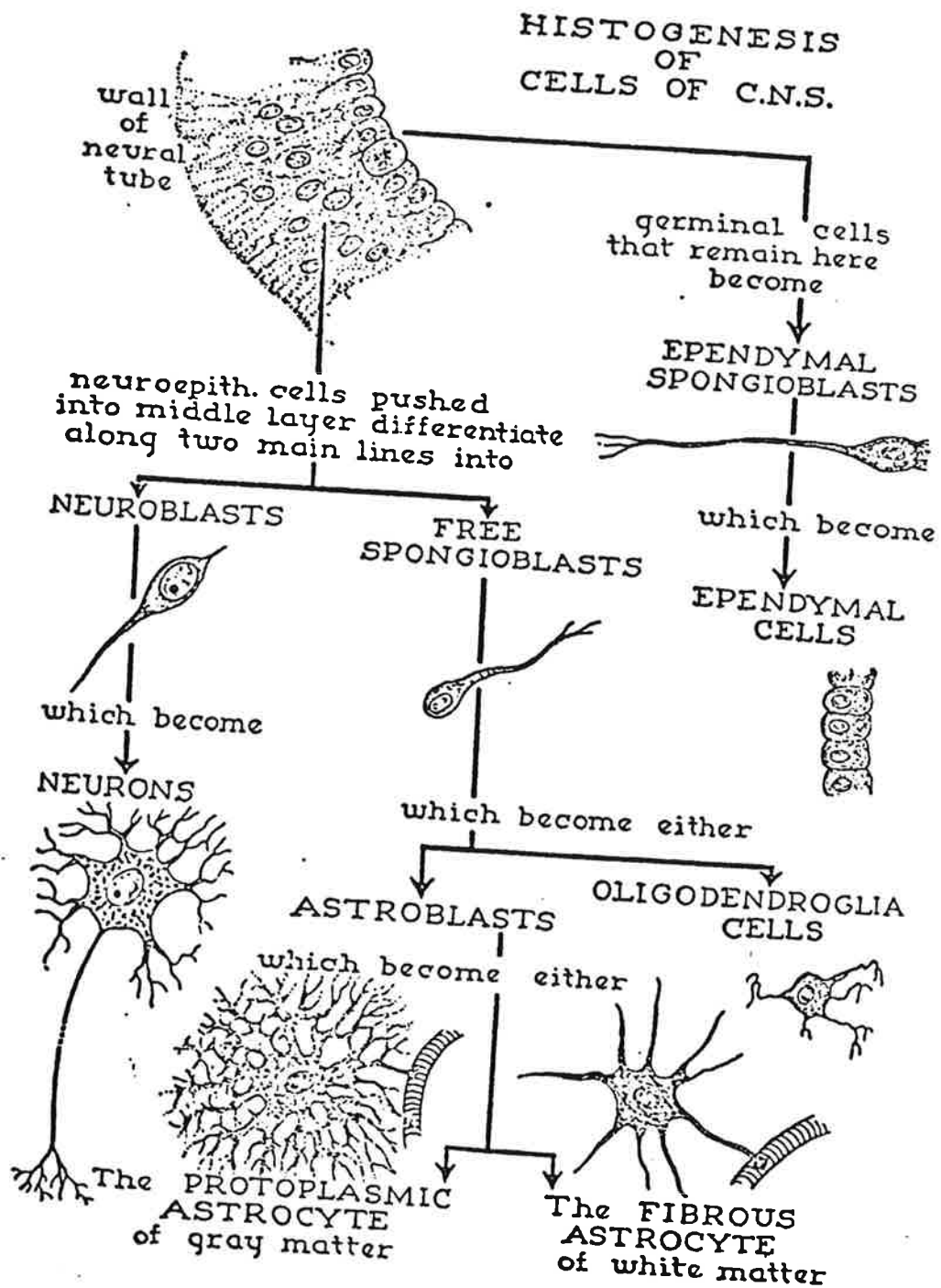


FIG. XXII. 1. Histogenesis of cells of central nervous system. Diagram showing main lines along which neuroectodermal cells of the neural tube differentiate. (Fig. 227 from

Ham's Histology, by courtesy of Dr. A. W. Ham and Lippincott Co., Philadelphia.)

TABLE 2*

SYNONYMS

GLIOMAS

- | | |
|--|--|
| <p>1. Astrocytoma
 Astrocytic glioma
 Astrocytoma, Grade 1 (Kernohan et al.)
 Astroma (Lenhosse.)
 Fibrillary astrocytoma
 Glioma (Golgi)
 Piloid astrocytoma
 Protoplasmic astrocytoma
 Sternzellengliom (Stroebe)</p> <p>2. Astroblastoma
 Astrocytoma, Grade 2 (Kernohan et al.)</p> <p>3. Ependymoma
 Blastoma ependymale (Marburg)
 Ependymal glioma
 Gliocpendymome (Masson)</p> <p>4. Ganglioneuroma
 Gangliocytoma
 Ganglioglioma
 Ganglion cell tumor
 Glioneuroma
 Neuroastrocytoma (Kernohan et al.)
 Neurocytoma
 Neurofibroma ganglionare</p> | <p>5. Glioblastoma multiforme
 Astrocytoma, Grades 3 and 4 (Kernohan et al.)
 Gliome polymorphe (Roussy, Lhermitte and Cornil)
 Gliosarcoma (Ewing, Borst)
 Spongioblastoma multiforme (Globus and Strauss)</p> <p>6. Medulloblastoma
 Glioma sarcomatodes (Borst)
 Glioblastoma isomorphe (Horteg)
 Neuroblastoma (Wright)
 Neurogliocytome embryonnaire (Masson)</p> <p>7. Neuroepithelioma
 Blastoma ependymale (Marburg)</p> <p>8. Oligodendroglioma
 Gliome à petites cellules rondes (Roussy, Lhermitte and Cornil)
 Oligodendrocytoma</p> <p>9. Spongioblastoma polare
 Neurinoma centrale (Joseph, MacPherson)
 Astrocytoma, Grade 2 (Kernohan et al.)</p> |
|--|--|

MENINGIOMAS

- | | |
|--|---|
| <p>Arachnoidal fibroblastoma (Mallory)
 Dural endothelioma</p> | <p>Meningeal fibroblastoma (Penfield)
 Mesothelioma of meninges</p> |
|--|---|

- 2 -

CONGENITAL TUMORS

- | | |
|---|---|
| 1. Craniopharyngioma
Adamantinoma
Epidermoid tumor of craniobuccal pouch
Papilloma of Rathke's pouch
Suprapituitary cystic epithelial tumor | 3. Pinealoma
Dysgerminoma
Chorioma (Askanazy) |
| 2. Cholesteatoma
Epidermoid
Pearly tumor
Tumereurs perlées (Cruveilhier) | 4. Chordoma
Ecchondrosis physifaliphora |
| | 5. Cyst of third ventricle
Colloid cyst of third ventricle
Pa aphysial cyst |

TUMORS OF PERIPHERAL NERVES

- | | |
|---|---|
| 1. Perineurial fibroblastoma
Neurilemmoma
Schwannoma
Acoustic neurinoma (if on eighth cranial nerve) | 3. Neurofibroma
Neuroma
Neurinoma |
| 2. Neurofibromatosis
von Recklinghausen's disease | 4. Neurogenic sarcoma
Malignant Schwannoma
Neurofibrosarcoma
Malignant neuroma |

TUMORS OF SYMPATHETIC GANGLIONS

- | | |
|---------------------------------------|--|
| 1. Ganglioneuroma
Sympathicocytoma | 2. Neuroblastoma
Sympathicoblastoma
Sympathoma embryonale
Sympathogonioma |
|---------------------------------------|--|

TUMORS OF PARAGANGLIONIC CELLS

- | |
|--|
| 1. Pheochromocytoma
Chromaffin tumor
Functionally active paraganglioma |
|--|

*Reproduced with permission from the publishers from Zimmerman, H. M., Netsky, M. G., and Davidoff, L. M. (1956) Atlas of Tumors of the Nervous System, Philadelphia, Lea & Febiger, Table IV, pp. 186-187.

of cell specificity and, as Scherer (1940) showed, may exhibit a varied cytologic picture, depending on which part of the tumor is examined; (4) recognition that the silver and gold impregnation methods of Cajal and Hortega, long regarded as essential for demonstrating differentiations of tumors, are not utterly specific. The views of authors quoted above should be digested by those desirous of further explanation. Willis stresses that varying degrees of anaplasia in cell growth do not spell out different neural tumors, - a consideration which applies to cancer in general. These are not just academic points; they apply equally to the diagnosis of brain tumors in animals. Zimmerman et al. also remarked that, because of the above complexities, the general pathologist has shied away from studies of brain tumors; yet it is surprising what can be accomplished in the way of diagnosis using routine staining methods.

To counterbalance the above, readers should consult the books by Zulch (1957) and by Dorothy S. Russell and L. Rubinstein (1971) - both of which have been referred to in reviews as classics of their kind. The work of Zulch is based on a personal analysis of some 4000 human cases and contains more than 1300 references. There are many pertinent historic facts on the development of knowledge and present classification of brain tumors which can do much to ease the perplexities of pathologists who may get a little bemused by the variety of names.

In 1932, Cushing stated: "Time, however, will bring order, agreement and simplification out of existing confusion. What is important for the surgeon to know is the kind of glioma he has brought to view, whatever its 'alias.' A medulloblastoma by any other name is just as unfavorable." Zulch remarked, "when I started to compare canine cases with human tumors and classify them accordingly, I realized the difficulties of this task".

Zulch states that the following ten types - found in all modern classifications, although sometimes under different names - are adequate for cataloguing the central and peripheral derivatives (tumors) of neuro-epithelium:

Medulloblastoma	Ependymoma
Spongioblastoma	Plexus papilloma
Oligodendroglioma	Pinealoma
Astrocytoma	Neurinoma
Glioblastoma	Gangliocytoma

He assembles these into four main groups according to their kinships and tissue differentiation, viz, medulloblastoma, gliomas, paragliomas, and gangliocytomas.

Russell and Rubinstein's classification is as follows, but they say no classification can satisfy all requirements:

- A. Tumors of glial series
 - I. Astrocytic group
 - 1. Astrocytoma
 - 2. Astroblastoma
 - 3. Polar spongioblastoma
 - II. Oligodendroglia
 - Oligodendroglioma
 - III. Ependyma and its homologues
 - 1. Ependymoma, subependymoma
 - 2. Choroid-plexus papilloma
 - 3. "Colloid cyst"
 - IV. Glioblastoma multiforme
- B. Pineal parenchyma
 - 1. Pinealoblastoma
 - 2. Pinealocytoma
- C. Retina (primitive epithelium)
 - Retinoblastoma
- D. Tumors of neuron series
 - 1. Medulloblastoma
 - 2. Medulloepithelioma
 - 3. Neuroblastoma
 - 4. Ganglioneuroma and ganglioglioma

For the comparative pathologist, it will be noted that some neural tumors known in man have not so far found their way into veterinary literature, e.g. cerebellar hemangioblastoma (part of Lindau's disease) and neurofibromatosis (Recklinghausen's disease); also there is little on neural heterotopias. Innes and Saunders (1962) do not deal with tumors of the skull or vertebral column; granulomas are dealt with elsewhere in Innes and Saunders, as are tumors of the pituitary gland. The encephalitis and carcinomatous neuropathy described by Russell and Rubinstein - with inflammatory and degenerative changes resembling those accompanying viral infection, and mainly following pulmonary, mammary, and ovarian cancer - has not been found so far in animals.

Finally, it should be stressed that there are many tumors of the nervous system of lower animals (dog, rats and mice) which cannot be classified into any of the recognized human varieties.

We do not possess reliable evidence of the incidence of spontaneous cerebral tumors in the various inbred and random bred strains of laboratory rats at varying ages; available data certainly does not cover examination of tens of thousands of rat brains, which would enable comparison with statements that in the human being between 2-5% of all tumors affecting the body are intracranial and intraspinal. Brain tumors of mice have been fully covered in the document by Zimmerman and Innes (1973). However, because of the relative rarity of published accounts on spontaneous neural tumors in mice, the latter publication is largely concerned with experimentally produced tumors. Dr. Zimmerman was a pioneer investigator of this subject decades ago. In his life time he has studied over 5000 human brain tumors. Much of this experience formed the basis of the Atlas of Tumors of the Nervous System (1956); it also formed the framework for the Zimmerman and Innes study.

MALIGNANCY VERSUS BENIGNANCY

Tumor simply means a swelling. A benign tumor means a growth which, if removed surgically, will not recur and never exhibits invasiveness and secondary spread. Malignancy means the opposite behavioral qualities of local invasiveness and then secondary spread with metastatic lesions by many different routes - viz. by expansion, infiltration, contiguity along tissue spaces, by lymphatic vessels, veins, arteries, and in coelomic cavities, and within the cranial cavity and vertebral canal along the cerebrospinal spaces.

To get involved in the perennial question of malignancy versus benignancy is a little like getting onto ground where angels fear to tread. One could be iconoclastic and deny a place for so-called benign tumors in books dealing with cancer, for then it would be essential to start by defining cancer or carcinoma. This was dealt with recently in the Bulletin Soc. Pharmac. Environmental Pathologists, 1973, No. 3, p. 10-11. The best and most profound discourse on this controversial subject was by L. J. Foulds (1969).

It is appropriate to mention that the ordinary canons on the differences between benign and malignant growth as applied to cancer elsewhere in the body, do not apply to brain tumors. Such tumors rarely metastasize at all, and only uniquely so outside the bony coverings; meningeal implantations may occur. Some varieties are, yet, extremely malignant in the sense of rapidity of growth and undifferentiated cellular characteristics, and the more so as they kill the host because of localization in some vital part and/or are inaccessible to surgical removal.

The problem of extracranial, or extraspinal metastases is an intriguing one and was analyzed by Rubinstein (1959). Deposits of glial tumors outside the neuraxis are so infrequent that their actual existence remained for long in doubt. Generally, it is believed that glial tumor cells are unable to penetrate the cerebral vessels and that the central nervous system is isolated from the lymphatic pathways.

Many experimental pathologists and regulatory agencies have traditionally insisted on classifying tumors as either benign or malignant, based on morphologic appearances. This is frequently an impossible task. In laboratory rodents there are many different tumors which show, according to some accepted standards, the pathologic features associated with malignant behavior but which do not pass into the stages of secondary spread, e.g., experimentally induced tumors of the Harderian glands of mice, so-called thyroid carcinomas and several different types of mammary tumors of both rats and mice. Hepatoma and actual hepatogenic carcinoma may frequently spread by venous pathways to the lungs of rats, but do so much less frequently in mice. Many experimentally induced tumors in rats and mice are transplantable and so are regarded by many authorities as being malignant.

In conclusion, all tumors involving the brain are malignant in the sense that they will eventually kill the animal. All types of such tumors which have been induced experimentally in rats or mice have also been observed to occur spontaneously in untreated control animals.

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