Histochemistry of Rathke pouch tumours

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A variety of names have been given to solid and cystic epithelial tumours anatomically related to the pituitary gland and its stalk. By ordinary staining techniques they closely resemble epidermoid cysts elsewhere, but differ from simple epidermoid or dermoid cysts when these occur in the brain, by provoking an unusual reaction in surrounding glia. Astrocytic cells near the epithelial cells of Rathke pouch tumours show marked eosinophilia of the fibres, which are often expanded to form tadpole-shaped Rosenthal bodies; an epidermoid cyst is enclosed by an unremarkable gliosis. It was felt that these differences might reflect different enzymatic activity in these superficially similar epithelial tumours.

This paper reports a survey of the activity of a number of enzymes as compared in four craniopharyngiomas, an ameloblastoma of the jaw, normal squamous epithelium from six sites, and epithelium from three epidermoid cysts.

CASES I-III

Three craniopharyngiomas, from a woman aged 36, a woman of 18, and a girl of 11. Histologically the tumours were all very similar, consisting of solid trabeculae of epithelium with a basal columnar layer and a central zone of squamous cells forming keratin and pearls in places. The basal columnar layer rested upon a basement membrane supported by a loosely cellular, vascularised connective tissue.

CASE IV

A more active craniopharyngioma from a man aged 69 who presented with progressive deterioration of vision over a period of three weeks. Histologically this tumour also consisted of trabeculae of squamous epithelium with a basal columnar layer separated from a loosely cellular connective tissue stroma by a collagenous basement membrane. This case differed from the other three craniopharyngiomas in that in many places the connective tissue stroma was invaded by strands and clumps of epithelial cells, showing a moderate degree of pleomorphism and an occasional mitotic figure.

CASE V

An ameloblastic tumour from the mandible in the region

of the 5th, 6th, and 7th teeth of a girl aged 11 years. Histologically the tumour consisted of clumps and trabeculae of epithelial cells, the outermost layer of cells resembling those of the enamel epithelium of the developing tooth-bud—that is, the ameloblastic—layer; the cells of this layer are tall columnar cells and are separated from the stromal connective tissue by a collagenous basement membrane. In places in the tumour long strands and clumps of epithelial cells had broken through the collagenous basement membrane and were invading the stroma. These invasive cells were surrounded by an infiltrate of lymphocytes and plasma cells.

TECHNIQUE

Blocks of fresh tissue 0.5-1.0 cm diameter were frozen onto chucks by immersion in liquid nitrogen. Sections were then cut at -20°C in a cryostat, and unfixed sections were used for the demonstration of enzymes. In all cases the sections were washed after completion of the histochemical reaction, and mounted in glycerin-jelly. The following histochemical techniques were used:

DEHYDROGENASES The technique was that of Pearse (1960) using the following substrates: sodium L-glutamate, sodium DL- β -hydroxybutyrate, glucose-6-phosphate disodium salt, 6-phosphogluconic acid barium salt, sodium DL- α -glycerophosphate, sodium DL-isocitrate, sodium lactate, sodium succinate, and sodium malate. A fresh solution of 0.1 M triphosphopyridine nucleotide was used for the pentose-shunt enzymes, no coenzyme was used for succinic dehydrogenase, and 0.1 M diphosphopyridine nucleotide was used for the rest. Sodium cyanide (0.1 M) was used as a respiratory inhibitor for all enzymes except the pentose-shunt enzymes where 0.1 M sodium azide was used instead. Sections were incubated for 45 min at 37°C .

DIAPHORASES Sections were incubated in a medium containing 0.1 M reduced diphosphopyridine nucleotide (DPNH) or reduced triphosphopyridine nucleotide (TPNH) and nitroblue tetrazolium at pH 7.4 for 45 min.

CYTOCHROME OXIDASE The method of Burstone (1960) using p-aminodiphenylamine and 3-amino-9-ethyl-carbazole. Sections were incubated for one hour before chelation in cobaltous acetate.

ALKALINE PHOSPHATASE A Naphthol AS-TR phosphate method was used (Burstone, 1958a). The pH of the incubating medium was 8.9 and Red Violet L-B salt was used as coupling agent.

TABLE I

Enzyme	ENZYMES FOUND IN DIFFERENT TISSUES EXAMINED				Normal squamous Epithelium	Epidermoid cyst Epithelium
	Craniopharyngiomas		Ameloblastomas			
	Epithelium: Regular	Invasive	Epithelium: Regular	Invasive		
Dehydrogenases—						
6-phospho-gluconic acid	+	↑	+	↑	+	+
glucose-6-phosphate	+	↑	+	↑	+	+
α-glycero-phosphate	+	+	+	+	+	+
lactic acid	+	↑	+	↑	+	+
isocitric acid	+	+	+	+	+	+
succinic acid	+	+	+	+	+	+
malic acid	+	+	+	+	+	+
glutamic acid	+	+	+	+	+	+
β-hydroxy-butyric acid	+	+	+	+	+	+
DPNH diaphorase	+	+	+	+	+	+
TPNH diaphorase	+	+	+	+	+	+
Cytochrome oxidase	+	+	+	+	+	+
Acid phosphatase	+	↑	+	↑	+	+
Non-specific esterase	+	+	+	+	+	+
Leucine amino-peptidase	0	+	0	+	0	0
Alkaline phosphatase	+	+	+	+	0	0

0 = absent. + = present. ↑ = activity increased.

FIG. 1. An acid phosphatase stain on the craniopharyngioma from Case 1. There is a strong reaction in the basal columnar layer of cells (arrow) and a weaker reaction in the intermediate zone of cells. × 150.

FIG. 2. An acid phosphatase stain on the ameloblastoma showing a moderate reaction in the basal layer of cells (arrow) and a weak reaction in the remainder of the tumour cells. × 150.

FIG. 3. A lactic acid dehydrogenase preparation of the craniopharyngioma from Case 1. The basal columnar layer of cells (arrow) is slightly stronger than the intermediate zone. $\times 120$.

FIG. 4. A lactic acid dehydrogenase stain on the ameloblastoma. There is a slightly stronger reaction in the basal layer of cells (arrow) than in the intermediate zone. $\times 150$.

ACID PHOSPHATASE A similar method to that used for alkaline phosphatase was used except that the pH of the incubating medium was 5.2.

NON-SPECIFIC ESTERASE The method was based on Gomori's modification of the technique of Nachlas and Seligman (1949) using α -naphthyl acetate as substrate, and Fast Blue BB salt as coupling agent.

LEUCINE AMINOPEPTIDASE The method was that of Burstone and Folk (1956) using L-leucyl- β naphthylamide as substrate. Sections were incubated for one hour at pH 7.1.

RESULTS

The Table shows the enzymes found in the different tissues examined.

All enzymes, with the exception of alkaline phosphatase and leucine aminopeptidase, were slightly more active in the basal columnar layer than in the intermediate zone of squamous epithelium in all five

tumours examined (Figs. 1-4). Alkaline phosphatase was very strong in the intermediate zone, but was almost absent in the basal layers of cells adjacent to the connective-tissue stroma (Figs. 5 and 6).

The clumps and columns of cells invading the stroma of the active craniopharyngioma from Case IV and of the ameloblastoma were the only sites to show a moderate degree of leucine amino-peptidase activity (Figs. 8 and 9). This was completely absent from the first three craniopharyngiomas, and was not found in the differentiated and non-invasive cells of the other two tumours.

The invasive cells of the active craniopharyngioma and the ameloblastoma also showed stronger acid phosphatase (Fig. 10) and dehydrogenase activity than the more differentiated parts of the tumour. This increased activity was most marked in the case of lactic acid dehydrogenase, and the pentose-shunt enzymes, and was not seen in the citric-acid cycle enzymes.

Squamous epithelium and the three epidermal

FIG. 5. *Craniopharyngioma (Case No. 1): alkaline phosphatase stain showing a strong reaction in the intermediate zone of epithelial cells (white arrow) and a negative reaction in the basal columnar layer of cells (black arrow) and in the keratinous pearls.* $\times 120$.

cysts contained the same enzymes as the craniopharyngiomas and the ameloblastoma, with the striking exception that there was no alkaline phosphatase (Fig. 7) or leucine aminopeptidase activity. All enzymes were slightly stronger in the basal layer (Fig. 11) with the exception of β -hydroxybutyric dehydrogenase, which was stronger in the stratum granulosum.

DISCUSSION

The nature of the epithelium found in tumours commonly called craniopharyngiomas is still in doubt. Some tumours contain cornifying squamous epithelium, but more usually they show an epithelium resembling that of the embryonic enamel organ or the ameloblastoma found in the jaw. Nevertheless, Willis (1953) and Russell, Rubinstein, and Lumsden (1959) state that these tumours are not distinguishable from epitheliomas in which squamous and basal cells are both present.

The presence of a high alkaline phosphatase

FIG. 6. *Ameloblastoma: alkaline phosphatase stain showing a strong reaction (arrow).* $\times 120$.

activity in the epithelium of the four suprasellar tumours and in the ameloblastoma suggests a closer link between these than with squamous epithelium and epidermal cysts, from which this enzyme was absent. Alkaline phosphatase appears to indicate a certain kind of epithelial differentiation. It has been shown to be present in the stratum intermedium of the developing tooth bud (Sasso and Castro, 1957), but there are conflicting reports on its presence in the basal layer of ameloblastic columnar cells (Engel and Furuta, 1942; Horowitz, 1942; Gomori, 1943; Bevelander and Johnson, 1945; Morse and Greep, 1947; Bevelander and Johnson, 1949; Harris, 1950; Wislocki and Sognnaes, 1950; Sasso and Castro, 1957). It does appear, then, that the craniopharyngioma corresponds histochemically to ameloblastomas and that they both have features in common with the developing tooth bud. The presence of a more typically squamous epithelium with areas of keratinization does not detract from the hypothesis that the epithelium is of dental origin; the enamel organ develops as a downgrowth of the overlying ectoderm, which later becomes separated

FIG. 7. Squamous epithelium: the epithelium stains negatively for alkaline phosphatase. There is a strong reaction in capillaries (arrow). $\times 120$.

FIG. 9. A leucine aminopeptidase preparation of craniopharyngioma (Case No. 4) showing strong activity in some of the invasive cells. $\times 350$.

FIG. 8. A leucine aminopeptidase preparation on the ameloblastoma showing a strong reaction in the columns of cells growing down into the connective tissue stroma. $\times 120$.

from the oral mucosa by a breaking-up of the more superficial part of the dental lamina. Isolated masses of epithelium are left, some of which remain uncornified and appear as small clumps of small, darkly staining cells; others become large rounded keratinized pearls. The present study gives no indication as to whether craniopharyngiomas are of developmental origin or whether they arise as metaplasia and reversion of anterior pituitary cells. Some support for the latter idea was given by Hunter (1955), who found increasing evidence of islands of squamous epithelial cells in the pituitary stalk with increasing age.

The development of leucine aminopeptidase activity in the invading cells of the fourth craniopharyngioma and of the ameloblastoma is of considerable interest, and is perhaps an indication of the increased proteolytic activity necessary for invasion. There have been several reports of high peptidase activity in peripheral portions of other tumours associated with invasion (Sylvén and Malmgren,

FIG. 10. An acid phosphatase stain on the craniopharyngioma from Case 4. There is a very strong reaction in the irregular columns of epithelial cells invading the stroma. $\times 350$.

FIG. 11. A lactic acid dehydrogenase stain on normal squamous epithelium. There is a slightly stronger reaction in the basal layer than in the more superficial layers of cells. $\times 350$.

1955; Burstone, 1956; Glenner, Burstone, and Meyer, 1959; Wattenberg, 1959; Willighagen and Planteydt, 1959; Rosenholtz and Wattenberg, 1961). There have also been reports of malignant tumours showing groups of cells with strong reactions for acid phosphatase (Burstone, 1958b), but the significance of this is uncertain. It may be that increased activity of acid phosphatase seen in the invasive clumps of cells are associated with another feature of invasion—increased phagocytic activity.

The increased activity of dehydrogenases in the same cells is also of interest. The change was most marked in the case of lactic acid dehydrogenase, but was also seen in the pentose-shunt enzymes. The former indicates a more anaerobic type of metabolism in these cells, and the latter may be associated with an increased demand for ribose-5-phosphate in the synthesis of nucleic acids.

SUMMARY

A variety of enzymes have been studied in four

craniopharyngiomas, an ameloblastoma, squamous epithelium and epidermal cyst epithelium. Craniopharyngiomas differed from squamous epithelium and epidermal cyst epithelium in that they contained a high activity of alkaline phosphatase and, in this respect, they resemble more closely the ameloblastoma and the developing tooth bud.

The epithelium in one of the craniopharyngiomas and the ameloblastoma had broken through the basement membrane in places and was invading the connective-tissue stroma. This epithelium showed an increased activity of acid phosphatase, the pentose-shunt dehydrogenases, and lactic acid dehydrogenase. The same epithelium also showed marked leucine aminopeptidase activity which was not found in any of the other epithelial types.

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