The role of mesenchyme in the morphogenesis and functional differentiation of rat salivary epithelium

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SUMMARY

The ability of foetal rat salivary epithelium, particularly from the parotid gland, to develop morphogenetically and functionally (amylase activity) in various mesenchymes, and the quantitative effects of altering mesenchymal mass on the development of the parotid epithelium, have been studied in vitro.

Both parotid and submandibular epithelial rudiments were able to undergo morphogenesis and subsequent cytodifferentiation in their own and in the reciprocal mesenchyme. The growth of the explant and the arrangement of the acini were governed by the mesenchyme, submandibular mesenchyme supporting the development of more acini, which were more closely packed, than parotid mesenchyme. The functional product of the epithelium was not qualitatively affected, amylase activity being developed only by parotid epithelium, whether in its own or in submandibular mesenchyme.

Amylase activity was greater when the epithelium from a single parotid rudiment was recombined with submandibular mesenchyme than with its own mesenchyme. Increasing the initial mass of either salivary mesenchyme also led to the development of more amylase activity.

Parotid epithelium was able to develop in lung mesenchyme, but not so well as in its own mesenchyme. Stomach and pancreatic mesenchyme could support only limited histogenesis of parotid epithelium.

The results are interpreted in terms of morphogenetic and growth control of salivary epithelium by mesenchyme, the subsequent cytodifferentiation of the terminal buds being typical of the organ from which the epithelium was derived.

INTRODUCTION

Since the pioneering work of Borghese (1950) and Grobstein (1953a–c) demonstrating the morphogenetic dependence of mouse submandibular epithelium on its capsular mesenchyme, the salivary system has become recognized as the classic example of high mesenchyme specificity amongst the many organ systems which depend upon epithelial–mesenchyme interactions for their development (Grobstein, 1967a; Wessells et al. 1971). Apart from mouse submandibular mesenchyme, only parotid (Grobstein, 1967b) or chick submandibular (Sherman, 1960) mesenchyme have been shown to evoke any morphogenetic response from mouse submandibular epithelium.

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The emphasis in previous work was on the initial production of adenomeres; the aim of the present work was to investigate the role of mesenchyme in the further growth and development of the salivary buds. Experiments using salivary epithelium recombined in vitro with mesenchyme from various sources have been directed towards: (1) the question of mesenchyme specificity in the functional development of salivary epithelium, and (2) the role of mesenchyme in controlling the rate of growth and differentiation of the submandibular and parotid glands, since in the foetus the submandibular gland grows and differentiates faster than the parotid, at least in the rat (Lawson, 1970; Redman & Sreebny, 1970).

The rat salivary system was chosen in preference to that of the mouse, used by previous workers, because in the rat one well-defined and measurable functional product, the enzyme amylase, is present in appreciable quantities only in the parotid (Schneyer & Schneyer, 1960; Tremblay, 1963; Shear & Pearse, 1963; Lawson, 1970), whereas in the mouse both the parotid and the submandibular gland have amylase activity (Junqueira & de Moraes, 1965; Rutter & Weber, 1965); interactions within the salivary system with respect to functional end product could therefore be identified more clearly in the rat.

MATERIALS AND METHODS

In calculating the embryonic age of the albino rats used the morning, after overnight mating, on which sperm were found in the vagina was counted as day 1.

Tissue culture. Mesenchyme was loosened from the epithelium of 17-day parotid, 16-day submandibular, and 13- and 14-day lung, dorsal pancreas and stomach rudiments, by incubating for 3–5 min at 37 °C in Tyrode's solution containing 0.25 % crystalline trypsin (Worthington) and 1 % pancreatin (Difco). The epithelial rudiment was then eased out of the surrounding mesenchyme with tungsten needles. After rinsing three times with 10 % cock serum the epithelium from one parotid rudiment was recombined with the mesenchyme from two parotids or submandibulars unless otherwise stated, or with the mesenchyme from three of the other organs. In some experiments also the epithelium from one submandibular was similarly recombined with twice its own mesenchyme, or with mesenchyme from two parotids. The tissues were held under 5 % CO₂ in air during these procedures, except when being handled (Grobstein, 1953b). The recombinates were cultivated as previously described for intact parotid rudiments (Lawson, 1970). They were supported by a thin agar film on a clot of fowl plasma and chick embryo extract and cultivated at 37 °C for 15 days; for the first 9 days the gas phase was 5 % CO₂ in air and thereafter it was a mixture of 50 % O₂, 45 % N₂ and 5 % CO₂.

Histology and staging. Histological procedures and classification of the explants according to the histogenetic stage reached by the parotid epithelium were as previously described (Lawson, 1970). An additional stage, stage O, was
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used to indicate that only a cyst of, usually pluristratified, epithelium was formed. In experiments in which the submandibular epithelium also was involved the classification was modified as follows: (1) buds with no visible cytodifferentiation (parotid, stage I); (2) traces of PAS (periodic acid-Schiff) positive material in the terminal buds (parotid, stage II); (3) more advanced cytodifferentiation (parotid, stages III and IV; in the terminal buds of the submandibular epithelium the cells are polarized and the apical cytoplasm stains brilliantly with PAS, as in the submandibular glands of the late foetus and newborn).

Amylase. Amylase activity (Bernfeld’s method) and the total organic solids content of the explants were estimated as previously described (Lawson, 1970).

Amylase activity was localized in some recombinates using a starch film method (modified from Shear & Pearse, 1963). The explants were quenched in liquid nitrogen and 6 μm cryostat sections were incubated on an unfixed film of hydrolysed starch in a moist atmosphere for 0.5–30 min at 21 °C, fixed in a mixture of methyl alcohol, acetic acid and water (5:1:5), stained with I₂, mounted in glycerine saturated with chloral hydrate (Romeis, 1948), and examined both with normal optics and with phase contrast. The blue colour of the undigested starch film in such preparations did not fade appreciably for several weeks. Every tenth section was fixed and stained for routine histology. Permanent preparations were made by oxidizing the starch film with periodic acid before incubating with the section (Shear & Pearse, 1963). The undigested starch was subsequently stained with Schiff’s reagent and the section stained with haemalum. The oxidized starch film is less sensitive to amylase action than the unoxidized film.

Experimental design and statistical analysis. The capacity of parotid and submandibular epithelium to develop in each other’s mesenchyme was tested in experiments in which reciprocal recombinates of 16-day submandibular epithelium with 17-day parotid mesenchyme and of 17-day parotid epithelium with 16-day submandibular mesenchyme were made; homotypic recombinates were cultured as controls. Material for histological study was taken from 16 experiments, eight of which were also analysed biochemically.

In order to compare the effect of mesenchyme from various sources (parotid, submandibular, lung, stomach, pancreas) on the development of the parotid epithelium, the experiment was set up in randomized blocks with ten replicates. In any one replicate a single explant from each recombination was fixed for histological examination, the remainder being pooled for amylase estimation.

When the effect of three different masses of mesenchyme from the parotid and submandibular were compared the experiment was set up in a 3 × 2 factorial design in seven replicates.

Analyses of variance were carried out on the biochemical results after transformation of the values to the logarithmic scale. Since there were no duplicate samples in any one replicate the error variance was estimated from the replicate × treatment interaction. The 95% confidence limits for comparing any two means
Table 1. *Morphogenesis and cytodifferentiation of salivary gland epithelium in homotypic and reciprocal recombinates after 15 days in vitro*

<table>
<thead>
<tr>
<th>Epithelium</th>
<th>Mesenchyme</th>
<th>Epithelial development*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid</td>
<td>Parotid</td>
<td>0 2 2 14 18</td>
</tr>
<tr>
<td>Parotid</td>
<td>Submandibular</td>
<td>1 1 4 15 21</td>
</tr>
<tr>
<td>Submandibular</td>
<td>Parotid</td>
<td>1 3 5 11 20</td>
</tr>
<tr>
<td>Submandibular</td>
<td>Submandibular</td>
<td>3 0 3 12 18</td>
</tr>
</tbody>
</table>

* See Materials and Methods.

Table 2. *Size and amylase activity of homotypic and reciprocal recombinates, N = 8; each sample contained one to four explants*

<table>
<thead>
<tr>
<th>Epithelium</th>
<th>Mesenchyme</th>
<th>Total organic solids/explant (µg ± S.E.)*</th>
<th>Amylase (units/mg ± S.E.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid</td>
<td>Parotid</td>
<td>94.2 ± 15.3</td>
<td>0.89 ± 0.17</td>
</tr>
<tr>
<td>Parotid</td>
<td>Submandibular</td>
<td>189.0 ± 26.3</td>
<td>2.99 ± 0.71</td>
</tr>
<tr>
<td>Submandibular</td>
<td>Parotid</td>
<td>76.2 ± 33.0</td>
<td>&lt;0.04 ± 0.01</td>
</tr>
<tr>
<td>Submandibular</td>
<td>Submandibular</td>
<td>178.7 ± 26.4</td>
<td>&lt;0.03 ± 0.01</td>
</tr>
</tbody>
</table>

* S.E. calculated for each combination separately, i.e. not via analysis of variance.

RESULTS

*Parotid and submandibular epithelium in homotypic and reciprocal recombination with mesenchyme*

In the absence of mesenchyme, salivary epithelium tended to disintegrate on the agar substrate used in these experiments and was not viable. On Millipore filter it was able to spread as a flat sheet of cells which did not develop further.

The histogenetic stage reached by the recombinates is shown in Table 1. There was no statistically significant difference between the different recombinates (χ² = 11.80, degrees of freedom = 9, P > 0.2) and the majority in all groups had a branched and budding epithelium with overt cytodifferentiation which, when sufficiently advanced to be identifiable, was characteristic of the gland of origin of the epithelium (Fig. 2A–D). On the other hand, the packing and arrangement of the acini appeared to be dependent on the source of the
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Fig. 1. Sections of recombinates after 15 days in vitro.

(A) Parotid epithelium in parotid mesenchyme.
(B) Parotid epithelium in submandibular mesenchyme.
(C) Submandibular epithelium in parotid mesenchyme.
(D) Submandibular epithelium in submandibular mesenchyme.
(E) Parotid epithelium in lung mesenchyme.
(F) Parotid epithelium in stomach mesenchyme.
(G) Parotid epithelium in pancreatic mesenchyme.

Mesenchyme: the arrangement of abundant, closely packed acini produced by parotid epithelium in submandibular mesenchyme (Fig. 1B) was indistinguishable from that formed by submandibular epithelium in its own mesenchyme (Fig. 1D), in contrast to the more scattered acini typical of parotid epithelium in its own mesenchyme (Fig. 1A) and also of submandibular epithelium in parotid mesenchyme (Fig. 1C).
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The final mass of the explants was dependent on the mesenchyme component (Table 2); the presence of submandibular mesenchyme ensured a larger explant. Amylase activity was demonstrable only in recombinates containing parotid epithelium; submandibular mesenchyme supported the development of greater activity than did parotid mesenchyme (Table 2). No amylase activity could be detected in the recombinates of submandibular epithelium either with its own or with parotid mesenchyme.

Localization of amylase activity

Experiments to localize amylase activity in the recombinates were carried out: (1) to test the assumption that amylase is only produced by salivary epithelium, as in the adult (Tremblay, 1963; Shear & Pearse, 1963), and not by the mesenchyme, even in heterotypic combinations; (2) to establish whether the higher amylase activity found when parotid epithelium was recombined with submandibular mesenchyme instead of its own mesenchyme, was due solely to an increased number of acini or also to increased activity per acinus or per acinar cell.

Amylase activity was found only under sections of acini formed by parotid epithelium or in the lumina of tubules associated with these acini (Fig. 2G, H). No activity was found under submandibular epithelium. After relatively long incubation the starch film was digested under parotid or submandibular mesenchyme immediately surrounding active parotid acini in the same section but not in adjacent sections. It could therefore be concluded that neither parotid nor submandibular mesenchyme produces significant amounts of amylase, even in association with active parotid acini.

No significant difference could be found in the average time required to reach a standard end point in the histochemical test (judged visually) between parotid acini developing in parotid or in submandibular mesenchyme. This indicates that the amylase activity per acinus is not greater in recombinates with submandibular mesenchyme. However, the small number of samples ($N = 23$),

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Fig. 2. (A–F) Detail of structure of recombinates after 15 days in vitro.
(A) Parotid epithelium in parotid mesenchyme.
(B) Parotid epithelium in submandibular mesenchyme. Magnification as in A.
(C) Submandibular epithelium in parotid mesenchyme. Magnification as in A.
(D) Submandibular epithelium in submandibular mesenchyme. Magnification as in A.
(E) Parotid epithelium in lung mesenchyme. Magnification as in A.
(F) Parotid epithelium in stomach mesenchyme. Magnification as in A.
(G) Amylase activity in recombinate of parotid epithelium with submandibular mesenchyme. Two min incubation: PAS-haemalum method. Unstained areas indicate amylase activity in overlying structures. Note variation in activity in different areas of section.
(H) High-power view of G. Amylase activity is restricted to terminal buds (arrows) and tubule lumina (stars). Magnification as in A.
Table 3. Histogenetic stage reached by parotid epithelium in recombinates after 15 days in vitro

<table>
<thead>
<tr>
<th>Histogenetic stage*</th>
<th>O</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undissociated parotid</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Epithelium Mesenchyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Day parotid</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>16-Day submandibular</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>13-, 14-Day lung</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>13-, 14-Day stomach</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>13-, 14-Day pancreas</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* O = Epithelial cyst only; for stages I—IV see Lawson (1970).

Braces at right enclose combinations between which there is no statistically significant difference (P > 0.05) in the frequency distribution according to histogenetic stage.

The effect of mesenchyme from different sources on the development of parotid epithelium

The development of recombinates of parotid epithelium with its own mesenchyme did not differ from that of the intact gland in vitro, either in histogenesis (Fig. 1 A; cf. fig. 2 G in Lawson, 1970; Table 3) or in growth and amylase activity (Fig. 3 Aa, b, 3 Ba, b, 3 Ca, b). Development of the parotid epithelium in the presence of heterotypic mesenchyme varied with the origin of the mesenchyme: in submandibular mesenchyme the results were similar to those described in the first section, the histogenetic stage reached by the branching parotid epithelium not differing significantly from that reached in homotypic mesenchyme (Table 3); however, the acini were more numerous and closely packed, the explants were twice as large (Fig. 3 Ac), contained five times as much amylase activity per culture (Fig. 3 B c) and about three times as much amylase per mg total organic solids (Fig. 3 C c). Parotid epithelium underwent morphogenesis in lung mesenchyme (Figs. 1 E, 2 E) but the histogenetic stage reached by the terminal buds was less advanced than in homotypic mesenchyme (Table 3), the cultures were smaller (Fig. 3 A d) and contained less amylase activity, both totally and per mg (Fig. 3 Bd and 3 Cd). In addition to the parotid-like terminal buds 40% of these explants contained columnar epithelium with plentiful material staining brilliantly with PAS (Figs. 1 E, 2 E). Stomach and pancreatic mesenchyme supported no, or limited, budding and histogenesis of the parotid epithelium (Table 3, Figs. 1 F, 2 F); on the rare occasions when the epithelium did proliferate in pancreatic mesenchyme, other than as a cyst, the morphology was reminiscent...
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Fig. 3. Effect of recombining parotid epithelium with mesenchyme from different sources on the total organic solids (A), total amylase activity (B), and specific amylase activity (C), of the recombinates after 15 days in vitro. Values are given for the undissociated parotid (a) and for parotid epithelium recombined with mesenchyme from parotid (b), submandibular (c), lung (d), stomach (e) and pancreas (f). Vertical lines represent the 95% confidence limits for comparing any two means, derived from the analyses of variance.

of the indented, compact acinar structure of the pancreas (Fig. 1G). The explants containing stomach or pancreatic mesenchyme were smaller than the other recombinates (Fig. 3Ae, f), and in the recombinates with pancreatic mesenchyme the mean amylase activity per mg did not rise significantly above that found in the intact parotid rudiment before cultivation (Fig. 3 Cf; cf. fig. 3 in Lawson, 1970).

These results indicate that parotid epithelium is able to undergo morphogenesis and subsequent cytodifferentiation in non-salivary mesenchyme, but that
Table 4. Growth of parotid and submandibular mesenchyme during 9 days in vitro

<table>
<thead>
<tr>
<th></th>
<th>Initial ± s.e.</th>
<th>N</th>
<th>Final ± s.e.</th>
<th>N</th>
<th>Relative increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-Day parotid</td>
<td>15.4 ± 1.2</td>
<td>4</td>
<td>35.2 ± 2.7</td>
<td>3</td>
<td>2.3</td>
</tr>
<tr>
<td>16-Day submandibular</td>
<td>13.8 ± 2.6</td>
<td>3</td>
<td>133.0 ± 8.3</td>
<td>6</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of recombining parotid epithelium with different masses of parotid (●) or submandibular (○) mesenchyme. Final size (A), total amylase activity (B) and specific amylase activity (C) of the recombinates are plotted. Vertical lines represent the 95% confidence limits for comparing any two means, derived from the analyses of variance.
Table 5. Summary of analyses of variance: the effect of mesenchyme mass from different sources

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Total organic solids</th>
<th>Total amylase explant</th>
<th>Amylase/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS*</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Replicates</td>
<td>6</td>
<td>0.0417</td>
<td>4.16</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Origin of mesenchyme (A)</td>
<td>1</td>
<td>1.5776</td>
<td>157.60</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mass of mesenchyme (B)</td>
<td>2</td>
<td>0.7665</td>
<td>76.47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Interaction A x B</td>
<td>2</td>
<td>0.0164</td>
<td>1.64</td>
<td>—</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.0100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean squares (MS) and variance ratio (F) from analysis of variance performed on data transformed to logarithms.
the growth of the recombinates, the histogenesis of the epithelium, and the amount of differentiation product produced, differ in different mesenchymes.

*The effect of different masses of mesenchyme on the development of the parotid epithelium*

To disentangle effects on the development of the parotid epithelium arising from the nature of the mesenchyme with which it is recombined and possible effects due to differences in the mass of available mesenchyme, single parotid epithelial rudiments were recombined with mesenchyme from 1, 2 or 4 parotid or submandibular rudiments. In addition, the content of total organic solids in fused pairs of parotid or submandibular mesenchymes before culture and after 9 days *in vitro* was estimated.

While the starting masses of parotid and submandibular mesenchyme were comparable, the parotid mesenchyme alone grew much more slowly than the submandibular mesenchyme alone (Table 4).

The results for the recombinates are shown in Fig. 4 and the relevant analyses of variance in Table 5. As expected, the final size of the explant increased with the initial mass of the mesenchyme provided (Fig. 4 A). In addition, the recombinates with submandibular mesenchyme were two to three times the size of those with the same initial amount of parotid mesenchyme.

The total amylase activity developed by a single parotid epithelial rudiment varied both with the initial amount of associated mesenchyme and with its source (Fig. 4B): the more mesenchyme, the greater was the amount of amylase; more than five times as much amylase activity was produced in the presence of submandibular as in the presence of the same starting mass of parotid mesenchyme. The first order interaction in the analysis of variance (Table 5) was statistically significant (*P* < 0.05) and is interpreted as a greater effect produced by altering the mass of parotid mesenchyme than of submandibular mesenchyme.

When the specific activity of amylase, i.e. amylase per mg total organic solids, was examined, the difference between the amylase activity of recombinates with parotid and with submandibular mesenchyme was still striking (Fig. 4C). In addition, whereas the amylase concentration in the recombinates with parotid mesenchyme varied with the initial mass of mesenchyme, this difference was absent in recombinates with submandibular mesenchyme; the result is reflected in the significant (*P* < 0.001) statistical interaction in the analysis of variance (Table 5).

**DISCUSSION**

The ability of rat parotid epithelium to undergo morphogenesis and subsequent cytodifferentiation in non-salivary mesenchyme, conflicts with the view that salivary epithelium can form adenomeres only in salivary mesenchyme. Although the level of histogenesis achieved in lung and particularly in stomach and pancreatic mesenchyme was lower than in salivary mesenchyme, it was not
negligible. Epithelial budding had been initiated in more than 50% of the recombinates with stomach and pancreatic mesenchyme and stage II, representing a level of cytodifferentiation only attained by the parotid at birth, or by the 17-day intact rudiment after about 9 days in vitro, was reached by 30–40% of these recombinates after 15 days in vitro. There are several possible explanations for the discrepancy between the present results and the interpretation of previous work: (1) Developmental processes in mouse and rat salivary systems are not comparable. (2) Generalizations from the submandibular gland do not apply to other salivary glands. (3) The culture conditions and the relatively long culture period required for the rat parotid were more favourable for the expression of an epithelial response. There is at the moment insufficient comparative information to judge the weight of these possibilities. (4) Although the epithelia looked clean under the dissecting microscope the possibility that parotid mesenchyme cells were carried over unnoticed into the recombinates cannot be rejected, but it must then be assumed that the non-salivary mesenchymes supported growth of the contaminating parotid mesenchyme cells to different degrees, in order to account for the different results with the various heterotypic mesenchymes and the non-viability of the epithelial rudiment without additional mesenchyme.

Once terminal buds had been formed, their further differentiation was typical of the epithelial source: cytodifferentiation in terminal buds formed by parotid epithelium in various mesenchymes was recognizably parotid in character, all stages from early, undifferentiated buds to polarized cells associated with an increase in amylase activity being found. Only in lung mesenchyme were the terminal buds sometimes accompanied by substantial quantities of epithelium showing atypical cytodifferentiation. Although the PAS-positive material produced by these columnar cells was more abundant than that found in the tubule lumina of the normal parotid it is not known whether its chemical composition differs from that of mucopolysaccharide normally produced by the parotid epithelium. Thus the cytodifferentiation potential of parotid epithelium is already restricted at a stage in the embryo when budding has just begun and tends to remain so, even after prolonged association with and growth in heterotypic mesenchyme. The indications are that submandibular epithelium is similarly restricted: the staining characteristics of the stage C terminal buds formed by this epithelium in parotid mesenchyme and in its own mesenchyme were indistinguishable, and there was no measurable amylase activity in either.

In contrast to the cytodifferentiation of the terminal buds, their initial formation and the continued branching growth of the epithelium varied with the source of the associated mesenchyme. The heterotypic mesenchymes were used at a stage when they are morphogenetically active in situ, at least in the mouse (Table 6), but the type of morphogenesis which they normally support differs: the requirements for acinar formation by lobulation in the pancreas are more easily satisfied (Golosow & Grobstein, 1962; Rutter, Wessells & Grobstein,
Table 6. Developmental ages of various mouse organs at stages when the mesenchyme is morphogenetically active, tabulated against the comparable morphogenetic stage in the rat

<table>
<thead>
<tr>
<th>Organ</th>
<th>Gestational age (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse†</td>
<td>10 — 11 — 12 — — — —</td>
<td>Wessells &amp; Cohen (1967), Grolosow &amp; Grobstein (1962)</td>
</tr>
<tr>
<td>Submandibular gland</td>
<td>— — — — — — — — — — 13 — 14</td>
<td>Borghese (1950), Grobstein (1953a)</td>
</tr>
</tbody>
</table>

* Sperm positive, ± copulation plug = day 1. † + Copulation plug = day 0.

1964; Wessells & Cohen, 1968; Wessells, 1970) than are those for the formation of branched structures such as the lung (Dameron, 1961, 1968; Alescio & Cassini, 1962; Taderera, 1967; Wessells & Cohen, 1968; Spooner & Wessells, 1970) and the salivary glands (Grobstein, 1953b; Grobstein & Cohen, 1965). The present experiments provide complementary evidence in that only lung mesenchyme allowed a degree of development of parotid epithelium approaching that supported by salivary mesenchyme.

There are a number of illustrations in the literature of an altered epithelial morphogenesis in response to foreign mesenchyme (Auerbach, 1960; Golosow & Grobstein, 1962; Dameron, 1961, 1968; Spooner & Wessells, 1970); in the case of mouse lung epithelium responding to chick mesenchyme (Taderera, 1967) and of mouse mammary epithelium in the presence of submandibular mesenchyme (Kratochwil, 1969) there are clear indications that the branching pattern is modified in the direction of that of the epithelium homologous for the mesenchyme. In the present experiments the most consistent modifications were found in the reciprocal recombinations within the salivary system and took the form of an increased number and closer packing of the terminal buds in submandibular mesenchyme. During normal development of the salivary glands the submandibular and sublingual epithelia branch rapidly in the same mesenchyme capsule to produce a bushy structure in contrast to the more tree-like form of the parotid (Lawson, 1970). The early morphogenetic branching patterns of the parotid and submandibular glands must be precisely analysed before it can be decided whether there are specific parotid and submandibular patterns imposed by the mesenchyme or whether the differences are due to quantitative variations on a basic salivary theme.
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Graphical data (fig. 5 in Lawson, 1970) indicate that the submandibular gland grows faster than the parotid throughout foetal life. These data have since been analysed statistically and the difference between the glands has been found to be significant ($P < 0.001$). The present experiments *in vitro* indicate that the mesenchymal component of the gland is responsible for the difference in growth rate: the final size of homotypic and reciprocal recombinates differed only when the source of the mesenchyme was different. Not only did the submandibular mesenchyme alone grow faster than the parotid mesenchyme but it encouraged the formation of more, densely packed terminal buds. The higher amylase activity per epithelial rudiment and per mg explant of recombinates of parotid epithelium with submandibular mesenchyme compared to the same epithelium in its own mesenchyme presumably are reflexions of this morphology, since no significant difference could be found in the degree of differentiation of the buds, either histologically or histochemically; however, the quantitative criteria used in both these methods are crude in comparison with the biochemical estimation, and this may have obscured small differences in acinar maturation.

Mesenchyme from two parotids was necessary in recombinates to support epithelial development comparable to that found in the intact gland under the same conditions *in vitro*. Less total amylase activity was produced in the presence of a single mass of mesenchyme and more in the presence of four, suggesting a close relationship between mesenchyme mass and epithelial growth rate. Such a relationship exists in mouse lung, where not only is growth of the bronchial epithelium reduced after X-irradiation of the mesenchyme (Alescio, Cassini & Ladu, 1963), but growth of the bronchial tree is promptly increased by supplying additional bronchial mesenchyme to an intact lung rudiment *in vitro* (Alescio & Colombo Piperno, 1967); this increased growth rate is associated with an overall increase in mitotic activity (Alescio & di Michele, 1968), although to date no evidence has been found for selectively higher mitotic activity in budding regions (Wessells, 1970).

On the basis of approximately equal initial masses of mesenchyme at least four times as much parotid as submandibular mesenchyme was necessary to produce the same end result in terms of amylase activity. Increasing the initial amount of submandibular mesenchyme also led to a higher final amylase activity per explant, but not to an increased amylase activity per mg, suggesting that epithelium buds to fill the available mesenchyme until an optimal epithelium–mesenchyme ratio is reached. The suggestion of a growth-regulating role of mesenchyme is supported by the work of Auerbach (1964) who showed that the ability to undergo budding morphogenesis is latent in mature adenomeres of the mouse submandibular gland and can be re-activated by recombining the acini with foetal capsular mesenchyme.

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