CULTURED HUMAN RESPIRATORY EPITHELIUM: ITS USE IN THE COMPARISON OF THE CYTOTOXIC PROPERTIES OF LOCAL ANESTHETICS

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In the search for local anesthetic drugs with optimal potency and minimal toxicity, new testing methods which are more sensitive and objective are being developed. Clinical methods of evaluation can be superior to laboratory techniques provided they are properly devised and standardized. On the other hand, an experimental method in which conditions may be deliberately varied can be a vastly more efficient means of analysis.

We have utilized tissue culture techniques and studies of the effects of drugs upon cell function as a method for comparing toxic properties of local anesthetic agents. The proposal to expose cultured tissue to local anesthetics and to observe the effect of these drugs on cell activity is based on the assumption that local anesthetics act as protoplasmic poisons. These drugs apparently affect the metabolism of the cell, disturbing its function. This can either be transient and completely reversible or it can result in permanent damage to the cell structure. Irreversible injury depends upon the chemical structure of the local anesthetic, its concentration, and the amount and rapidity of absorption by the cell.

Ciliated epithelium appeared to be most valuable for such cytotoxic studies, since the ciliary beat can serve easily as an index of cellular activity. In the past, many investigators have been interested in the effect of chemicals on ciliary action. The experiments were usually performed with excised strips of tracheal, esophageal or pharyngeal mucosa of animals (frog, rabbit, rat, horse, ox, sheep). The gill of the oyster has also been used for this purpose. Recently, studies have been reported on the action of drugs on ciliary activity when topically applied to the upper respiratory tract of man. The effect of changes in temperature and the influence of different ions and changes in pH on ciliary action have also been investigated.

In 1939 Proetz and Pfingsten described a method for cultivating ciliated nasal mucosa of the guinea pig fetus. In 1949 a tissue culture study of human ciliated nasal epithelium was reported by Rose, Fomerat, and Danes. Since 1956 successful cultivation of human tracheal and bronchial epithelium has been performed in the Tissue Culture Laboratory of the University of Texas Medical Branch, Galveston.

When electrolyte balance and other physiological factors such as osmotic equilibrium are maintained, cultures of human respiratory epithelium remain in a steady state for several days. Increased or decreased strength or rapidity of ciliary beat or disturbance of coordination of ciliary motion following exposure of the cells to the drugs under study.
may, therefore, be interpreted as a cytotoxic effect of the local anesthetic agent. The observations of such tissue with phase-contrast photomicrography and, particularly, with cinematography offer ideal conditions for observing and recording the results of such investigations.

Methods and Materials

Since a detailed description of the method has been published elsewhere only a brief summary of the technique as well as certain modifications are given here.

Explanted from punch biopsies of human tracheal and bronchial epithelium were cul-

Fig. 1. Rotating globe of human tracheal epithelium. Photograph was made on the eighth day of incubation at 37°C. Magnification X 720.
Fig. 2. Cell mass of human tracheal epithelium (rotating speed 5/minute) One complete rotation is shown over a period of 12 seconds. Film was made on second day of incubation at 37 C. Magnification × 400.
vated in simple hanging drop preparations. After 2 to 5 days of incubation the tissue was transferred to a perfusion chamber and carefully sealed in with a mixture of paraffin and beeswax. The nutrient was introduced through an inlet tube, passed across the central area containing the ciliated cells and removed through a capillary outlet tube. The chamber was placed on the stage of a microscope and effects recorded by cinematography at a rate of sixteen frames per second.

Under conditions in which liquefaction of the clotted medium occurred, small epithelial explants curled up to form globes with their cilia on their outside surface (fig. 1). Since these cell clusters rotated for several days at a relatively uniform rate, they became useful in the study of changes in the chemical environment. The rotating ciliated epithelial globe which was transferred into the perfusion chamber filled with Gey's balanced salt solution was allowed to stabilize until rotational movements persisted at the same speed (fig. 2). The number of rotations per minute was clocked with a stopwatch.

Local anesthetic solutions to be perfused through the chamber were prepared by dissolving the crystals of each drug in Gey's balanced salt solution (BSS) since, as a rule, commercially available local anesthetic solutions, tables or powder contain preservatives and binding substances which generally have a deleterious effect on the cells in culture. It was possible to determine for each local anesthetic under observation the concentration which (a) did not affect ciliary activity, (b) caused persistent stimulation, (c) stopped ciliary motility but, after reperfusion with BSS, resulted in revival of the tissue, and (d) arrested ciliary action with permanent cell injury so that reperfusion with BSS failed to reactivate the ciliary beat. Two thousand fifty-nine explants of tracheal and bronchial tissue taken from 119 patients were cultured and examined. To obtain the data presented here, a total of 102 perfusion experiments were performed.

### Results

Table 1 presents a comparison of the effects of six clinically useful local anesthetic drugs upon human ciliated epithelium in tissue culture. The agents tested were procaine, chloroprocaine (Nesacaine), lidocaine (Xylocaine), cocaine, tetracaine (Pontocaine) and dibucaine (Nupercaine). As revealed by this table, solutions of procaine hydrochloride (0.05 per cent) and lidocaine hydrochloride (0.01 per cent) failed to exert any visible effect on ciliary function. Both drugs caused persistent stimulation at concentrations as low as 0.1 per cent and temporary loss of ciliary motility was observed with procaine and lidocaine in concentrations ranging from 5 to 20 per cent.

Chloroprocaine hydrochloride did not change the ciliary beat when the drug was diluted to 0.005 per cent. The drug began to exhibit a stimulatory action on the rotating cell cluster at 0.01 per cent concentration, while ciliary arrest occurred at concentrations ranging from 0.5 per cent to 3.0 per cent. Attempts to produce solutions higher than 3.0 per cent in

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### Table 1

**Response of Ciliary Movement of Cultured Human Respiratory Epithelium to Local Anesthetic Drugs**

<table>
<thead>
<tr>
<th>Response of Ciliated Cell</th>
<th>Procaine HCl (per cent)</th>
<th>Chloroprocaine HCl (per cent)</th>
<th>Lidocaine HCl (per cent)</th>
<th>Cocaine HCl (per cent)</th>
<th>Tetracaine HCl (per cent)</th>
<th>Dibucaine HCl (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No effect</td>
<td>0.05</td>
<td>0.005</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Persistent stimulation</td>
<td>0.1–2.0</td>
<td>0.01–0.1</td>
<td>0.1–2.0</td>
<td>0.1–0.5</td>
<td>0.015</td>
<td>0.003</td>
</tr>
<tr>
<td>Stoppage of ciliary activity reversible</td>
<td>5.0–20.0</td>
<td>0.5–3.0</td>
<td>5.0–20.0</td>
<td>10.0 (? )</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Stoppage of ciliary activity irreversible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disturbance of ciliary coordination</td>
<td>1.0–20.0</td>
<td>0.1–3.0</td>
<td>0.5–20.0</td>
<td>0.5–20.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
concentration were unsuccessful, because the
dissolving of the chlorprocaine crystals in the
balanced salt medium resulted in a super-
saturated solution.

Perfusion with tetracaine and dibucaine
hydrochloride solutions showed no effect on
ciliary movement at concentrations up to 0.001
per cent (dibucaine) and up to 0.01 per cent
(tetracaine), whereas the first stimulating re-
response was noted with dibucaine at 0.003 per
cent and with tetracaine at 0.015 per cent.
On exposing the tissue to solutions of tetra-
caine 0.15 per cent or to dibucaine 0.1 per cent
a rapid decrease and subsequent stopping of
ciliary action was observed. Ciliary action
failed to return when Gey's BSS was
reperfused.

Cocaine accelerated rotary activity of the
epithelial cell mass at concentrations of 0.1
to 0.5 per cent while a 10 per cent solution
arrested ciliary action, usually with incom-
plete recovery. Cocaine at a concentration of
20 per cent rendered the cell damage ir-
reversible.

The onset of the inhibitory effect with con-
centrations resulting in reversible ciliary ar-
est was observed to be considerably more
rapid with chlorprocaine and lidocaine than
with procaine, cocaine, tetracaine or dibucaine.
While at various effective concentrations chlo-
roprocaine and lidocaine need an average time
of fifteen to thirty minutes to completely stop
ciliary activity, the remaining compounds ex-
erted the same within 60 to 75 minutes.
Similar observations were made in regard to
the time needed for recovery after removal of
the agent.

At effective concentrations procaine, chlor-
procaine, lidocaine and cocaine caused a
peculiar vibration of the whole tissue which
was not observed with tetracaine and dibu-
caine.

**DISCUSSION**

The type of response of the epithelial globe
following exposure to the various local an-
esthetic agents at different concentrations was
believed to represent the toxic effect on the
cell. An increase of ciliary action appeared
to be the result of a stimulatory effect which
might persist for some time, or might be only
a transient phenomenon. A depressant action
resulted in a decrease of ciliary activity and
eventual stoppage. The suppression of ciliary
motion was either reversible, indicating a
temporary, nonlethal effect on protoplasmic activity or irreversible in which case
the reperfusion with Gey's BSS either failed
or only partially succeeded in reviving ciliary
function.

The intensity with which the local anes-
ethetics affect cell function as reflected by the
change of ciliary activity of cultured human
respiratory epithelium appeared to differ sig-
nificantly with the various agents. Procaine,
chlorprocaine and lidocaine had a remarkably
wide range of discernible effect on cell activity
without causing permanent damage to the
cell. None of these three drugs at clinically
useful concentrations was observed to render
the complete ciliary arrest irreversible. In
contrast to this group tetracaine and dibucaine
not only stopped ciliary action at considerably
lower concentrations but appeared to have a
markedly smaller margin of safety with regard
to their least effective concentrations as com-
pared to those solutions causing irreversible
damage to the tissue. Permanent cell damage
with signs of disintegration of the epithelial
mass occurred with tetracaine at 0.15 per
cent and with dibucaine at 0.1 per cent which
are concentrations commonly used for pro-
ducing regional anesthesia in man.

The onset of the inhibitory effect upon
cellular activity was observed to be almost
twice as fast with chlorprocaine and lidocaine
as compared to procaine, cocaine, tetracaine
and dibucaine. The considerably faster mani-
festation of cellular depression and the more
prompt reversibility of the effect as noted
with chlorprocaine and lidocaine suggested a
higher diffusibility and penetrating power of
these agents as compared to that of procaine,
cocaine, tetracaine, and dibucaine.

The peculiar trembling and shaking of the
whole tissue as observed with procaine, chlor-
procaine, lidocaine and cocaine at certain con-
centrations were believed to represent a dis-
turbance in the mechanism of ciliary coordi-
nation. Perfusions with tetracaine and dibucaine
failed to show this unusual phenomenon.
SUMMARY

Cultures of human ciliated respiratory epithelium obtained from the trachea and bronchus were utilized for the study of the effects of local anesthetic drugs upon the cell. The ciliary beat which remained undisturbed when the explant of respiratory epithelium was transferred into a balanced salt medium served as an index of cell activity. With exposure to different concentrations of local anesthetic drugs ciliary activity underwent changes such as acceleration, deceleration, arrest or disturbance of coordination of the ciliary beat. The effect was either reversible or resulted in permanent cell injury.

This method was used for a comparative study of procaine, chlorprocaine, lidocaine, cocaine, tetracaine and dibucaine. The low toxicity and the remarkably broad spectrum of effectiveness of procaine, chlorprocaine and lidocaine upon the cultured cell was significant. All three drugs failed to produce permanent injury at clinically useful concentrations.

Tetracaine and dibucaine revealed a considerably higher toxicity to the cells. The margin of activity without causing permanent cell damage was observed to be considerably smaller and cellular activity was rendered irreversible at concentrations commonly used in producing regional anesthesia in man.

Cocaine appeared to occupy a position between the procaine-chlorprocaine-lidocaine group and the tetracaine-dibucaine group.

The onset of drug action resulting in ciliary arrest and the reversibility of the process with complete recovery of ciliary motion was noted to be almost twice as rapid using chlorprocaine and lidocaine perfuses as compared to the effects obtained with procaine, cocaine, tetracaine and dibucaine. This observation suggests a higher diffusibility and penetrating power of chlorprocaine and lidocaine.

This investigation was supported in part by the Medical Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-32, administered by C. M. Pomerat, Director of the Tissue Culture Laboratory of the University of Texas Medical Branch, Galveston. Grateful acknowledgment is made to Dr. C. M. Pomerat for helpful advice and to Messrs. C. G. Lefebre, E. E. Pitsinger and D. Pearson for their very generous assistance with photographic work.

REFERENCES

DIHYDROMORPHINON Therapeutic doses of Dihydromorphinon ("Dilaudid")-atropine caused a small decrease of respiratory minute volume, but blood gas values remained normal in 7 out of 8 healthy male volunteers. (Weimann, G., and Hermanz, N.: Concerning the Influence of d-Hydromorphinon-Atropine on Respiration, Der Anaesthesist 8: 351 (Dec.) 1959.)

PROMETHAZINE DRIP The danger of extravasations of blood and urine after suprapubic prostatectomy is increased in patients made restless by pain and by reflexes arising from the region of the bladder. Prolonged, heavy sedation with narcotics alone is undesirable in elderly patients. The possibility that better results might be obtained by a combination of a narcotic and promethazine hydrochloride was investigated in 82 patients who had undergone suprapubic prostatectomy under spinal anesthesia. All received meperidine or morphine as needed for pain, and 41 patients received in addition promethazine by intravenous drip (75 mg. promethazine per 1,000 cc. of solution, given at a rate of 30 drops per minute for a period of from 12 to 16 hours). Patients so treated remained free from nausea, vomiting, chills, and cold sweats; tolerated post-operative bladder irrigations without reflex spasms or hemorrhages; and required, on the average, less than half the usual dose of narcotics. No untoward effects were observed. This method may be a valuable aid in the postoperative management of any patient who must undergo a traumatic operation and who is in an age group in which the best physiological conditions do not prevail. (Sheiner, B., and Pinck, B. D.: Promethazine Drip in the Postoperative Management of Suprapubic Prostatectomy, J. A. M. A. 171: 1955 (Dec. 5) 1959.)

CARCINOID TUMORS In carcinoidosis the level of 5 hydroxy-tryptamine (5 H-T, serotonin, enteramine) may be markedly raised; it is depressed in phenylketonuria. Four main theories have been advanced concerning the function of 5 H-T in the body, the most exciting of which is that in the brain it is the synaptic mediator of the central parasympathetic nerves, in much the same way as noradrenaline is of the sympathetic. Carcinoid tumors develop from argentaffin cells and produce symptoms of abdominal pain, diarrhea and, in certain cases, flushing attacks, and valvular lesions of the heart affecting mainly the right side. The treatment of choice for carcinoid tumors is that for any malignant lesion, namely, wide resection of the primary growth and removal of the local lymphatics if technically possible. At operation, difficulty may develop during anesthesia since 5 H-T is a powerful broncho-constrictor, and high blood levels may be obtained by manipulation of the tumor. Such bronchoconstriction resists both ether and meperidine, and the safest anesthetic method appears to be epidural combined with pre-operative preparation with chlorpromazine, which is a 5 H-T antimetabolite. (Davies, A. J.: Carcinoid Tumours (Argentaffinomata), Ann. Roy. Coll. Surgeons England 25: 277 (Dec.) 1959.)