| Preliminary treatment | No. of chick | Age, 6 weeks | Age, 7 weeks | | Age, 13 weeks | |
|---|---|---|---|---|---|---|
| | | | Range | Mean ± SE | Range | Mean ± SE |
| Group I<br>Single i.v. injection of 16 mg HGG (12-days embryos) | 10 | One injection of 40 mg HGG | 64–512 | 217,6 ± 50,25 | | |
| Group II<br>Eight s. c. injection of 16 mg of HGG each at age 1–32 days | 10 | One injection of 40 mg HGG | 16–64 | 40 ± 6,54 | *64–512 | 234 ± 52,7 |
| Group III<br>None (control) . . . . . | 12 | One injection of 40 mg HGG | 64–512 | 213,3 ± 45,86 | 128–1024 | 298 ± 72,33 |

* Nine chicks tested

A single injection of antigen given to 12-days old embryos had no effect on the latter immune response. This data seems to support the view of SIMONSEN[4] that the day of injection may be of significance for reduction of immunological responsiveness.

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*Zusammenfassung*

Wiederholte Injektionen von menschlichem γ-Globulin in der ersten Nachgeburtsperiode, führte bei Kücken zu immunologischer Toleranz gegenüber diesem Antigen.

[4] M. SIMONSEN, Acta path. microbiol. scand. *39*, 21 (1956).

## Cholinesterase in Human Salivary Glands

In case of tumour in or near the salivary glands, surgical removal of the tumour and surrounding glandular tissue is the standard treatment. Advantage of this fact was taken to obtain fresh human salivary gland tissue for estimation of cholinesterase activity. Previously, the cat submaxillary and parotid gland (STRÖMBLAD[1]) have been found to contain cholinesterase.

*Methods.* Only apparently healthy tissue was used; the tumour was cut off with a wide margin. The tissue was carefully cleaned, washed in saline, weighed, minced with scissors, and ground in a glass homogenizer with Krebs' bicarbonate-Ringer. The volume in ml was then brought up to five times the weight of the tissue in g.

The cholinesterase estimations were made manometrically using Warburg flasks of conventional shape and size. The main compartment contained 0·2 ml of the homogenate and 1·5 ml of Krebs' bicarbonate-Ringer solution. The substrates, in the side bulb in 0·3 ml, were acetylcholine, methacholine, or benzoylcholine in concentrations giving final reaction molarities of 0·011, 0·035, and 0·290, respectively. The substrates were added after 15 min of temperature equilibration. The gas was $N_2$ with 5% $CO_2$ and the temperature 37° C. Readings were taken every 5 min for 40 min. In all experiments, a thermobarometer and enzyme blank were used. Corrections were made for changes in these flasks and for non-enzymic hydrolysis. All estimations were made in duplicate. The activity was expressed in μl $CO_2$ evolved / 30 min / g of tissue.

In some experiments, a small volume of eserine salicylate giving a reaction mixture molarity of $3{\cdot}63 \times 10^{-6}$ was tipped into the main chamber 25 min after estimation had begun. The $CO_2$ evolution was almost completely abolished and this was taken as evidence that the reactions studied were enzymic breakdown of choline esters.

*Table*

| | Acetylcholine | Methacholine | Benzoylcholine |
|---|---|---|---|
| Parotid glands | ♂ 1335 ± 97·7 ($n$ = 8) | 493 ± 49 ($n$ = 7) | 75 ± 233 ($n$ = 6) |
| | ♀ 1035 ± 64·8 ($n$ = 8) | 405 ± 30 ($n$ = 5) | 70 ± 20·0 ($n$ = 5) |
| Submaxillary glands | ♂ 1463 ± 29·8 ($n$ = 6) | 573 ± 28·6 ($n$ = 5) | 133 ± 26·9 ($n$ = 5) |
| | ♀ 1183 ($n$ = 3) | 388 ($n$ = 3) | 50 ($n$ = 3) |

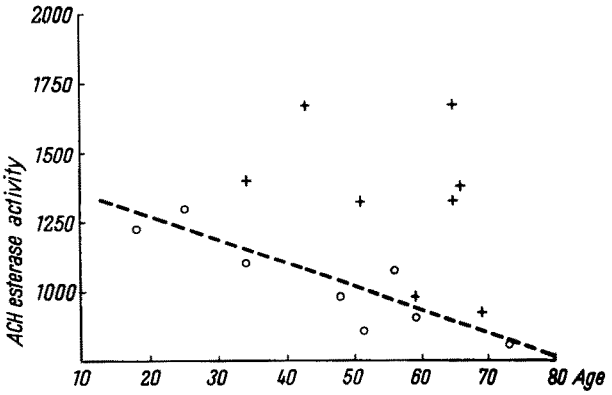
*Results and Discussion.* The human parotid and submaxillary glands showed a moderately high cholinesterase activity (see Table). The activity with acetylcholine and methacholine was much higher than with benzoylcholine, in fact in some cases no enzymic splitting of benzoylcholine was found. The substrate concentration-activity curve showed an almost regular bell-shaped form. Thus the cholinesterase in the human salivary glands is mainly true cholinesterase (acetylcholine esterase). The same is true for cat glands (STRÖMBLAD[1]).

The acetylcholine splitting activity was higher for male parotids than for female parotids when analyzed with Student's $t$-test ($P < 0{\cdot}05$). When the acetylcholine-splitting activity of male and female glands was plotted against the age of the patients, a correlation was found for females between age and activity ($r = 0{\cdot}807$, $P < 0{\cdot}01$). No such close correlation could be demonstrated for the male parotid glands. Analysis of variance

[1] B. C. R. STRÖMBLAD, Acta physiol. scand. *41*, 118 (1957).

was applied and the difference between males and females was then significant on the level of $P < 0{\cdot}01$.

LACASSAGNE[2] found histological differences between the male and female submaxillary glands from rats. He was also able to change the appearance of the structures by injections of sex hormones. It is therefore possible that the difference in enzymic activity in human male and female parotid glands is due to sex hormonal influence.




The Figure shows the acetylcholine splitting activity (ordinate) plotted against the age (abscissa) for male (+) and female (o) parotids. The regression line for female glands ($k = -0{\cdot}334$) is drawn.

Since usually only a small part of the gland was obtainable, it was thought of interest to know whether any differences found between glands could be due to differences in activity in different parts of the gland. This possibility was tested in cases where much tissue was at disposal. The tissue was divided into several pieces and the activity of each estimated. It was found that the results obtained with different pieces from one gland were very similar. One, almost whole, parotid gland, for example, was divided into 16 parts and the acetylcholine splitting activity was estimated. The standard error in this series was $\pm$ 2·1 %, that is of an order which might be due to the error of the method used. Thus the cholinesterase in parotid glands seems to be evenly distributed. The activity was about the same in human parotid and submaxillary glands (see Table). In the cat, the activity in the submaxillary gland is about double that of the parotid gland (STRÖMBLAD[1]).

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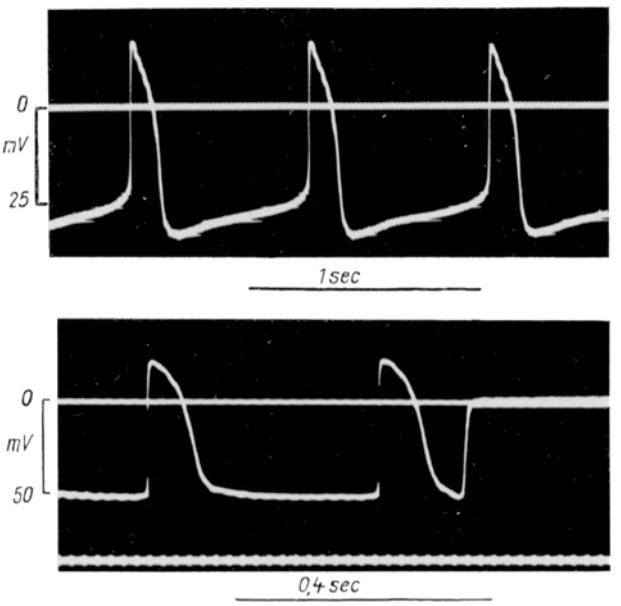
*Zusammenfassung*

Operativ frisch entnommene menschliche Speicheldrüsen (Submaxillaris und Parotis) zeigen Cholinesteraseaktivität. Hauptsächlich werden spezifische Esterasen gefunden. Die Aktivität der männlichen Parotis ist höher als die der weiblichen. Letztere zeigt eine Aktivitätsabnahme mit zunehmendem Alter.

[2] A. LACASSAGNE, C. R. Soc. Biol. *133*, 180 (1940).

## Early Functional Differentiation of Heart Muscle Cells[1]

Tissue cultures from heart muscle cells contract spontaneously. According to FÄNGE *et al.*[2], all cells show an electrical behaviour characteristic of pacemaker regions, the so-called prepotentials. The pacemaker properties are evident even if the material is taken from parts of the embryonic heart that would differentiate into ventricular tissue, provided the embryos are younger than 192 h (8 days). The question arises whether *in vivo* all heart fibres at an early stage of differentiation show pacemaker activity[3].




Upper record: transmembrane potential of sinus region of a 42-h old chick embryo. Lower record: transmembrane potential of cardiac tube (future ventricle)

Fertile chick eggs, incubated for 37 to 67 h at 38°C, were used. The yolk was removed by suction under warm Locke's solution. The embryo, with intact area vasculosa, was transferred to a Perspex chamber. This contained Locke's solution thermostatically controlled at 37°C and aerated with oxygen (95%) and $CO_2$ (5%). With the help of a binocular microscope, the membranes overlying the heart were dissected, and a single muscle fibre was impaled with a glass capillary microelectrode (tip < 0,5 µ).

The potential difference between the microelectrode and an indifferent electrode was applied to a cathode-follower with a low grid current[4], amplified, displayed on a cathode ray tube and recorded on moving film. The second beam of a dual beam scope was used to indicate the level of zero potential difference. Embryos from successful experiments were kept in order to check their age and to measure the diameter of their cardiac fibres.

[1] Aided by a grant from the Italian 'Consiglio Nazionale delle Ricerche'.

[2] R. FÄNGE, H. PERSSON, and S. THESLEFF, Acta Physiol. Scand. *38*, 173 (1957).

[3] See also the comparative physiological researches on the isolated embryonic fishheart by HJ. HUGGEL (Z. vgl. Physiol. *42*, 63 (1959)), which showed by the ligaturemethod the early development of pacemaker activity.

[4] E. MEDA, Arch. Fisiologia *58*, 404 (1958).