The enzymohistochemical pattern is, on the contrary, similar to that observed in the animals killed 4 days after hypophysectomy.

As far as 3β-HSD is concerned the above results are in agreement with the conclusions by LEVY et al.[9], TAYLOR[10], and FUHRMANN[11], i.e. that the presence of these enzymes in the ovaries depends on the hypophysial incretion. It is to be pointed out that the loss of this enzymatic activity runs at different rates in the different ovarian structures. The same is to be said for the G-6-PD.

First of all the ovarian granulosa, blocked in its maturation, do not acquire the 3β-HSD and the G-6-PD activities. Then the thecal and interstitial cells show a reduction of their enzymatic activities. The C.L. enzymes are, according to LEVY[9], the most resistant. Only later is 3β-HSD activity strongly reduced in C.L. as observed by TAYLOR[10] in rats 3 months after hypophysectomy.

As for 20α-HSD activity it must be said that hypophysectomy does not prevent the onset of new C.L. 20α-HSD negative when the animals are hypophysectomized in estrus or in proestrus, whereas it blocks the ovulation and the consequent formation of C.L. in those animals submitted to hypophysectomy in metestrus and diestrus. In no animal does the hypophysectomy inhibit the appearance of the 20α-HSD activity both in the C.L. already present at the time of the operation and in those that appear after it (rats in estrus and proestrus).

Looking at these results, consistent with the fact that the C.L. of pregnancy are 20α-HSD negative[1,4], we may suggest the possibility that the gonadotropins will play their role mostly in inhibiting rather than inducing the onset of 20α-HSD activity in the C.L.[12].

Corpora lutea (C.L.) scored in ovaries of hypophysectomized rats

Cycle phase at the moment of hypophysectomy	2 days after hypophysectomy			4 days after hypophysectomy		
	No. of animals	C.L./ovary		No. of animals	C.L./ovary	
		Total[a]	20α-HSD negative		Total[a]	20α-HSD negative
Proestrus	5	45.1	3.4	5	41.4	0
Estrus	5	38.4	5.7	5	36.2	0
Metestrus	5	42.3	1.8	5	35.7	0
Diestrus	5	39.4	0	5	36.7	0

[a] Identified by the visualization of the 3β-HSD activity.

Riassunto. È stato studiato il quadro enzimatico dei corpi lutei (C.L.) di recente formazione nelle ovaie di ratte ipofisectomizzate. In particolare si è constatato come la comparsa dell'attività 20α-idrossisteroide deidrogenasica non sia impedita dall'ipofisectomia. Si suggerisce la possibilità che l'ipofisi eserciti una azione inibitrice sulla comparsa di questo enzima.

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Hydrolysis of Amino Acid β-Naphthylamides by Aminopeptidases in Human Parotid Saliva and Human Serum

The enzymatic hydrolysis of leucyl β-naphthylamide in serum[1], urine[1], and cerebrospinal fluid[2] had been reported. MÄKINEN[3] recently found in whole human saliva, and in human dental plaque, the aminopeptidases which hydrolyze various amino acid β-naphthylamides including leucyl β-naphthylamide. He suggested that the aminopeptidases are mostly produced by the plaque organisms. However, specific data on the presence of an enzyme hydrolyzing amino acid β-naphthylamides in fluid from the salivary glands is lacking. We tried to measure the activities of aminopeptidases in human parotid salivary secretions by means of a photometric procedure[1], but the activities were too low to be measured by this method. We subsequently found that the activity could be exactly measured by a sensitive fluorometric assay[4,5]. This communication reports the presence of aminopeptidases in human parotid saliva and the comparison of the substrate specificities with the enzymes in human serum.

Parotid saliva was collected from 4 subjects aseptically by means of a cannula devised by UMEMOTO et al.[6]. Salivary flow was elicited by the stimulation of dilute acetic acid. The substrate amino acid β-naphthylamides, which were synthesized as described by GLENNER et al.[7], were kindly supplied from Dr. G. G. GLENNER. The incubation mixture contained 90 μmoles *Tris*-maleate buffer, pH 7.0, 0.15 ml of parotid saliva, 0.45 μmole amino acid β-naphthylamide and water to 0.90 ml. The activity for the hydrolysis of α-L-aspartyl β-naphthylamide and α-L-glutamyl β-naphthylamide was measured in the presence of 10 m*M* of Ca^{2+}. Incubation was carried out at 37 °C for 60 min. Increase of fluorescence intensity

[1] J. A. GOLDBARG and A. M. RUTENBURG, Cancer *11*, 283 (1958).
[2] J. B. GREEN and M. PERRY, Neurology *13*, 924 (1963).
[3] K. K. MÄKINEN, Acta odont. scand. *24*, 579 (1966).
[4] L. J. GREENBERG, Biochem. biophys. Res. Commun. *9*, 430 (1962).
[5] M. ROTH, in *Enzymes in Clinical Chemistry* (Ed. Y. RUYSSEN and L. VANDENDRIESSCHE, Elsevier Publ. Co., Amsterdam 1965), p. 10.
[6] Y. UMEMOTO, M. MORI, Y. KAKUDO, M. KUWAGATA and Y. HIDAKA, J. Osaka odont. Soc. *14*, 14 (1950).
[7] G. G. GLENNER, L. A. COHEN and J. E. FOLK, J. Histochem. Cytochem. *13*, 57 (1965).

of 410 nm of β-naphthylamine released by enzymatic hydrolysis of amino acid β-naphthylamides was measured with the excitation light at 335 nm using an Aminco-Bowman Spectrophotofluorometer[8]. A blank without substrate was run for each sample. All values were corrected for spontaneous hydrolysis of amino acid β-naphthylamide[7] by substracting the value in a control solution containing water instead of the enzyme sample. Serum enzyme activities were measured colorimetrically. Incubation was stopped by the addition of 0.3 ml of 10% Tween-20 in 1 *M* acetate buffer pH 4.2 containing 0.45 mg of stabilized diazonium salt Fast Garnet GBC.

Results are shown in the Table. Although the activities in human parotid saliva were very low compared with those in serum, enzymes responsible for the hydrolysis of various kinds of amino acid β-naphthylamide were found in human parotid saliva. Substrate specificities of hydrolysis of various amino acid β-naphthylamides by human parotid saliva were similar to those by human serum. Naphthylamide derivatives of alanine, leucine, methionine, and norleucine were good substrates for both salivary and serum enzymes. The hydrolysis of lysine and arginine may be catalyzed by aminopeptidase B[9] in parotid saliva. This enzyme activity may have significance in the formation of bradykinin from kallidin-10[10] in saliva. Very low activity of the hydrolysis of α-L-aspartyl β-naphthylamide and α-L-glutamyl β-naphthylamide was detected in saliva only in the presence of Ca^{2+}. This activity may be catalyzed by aminopeptidase A[11] in parotid saliva. This enzyme in serum is responsible for the destruction of angiotensin II[12]. It is interesting that the hydrolysis of glycyl-prolyl β-naphthylamide in parotid saliva was relatively higher than those of other amino acid β-naphthylamides, indicating the presence of a newly described enzyme[13] in parotid salivary fluid[14].

Hydrolysis of amino acid β-naphthylamides by human parotid saliva and serum

Amino acid β-naphthylamide	Aminopeptidase activity (mean ± S.D.)	
	Parotid saliva mμmoles/min/l	Serum μmoles/min/l
Ala	87.0 ± 5.7	78.3 ± 19.0
Arg	7.5 ± 3.5	34.8 ± 18.0
Asp-NH_2	7.3 ± 3.0	0.9
α-L-Asp	0.8 ± 0.9	0.8 ± 0.5
β-Asp	0.0	0.0
Glu-NH_2	18.8 ± 4.5	20.0
α-L-Glu	1.2 ± 1.6	8.6 ± 3.6
D-Glu	0.0	0.0
γ-Glu	0.0	0.0
Gly	4.7 ± 6.4	9.8
Gly-Phe	10.0 ± 4.4	9.6 ± 4.5
Gly-Pro	1120 ± 692	25.1
Ile	23.7 ± 10.8	8.4 ± 3.5
Leu	94.3 ± 10.8	45.0 ± 18.4
Lys	16.8 ± 5.7	10.3
Met	101.3 ± 16.5	65.5 ± 27.2
Norleu	125.3 ± 71.9	34.0 ± 19.3
Norval	46.3 ± 19.8	26.6
Phe	65.0	34.0
Pro	6.8 ± 7.9	1.8 ± 1.9
Ser	0.0	4.6 ± 3.0
Val	5.5 ± 2.5	7.6 ± 4.1

Zusammenfassung. Die Aktivität der Aminopeptidasen im menschlichen Speichel von Ohrspeicheldrüsen wurde durch Fluoreszenzanalyse gemessen. Die Substratspezifität der Speichelaminopeptidasen war derjenigen der Serumenzyme ähnlich. Das Glycyl-Prolin β-naphthylamidspaltende Enzym war jedoch im Speichel von Ohrspeicheldrüsen in relativ grösserer Menge vorhanden.

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[8] The Aminco-Bowman spectrophotofluorometer was purchased by United States Public Health Service Research Grant No. 7R05 TW-00219-01A1 for T. NAGATSU, which is gratefully acknowledged.

[9] V. K. HOPSU, U. M. KANTONEN and G. G. GLENNER, Life Sci. *3*, 1449 (1964).

[10] V. K. HOPSU-HAVU, K. K. MÄKINEN and G. G. GLENNER, Nature *212*, 1271 (1966).

[11] G. G. GLENNER, P. J. MCMILLAN and J. E. FOLK, Nature *194*, 867 (1962).

[12] I. NAGATSU, L. GILLESPIE, J. E. FOLK and G. G. GLENNER, Biochem. Pharmac. *14*, 721 (1965).

[13] V. K. HOPSU-HAVU and G. G. GLENNER, Histochemie *7*, 197 (1966).

[14] The authors are grateful to Dr. G. G. GLENNER (National Institutes of Health, Bethesda) for the gift of amino acid β-naphthylamides and for the criticism and the correction of the manuscript.

Receptive Fields of Cells in the Human Visual Cortex

Microelectrodes developed for neurosurgical use[1] were adapted for chronic implantation into the brain of patients with intractable seizures[2], in addition to the usual macro-electrodes of 'depth electrography'[3]. By means of these indwelling microelectrodes scores of single units in the visual cortex have been observed, many showing no change of activity to any of a wide range of stimuli. Many other units were responsive to general visual stimulation (by the movement of random sized discs and bars in various orientations on contrasting backgrounds), but in each of these we could not find a specific or key feature of the stimulus which would allow us to plot the receptive field. This is defined as a spatial plot of the visual stimuli which influence the firing of the unit under observation.

A number of receptive fields belonging to both cell bodies and axons were found in several patients. We had the opportunity to study 5 of them in 2 alert and co-operative patients. The patient viewed a 'grain of wheat' lamp or an ink dot accurately and without difficulty. Plotting was done directly on a large white or black sheet of cardboard at a distance of 1 m with the aid of various sized black and white discs and bars held on thin, stiff, wire handles (Fig. 1). Similar wands were used in 4 colors, red, yellow, green and blue. Spectrophotometric curves of these colors are unimodal, revealing good purity of hue.