Table II. Units of cholinesterase activity found in normal adult females and in those suffering from neoplastic diseases

	Plasma		Whole blood		Blood cells	
	Normal ♀	Cancer ♀	Normal ♀	Cancer ♀	Normal ♀	Cancer ♀
Average UCh activity	94	68	139	105	200	161
Standard deviation	± 10.5	± 5.4	± 8.7	± 10.3	± 13.1	± 13.3
Standard error	± 2.3	± 1.3[a]	± 1.9	± 2.5[b]	± 2.9	± 3.3[c]
Number of cases	20	16	20	16	20	16

[a] $P < 0.001$. [b] $P < 0.001$. [c] $P < 0.001$.

the suprarenal function and our present investigation, because up to the present there is no proof of diminution of the corticosuprarenal function in neoplastic diseases. On the contrary, in certain types of tumours, the suprarenal function is increased[6] and, according to other authors, cortisol metabolism is also changed[7]. In any case, it is fairly probable that the mechanism that produces the fall in cholinesterase activity in cancer is not in any way related to the suprarenal hormones; it is possible that other factors as yet unknown play a part in this. There is also a difference in the diminution of cholinesterase activity caused by the suppression of adrenal hormones, and the diminution found in neoplastic diseases. In the first case, the greatest diminution is found in the fraction corresponding to the blood cells, the variation in plasma being much less. The diminution of cholinesterase activity in neoplastic diseases is more homogenous, because both the plasma and cell fractions are intensely diminished.

Résumé. On a étudié l'activité cholinestérasique dans le plasma, le sang total et les cellules sanguines chez 23 adultes de sexe masculin et chez 20 adultes de sexe féminin apparemment sains, et dans les mêmes fractions, chez 22 hommes adultes et chez 16 femmes adultes, malades du cancer. On a constaté une diminution très marquée de l'activité cholinestérasique dans le plasma, le sang total et les cellules sanguines chez tous les malades. La différence a été hautement significative ($P < 0{,}001$). Cette détermination peut avoir une valeur présomptive dans le diagnostic du cancer.

J. R. VACCAREZZA and J. A. WILLSON

Laboratorio de Investigaciónes Fisiopatológicas, Paraná 981, Buenos Aires (Argentina), and Laboratorio Central del Hospital Muñiz, January 13, 1965.

[6] H. J. WETSTONE, R. V. LAMOTTA, A. BELLUCCI, R. TENNANT, and B. V. WHITE, Am. J. int. Med. *52*, 102 (1960).
[7] E. E. WERK, L. J. SHOLLITON, and R. T. MARNELL, Am. J. Med. *34*, 192 (1963).

Regional changes of the Cholinergic System in the Guinea-Pig's Brain after Physostigmine

It is well known that cholinesterase inhibitors (ChEI) desynchronize the EEG[1,2]; however, the activation is limited to the cortical areas[3,4]. Moreover, the ChEI cause dissociation between behaviour[5] and ACh content of the brain: the signs of excitatory state are associated with increased ACh levels which are, instead, characteristic of reduced nervous activity[6,7].

To obtain further information on the central effects of physostigmine, we studied the changes the drug produced in the cholinergic systems in the different areas of the guinea-pig's brain.

Methods. Physostigmine sulphate was given i.p. to guinea-pigs of both sexes weighing 250–350 g. The animals were decapitated 20 min, 1 h, and 3 h after 0.2–1 mg/kg and, as soon as convulsions developed (20–30 min), after 5 mg/kg. Different areas of the brain (see Tables) were quickly removed and submitted to the extraction procedure[8] and the ACh content was bio-assayed on the eserinized frog's rectus muscle.

Other animals were treated for a week with physostigmine 100 γ/kg i.p. 24 h after the 7th injection the animals were killed to determine (a) the ACh content; (b) cholinoacetylase activity (ChA), according to the method of BULL et al.[9] at 37°C; (c) total cholinesterase activity (ChE), according to AMMON's method[10], as described by AUGUSTINSSON[11] at 37°C.

Results. Normal values of total ACh, ChE, ChA are reported in Table I. We also calculated (a) the minimum theoretical time required to synthesize (ST) and hydrolise (HT) the total amount of transmitter present in a given

[1] E. K. KILLAM, Pharmacol. Rev. *14*, 175 (1962).
[2] C. LOEB, F. MAGNI, and G. F. ROSSI, Arch. ital. Biol. *98*, 294 (1960).
[3] M. MONNIER and W. ROMANOWSKY, Electroenceph. clin. Neurophysiol. *14*, 486 (1962).
[4] C. SERGIO and V. G. LONGO, in *Neuropsychopharmacology*. Proceedings of the 1st International Congress of Neuropsychopharmacology, Roma (1958) (Elsevier Publ. Co., 1959), p. 411.
[5] P. B. BRADLEY and A. N. NICHOLSON, Electroenceph. clin. Neurophysiol. *14*, 824 (1962).
[6] W. FELDBERG, Arch. int. Physiol. *59*, 544 (1951).
[7] F. CROSSLAND, J. Pharm. Pharmacol. *12*, 1 (1960).
[8] L. BEANI and C. BIANCHI, J. Pharm. Pharmacol. *15*, 281 (1963).
[9] G. BULL, C. D. HEBB, and D. RATKOVIC, Biochem. biophys. Acta *67*, 138 (1963).
[10] R. AMMON, Pflügers Arch. ges. Physiol. *233*, 486 (1934).
[11] K. B. AUGUSTINSSON, Acta physiol. scand., Suppl. *15*, 52 (1948).

area and (b) the ratio ChE/ChA. These values may give some information on the regional ACh metabolism.

In Table II, total ACh amounts after a single injection of the drug are given. Physostigmine significantly increases the ACh levels in every area, the rate and the extent of the effect being dose-dependent. 20 min after 5 mg/kg the ACh content is nearly doubled and an excitatory state appears, followed by convulsions and death. No change in total ACh takes place in seven-day-treated animals killed 24 h after the last injection (values not given). On the contrary, some changes are observed in the ChE and ChA activities. ChE activity (expressed in mg ACh split/g fresh tissue/h $\pm$ S.D.) is significantly reduced in the olfactory lobes (23.89 $\pm$ 3.41; 21 experiments; $0.01 > P > 0.001$), in the cerebellar vermis (110.55 $\pm$ 12.61; 21 experiments; $0.05 > P > 0.02$), in the thalamus (56.90 $\pm$ 6.80; 19 experiments; $P < 0.001$), in the caudate nucleus (148.5 $\pm$ 19.9; 19 experiments; $P < 0.001$). The enzymatic activity is not statistically different from the control values in the cerebral cortex (35.26 $\pm$ 3.28; 21 experiments; $P < 0.05$).

ChA activity (expressed in mg ACh syn./g fresh tissue/h $\pm$ S.D.) is significantly higher only in the parietal cortex (1.335 $\pm$ 0.346; $P < 0.001$) and in the cerebellar vermis (0.148 $\pm$ 0.030; $P < 0.001$). Even in the other areas, however, there is an increase in ChA, although not statistically significant: in the olfactory lobes the enzymatic activity reaches the value 0.880 $\pm$ 0.208, in the thalamus 1.321 $\pm$ 0.414, and in the caudate nucleus 5.700 $\pm$ 1.327 (average of 8 experiments).

Discussion. Unlike scopolamine, which decreases ACh only in the telencephalic areas[12], physostigmine increases ACh more diffusely. The increase, however, is greater in the telencephalon than in the cerebellar vermis and thalamus. This fact may depend on the different ACh metabolism and disposition[13]. The ST and ChE/ChA ratio (see Table I) are higher in the cerebellar vermis and thalamus, suggesting a lower ACh metabolic rate.

[12] L. Beani, C. Bianchi, and P. Megazzini, Exper. *20*, 677 (1964).
[13] C. O. Hebb and D. Ratkovic, in *Comparative Neurochemistry*. Proceedings of the 5th International Neurochemistry Symposium, Austria 10–15 June (1962) (Pergamon Press, Oxford 1964), p. 347.

Table I. Normal values of total ACh, ChE and ChA in different areas of the guinea-pig's brain. In brackets the number of experiments

Area	Total ACh γ/g	ChA activity ACh synthesis mg/g fresh tissue/h	ChE activity ACh hydrolysis mg/g fresh tissue/h	Minimum synthesis time (ST) sec	Minimum hydrolysis time (HT) sec	ChE/ChA ratio
Olfactory lobes	2.090 $\pm$ 0.550 (40)	0.721 $\pm$ 0.187 (22)	27.06 $\pm$ 2.82 (29)	10 4/10	27.8/100	37.5
Parietal cortex	2.670 $\pm$ 0.560 (40)	0.924 $\pm$ 0.166 (21)	35.26 $\pm$ 3.28 (29)	10 4/10	27.2/100	38.1
Cerebellar vermis	0.539 $\pm$ 0.142 (36)	0.106 $\pm$ 0.024 (21)	120.4 $\pm$ 15.70 (28)	18 3/10	1.6/100	1135
Thalamus	5.32 $\pm$ 0.97 (41)	1.231 $\pm$ 0.211 (19)	63.89 $\pm$ 7.24 (35)	15 5/10	29.9/100	51.9
Caudate nucleus	4.820 $\pm$ 0.97 (40)	5.168 $\pm$ 0.854 (21)	172.30 $\pm$ 19.29 (32)	3 3/10	10.0/100	33.3

Table II. Effect of physostigmine (single injection) on the total ACh of the guinea-pig's brain. In brackets the number of experiments

Area	200 γ/kg			1 mg/kg			5 mg/kg
	20 min	1 h	3 h	20 min	1 h	3 h	20–30 min
Olfactory lobes	2.710 $\pm$ 0.520[c] (9)	2.510 $\pm$ 0.450[c] (16)	2.840 $\pm$ 0.83[c] (10)	4.610 $\pm$ 1.210[d] (12)	2.550 $\pm$ 0.440[d] (19)	2.580 $\pm$ 0.440[b] (10)	4.060 $\pm$ 0.900[d] (12)
Parietal cortex	3.600 $\pm$ 0.630[d] (11)	3.020 $\pm$ 0.440[a] (16)	3.530 $\pm$ 0.53[d] (10)	5.010 $\pm$ 1.100[d] (14)	3.040 $\pm$ 0.640[b] (19)	3.310 $\pm$ 0.510[d] (10)	5.170 $\pm$ 0.920[d] (12)
Cerebellar vermis	0.635 $\pm$ 0.226 (11)	0.562 $\pm$ 0.123 (16)	0.699 $\pm$ 0.13[c] (11)	0.972 $\pm$ 0.244[d] (11)	0.616 $\pm$ 0.087[a] (18)	0.699 $\pm$ 0.115[c] (8)	0.905 $\pm$ 0.261[d] (12)
Thalamus	6.330 $\pm$ 0.860[d] (12)	5.660 $\pm$ 0.510 (16)	5.750 $\pm$ 0.66 (11)	6.650 $\pm$ 1.170[d] (14)	6.120 $\pm$ 1.200[c] (19)	5.870 $\pm$ 0.920 (12)	8.130 $\pm$ 1.060[d] (12)
Caudate nucleus	6.960 $\pm$ 1.230[d] (12)	6.130 $\pm$ 1.290[d] (16)	6.230 $\pm$ 1.22[d] (11)	8.960 $\pm$ 1.750[d] (13)	6.970 $\pm$ 1.410[d] (19)	5.330 $\pm$ 0.930 (10)	10.520 $\pm$ 2.890[d] (12)

[a] These values differ significantly from the controls at a level of $0.05 > P > 0.02$. [b] These values differ significantly from the controls at a level of $0.02 > P > 0.01$. [c] These values differ significantly from the controls at a level of $0.01 > P > 0.001$. [d] These values differ significantly from the controls at a level of $P \leq 0.001$.

Following physostigmine subacute treatment, total ACh is the same as in the controls, but ChE activity is decreased and ChA increased almost everywhere.

The ChE reduction is obviously due to the peculiar pharmacological effects of the drug[14]. The ChA increase suggests, instead, that the subacute treatment may stimulate the cholinergic neuronal pool. Probably, the ACh increase in some synaptic areas activates non-cholinergic chains which, in turn, stimulate the cholinergic neurons. Our hypothesis agrees with the well-known desynchronizing effect of the drug[1].

The conclusions drawn from our experiments are therefore as follows: (1) The drug increases total ACh, mostly in those areas where the neurotransmitter metabolic rate is high. (2) Sub-acute treatment increases ChA activity, probably through indirect activation of the cholinergic neuronal pool.

Riassunto. L'eserina 0.2–5 mg/kg aumenta la ACh totale del cervello di cavia soprattutto nel telencefalo. Dopo trattamento sub-acuto, l'attività colinoacetilasica aumenta, forse per stimolazione mediata dei neuroni colinergici.

PAOLA MEGAZZINI, GIUSEPPINA BERNARDI, and PAOLA L. BALLOTTI

Istituto di Farmacologia e Clinica Tossicologica della Università di Firenze (Italy), January 25, 1965.

[14] F. KALSBEEK and J. COHEN, Biochem. biophys. Acta *4*, 559 (1950).

Salivary Secretion of the Major Sublingual Gland of Rats

Isoprenaline, given repeatedly to rats, causes a marked enlargement of the submaxillary and parotid glands[1], while the weight increase of the sublingual gland is slight or absent[2,3]. Isoprenaline evokes a marked flow of saliva from the submaxillary gland of rats[4]. In the present investigation, the secretory responses of the rat sublingual gland to isoprenaline, as well as to some other sialagogue drugs, are studied. A continuous flow of saliva from the sublingual gland was observed at the beginning of all experiments. This continuous secretion was further studied.

Twenty-four male rats (Sprague-Dawley) weighing 255–385 g were used. The rats were anaesthetized with chloralose (100 mg/kg) intravenously after ether induction. The sublingual ducts, lateral to the submaxillary ducts, were exposed in the neck and cannulated using fine glass cannulae. The cannulae gave about 100 drops out of 1 ml of distilled water. Secretion appearing at the tip of the cannula was noted and registered on a smoked drum. The secretory responses to different doses of isoprenaline (isopropylnoradrenaline) sulphate and of the hydrochlorides of adrenaline and methacholine given intravenously were estimated. To increase the sensitivity to secretory drugs, the sublingual gland was parasympathetically denervated in 7 rats by section of the chorda-lingual nerve 3 weeks in advance. For the study of the continuous secretion, atropine sulphate and dihydroergotamine methansulphonate were given intravenously; in 5 rats the sublingual glands were sympathetically denervated by bilateral excision of the superior cervical ganglion about 2 weeks in advance.

A slow continuous flow of sticky saliva from the sublingual gland was observed. This flow of saliva was found to go on for hours at a constant rate. The rate varied, however, from one animal to another; it was calculated to correspond to about 3 μl/h (range 1–5 μl). This flow of saliva was produced by sublingual glands weighing 47 ± 2.1 mg ($n = 10$). The continuous secretion was not altered by preganglionic parasympathetic or post-ganglionic sympathetic denervation in combination with removal of the adrenals; and neither atropine 1 mg/kg nor dihydroergotamine 0.5 mg/kg abolished or changed the secretion.

The secretory responses of the sublingual gland to sialagogue drugs were always small, usually much less than 1 drop of saliva, and difficult to evaluate also because of the continuous secretion.

The rat's sublingual gland was found to be very insensitive to isoprenaline. Even after 100 μg isoprenaline/kg, no significant increase of the rate of the continuous secretion was noticed. Similarly the sensitivity to adrenaline was very low; a secretory response to adrenaline was not regularly observed, even after 20 μg/kg. Methacholine, on the other hand, evoked small but obvious secretory responses. The threshold dose of methacholine was found to be about 0.5 μg/kg. The secretory responses were augmented with increasing doses; 10 μg methacholine/kg was found to give about $^1/_3$ drop of saliva. The sensitivity of the sublingual gland to these secretory drugs was increased after parasympathetic denervation. Isoprenaline 100 μg/kg was found to elicit a small secretory response from the denervated sublingual gland while a higher dose, 1 mg/kg, evoked a long-lasting flow of saliva up to about 1 drop in $^1/_2$–$1\,^1/_2$ h. The denervated gland was still very insensitive to adrenaline, but 20 μg adrenaline/kg usually caused a small secretory response. The threshold dose of methacholine was markedly lowered, to about 0.05 μg/kg, after denervation and the secretory response to 10 μg methacholine/kg was increased by about 50%.

The sublingual gland of rats has thus been found to secrete spontaneously, like the sublingual gland of cats[5] and the submaxillary gland of rabbits[6]. When comparing the rate of this spontaneous secretion of these three salivary glands, considering the differences in gland weight, it may be noted that the flow rate is highest in cats and lowest in rabbits.

The sublingual gland of rats is very insensitive to both adrenaline and isoprenaline. A secretory response to these catecholamines is usually seen after parasympathetic denervation. Activation of the adrenergic receptors, both

[1] H. SELYE, R. VEILLEUX, and M. CANTIN, Science *133*, 44 (1961).
[2] CHARLOTTE A. SCHNEYER, Am. J. Physiol. *203*, 232 (1962).
[3] C. PEREC, unpublished observation (1964).
[4] P. OHLIN, Acta Univ. Lund. II, *17* (1964).
[5] N. EMMELIN, Acta physiol. scand. *30*, Suppl. 111, 34 (1953).
[6] I. NORDENFELT and P. OHLIN, Acta physiol. scand. *41*, 12 (1957).