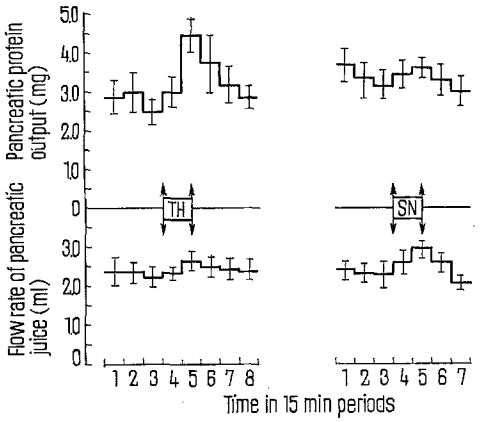

Fig. 1. The effect of an i.v. infusion of theophylline (TH) (6 mg/kg over 20 min) and supplementary dose of secretin (SN) (4 U/20 min) on output of protein (above) and flow rate (below) of pancreatic juice (± S. E.). Throughout each experiment, background pancreatic secretion was maintained by continuous i.v. infusion of secretin (11.6 U/h) and PZ (24 U/kg/h). For statistical evaluation of the effects of theophylline and the supplementary dose of SN, values obtained in the 15 min period preceeding the infusions were compared (*t*-test for paired values) with the values obtained in the second period after the beginning of the infusion.

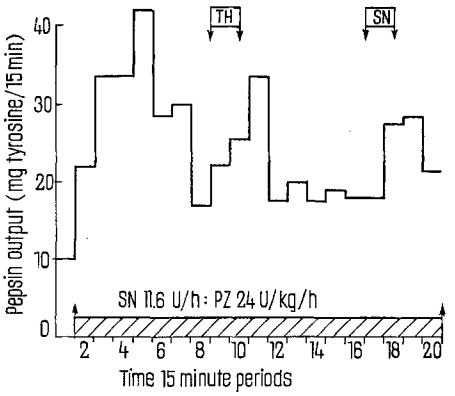

Fig. 2. The effect of an i.v. infusion of theophylline (TH) (6 mg/kg over 20 min) and a supplementary dose of secretin (SN) (4 U/20 min) on the output of pepsin. Throughout the experiment continuous i.v. infusions of secretin (11.6 U/h) and PZ (24 U/kg/h) were maintained.

finding that theophylline did not cause any significant change in the flow rate of pancreatic juice may be that flow rates stimulated by a continuous dose of SN, were already at maximal levels. To determine if the flow rate of pancreatic juice was indeed maximal, the dose of SN was doubled for a period of 20 min, 2–3 h after the injection of theophylline. This supplementary dose of SN did not result in any significant change in either flow rate ($p > 0.05$) or protein output ($p > 0.1$) of pancreatic juice (Figure 1). This suggests that the flow rate, prior to the administration of theophylline was maximal. Under the experimental conditions described in this paper, it would appear that i.v. administration of theophylline enhances the pancreatic protein stimulating effect of PZ, and that this effect is not dependent on increased flow rate.

In 5 out of 6 experiments, pepsin output was increased in response to theophylline, and in all experiments in response to the supplementary dose of SN. The results of one experiment are shown in Figure 2.

On the basis of the known action of theophylline on the cyclic AMP system[2], the above evidence suggests that cyclic AMP may be involved in mediating the pancreatic action of PZ and the pepsin stimulating action of SN.

Résumé. Chez le chat, l'injection intravéneuse de théophylline qui, comme on sait, produit une accumulation de l'AMP cyclique, augmente aussi l'effet de la pancréozymine sur la production des protéines pancréatiques et de la sécrétine sur celle de la pepsine.

R. A. PEDERSON[10], J. A. PEARSON and J. C. BROWN[11]

Department of Physiology, University of British Columbia, Vancouver 8 (British Columbia, Canada), 4 March 1970.

[10] Canadian Medical Research Council Scholar.

[11] This work was supported by Grant No. MA3093 from the Medical Research Council of Canada to Dr. J. C. BROWN.

Disposition of the Portal Vessels of the Avian Pituitary in Relation to the Median Eminence and the Pars Distalis

The existence of distinct anterior and posterior groups of hypophysial portal vessels has recently been demonstrated in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*[1]. The anterior group of portal vessels originates from the primary capillary plexus in the anterior division of the median eminence and the posterior group of portal vessels originates from the primary capillary plexus in the posterior division of the median eminence. The anterior and posterior groups of portal vessels branch into the sinusoids of the cephalic and caudal lobes of the pars distalis respectively. It is postulated that this regional distribution of portal vessels in the white-crowned sparrow provides the anatomic basis for individual neuroendocrine controls by the anterior and posterior divisions of the median eminence over the cephalic and caudal lobes of the pars distalis[2]. In a recent study, we have demonstrated the presence of distinct anterior and posterior groups of portal vessels supplying respectively the cephalic and caudal lobes of the pars distalis in 15 species of birds and suggested that this type of arrangement may be widespread among birds[3].

[1] A. VITUMS, S. MIKAMI, A. OKSCHE and D. S. FARNER, Z. Zellforsch. *64*, 541 (1964).

[2] D. S. FARNER, F. E. WILSON and A. OKSCHE, in *Neuroendocrinology* (Ed. L. MARTINI and W. F. GANONG; Academic Press, New York 1967), vol. 2, p. 529.

[3] C. J. DOMINIC and R. M. SINGH, Gen. comp. Endocr. *13*, 22 (1969).

In the course of a study on the comparative anatomy of the avain pituitary gland[4], we have observed the presence of distinct, non-interconnected, anterior and posterior groups of portal vessels in over 30 species of birds, belonging to 9 orders. The primary capillary plexus forms a dense covering on the ventral surface of the median eminence. In species like *Passer domesticus* Linn. (Figure 1), *Lonchura malacca* Linn., *Psittacula cyanocephala* Linn. and *Upupa epops* Linn., there are distinct, though to some extent interconnected anterior and posterior primary capillary plexus in the median eminence. In others, e.g., *Acridotheres tristis* Linn., *Sturnus contra* Linn., *Turdoides malcolmi* Sykes and *Merops philippinus* Linn., the primary capillary plexus is undivided. Irrespective of whether the primary capillary plexus is single or divided, there are invariably present in all species distinct anterior and posterior groups of portal vessels originating respectively from the anterior and posterior regions of the median eminence. The anterior group of portal vessels follows the anterior margin of the pars tuberalis, enters the pars distalis dorsally at the junction of the cephalic and caudal lobes and then bends towards the cephalic lobe where it breaks up into capillaries. Similarly, the posterior group of portal vessels traverses the posterior margin of the pars tuberalis and breaks up into capillaries in the caudal lobe of the pars distalis (Figure 1).

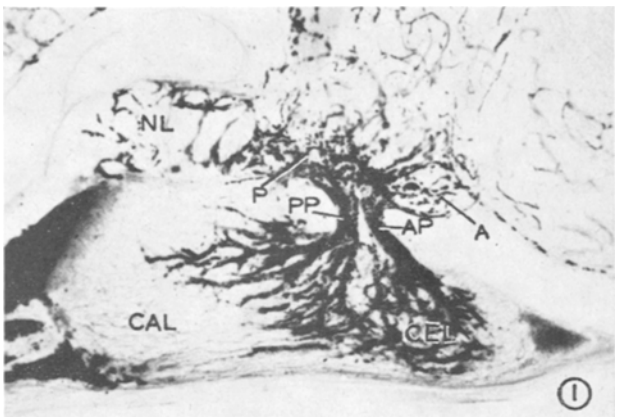

Fig. 1. Sagittal section (circa 100–200 µ thick) through India ink-injected pituitary of *Passer domesticus*, showing anterior (A) and posterior (P) plexus in the median eminence, the anterior (AP) and posterior (PP) groups of portal vessels supplying respectively the cephalic lobe (CEL) and caudal lobe (CAL) of the pars distalis. NL = neural lobe. The anterior and posterior primary capillary plexus are not clearly demarcated in the photograph. × 53.

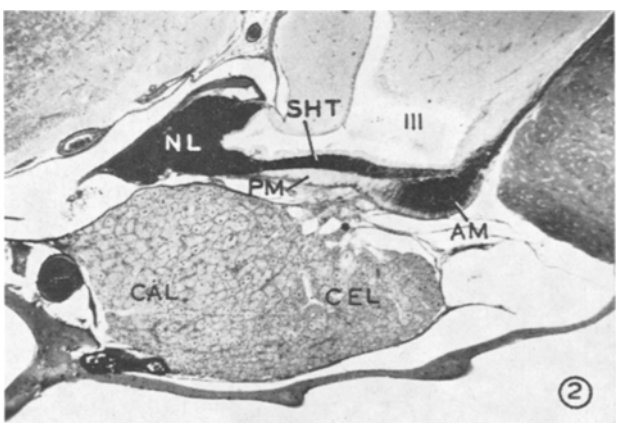

Fig. 2. Mid-sagittal section (15 µ thick) through the pituitary of *Passer domesticus*, stained in Gomori's Aldehyde-Fuchsin, showing the Gomori-positive anterior region (AM) and Gomori-negative posterior region (PM) of the median eminence. SHT = supraoptico-hypophysial tract; III = third ventricle. × 42.

It is likely that the widespread occurrence of this type of regional distribution of hypophysial portal vessels in birds may be correlated with the histological bipartition of the median eminence and of the pars distalis. The avian median eminence contains Gomori-negative fibres of the tubero-infundibular tract and Gomori-positive fibres of the supraoptico-paraventricular tract[5]. The anterior part of the median eminence is particularly rich in Gomori-positive fibres of the supraoptico-paraventricular tract; on the contrary, the posterior part of the median eminence is mostly composed of Gomori-negative fibres of the tubero-infundibular tract[2]. A Gomori-positive anterior division and a Gomori-negative posterior division of the median eminence have been observed by us in over 40 species of birds[4], including all species in which distinct anterior and posterior groups of portal vessels have been demonstrated (Figure 2).

One of the most important features of the avian pituitary is the cytological differentiation of the pars distalis into the cephalic and caudal lobes[6,7]. Such a differentiation has also been confirmed in over 60 species of birds by the present authors[4]. A functional differentiation between the cephalic and caudal lobes of the pars distalis of the fowl has been demonstrated[8]. Electron microscopic studies on the adenohypophysis of the white-crowned sparrow indicate that there is regional distribution of the secretory cells in the pars distalis[9]. From the point of view of the neuroendocrine regulation of adenohypophysial function, the histological differentiation of the median eminence and of the pars distalis assumes great significance in the light of the regional distribution of the portal vessels. Since there are no important interconnections between the two groups of portal vessels, it is likely that blood from the Gomori-positive anterior division of the median eminence passes to the cephalic lobe and blood from the Gomori-negative posterior division of the median eminence passes to the caudal lobe of the pars distalis. Hence it seems probable that the secretory activity of the cephalic lobe cells is regulated primarily by the neurohormones from the supraoptic and paraventricular nuclei travelling via the anterior group of portal vessels and that of the caudal lobe cells is regulated primarily by neurohormones from the tuberal nuclei travelling via the posterior group of portal vessels. Experimental investigations on the regional distribution of portal vessels have not been reported in birds. How-

[4] C. J. Dominic and R. M. Singh, unpublished results (1970).
[5] A. Oksche, Mem. Soc. Endocr. *12*, 199 (1962).
[6] H. Rahn and B. T. Painter, Anat. Rec. *79*, 297 (1941).
[7] K. G. Wingstrand, *Structure and Development of the Avian Pituitary* (Gleerup, Lund, 1951).
[8] S. Mikami, J. Fac. Agr. Iwate Univ. *3*, 473 (1958).
[9] S. Mikami, A. Vitums and D. S. Farner, Z. Zellforsch. *97*, 1 (1969).

ever, in rats, goats and sheep, complete transection of the pituitary stalk induces extensive bilateral necrosis of the pars distalis, whereas partial transection of the stalk causes only unilateral necrosis on the corresponding side of the pars distalis[10]. This possibly suggests that certain groups of portal vessels supply restricted areas in the pars distalis. It is likely that such a situation may also prevail in birds[11].

Zusammenfassung. Die ermittelte Gefässversorgung der Vogelhypophyse beweist die Existenz eines doppelten Portalsystems der Pars distalis, womit auf zwei hypothalamo-hypophysäre Mechanismen hingewiesen wird.

RAM MURAT SINGH and C. J. DOMINIC

Department of Zoology, Banaras Hindu University, Varanasi 5 (India), 22 January 1970.

[10] J. H. ADAMS, P. M. DANIEL and M. M. L. PRICHARD, Endocrinology *75*, 120 (1964).

[11] Thanks are due to Prof. S. P. RAY CHAUDHURI for encouragement. This investigation was in part supported by Grant No. M67-0139 from the Population Council, New York.

Choline Acetyltransferase and Acetylcholinesterase in Myo-Tendinous and Neuro-Muscular Junctions of Mouse Skeletal Muscle

Acetylcholine (ACh), choline acetyltransferase (ChAc) and acetylcholinesterase (AChE), are highly concentrated at the neuro-muscular junctions of skeletal muscle fibres[1,2]. It has been shown further that AChE is highly concentrated also at the junctions between skeletal muscle fibre and tendon[3–8], and that in some muscles the membrane is more sensitive to locally applied ACh in this region than elsewhere (except in the neural zone)[9–11]. It is unknown, however, whether ACh has a function at the myo-tendinous junctions. As preformed ACh probably cannot diffuse to the myo-tendinous region from other places, we wanted to determine the activity of ChAc, the enzyme synthesizing ACh, in this region.

The external oblique abdominal muscle of albino mice was used. In this thin segmental muscle, both neuromuscular and myo-tendinous junctions are located in discrete, narrow bands. The muscular abdominal wall was pinned on carboard coated with aluminium foil and frozen by floating on liquid N_2. Such treatment makes neuro-muscular and myo-tendinous junctions easily visible[12]. Stable and easily handled preparations were obtained on freeze-drying. Approximately 0.3×3 mm samples of the external oblique muscle were dissected under a stereo microscope (Figure 1) by means of razor blades and pointed tweezers. Adhering pieces of underlying muscles were easily recognized and removed from the samples. The dissection was occasionally controlled by staining the remaining tissue for AChE with a modified Koelle-Friedenwald technique[13]. The samples were weighed (10–60 µg) on a Cahn Gram electrobalance. Samples containing neuro-muscular junctions, myo-tendinous junctions and non-junction muscle were assayed for AChE and ChAc by radiochemical methods based on the hydrolysis of [1-^{14}C]acetylcholine chloride (The Radiochemical Centre, Amersham, England) and on the production of ACh from [1-^{14}C]acetyl-CoA (New England Nuclear Corp., Boston, Mass.)[14]. For AChE the samples were incubated for 30 min at 25 °C in 25 µl of incubation mixture, for ChAc the figures were 60 min, 37 °C and 25 µl. Triton X-100 was added at 0.5% (v/v) final concentration to release all enzyme activity. Other details were as earlier described[14].

The AChE activity was 4–9 times higher in the neuromuscular and myo-tendinous preparations than in those from non-junction muscle (Figure 2). For ChAc the ratios of the mean activities of samples from neuro-muscular junctions, non-junction muscle and myo-tendinous junctions were 100:6:4 (Figure 2). The same activity ratios (100:7:5) were obtained in additional experiments with a method using [1-^{14}C]acetate and an enzyme system to generate [1-^{14}C]acetyl-CoA in situ[14], although in these experiments the absolute activities were somewhat higher. With either method the activity of the myo-tendinous

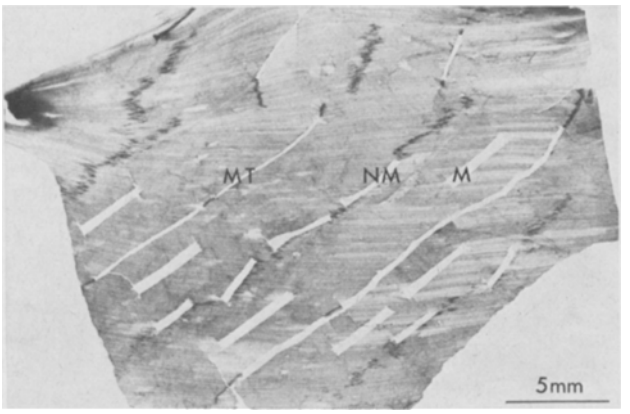

Fig. 1. Photomicrograph of the freeze-dried external oblique abdominal muscle after dissection of samples containing neuro-muscular junctions (NM), non-junction muscle (M) and myo-tendinous junctions (MT). Note that the MT could be dissected thinner than the NM and therefore were less diluted with muscle. Note also that the MT contained the junctions from 2 adjacent segments.

[1] C. O. HEBB, K. KRNJEVIĆ and A. SILVER, J. Physiol. *171*, 504 (1964).
[2] M. ISRAËL and J. GAUTRON, Proc. int. Soc. Cell Biol. Symp. Vol. 7. Cellular Dynamics of the Neurone (Ed. S. H. BARONDES; Academic Press, N. Y.), p. 137.
[3] R. COUTEAUX, C. r. Soc. Biol., Paris *147*, 1974 (1953).
[4] M. A. GEREBTZOFF, Acta physiol. pharmac. neerl. *6*, 419 (1957).
[5] G. H. SCHWARZACHER, Arch. int. Pharmacodyn. *128*, 330 (1960).
[6] R. COUTEAUX, Bibl. anat. *2*, 207 (1961).
[7] L. LUBIŃSKA and J. ZELENÁ, J. Anat. *101*, 295 (1967).
[8] H. TERÄVÄINEN, Experientia *25*, 524 (1969).
[9] R. MILEDI and J. ZELENÁ, Nature *210*, 855 (1966).
[10] M. PÉCOT-DECHAVASSINE, Arch. int. Pharmacodyn. *176*, 118 (1968).
[11] E. X. ALBUQUERQUE and R. J. McISAAC, Life Sciences *8*, 409 (1969).
[12] R. CREESE, J. M. ENGLAND and D. B. TAYLOR, J. Physiol., London *200*, 7 P (1969).
[13] J. STORM-MATHISEN, J. Neurochem., in press.
[14] F. FONNUM, Biochem. J. *115*, 465 (1969).