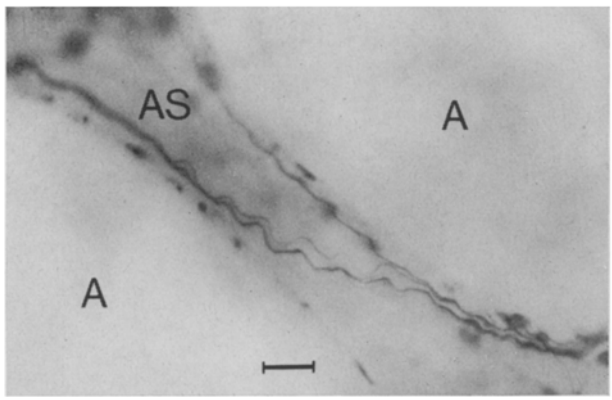

Fig. 1. Photomicrograph of the innervation of the atria (A) of the lungs of *G. domesticus*. Plexus of fine nerve fibres in an interatrial septum (AS) (a wall shared by 2 atria). Scale = 10 μm. Modified Bielschowsky-Gros silver technique.

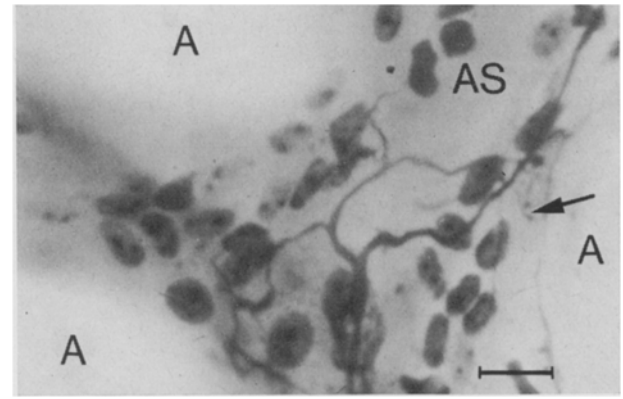

Fig. 2. Photomicrograph of a nerve fibre in the interatrial septa of the lungs of *G. domesticus* which is distributed in a way suggestive of a free sensory nerve ending; some of the finest branches of the ending appear to terminate in knob-like swellings (arrow). Scale = 10 μm. Modified Bielschowsky-Gros silver technique.

The airway walls of the tertiary bronchus and the atria opening into it were innervated by the same nerve plexus consisting mainly of fine fibres less than 1.5 μm in width (Figure 1). Occasionally, however, thicker nerve fibres which were considered to be afferent, left the plexus and were distributed separately. The structure and distribution of the terminal branches of these fibres strongly suggested that they were free sensory nerve endings. The most convincing evidence for these supposedly afferent endings was obtained with the silver stain (Figure 2). Each thick fibre divided rapidly several times into successively finer fibres which usually extended in opposite directions to each other and sometimes appeared to end in knob-like swellings. Although these endings innervated relatively large areas of the airway walls, their branches were distributed in several planes and it was only possible in a single photograph to demonstrate a small part of an ending. Encapsulated endings and neurite-receptor cell complexes were not seen.

This seems to be the first observation with the light microscope of possible afferent nerve endings in the walls of the tertiary bronchi and atria of the avian lung. Possibly they are the source of some of the unit activity in the cervical vagus in phase with resting breathing[4]. They may also be the intrapulmonary receptors that are sensitive to the concentration of CO_2 in the airways[5]. Further work, is in progress to establish precisely the structure and function of these afferent endings[15].

Résumé. Des examens au microscope optique on montré l'existence de terminaisons nerveuses, dont on supposait la présence, dans les structures des bronchioles tertiaires et des atrium des poumons de la poule domestique (*G. domesticus*). Les caractères morphologiques en sont décrits. On pense qul'il s'agit de la première preuve de l'existence de terminaisons afférentes dans les «conduits» d'air, réalisée à l'aide du microscope optique.

J. McLelland

Department of Veterinary Anatomy,
University of Liverpool,
P.O. Box 147, Liverpool L69 3BX (England),
26 July 1971.

[15] This work was supported by a grant from the British Egg Marketing Board.

The Effect of Plasma and Transferrin on the Hemin Inhibition of Iron Uptake by Reticulocytes

Hemin added to reticulocytes incubated in vitro inhibits heme synthesis[1]. Moreover, our experiments demonstrated that $10^{-4}M$ hemin concentration decreases reticulocyte uptake of iron[2]. In our incubation mixture[2], plasma was used as a donor of transferrin. Some studies[3,4] proved that various plasma proteins bind heme and may, in this way, release the effect of hemin on certain biochemical reactions[5]. The present study compares the effect of hemin on the reticulocyte uptake of iron which is bound either to the purified transferrin or to transferrin in plasma.

Methods. Reticulocyte-rich erythrocytes (referred to as reticulocytes) were obtained from three-times bled rabbits, washed and incubated for 60 min in a medium[2] containing ^{59}Fe bound either to purified transferrin or to transferrin of rabbit plasma. 0.3 ml of reticulocytes were incubated in the final incubation mixture containing 0.5 ml of rabbit plasma or 1.25 mg of rabbit transferrin. Before use, both

[1] D. Karibian and I. M. London, Biochem. biophys. Res. Commun. *18*, 243 (1965).
[2] P. Poňka and J. Neuwirt, Blood *33*, 690 (1969).
[3] N. H. Fairley, Q. J. Med. *10*, 115 (1941).
[4] D. A. Sears, J. Lab. clin. Med. *71*, 484 (1968).
[5] P. L. Scholnick, L. E. Hammaker and H. S. Marver, Proc. natn. Acad. Sci. USA *63*, 65 (1969).

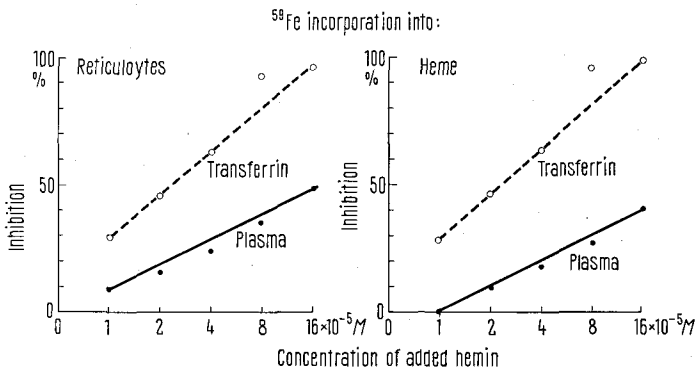

Fig. 1. The effect of various hemin concentrations on the incorporation of ^{59}Fe bound to plasma or purified transferrin into reticulocytes and heme.

transferrin and plasma were dialyzed to remove iron[6]. After dialysis standard amount of iron was added to plasma or transferrin. Final concentration of iron in both, plasma and transferrin solution (25.0 mg of transferrin dissolved in 10 ml of medium) was 100 µg/100 ml. All other conditions of incubation, preparation of cells and determination of specific activity of heme were the same as those described recently[2].

In some experiments the uptake of labeled hemin by reticulocytes was determined. Highly labeled ^{59}Fe-hemin was prepared using previously described methods[2]. The conditions of incubation were similar to those used in the iron uptake experiments but ^{59}Fe was excluded from the incubation mixture and replaced by an appropriate hemin concentration.

Hemin was obtained from Calbiochem and dissolved as usual[2]. ^{59}Fe (ferric citrate, specific activity about 10 mC/mg) was delivered from the Zentralinstitut für Kernforschung, Dresden. Rabbit transferrin was kindly given by Koch and Light Laboratories[7]. Incubation medium was purchased from USOL, Prague.

Results and discussion. Figure 1 demonstrates that the same molar concentration of hemin inhibits ^{59}Fe uptake by reticulocytes to a much higher extent if radioiron is bound to purified transferrin. $2 \times 10^{-5} M$ hemin concentration has a minute effect on radioiron incorporation into heme if plasma is present in the incubation mixture. This concentration of hemin, however, inhibits ^{59}Fe incorporation into heme to about 50% if the medium contains only purified transferrin. Approximately 10 times higher hemin concentration inhibits ^{59}Fe incorporation into heme from plasma to 50%.

The amount of heme taken up by reticulocytes is markedly decreased if plasma is present in the incubation mixture (Figure 2). This experiment (Figure 2) suggests that plasma binds part of heme present in the incubation mixtures. As a consequence, the concentration of free heme in the medium decreases as can be judged from smaller uptake of heme by cells. In a separate experiment it was proved that corresponding amount of labeled heme cannot be removed from the medium containing plasma after its chromatography on Sephadex G-25.

The present results demonstrate that the presence of plasma in the incubation mixture decreases the inhibitory effect of hemin on iron uptake and heme synthesis in reticulocytes. In reticulocytes incubated with purified transferrin without plasma, the hemin concentration 10^{-5} M is sufficient to inhibit heme synthesis.

On the basis of the present experiments, it is necessary to reevaluate the results of our first communication[2] in which the effective hemin concentration about $10^{-4} M$ was reported. The addition of exogenous hemin to erythroid cells is used in many studies of the regulation of hemoglobin synthesis and the observed diminution of the heme effect by plasma may explain some discrepancies in the reported results[7].

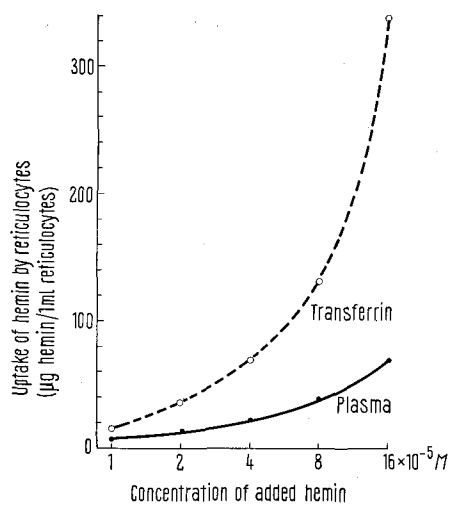

Fig. 2. The uptake of ^{59}Fe-labeled heme by reticulocytes from the incubytion mixture containing plasma or purified transferrin. The quantity of heme taken up by given volume of cells was calculated from the specific activity of ^{59}Fe-heme, amount and radioactivity of cells in the incubation mixture.

Zusammenfassung. Es wird gezeigt, dass die Anwesenheit von Plasma die Hemmwirkung von Hämin auf die Eiseninkorporation in Reticulocyten vermindert und dass dieser Effekt durch Bindung von Hämin an Plasmaproteine zustande kommt.

P. POŇKA and J. NEUWIRT

Department of Pathological Physiology, Faculty of General Medicine, Charles University, Praha 2 (Czechoslovakia), 2 August 1971.

[6] P. POŇKA and J. NEUWIRT, Br. J. Haemat. *19*, 593 (1970).

[7] The authors wish to thank Koch and Light Laboratories for their kind delivery of rabbit transferrin.