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A NOTE ON THE DISTRIBUTION OF [^{14}C]-HISTAMINE ADDED TO BLOOD

BY

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^{14}C-Labelled histamine was incubated with canine or human blood at 37° C. After 1 hr 80 to 100% of the added histamine could be recovered in unchanged form. When [^{14}C]-histamine was added to whole blood *in vitro* it tended to become equally distributed between the cell and plasma fraction of the blood, and when the cell fraction of blood containing [^{14}C]-histamine was suspended in [^{14}C]-histamine-free plasma the labelled histamine tended to become equally distributed between cells and plasma. The pattern of distribution of intravenously injected [^{14}C]-histamine in the blood of anaesthetized dogs seemed to be the same as that of histamine added *in vitro*. The injected histamine entered the cell fraction of the blood at a slow rate. These experiments indicate that in dog and man the blood cells are of little or no importance for the inactivation of histamine released into the circulating blood.

Anrep & Barsoum (1935) and Anrep, Barsoum, Talaat & Wieninger (1939) studied the distribution of histamine added to blood. They found that both red and white blood cells easily took up considerable quantities of histamine added *in vitro*, but only when the histamine concentration of the plasma was higher than that of the cells. They also reported that the histamine taken up by the cells was not, or only to a small extent, released again when the cells were resuspended in histamine-free plasma. Since the quantities of histamine used were rather large, this might mean that the blood cells could take part in the inactivation of histamine reaching the blood in the living organism. Similar experiments have now been done with ^{14}C-labelled histamine, assayed by the isotope dilution technique developed by Schayer (Schayer & Cooper, 1956). This made it possible to assay for the added histamine in the presence of histamine originally present in the blood. It was possible to work with smaller more " physiological " quantities of histamine (0.03 to 0.05 μg histamine base per ml. blood). Three types of experiments were carried out: (1) Studies of the inactivation of [^{14}C]-histamine added to canine or human blood *in vitro*. (2) Studies of the distribution of [^{14}C]-histamine added to canine or human blood *in vitro*. (3) Studies of the distribution in the blood of [^{14}C]-histamine injected intravenously into dogs.

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METHODS

Experiments on blood incubated in vitro. Blood from 5 dogs and one man was used. Canine blood was taken with a polythene catheter inserted into a femoral artery under local anaesthesia. Human blood from a cubital vein was obtained with an internally polished cannula of stainless steel. The blood was collected in polythene tubes containing 0.15 ml. of a heparin solution (5 mg/ml.) per ml. blood. After adding histamine labelled with ^{14}C in the 2 position of the imidazole ring (specific activity 4.75 mC per mM; Radiochemical Centre, Amersham, England), the sample was well mixed and then placed in a shaking incubator at 37° C for varying periods of time. To separate cells from plasma the blood was centrifuged for 10 min at a rate of 10,000 rev/min in a refrigerated centrifuge (the diameter of the centrifuge head was about 20 cm). Samples of the blood were taken with silicone-treated pipettes, and the isotope dilution assays were carried out as described by Lindell & Schayer (1958).

Distribution of histamine in vivo. Two dogs (one male and one female) weighing 9 to 12 kg were fasted overnight and anaesthetized with sodium pentobarbitone (30 mg/kg) intravenously.

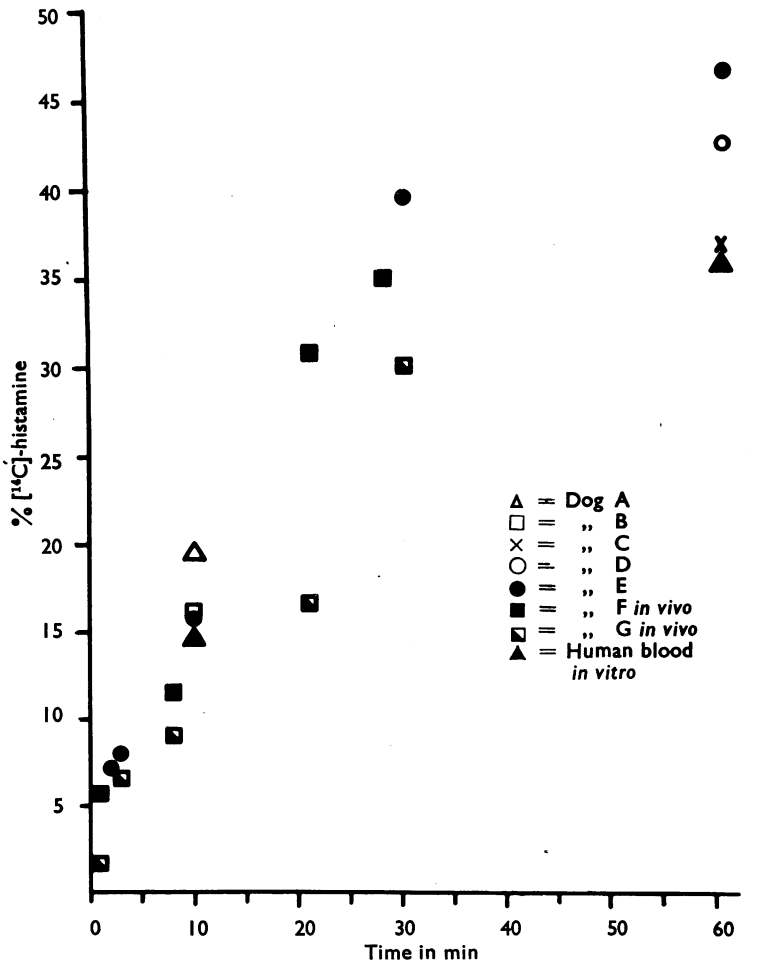

Fig. 1. The distribution between cell and plasma fraction of [^{14}C]-histamine added to human and canine blood. Abscissa: Duration of incubation of [^{14}C]-histamine with whole blood (shaking incubator at 37° C). Ordinate: The amount of [^{14}C]-histamine found in cell fraction (expressed as % of [^{14}C]-histamine content of whole blood).

[^{14}C]-Histamine dissolved in saline was infused with a motor-driven syringe into a catheter tied into a branch of the left femoral vein, at a rate of 0.6 to 1.2 μg/kg/min histamine base. Arterial blood was collected from a polythene catheter in the right femoral artery and transferred to the centrifuge as quickly as possible.

RESULTS

When [^{14}C]-histamine had been incubated with canine or human blood for 1 hr at 37° C, 80 to 100% of the added histamine could be recovered in unchanged form. Since the quantity of labelled histamine was 0.03 to 0.05 μg/ml. blood, this would mean that 0.01 μg histamine or less was metabolized in 1 hr by 1 ml. blood.

Fig. 1 shows the distribution of [^{14}C]-histamine added to whole blood. It may be seen that the amount of [^{14}C]-histamine in the cell fraction of the blood increased with increasing time of incubation. After 5 min of incubation about 5% of the labelled histamine was in the cell fraction, and after 1 hr 35 to 47% of the added histamine was found in the cell fraction of the blood. The distribution of added histamine seemed to be the same in human blood as in canine blood. The distribution of injected [^{14}C]-histamine in the blood of two dogs is also shown in the figure.

In the next series (Fig. 2) [^{14}C]-histamine was incubated with whole blood for 1 hr. The blood was then centrifuged and the cell fraction suspended in [^{14}C]-

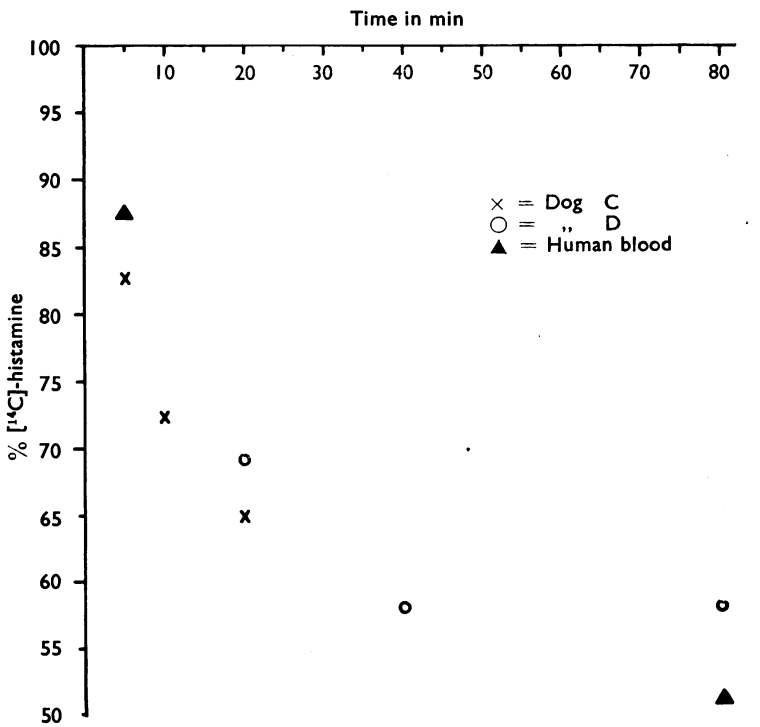

Fig. 2. Release of [^{14}C]-histamine from cell fraction of blood suspended in [^{14}C]-histamine-free plasma. Abscissa: Duration of incubation of [^{14}C]-histamine containing cell fraction in [^{14}C]-histamine-free plasma (shaking incubator at 37° C). Ordinate: The amount of [^{14}C]-histamine found in cell fraction (expressed as % of [^{14}C]-histamine content of whole blood).

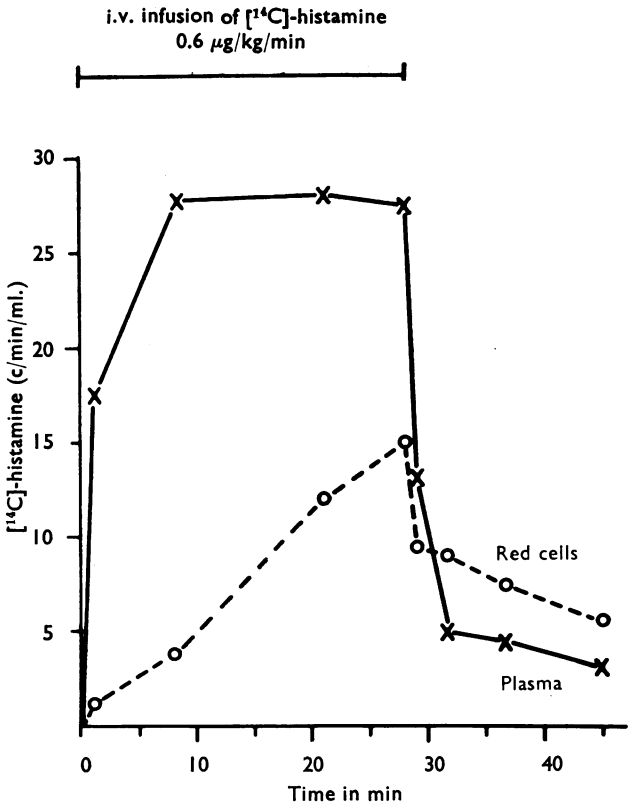

Fig. 3. Distribution of intravenously injected [^{14}C]-histamine between plasma and cell fraction of arterial blood from a male dog weighing 9 kg. Abscissa: Duration of experiment. Ordinate: [^{14}C]-Histamine content of cell and plasma fraction.

histamine-free plasma. After thorough mixing this blood was placed in the shaking incubator and samples removed for isotope dilution assay at various intervals. After 5 min of resuspension in histamine-free plasma the cell fraction contained 80 to 90% of the labelled histamine, and after 80 min 50 to 60% of the radioactive histamine was in the cell fraction.

Figs. 3 and 4 show the results of two experiments *in vivo*. During the intravenous infusion of [^{14}C]-histamine the concentration of labelled histamine in the blood plasma rose rapidly, but that of the cell fraction rose much more slowly. When the infusion was stopped the concentration of [^{14}C]-histamine in the plasma fell very rapidly; that in the cell fraction of the blood also dropped, but more slowly.

DISCUSSION

It was found that of [^{14}C]-histamine added to canine or human blood *in vitro* very little, if any, was metabolized. There was no evidence of any significant enzymic inactivation of histamine in heparinized whole blood. It should be mentioned that Blaschko, Friedman, Hawes & Nilsson (1959) found that dialysed

dog serum sometimes oxidized histamine. Later the same year Blaschko expressed doubts about the physiological significance of the serum enzymes for the inactivation of histamine.

[^{14}C]-Histamine added to whole blood tended to become equally distributed between the cell and the plasma fractions. This result is similar to that of Anrep & Barsoum (1935) and Anrep *et al.* (1939), who worked with non-labelled histamine in much higher concentrations.

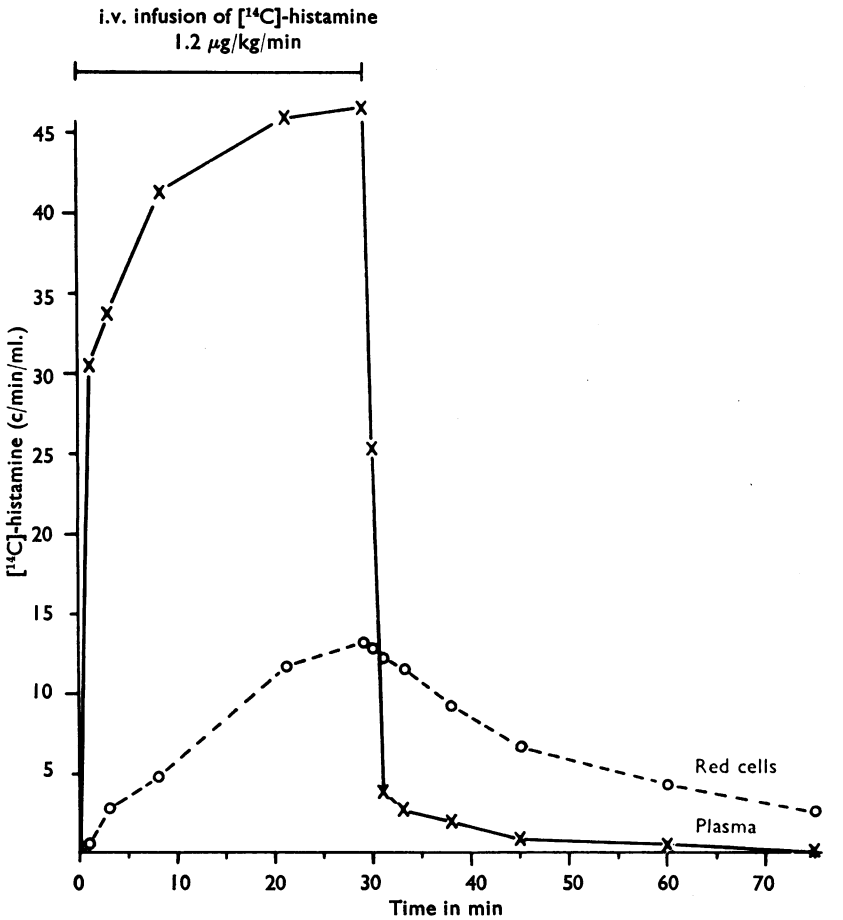

Fig. 4. Female dog weighing 12 kg. Conditions otherwise as described in Fig. 3.

When the cell fraction from blood containing [^{14}C]-histamine was resuspended in [^{14}C]-histamine-free plasma, radioactive histamine moved into the plasma and tended to become equally distributed between the cell and plasma fraction of the blood. This is not in agreement with the results of Anrep & Barsoum and their colleagues. It might be argued that in the present experiments the cell fraction released histamine into the plasma as a consequence of cell damage. However, the results of the experiments *in vivo* do not support such an assumption.

It would be reasonable to assume that some exchange of added histamine took place between plasma and cell fraction during the centrifugation procedure. However, only 5% of the histamine added to whole blood was found in the cell fraction after incubation for 5 min and centrifugation for 10 min. This indicates that any exchange of labelled histamine between cells and plasma during the centrifugation was small.

The infusion experiments indicate that the uptake of histamine by blood cells is too slow to be of significance in the inactivation of histamine that has been released into the blood. The cell fraction released [^{14}C]-histamine which it had taken up only when the [^{14}C]-histamine concentration in the plasma fell below that of the cell fraction. These observations are in agreement with the finding by Schayer & Cooper (1956) and by Nilsson, Lindell, Schayer & Westling (1959) that after the injection of ^{14}C-labelled histamine in man the radioactivity is rapidly excreted in the urine.

REFERENCES

ANREP, G. V. & BARSOUM, G. S. (1935). Distribution of histamine between plasma and red blood corpuscles. *J. Physiol. (Lond.)*, **85**, 36*P*.

ANREP, G. V., BARSOUM, G. S., TALAAT, M. & WIENINGER, E. (1939). Effect of clotting and of addition of histamine on its distribution in blood. *J. Physiol. (Lond.)*, **96**, 130–138.

BLASCHKO, H. (1959). Oxidation of 1,4-methylhistamine by mammalian plasma. *J. Physiol. (Lond.)*, **148**, 570–573.

BLASCHKO, H., FRIEDMAN, P. J., HAWES, R. & NILSSON, K. (1959). The amine oxidases of mammalian plasma. *J. Physiol. (Lond.)*, **145**, 384–404.

LINDELL, S.-E. & SCHAYER, R. W. (1958). The renal removal of injected [^{14}C]-histamine from the blood in dogs. *Brit. J. Pharmacol.*, **13**, 44–51.

NILSSON, K., LINDELL, S.-E., SCHAYER, R. W. & WESTLING, H. (1959). Metabolism of ^{14}C-labelled histamine in pregnant and non-pregnant women. *Clin. Sci.*, **18**, 313–319.

SCHAYER, R. W. & COOPER, J. A. D. (1956). Metabolism of [^{14}C]-histamine in man. *J. appl. Physiol.*, **9**, 481–486.