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The Binding of Chromate to Red Corpuscles, Haemoglobin and Globin

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This investigation has shown that the presence of calcium in the incubation fluid promotes ^{51}Cr loss by chromate-labelled erythrocytes and blocks chromate uptake by red corpuscles. A site is suggested for the binding of chromate to the globin molecule.

The practice of labelling red corpuscles with radio chromate has become considerably wide-spread in recent years [1]. In contrast to other *in vitro* labelling methods employing radioactive substances — such as have been applied in the determination of the circulating red corpuscle mass [2–5] — the binding of ^{51}chromate to the red corpuscles is so stable that not only the corpuscle volume can be determined, making use of ^{51}chromate-labelled red corpuscles, but even information concerning the lifespan of the erythrocytes can be obtained [6,7].

The chromate loss from the circulating red corpuscles is, however, only a relative measure of the lifespan of the erythrocytes owing to a leakage of about 1 % per day from viable erythrocytes, apart from the 0.85 % loss per day due to red corpuscle physiological destruction [8].

In the healthy human organism, half of the chromate taken up by the red corpuscles is given off in the course of about one month, whereas in many pathological cases the chromate loss from the erythrocytes has been found to be far more rapid [7].

In vivo-labelling of red corpuscles by radiochromate shows an uptake of about 10 % only of the amount injected [9–11].

The uptake of radiochromate by red corpuscles *in vitro* increases with increasing temperature and increasing acidity [8,12,13]. It is also increased when an isotonic acid citrate solution (called ACD solution) is used instead of heparin as an anticoagulant [14,15].

It is reported that when ^{51}chromate-labelled red corpuscles are incubated in plasma, the ^{51}Cr loss is larger than when incubated in saline [16].

One of the aims of this investigation is to explain the above-mentioned difference in the rate of ^{51}Cr loss by the erythrocytes. The other is to determine the site of the binding of the chromate in the globin molecule, which is known to be responsible to a very large extent for the fixation of the chromate.

Gray and Sterling found already that, under optimal conditions *in vitro*, 80—90 % of the added radiochromate is taken up by the erythrocytes [6]. About 97 % of the radiochromate taken up by the erythrocytes is bound to the haemoglobin and only 2 % to the stroma.

EXPERIMENTAL

Materials. Blood was drawn by heart puncture from rats and rabbits of our laboratory strain. Horse blood was supplied by the Royal Veterinary Institute and human blood was supplied by the Karolinska Hospital. The radiochromate used had a specific activity of 1 mC per 23.76 μg chromium.

Determinations. Protein concentration of the globin solutions was determined according to the method of Warburg as modified by Kalckar [17]. The haemoglobin was determined as alkaline oxyhaemoglobin as described by Evelyn [18]. While the molecular weight [19] of haemoglobin is 66 700, that of globin is 66 000. Radioactivity was determined in a scintillation crystal counter, the samples having a volume of 10 ml.

Preparations. Haemoglobin was prepared by haemolysing the red corpuscles after repeated washings. Haemolysis was carried out by adding the same volume of distilled water and 0.2 volume of toluene. The haemolysate was centrifuged and the clear supernatant withdrawn. Rat and horse haemoglobin was crystallized from the clear haemolysate as described by Drabkin [20]. The haemolysates prepared from human and rabbit blood were filtered through Celite No. 545, as described by Boyd [21].

Native globin was prepared according to the method described by Rossi-Fanelli *et al.*[22], at a temperature of −15°C.

Activation. 5—12 ml whole blood were incubated for 1 h at room temperature with sodium chromate having an activity of 0.125—0.250 μC. After incubation, the percentage of uptake of sodium chromate by the erythrocytes was determined.

5—10 ml of haemoglobin and globin solutions were incubated in the same way as blood (above). The pH of the acid globin solutions was adjusted to 7.4 by 1 N NaOH.

After 1 h of incubation at room temperature the percentage of protein-bound chromium was determined, as described by Clegg [23], the sample being centrifuged for 1.5 h at 6 000—7 000 r.p.m.

RESULTS

Table 1 demonstrates that no difference can be detected in the uptake of chromate by the red corpuscles and by globin isolated from human, rabbit, horse or rat blood. It can be seen from Fig. 1 how the presence of 10 mg of

Table 1. Comparison of chromate binding of red corpuscles and the corresponding amount of globin.

Animal	% Chromate bound to red corpuscles	% Chromate bound to globin
Human	58.5	62.5
Rabbit	59.0	63.2
Horse	61.1	64.2
Rat	60.7	63.6

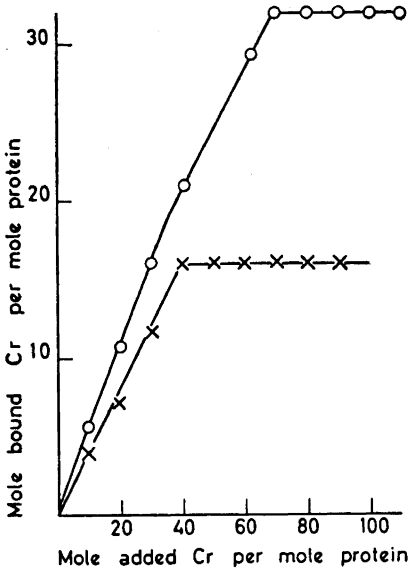

Fig. 1. The binding of ^{51}Cr by red corpuscles incubated in the presence of various amounts of chromate and in the presence and absence of calcium. Whole blood, haemoglobin and globin in the absence of calcium —O—O—; whole blood, haemoglobin and globin in the presence of calcium —×—×—.

calcium per 100 ml solution influences the uptake of chromate by human red corpuscles, haemoglobin and globin. The amount of chromate bound per molecule protein increases with increasing amounts of added chromate. In these experiments also, the presence of calcium ions reduces the amount of ^{51}chromium bound to about half of the amount fixed in the absence of cal-

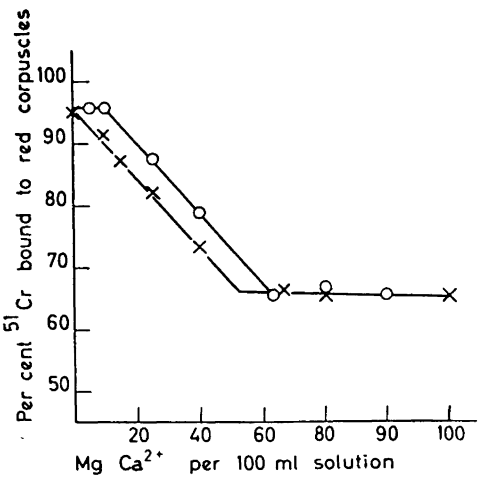

Fig. 2. The influence of varying amounts Ca^{2+} on the uptake of chromate by the red corpuscles. Saline —×—×—; ACD-plasma —O—O—.

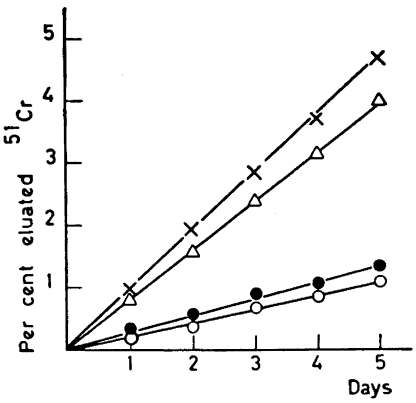

Fig. 3. Elution of ^{51}Cr from chromate labelled red corpuscles in the presence and absence of Ca^{2+} at 5°C. Saline —O—O—; Ca^{2+}-saline —×—×—; ACD-plasma —●—●—; Ca^{2+}-ACD-plasma —△—△

cium. In the presence of calcium ions, one molecule of protein can bind up to 16 molecules of chromate. In the absence of calcium, one molecule of protein can bind as much as 32 molecules of chromate.

The influence of varying amounts of calcium ions on the uptake of chromate by human red corpuscles in saline and ACD plasma is shown in Fig. 2. In calcium-free medium, the red corpuscles take up almost 100 % of the added chromate. Increasing amounts of calcium ions reduce the uptake of chromate until a plateau is reached at 60 %. In saline, this point is reached at a calcium concentration of 52 mg per 100 ml blood, and in ACD plasma at a calcium concentration of 64 mg per 100 ml. The fact that a part of the calcium is bound to the citrate of ACD-plasma explains the above difference. The amount of ionised calcium bound to ACD plasma was determined by conductometrical titration to be 12 mg per 100 ml.

Fig. 3 shows the elution of chromium from ^{51}Cr-labelled human red corpuscles incubated in different media at 5°C. Corrections were made for the small haemolysed fraction. The experiments, which took 5 days, were carried out at a low temperature as, when 37°C was sustained for 5 days, a marked haemolysis could not be avoided. The uptake of chromate by erythrocytes is known to depend very little on temperature. The elution rate in saline and ACD plasma was found to be 0.2 % per day. In saline containing 10 mg of calcium per 100 ml blood, the elution rate was 0.8 % per day, thus very much larger, and in ACD plasma containing 10 mg free calcium per 100 ml, the percentage was 0.75 per day.

DISCUSSION

We found that the presence of calcium in blood reduces the uptake of chromate by red corpuscles [15]. The uptake of chromate by haemoglobin and globin is also reduced to the same extent in the presence of calcium. This result suggests that globin and calcium compete for the chromate present and correspondingly the erythrocytes take up more chromate in the absence of calcium. This conclusion is supported by the observation that the elution

of chromate from the red corpuscles is increased when calcium is added to the blood. Cohn *et al.*[24] and Cannan [25] have determined the amino-acid groups present in haemoglobin and found among other amino-acid groups present a total of 33 histidine groups, all of which were dissociated at a physiological pH.

This result, and our finding that the number of chromate molecules bound by the globin molecule amounts to 32, suggests that the chromate is fixed to the histidine groups.

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