fibrillar center does not present any connection with a chromosomal structure. The rupture of the normally existing relations with the nucleolar chromosome is particularly evident when the fibrillar centers are situated at the periphery of the rearranged nucleolar mass (figure 4). Indeed, in the normal state, penetration of a microchromosome into the fibrillar center is always observed in this case[3].

Hypertrophy of the fibrillar center after treatment with actinomycin D recalls that described by Bassleer et al.[9] using daunomycin. In the case where the fibrillar center resulting from nucleolar rearrangement is both single and voluminous, a process of fusion of 2 centers cannot be excluded. Such fusion is obviously not involved when 2 or 3 large fibrillar centers can be observed.

The rearrangement of nucleolar structure results from displacement of its constituents with respect to one another. Such displacement could possibly explain the disappearance of the connections between the fibrillar center and the chromosomal fibres. Rupture of these relations is undoubtedly facilitated by alterations of the deoxyribonucleoprotein fibres under the action of the drug. It is remarkable to note that reorganization of the nucleolus leaves intact the relations between the fibrillar center and the dense fibrils which normally surround it. This observation suggests that the fibrillar center and its dense fibrillar crown may constitute a functional unit.

9 R. Bassleer, G. Goessens, A. Lepoint, C. Desaive and C. Kinet-Denoel, Beitr. Path. *150*, 261 (1973).

Haemolysis of dog erythrocytes by sorbose in vitro

A. Kistler and P. Keller[1]

Biological Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd, CH-4002 Basle (Switzerland), 20 April 1977

Summary. In vitro, L-sorbose induces in dog erythrocytes haemolysis which is dependent upon time of incubation, temperature, pH and the concentration of sorbose. The in vitro susceptibility to sorbose-induced haemolysis is different in various species.

In the companion paper[2], we reported on the susceptibility to haemolysis which was observed after sorbose administration in dogs but not in rats. In this study we have investigated in vitro whether L-sorbose acts directly on erythrocytes, and if there are species differences in the susceptibility of erythrocytes to sorbose.

Materials and methods. Swiss Beagle dogs, rabbits and cats and outbred stocks of Fü-albino SPF rats and mice were obtained from the Institute of Biological and Medical Research (Füllinsdorf BL). Further, blood from a standardbred horse and from a Swiss Simmental cow was obtained from the School of Veterinary Medicine, University of Berne, and human blood was made available from one of the investigators.

Blood samples were collected in heparinized tubes and the red blood cells washed 2–3 times with physiological NaCl solution. The red blood cells were incubated in Hanks balanced salt solution (without phenol red, supplemented with 60 µg/ml penicillin and 100 µg/ml streptomycin) in cell culture dishes (Falcon, 5.5 cm, final volume 5 ml) in a H_2O-saturated 5% CO_2-air atmosphere. In some experiments the bicarbonate-buffer in the Hanks solution was replaced by 25 mM Hepes-buffer, pH 7.4. L(–)sorbose or D(+)glucose was added as indicated in the text. After incubation, the medium together with the cells were centrifuged and the haemoglobin (Hb) concentration was determined in the supernatant as described[2]. The Hb release into the medium was expressed as percent of the Hb content of the erythrocytes added to the medium.

Results. The incubation of dog erythrocytes (containing 10–14 mg Hb) at 34 °C in presence of L(–)sorbose (5.6–

1 The authors are greatly indebted to Prof. A. Studer, Prof. K. F. Gey and Dr H. Hummler for critical reading of the manuscript. The technical assistance of Mrs E. Stöckli and Mrs C. Villien and of Mr P. Back and Mr V. Loechleiter is gratefully acknowledged.

2 P. Keller and A. Kistler, Experientia *33*, 1380 (1977).

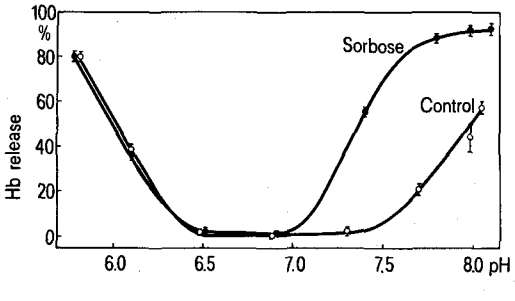

Fig. 1. Effect of pH on the haemolytic effect of sorbose in dog erythrocytes. Dog erythrocytes (containing 10.7 mg Hb) were incubated with or without sorbose (17 mM) in Hanks solution of different pH (range 5.0–9.0). Incubation was performed at 34 °C for 23 h and the pH determined in the medium. Results are expressed as percent of Hb release into the medium. Means of 4 determinations ± SD.

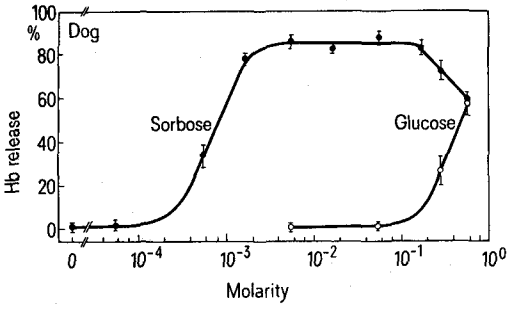

Fig. 2. Dependence of the haemolytic effect on sorbose concentration in dog erythrocytes (containing 13.6 mg Hb). 48-h-incubation in Hanks solution at 34 °C in the absence and presence of sorbose or glucose. The glucose concentrations were not corrected for the glucose content of 5 mM in the Hanks solution. Results are expressed as percent Hb release into the medium ± SD.

Species-specific sensitivity of erythrocytes to haemolysis induced by sorbose in vitro

Species	Control	Sorbose (m*M*)				Glucose (m*M*)		
		1.7	17	56	170	17	56	170
Mouse	2.5 ± 0.3	–	9.0 ± 0.5	44.2 ± 5.8	81.9 ± 3.3	2.7 ± 0.4	2.5 ± 0.1	< 2
Rat	< 2	–	26.8 ± 2.2	47.8 ± 1.3	54.5 ± 2.6	< 2	< 2	< 2
Rabbit	54.9 ± 3.0	58.2 ± 2.0	57.7 ± 0.8	54.4 ± 0.3	40.5 ± 1.1	51.1 ± 0.7	40.9 ± 1.3	23.4 ± 0.2
Cat	< 2	6.5 ± 0.1	19.2 ± 0.4	26.1 ± 0.2	14.7 ± 0.6	< 2	< 2	< 2
Cow	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Horse	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Man	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2

Freshly prepared erythrocytes from the various sources (containing 9.5–13 mg Hb) were incubated in Hanks solution (pH 7.4–7.6) at 34 °C in absence or presence of different concentrations of sorbose or glucose for 48 h. Results are expressed as percent of Hb release into the medium. The numbers indicate the mean ± SD of 3 determinations.

560 mM) resulted in an almost linear increase in haemolysis during the first 12 h, reaching total haemolysis after 24 h. In the absence of sorbose less than 2% Hb release was noted after 48 h of incubation. The effect of temperature on dog erythrocytes in the presence and absence of sorbose (17 mM) was examined. At 22, 34, 37 and 40 °C the Hb release (mean ± SD of 3 determinations) in presence of sorbose was 10.8 ± 0.2, 47.7 ± 0.8, 62.4 ± 3.0 and 75.4 ± 1.9%, respectively, after 27 h of incubation. Less than 3% Hb release was found in the absence of sorbose. The pH of the incubation medium markedly affected the sorbose-induced haemolysis in dog erythrocytes above pH 7.0 (figure 1). Figure 2 shows that sorbose concentrations as low as 1.7 mM caused an almost complete haemolysis of the erythrocytes after 48 h of incubation. In contrast, glucose concentrations up to 61 mM did not affect them.

Next, we examined whether or not the induction of haemolysis by sorbose is a dog-specific phenomenon (table). Of the species tested, only mouse erythrocytes showed a similar susceptibility to sorbose as dog erythrocytes, but to cause 50% haemolysis about 80 times higher concentrations of sorbose were needed. In rat and cat erythrocytes, some haemolysis induced by sorbose was also noted, however, to a much lower degree. Under the conditions used, rabbit erythrocytes lysed to about 50% in control medium, which might be due to a different pH-sensitivity of the rabbit erythrocytes. Since no further haemolysis was found in the presence of sorbose, no additional investigations were undertaken to clarify this observation. Sorbose had no effect at all on erythrocytes from a cow, a horse and a normal man.

Discussion. The present study clearly demonstrates that sorbose acts directly on dog erythrocytes to induce haemolysis. As discussed in the companion paper[2], it appears that the mechanism of action of sorbose in dog erythrocytes is different from that of glucose-6-phosphate-dehydrogenase deficiency and favism in man. The dependence of the haemolytic effect of sorbose in dogs upon temperature and pH suggests that sorbose acts on the red blood cell metabolism rather than directly on the cell membrane. The monosaccharide nature of sorbose would suggest an interaction of sorbose with glycolysis.

We have shown that dog erythrocytes are much more susceptible to haemolysis induced by sorbose than mouse, rat and cat erythrocytes, and that sorbose had no haemolytic effect on erythrocytes from rabbit, cow, horse and man. However, considering the potential role of sorbose as a food additive, further investigations are necessary to exclude the possibility that human erythrocytes with some specific deficiencies could be sensitive to sorbose-induced haemolysis. In view of the many common forms of haemolytic anemia in humans (particularly in the Mediterranean countries, Africa, America and the Far East), this latter possibility should be definitively excluded in man.

The haemolytic effect of sorbose in dogs

P. Keller and A. Kistler[1]

Biological Pharmaceutical Research Department F. Hoffmann-La Roche & Co., Ltd, CH-4002 Basle (Switzerland),
20 April 1977

Summary. The ingestion of L-sorbose, a sugar of the keto-hexose group, causes haemolysis in dogs, but not in rats. Heinz bodies were not found after sorbose administration in either animal species nor were the animals examined deficient in glucose-6-phosphate-dehydrogenase.

Among a number of hereditary haemolytic anaemias associated with intrinsic erythrocyte defects[2], the glucose-6-phosphate-dehydrogenase deficiency was mainly found to be responsible for drug-induced haemolysis in man and many potentially haemolytic drugs have been recorded[2–6]. Evidence of a susceptibility to drug-induced haemolytic conditions has also been reported in dogs[7], cats and horses[8]. In the present study, we shall report on a type of acute haemolytic anaemia occurring in dogs after the administration of a sugar, the L-sorbose, which belongs to a group of substances quite different from those already mentioned in connection with drug-induced haemolysis[6].

Materials and methods. Animals: Approximately 1-year-old Swiss Beagle dogs and adult Fü-albino SPF rats of