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

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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

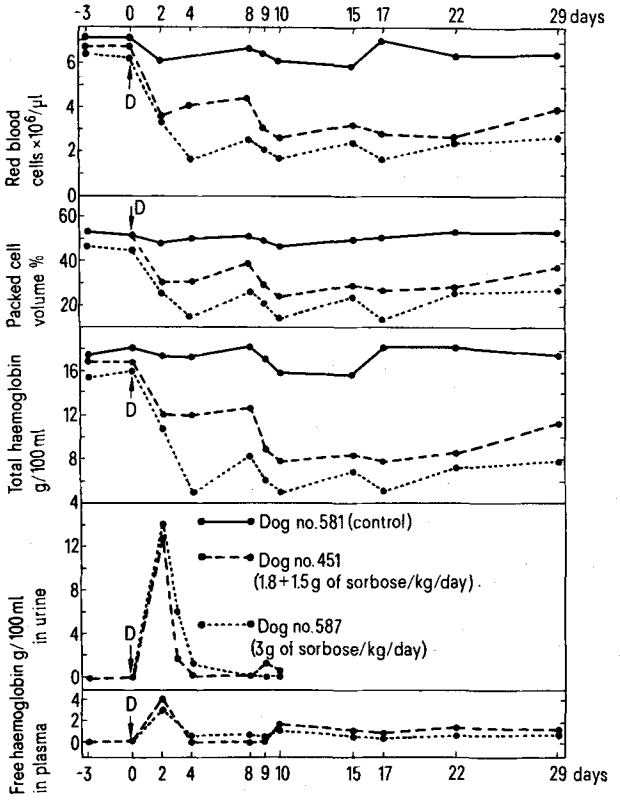
outbred stock, obtained from the Institute of Biological and Medical Research (Füllinsdorf BL), were used.

Experiments: 2 male dogs were given L-sorbose for a period of 29 days. Dog No. 587 received a daily dose of 3 g of sorbose/kg as an aqueous solution by stomach tube and dog No. 451 was dosed orally by means of an optimized dietary feed with 10% of its starch content replaced by sorbose (daily intake of sorbose expressed as mean ± SD: 1.8 ± 1.5 g/kg). The control dog No. 581 received normal dog cubes (Nafag No. 939/930).

The activities of glucose-6-phosphate-dehydrogenase, aldolase, pyruvate-kinase and lactate-dehydrogenase in haemolysates and the corresponding red blood cell parameters of man and nontreated dog and rat

Species*	Man (3)	Dog (26)	Rat (16)
RBC ($\times 10^6/\mu l$)	4.68 ± 0.16	6.75 ± 0.43	7.04 ± 0.60
PCV (%)	44 ± 2	49 ± 2	42 ± 4
Hb (g/100 ml)	15.2 ± 0.5	17.3 ± 0.9	14.5 ± 1.3
G6P-DH	3.63 ± 0.23	3.71 ± 0.92	7.94 ± 2.57
ALD	1.43 ± 0.49	1.25 ± 0.30	1.54 ± 0.43
PK	3.77 ± 1.39	1.76 ± 0.76	2.18 ± 1.47
LDH	94.5 ± 6.4	45.3 ± 7.0	171.0 ± 46.0

*Number of subjects in brackets. Values are given as mean ± SD. Enzyme activities are expressed as μmoles of substrate reacting per min per g of haemoglobin at 25 °C.



Course of haemolysis induced in dogs by oral sorbose administration. Red blood cell parameters (RBC count, PCV and total Hb) as well as free plasma haemoglobin were recorded over a period of 29 days and urine haemoglobin during the first 10 days. These parameters were also determined 3 days prior to and some 10 min before the first administration (D ↓). Dog No. 581 served as control, dog No. 587 was dosed by stomach tube and dog No. 451 ingested sorbose as feed-admix.

Further, a group of 8 male and 8 female rats was dosed for a period of 6 weeks with an optimized dietary feed containing 20% of sorbose (daily intake: mean = 17.5 g/kg; SD = 2.6 g). Control rats received the same feed without sorbose.

Methods. At various time intervals, blood samples from the animals were drawn into heparinized vials. Red blood cell counts (RBC) and determinations of the haemoglobin concentration (Hb) and the packed cell volume (PCV) were carried out with a Coulter Counter ZF with a haematocrit accessory. Blood films were stained with Brillant-Cresyl to visualize Heinz bodies.

Haemolysates were prepared from washed red blood cells of men and nontreated dogs and rats as cited by Richterich[9]. Activities of glucose-6-phosphate-dehydrogenase (G6P-DH; EC.1.1.1.49), pyruvate-kinase (PK; EC.2.7.1.40) and fructose-1,6-diphosphate-aldolase (ALD; EC. 4.1.2.13) were determined using Boehringer UV-test combinations and a Zeiss PMQ II spectrophotometer with a W+W recorder. Lactate-dehydrogenase (LDH; EC.1.1.1.27) was assayed in a Centrifichem centrifugal analyzer using Centrifichem UV-test combinations. Enzyme activities are expressed as μmoles of substrate reacting per min/g of haemoglobin at 25 °C.

Results. Dogs: 24 h after the first administration of sorbose marked haemoglobinuria was observed. In both treated dogs, urine haemoglobin concentration amounted to 14 g/100 ml and the plasma haemoglobin to 4 g/100 ml on the second day and diminished rapidly thereafter. The haemolytic phase, represented by the decrease of the RBC count, PCV and haemoglobin concentration, then developed to its full extent within about 4 days when sorbose was given at a constant dose level by stomach tube (figure, dog No. 587). A slight delay in the development of haemolysis, due to irregular feed and drug intake, was seen when sorbose was given as a feed-admix (figure, dog No. 451). The investigation of the blood films did not reveal the presence of Heinz bodies during this phase, nor were they found later. Apathy, decreased feed intake and loss of body weight, which are related to anaemia, were observed, but scleral icterus did not occur. After the 17th day of sorbose administration, all red blood cell parameters began to rise slowly. Thus, a recovery phase seemed to be attained although dosing was continued. After 29 days of sorbose administration, total Hb, PCV and RBC counts were still far below normal values in both dogs.

Rats: In rats with an average daily ingestion of 17.5 g of sorbose/kg, no change in total Hb, PCV and erythrocyet number was recorded over a period of 6 weeks and the values were similar to those found in the control rats.

RBC-enzymes: The RBC-enzyme activities and the corresponding red blood cell parameters of men and non-

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treated dogs and rats are summarized in the table. The activity of ALD and PK did not differ significantly in the haemolysates of normal red blood cells from man, dog and rat (table). The G6P-DH activity in red blood cells from dog was similar to that in man, but distinctly lower than in rats. The highest levels of LDH activity were found in erythrocytes of rats, followed by those of man and dog in decreasing order.

Discussion. The results described in the present study were confirmed in a 13-week toxicity study in dogs (6 animals/dose group) which showed a dose-dependent haemolytic anaemia[10]. The course of haemolysis in dogs after sorbose intake resembles that of drug-induced haemolytic disorders in man[2-6]. However, an important difference to the effect of oxidant drugs in individuals deficient in glucose-6-phosphate-dehydrogenase or to naphthalene-induced haemolysis in the dog[7] is the lack of Heinz body formation.

Within the red blood cell, oxidant substances, including naphthalene, impair all of the reductive processes dependent upon NADPH, which is not sufficiently regenerated because of the G6P-DH deficiency. Thus, Heinz bodies appear as the final stage of oxidative destruction of haemoglobin[3,6]. In favism in man haemolysis occurs without the formation of Heinz bodies, but involves extracellular immunological mechanisms and is also linked to G6P-DH deficiency[2,6].

In the case of sorbose, however, the chemical nature of the drug, which is a reducing substance, and the absence of Heinz bodies suggest that other mechanisms than those reported with oxidants are involved. That the haemolytic effect of sorbose in dogs is not linked to G6P-DH deficiency is also supported by the finding that the G6P-DH activity in red blood cells of our dogs is similar to that in normal man (table). The higher enzyme activities in rat erythrocytes found in this study are probably due to the fact that rats have a higher reticulocyte count than dogs and man. Younger red blood cells are reported to show higher activities of several RBC enzymes[3,4,9].

A plausible alternative for the cause of sorbose-induced haemolysis in dogs would be an effect of sorbose upon glycolysis in canine erythrocytes. This is at present being investigated by in vitro experiments which have already shown that sorbose has a direct haemolytic effect[11]. These studies should further clarify whether the haemolytic effect of sorbose is confined to the dog or also occurs in man and other mammals.

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Identification of ϱ-antigenic determinants on the surface of mouse T-lymphocytes

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Summary. A monospecific antiserum has been prepared in rabbits against purified ϱ-antigen, a 100,000 mol.wt glycoprotein found on the surface of mouse L-cells. This antiserum has been employed to demonstrate the presence of ϱ-antigenic determinants selectively on the surface of mouse T-, but not B-lymphocytes.

Lymphocytes involved in specific immunity fall into 2 classes depending upon whether they are dependent (T-cells) or independent (B-cells) of the thymus for their maturation[2]. These 2 lymphocyte populations can be distinguished operationally by the fact that they express quite different sets of cell surface antigens. For example, thy-1-, TL- and Ly-antigens[3-5] are found on the surface of mouse T-, but not B-cells while easily demonstrable surface immunoglobulins[6], FC receptors[7,8] and binding sites for C3 (the third component of complement)[9] are characteristic of B-cells. Here we report the presence of a new antigen called rho (ϱ) expressed on the surface of mouse T-, but not B-lymphocytes. ϱ-determinants are recognized in an immune cytolysis assay by cross-reaction with antiserum prepared against purified ϱ-antigen, a high mol.wt glycoprotein isolated from mouse L-cells. Our results indicate that ϱ is present on all mouse T-cells and that it is not identical to any previously described T-cell antigen.

The experimental methods we have employed to demonstrate the presence of ϱ on the T-cell surface are based on the use of an antiserum prepared against purified ϱ-antigen. ϱ is a 100,000 mol.wt glycoprotein found on the surface of mouse L-cells where it can function as a receptor for concanavalin A. ϱ was purified to molecular homogeneity by affinity chromatography on a column of con A-Sepharose according to the procedure of Hunt et al.[10]. Anti-ϱ serum was prepared by injecting adult male rabbits in the footpads with 300–600 μg of purified ϱ-antigen emulsified in complete Freund's adjuvant. Rabbits were bled at regular intervals thereafter and tested for the production of antibodies to ϱ in 3 ways. First, immune sera in the presence of complement were found to be toxic for L-cells in a dose dependent fashion as shown in table 1. This provides quite reasonable evidence that ϱ is in fact present on the L-cell-surface. Second, immune but not preimmune sera were found to combine specifically with purified ϱ-antigen as judged by an immune precipitation test. Third, immune sera were shown to precipitate ϱ-antigen specifically from solutions produced by dis-

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