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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solving whole L-cells or L-cell membranes in 2% Triton X-100. Details of these latter 2 assays will be published elsewhere.

Mouse lymphocytes were tested for the presence of ϱ-determinants by an immune cytolysis procedure identical to that described in table 1; lymphocytes from both the spleen and the thymus were examined. The results, as shown in table 2, indicate that whereas greater than 90% of thymus cells were sensitive to specific killing by anti-ϱ plus complement only 40–50% of spleen cells could be lysed. The level of spleen cell killing could not be increased by adding more anti-ϱ and the proportion (40–50%) of sensitive spleen cells corresponded well with the proportion of T-cells present in mouse spleen[11]. The pattern of sensitivity observed with rat thymus and spleen cells was quite similar to that observed with mouse. Greater than 90% of rat thymus but only 47% of rat spleen cells were sensitive to lysis by anti-ϱ plus complement. In contrast, guinea-pig spleen and human peripheral blood lymphocytes were all resistant to lysis in this assay.

The most straightforward explanation for these results was to assume that mouse and rat T-cells express ϱ-antigenic determinants on their surfaces while B-cells do not. 2 types of experiments were carried out to test this hypothesis. In the first case mouse and rat spleen cell populations were enriched for T-cells by passing them over a nylon wool column. B-cells are selectively adsorbed to nylon wool and the eluted cell population is correspondingly enriched for T-cells[12]. In our experiments, this procedure resulted in approximately a 2fold enrichment for T-cells which accounted for approximately 75% of the eluted cell population. A corresponding increase was observed in the proportion of cells containing ϱ-antigen as shown in table 2 and this supports our hypothesis that it is the T-cells which contain ϱ.

The second type of experiment designed to test the association of ϱ with T-cells also involved the use of mouse spleen cells. These cells were first exposed to anti-ϱ plus complement under conditions where all ϱ-containing cells should be lysed. The surviving cells were then exposed to antibodies to thy-1, a specific T-cell marker, plus complement in order to lyse any T-cells that may be remaining. An increase in the proportion of lysed cells

Table 1. Toxicity of anti-ϱ for mouse L-cells

Serum dilution	Dead cells (%) in NRS	anti-ϱ	C.I.
N	19.3	94.7	93.4
1:2	22.5	94.5	92.9
1:4	17.4	79.2	74.8

Suspension cultures of L-929 cells employed in these experiments were grown at 37 °C in F-14 SMEM medium (Grand Island Biological Co.) containing 5% fetal calf serum and antibiotics. Cells were washed twice in phosphate buffered isotonic saline (PBS) before use and greater than 95% were found to be viable at this stage. Cytotoxicity assays were performed by suspending 5×10^5 cells in 0.5 ml PBS and adding 0.1 ml anti-ϱ or normal rabbit serum (NRS) plus 30 µl guinea-pig serum (1:10 dilution) as a source of complement. This mixture was incubated at 37 °C for 60 min after which cell viability was determined by trypan blue dye exclusion. The results are expressed as percent of total cells dead and in the form of a cytotoxicity index (C.I.) designed to correct for background levels of killing.

$$\text{C.I.} = \frac{\text{dead cells (\%) in anti-}\varrho - \text{dead cells (\%) in NRS}}{100\% - \text{dead cells (\%) in NRS}}.$$

Table 2. Toxicity of anti-ϱ for thymus and spleen cells

	Thymus		Spleen Pre-nylon column		Post-nylon column	
Serum	Dead (%)	C.I.	Dead (%)	C.I.	Dead (%)	C.I.
A. C_3H-mouse						
NRS	4.8	–	4.5	–	5.6	–
Anti-ϱ	91.7	91.3	47.2	44.7	81.2	80.1
B. Sprague-Dawley rats						
NRS	0.5	–	5.0	–	5.2	–
Anti-ϱ	99.5	99.5	47.2	44.4	74.1	72.1

Thymus and spleen cell suspensions were prepared from 25 g C_3H-mice or from 75 g Sprague-Dawley rats by teasing the organs apart in Earle's balanced salt solution (BSS). Cells were passed through stainless steel and then nylon screens before being washed 3 times with BSS at 4 °C. Thymus cell suspensions prepared in this way were employed directly for immune cytolysis assays as described in table 1; sera were used in undiluted form. Spleen cells were divided into 2 equal samples of approximately 10^7 cells each. One was used directly for immune cytolysis while the other was enriched for T-cells by the nylon wool column method[12]. Cells suspended in 1 ml BSS containing 5% fetal calf serum were allowed to adsorb for 45 min to a 1×6 cm column of nylon wool (Fennall Laboratories, Morton Grove, Ill.) which had been equilibrated with BSS plus 5% fetal calf serum at 37 °C. Non-adherent cells were then eluted from the column with 20 ml of Eagle's Minimal Essential Medium containing 10% calf serum and tested in the immune cytolysis assay. Between 40% and 60% of the spleen cells applied to the column were recovered after this procedure and greater than 90% were found to be viable. In the case of mouse spleen cells enrichment for T-cells was confirmed by showing that 75% of the non-adherent cells possess thy-1, a specific T-cell surface antigen, while less than 45% of the original cell population were T-cells.

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Table 3. Sequential cytotoxicity assay

Stepwise additions	Cell viability Dead (%)	C.I.
1. Anti-ϱ plus complement	48.8	43.6
2. Anti-thy-1 plus complement	49.8	40.6

A cell suspension was prepared from the spleen of a 25 g C_3H-mouse as described in table 2. The suspension was divided into equal samples and treated with a series of stepwise additons. The first sample was treated as follows: 0.1 ml anti-ϱ was added to each of 4 tubes containing 2×10^5 cells in 0.1 ml BSS and incubated for 1 h at 4 °C. Guinea-pig complement (0.1 ml of a 1:10 dilution) was then added to each tube and incubation was continued for a further h at 37 °C. Cell viability, as determined by trypan blue dye exclusion, was determined at this stage on 2 of the tubes. 0.1 ml AKR-mouse anti-thy-1 (Bionetics Laboratories, Kensington, Md) plus 0.1 ml guinea-pig complement was then added to each of the remaining tubes and they were incubated for 1 h at 37 °C. A final viability count was performed and the results were expressed as described in table 2. The reverse experiment in which the order of addition was anti-thy-1 followed by anti-ϱ was performed on the second sample of cells. It yielded results quite similar to those shown above. A 1:10 dilution of the anti-thy-1 serum used in these experiments in the presence of complement was found to lyse greater than 90% of C_3H-mouse thymus cells when assayed by the procedure described here.

should result only if some T-cells had escaped killing by anti-ϱ. In practice, no increased killing was observed as shown in table 3. This demonstrates clearly that there are no thy-1 containing (T)-spleen cells which do not also contain ϱ.

Together these 2 experiments provide quite strong evidence that ϱ-determinants are present on the surface of mouse T-, but not B-lymphocytes. The sequential cytotoxicity experiment, in fact, suggests that ϱ is expressed by all mouse T-cells and not simply by a subpopulation of them. Both experiments, however, admit the possibility that a small but significant population of B-cells may express. Further studies will be required to clarify this point.

The demonstration of ϱ-determinants on the T-cell-surface raises the issue of whether ϱ may be identical to any of the previously described mouse T-cell specific antigens such as thy-1, TL, Ly and MTLA. So far, it appears that this is not the case. For example, if ϱ were the same as thy-1, then L-cells expressing ϱ ought to express thy-1 as well. Our experiments as shown in table 4 have demonstrated that this is not the case. Furthermore, ϱ antigen (mol.wt 100,000) and a protein (mol.wt approximately 120,000) recently precipitated specifically from solubilized mouse T-cell-membranes by anti-ϱ (S. Cancelosi and J. Brown, unpublished observations) are found to have considerably higher mol.wts than the thy-1 antigen molecule (mol.wt 27,000)[13–15]. Similar but less conclusive arguments can also be made in the case of TL-, Ly- and MTLA-antigens. Cohen et al.[16] have shown that L-cells do not express TL-antigens and Ly-antigens are expressed on the surfaces of lymphoid cells only[17,18]. MTLA has a mol.wt significantly different from ϱ[19,20]. These results are consistent with the view that ϱ is not identical to thy-1, TL, Ly or MTLA antigens.

The information we have about ϱ-antigen at the present time gives us very few clues about what its function may be. ϱ is found to be present on the surface of L-cells and of T-, but not B-lymphocytes from the mouse and rat. In addition, we have detected ϱ on the surface of Swiss mouse embryo fibroblasts, but not human or mouse erythrocytes. Since ϱ antigen is itself a con A-receptor and since con A is found to be mitogenic for T-, but not B-lymphocytes, the possibility exists that ϱ may be involved in the con A-induced mitogenesis of T-lymphocytes. Experiments employing anti-ϱ-serum are currently in progress to test this possibility.

Table 4. Resistance of mouse L-cells to anti-thy-1

Serum dilution	Dead cells (%) in NRS	anti-ϱ	anti-thy-1
N	3.0	88.7	10.3
1:10	–	–	8.4
1:20	–	–	6.2
1:40	–	–	5.7

L-cells grown as described in table 1 were washed free of serum and resuspended in Eagles Minimal Essential Medium at a concentration of 2×10^6 cells/ml. Varying concentrations of anti-thy-1, anti-ϱ or normal rabbit serum (NRS) were added to 0.1 ml samples of the cell suspension and incubated at 4 °C for 1 h. 0.1 ml guinea-pig complement (diluted 1:10) was then added to each tube and incubation was continued for a further one hour at 37 °C. Cell viability was determined by trypan blue dye exclusion.

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Early foetal thrombosis induced by Thalidomide in mouse: Possible explanation for teratogenicity

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Summary. Mouse foetuses were treated by Thalidomide on days 11–12 in order to verify whether the drug would induce blood abnormalities leading to circulatory troubles. About 18% of the treated foetuses showed both severe limb haemorrhages on day 14, and obvious alterations of the nucleated red blood cells of vitelline origin. These blood abnormalities, occurring suddenly during the well-known 'critical stage' of foetal development, could be responsible for circulatory blocks leading to necrosis.

Although the teratogenic effects of Thalidomide have been known for many years in man and animal, particularly in mice[1], and in rabbits[2], its mechanism still remains unknown. Among the different modes of action postulated, it has been suggested that this drug could be an antagonist of glutamic acid, considering the similarity of their formulae[3]. It has also been hypothesized that Thalidomide could be antagonistic to several vitamins of the B-group, particularly to riboflavin[4] or folic acid[5]. The fact that some antagonists of folic acid have been proved to be teratogenic supports the latter hypothesis[6]. Some hereditary limb amputations, greatly resembling the induced Thalidomide malformations, have been described in the rabbit[7]. It has been shown that these abnormalities could be induced by a blood defect appearing very early in the fetal life[8]. This trouble could lead to thrombosis which in turn induces hypoxia and then limb necrosis. However, these amputations can be prevented in utero by either hyperoxic treatment or treatments aiming at the reduction of abnormal erythro-