5. DISCUSSION

The ABLS used in these experiments and raised in horses according to the adjuvant method described by Monaco et al. (1966) had a maximal cytotoxic titer of 2500 and a maximal hemolytic titer of 64. We found that for these experiments the adjuvant method was an optimal method for producing ABLS. Kreeftenberg (personal communications), however, found that the use of BCG as adjuvant in producing ALS yielded ALS of better quality with higher potency. Its mechanism comprised the stimulation of the T cells by the acid fast Bacilli of the BCG, of which the T cells function as helper cells in the production of antibody.

In the preliminary experiment, injection of ABLS did not cause toxicity and anemia to the calves, although no inactivation (heated 56°C, 30 minutes) and absorption with bovine red blood cells was performed. That was the reason why it was decided to use fresh unabsorb ABLS for the whole experiments. The nontoxic effect of ABLS to bovine erythrocyt was indicated by the unaffected level of haematocrit percentage (Fig. 8) and red blood cell counts.

It was important to use lymphocyte antigen that is not contaminated with interstitial materials, erythrocyte fragments and thrombocytes. Such antigen can only be obtained if the lymphocytes were from organs that do not contain much interstitium or blood. Balner (1970) reported that lymphoid
cells from thymic gland and thoracic duct were the best to produce nontoxic ALS with high immunosuppressive activity. Lymphocytes from the spleen were usually contaminated with erythrocytes which yielded ALS with a very high haemolytic titer and caused anemia in the injected animals. Lymphocyte antigens from lymph nodes were usually mixed with interstitial material and the ALS obtained was generally toxic to the kidney basement membrane. Therefore we used thymocytes for the production of ABLS.

High doses of ABLS (7.5 - 10 ml per kg body weight) caused destruction of many lymphocytes in a short period of time and in this way a large amount of toxic material was built up in the blood stream, which increased the permeability of the blood vessels and caused haemorrhages as were found in the lymph nodes, kidney and especially the intestines of the calves. Another possible cause of the haemorrhages might be due to antibody-antigen reaction attached to the endothelial cells of the blood vessels, followed by the lysis of the antibody-antigen-endothelial cell complex by the presence of complement and polymorphonuclear cells. The vessels were then occluded by thrombocytes, which caused thrombosis and haemorrhages. The haemorrhages may have been the cause of death of the calves. Mortality was not due to infectious agents, because from 6 calves that succumbed the high doses of ABLS Salmonella was isolated from only two calves.
Low doses of ABLS (1 ml per kg body weight) appeared to be tolerated by the calves because lymphocytes destruction occurred gradually so that there was enough time to neutralize the toxic materials and to phagocytize lymphocyte fragments. The latter was enhanced by the increased numbers of neutrophils. Although the number of lymphocytes very rapidly decreased in calves receiving ABLS, the white cell counts increased to approximately twice the pretreatment level at the beginning of the ABLS administration. This increase was due to the increased number of neutrophils. This phenomenon was also observed by Gray et al. (1966) and Iwasaki et al. (1967). The possible cause of the initial neutrophilic reaction may have been the horse serum protein, because the same inflammatory response was also observed in control calves receiving 4 injections of normal horse serum (Fig. 4). All the calves also had a slight rise in temperature at the beginning of the experiment. Another possible cause of the inflammatory reaction was the cellular break down of the small circulating lymphocytes due to ALS as reported by Lance (1969) and Tyler et al. (1969). Neutrophils and macrophages were needed for phagocytosis of the death lymphocytes and their fragments. This was compatible with the observations of Taub and Lance (1968), Lance (1970), Everett et al. (1970), Tyler et al. (1959) and Hay et al. (1974) who saw macrophages containing phagocytized lymphocytes in the lymph nodes and liver after ALS injection.
The number of eosinophils increased especially at the end of the experiment. This is considered as an expression of allergic reaction to repeated injection of ABLS. ABLS injection stimulated the formation of antibody against horse serum and induced antibody-antigen reaction with eosinophilic proliferation.

We found that after ABLS administration the reduced number of lymphocytes did not return to the pretreatment level 2 weeks after the last ABLS administration. While Iwasaki et al. (1967) reported that in dogs the return of the peripheral lymphocyte counts were completed within 5-10 days. The difference in potency of sera might play a role. However, the difference in animal species where the sera were produced also determined the potency of the sera. Rabbit ALS was found to be ten times more potent than horse ALS (Kreeftenberg, personal communications). James and Anderson (1967) reported that rabbit anti-rat ALS produced more marked and prolonged lymphopenia than did horse antiserum. The duration of ALS administration and doses also determined the return of the number of lymphocytes. Animals injected with ABLS for longer period would take longer time to recover than animals only receiving a few injections of ALS.

Taub and Lance (1968b) and Tyler et al. (1969) found an increase in weight of the lymph nodes and spleen in mice due to cell hyperplasia after RAMLS administration. At necropsy we found that the lymph nodes of the ABLS treated
calves were slightly swollen, but in contrast the spleen was contracted as indicated by shrinkage of the capsule and cellular depletion. It is obvious that ABLS administration first caused lymphocyte depletion in the lymph nodes and later in the spleen. When the calves died cellular hyperplasia had started in the lymph nodes and the increase in sizes of the lymph nodes were due to congestion, haemorrhage, follicle germinal center reaction and histioreticular cell hyperplasia. This follicle germinal center reaction was an immune reaction which might also occur in the spleen as reported by Iawasaki et al. (1967) who found lymphoid hyperplasia in the lymph nodes and spleen of dogs treated with HADLS, particularly in animals treated 2 months or longer. It can be concluded that ABLS does not influence the blastogenesis in the lymph nodes. This is compatible with the findings of Lance (1968) and Denman and Frenkel (1968) who reported that ILG penetrated the thymus, spleen and lymph nodes to a limited extent.

Compared with the controls, the majority of the ABLS treated calves showed suppression of the immune response to SRBC. Only two calves were able to produce antibody to SRBC (Table 5) in low titers. This findings was compatible with the observation on the primary immune response to SRBC in mice and rats as reported by Monaco et al. (1965a, 1966), Denman et al. (1966), Curry and Ziff (1966), Bart et al.
James and Milne (1971, Reubens et al. (1971) and Lance (1975). However, these investigators observed that ALS suppressed the primary immune response only when it was given prior to the antigen injection. When given on the same day or after the antigen ALS was not effective. SRBC was already known as thymus dependent antigen (Fudenburg et al. 1966).

The depression on the immune response to SRBC indicated that the T cell function was disturbed. This was also indicated by the decrease number of rosette forming lymphocyte as a result of ABLS injection (Table 10). From this result it can be concluded that there is no T cell function as helper cells in the production of antibody, because ABLS administration killed T lymphocytes.

The B cell function was also disturbed as indicated by the depressed antibody formation against tetanus toxoid.

It is already known that bacterial polysaccharide is a thymus independent antigen (Fudenburg et al. 1966). So that no immune reaction to tetanus toxoid, indicated that ABLS disturbed or killed the B-cells as shown in table 10 where the B-cell population was decreased to zero after ABLS injection. Although the production of ABLS is by injection of thymocytes, it is also effective against B lymphocytes. The horse’s reaction to the thymocytes is directed to lymphocytes which originated from the haemopoietic stem cells. The differentiation to B and T cells is based on the marker of the cells, but both lymphocytes are originated from the haemopoietic stem cells.
This findings is in accordance with the findings of Field and Gibbs (1968) who reported that ALS produced from thymocytes in rabbits caused inactivation of both haemopoietic stem cells and spleen cells. They cited from Ford and Minklem (1963) that thymocytes are also derived from the bone marrow so the similarity in antigenic composition with haemopoietic stem cells may result from a common origin.

On the otherhand our own experienced indicated that lymphocytes derived from the thymus gland also contained approximately 3% B lymphocytes. This explain why the ABLS produced in horses by the injection of thymocytes is also effective against B lymphocytes. This is the reason why we are apted to use the name antilymphocyte serum instead of anti-thymocyte serum.

Our findings in the decrease of T cell population after ABLS administration was in accordance with the findings of Bishop et al. (1975) and Cosimi et al. (1976), Thomas et al. (1977) and Thomas et al. (1978) in monkey and in human beings treated with ATG. But in contrast to Thomas et al. (1977) we found that the ABLS treated calves had also a decreased number of B cells.

That there was a depression on the T cell function was strengthen by the facts that there was a significant inhibition of the delayed hypersensitivity reaction and prolonged graft survival in the ABLS treated calves as compared to the
controls. Waksman et al. (1961) found that the suppressive effect on the tuberculin reaction in guinea pigs was caused by the reduction in cellular infiltration in the skin. They reported that the suppression of the delayed hypersensitivity reaction was mostly observed in animals with lymphocyte counts below 4000/mm$^3$. In the course of our experiments the lymphocyte counts in the experimental calves were below 2000/mm$^3$.

Comparing our findings with that of Waksman et al. (1961) it can be concluded that the low number of lymphocytes population in the A3LS treated calves caused the inhibition of the delayed hypersensitivity reaction.

The decrease in the number of lymphocytes will also cause suppression of the cellular and humoral immune responses. This was reported by Gowans et al. (1962) who investigated the role of small lymphocytes in the immune response in rats. They observed that small lymphocytes initiated the response to skin homograft by first differentiating into large pyroniniphyllic cells which gave rise to immunologically activated small lymphocytes. These invaded the graft and caused rejection. They also found that lymphocyte depleted rats could not be sensitized by primary tetanus and SRBC injections, but the injection of thoracic duct cells into these rats would restore the immune response.

In the control calves the grafts were rejected within 8 days (Table 8). The process of rejection in these animals
were usually completed in 3 days. In the ABLS treated animals, however, this process took 7 days to complete. The average survival prolongation in the ABLS treated calves was 4.85 days. Graft survival time is usually used to evaluate ABLS potency. Although all the calves received the same treatment with ABLS not all of them responded the same way to the treatment. The longest graft survival was in calf number 158 followed by calves 156 and 161. Calves 158 and 161 also had complete immune suppression of the humoral immune response to SRBC and tetanus toxoid throughout the course of the experiment. So that, it can be concluded that there is an individual difference in response to ABLS. The time of graft survival in the ABLS treated calves was about as long as that in the rats and monkeys as reported by Woodruff and Anderson (1963) and Balner (1969). Monaco et al. (1966, 1966a) found that mice treated daily with RAMLS for 7 days until the day of grafting had a graft survival prolongation between 21 and 24 days, whereas if the administration of RAMLS was continued after grafting the graft would not be rejected.

The reactivity of the lymphocytes to PHA and PWM of the ABLS treated calves was completely inhibited (Table 9). One week after the administration of ABLS was discontinued the cell reactivity reappeared and two weeks after discontinuation the reactivity reached 50% of the pretreatment range. These were compatible with the results reported by
Tursi et al. (1969) in mice. In our experiment there existed the possibility that the lymphocytes from the ABLS treated calves were coated by the ABLS, so that they became insensitive and were not able to respond to the mitogen.

This experiment is the first pioneering work on the effects of ABLS in calves. In order to extend the use of ABLS in the future many aspects on the effects of ABLS are still needed to be studied. The mechanism of action of ABLS in cattle, the pathogenesis of the alterations in the lymph nodes and spleen due to ABLS and why ABLS affects the thymus gland to a limited extend are interesting features to be studied. On the other hand it is interesting to study the clinical use in Veterinary Medicine of ABLS and ALS against other animal species. The use of ABLS as an immunosuppressive agent in transmission studies of unknown diseases or in transplantation experiments are interesting subjects that still need to be propagated.