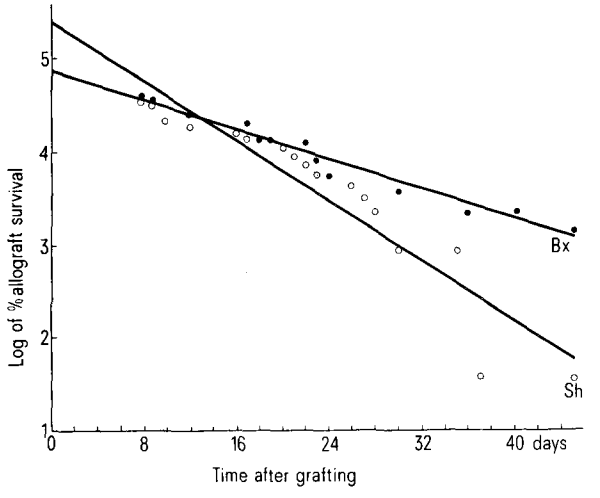
turned 180° so that the feathers grew anteriorly, making identification of the grafts easier. As anaesthetic, sodium pentobarbital (i.p. 30 mg/kg) was used during grafting. Examination of the grafts was not attempted until 1 week after grafting and then they were evaluated every 2 days during the following 7 weeks. A graft was considered as rejected when necrotic shrunken and/or crusty areas appeared. Autopsies were performed on several Bx and Sh chickens when 12 weeks old. The spleen and thymus were removed and stored in 10% buffered formalin until prepared for histological examination. The specimens were embedded in paraffin, sectioned and stained with haematoxyline and eosin. Careful necropsy was performed in search of bursal tissue remmants in bursectomized animals; none were found.



Per cent skin allograft survival in sham-operated (Sh) and bursectomized (Bx)/chicken. The difference between the 2 linear regressions is highly significant ($p<0.001$).

Effect of bursectomy on skin allograft survival in chickens

Group	Graft survival 10 days	20 days	30 days	40 days
Control sham-operated	16/21	12/21	4/21	1/21
Bursectomized	16/17	10/16	5/14	4/14

The data from all Bx and Sh birds are grouped together in the table. Percentage skin allografts survival in Sh and Bx birds showed 2 curviline functions that are plotted semilogarithmically. The differences between these 2 linear regressions are highly significant ($p < 0.001$; figure).

The outstanding feature of splenic morphology was the absence of germinal centers in the Bx birds in contrast to the Sh chickens. Cross sections of the thymus showed no clear-cut morphological differences between the 2 groups.

Our results clearly show that early bursectomy produces a delay of skin allograft rejection. This observation may imply an alteration in thymus function. The bursa of Fabricius in the chicken exports lymphoid cells to the thymus early in embryonic life. The very early export of cells from the bursa can explain the first appearance of immunoglobulin-containing cells in other lymphoid organs, on the 17th to 19th day of incubation[8]. Late surgical bursectomy, at 17 days of incubation[3] or later, leaves open the consideration of possible cellular[6] or humoral interactions preceding the operation that may affect thymus functions.

In contrast with the results obtained after late bursectomy, our experiments in early bursectomized chicken suggest some interaction between the bursa of Fabricius and the thymus or other elements of the immune system, which find their expression in a delayed rejection of skin allografts.

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Erythrocyte 2,3-diphosphoglycerate in anaemic sheep[1]

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Summary. Experimental anaemia resulted in an increase of red cell 2,3-DPG from 0.11 μM/g Hb to 0.99 μM in haemoglobin A sheep and from 0.21 μM to 1.5 μM in haemoglobin B sheep. Production of haemoglobin C as a result of anaemia was confined to haemoglobin A only. The results, therefore, appear to suggest that the rise in 2,3-DPG in the red blood cells of different haemoglobin types is independent of haemoglobin C.

A considerable amount of evidence now available[2–5] indicates that 2 organic phosphate compounds, 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) moderate haemoglobin function in man. 2,3-DPG causes a concentration dependent decrease in the oxygen affinity of haemoglobin by its binding to specific sites on the β-chain. ATP also reduces the affinity of haemoglobin for oxygen. However, 2,3-DPG is present in the erythrocyte in a much higher concentration than ATP and is, therefore, considered to be more important in moderating haemoglobin oxygen affinity.

The relationship between red cell 2,3-DPG and haemoglobin function has been investigated in several mam-

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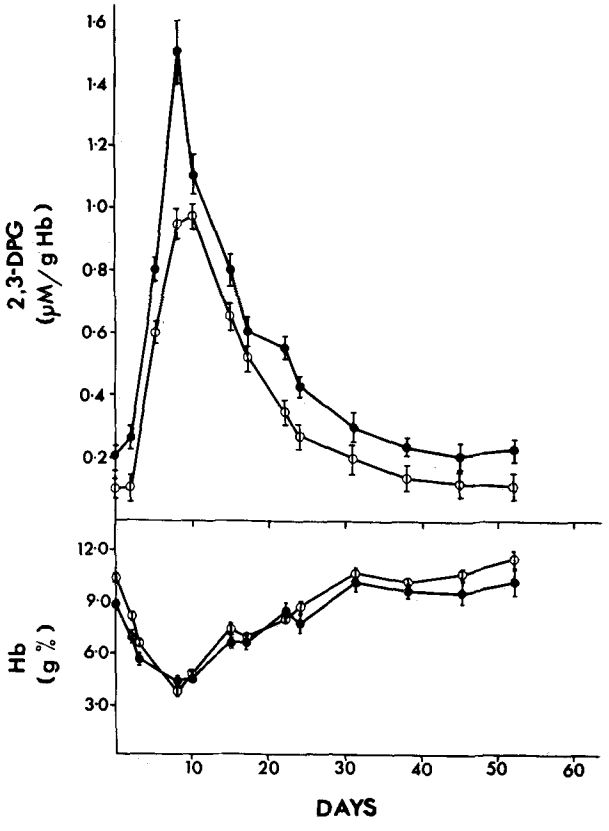
malian species[6,7]. Based on such studies, Bunn et al.[7] have classified mammals into 2 groups; the first group, which contains the majority of mammalian species tested, have abundant red cell 2,3-DPG and have a haemoglobin of high oxygen affinity that is markedly lowered in the presence of 2,3-DPG. The second group which includes sheep, goats, cattle and cats, is characterized by low levels of red cell 2,3-DPG and haemoglobins of low oxygen affinity which do not react strongly with 2,3-DPG. The lack of interaction of sheep haemoglobin with 2,3-DPG has been explained on the basis of the primary structure of the haemoglobin molecule[8]. There is a deletion of one amino acid residue at the N-terminal end of the β-chain; this results in an increased intramolecular distance and prevents 2,3-DPG from forming the linkage that stabilizes the deoxy conformation.

Sheep have 2 genetically determined haemoglobin types, Hb A and Hb B. Hb A has a greater affinity for molecular oxygen than Hb B. Haemoglobin A sheep have a larger blood volume, a higher haematocrit and haemoglobin concentration and are more resistant to hypoxia than Hb B type sheep[9]. A third haemoglobin, Hb C, is produced by Hb A sheep only. Although the appearance of this haemoglobin is usually related to hypoxic stress, the oxygen affinities of Hb A and C do not differ significantly, indicating that the reported resistance of Hb A type sheep to hypoxia is not due to the presence of Hb C[9]. The effect of anaemia on the concentration of 2,3-DPG in the red blood cells of Hb A and Hb B type sheep has not been investigated. The present study was undertaken to provide information on this subject. Blood was collected by jugular venepuncture from 8 adult Merino ewes, 4 of which were Hb A type and 4 Hb B type. Red blood cell 2,3-DPG was measured by an enzymic method[10].



Effect of experimental anaemia on the levels of Hb and 2,3-DPG in the red blood cells of Hb A (○) and Hb B (●) sheep.

After normal values had been established over a period of several weeks, the animals were made anaemic by removal of approximately 600 ml of blood on each of 4 days, designated day 0, 1, 2 and 4. The production of Hb C was monitored electrophoretically.

The results are shown in the figure. The phlebotomy resulted in a decrease in the haemoglobin level from 10.9g% to 4.2g% in Hb A and from 9.0g% to 4.5g% in Hb B sheep. Red cell 2,3-DPG levels increased from 0.11 μM/g Hb to 0.99 μM in Hb A and from 0.21 μM to 1.5 μM in Hb B sheep. Hb C appeared in the blood of Hb A sheep only and accounted for about 50% of the total haemoglobin by day 20. A brief reticulocytosis was observed but after day 17, virtually no reticulocytes were present.

It has already been well established that haemoglobin affinity for oxygen is unaltered during anaemia in sheep of either haemoglobin type[11,12]. With respect to the effect of 2,3-DPG on haemoglobin function in sheep, Bunn et al.[7] have shown that with haemoglobin that is stripped of organic phosphates, there is a rise in P_{50} from 16 to 21 mm Hg in Hb B and from 14 to 17.5 mm Hg in Hb AB sheep in the presence of 1 mM 2,3-DPG. When expressed as a percentage of the initial values, the rises in 2,3-DPG values in the present investigation were approximately 800% in Hb A and 600% in Hb B sheep. Although these rises seem impressive, the absolute increases were only 0.0008 mM/g Hb in Hb A and 0.0012 mM/g in Hb B sheep; i.e. several hundred times smaller than the values used by Bunn et al.[7].

An interesting feature of the present investigation is that the rise in 2,3-DPG in the red blood cells of anaemic sheep is similar in sheep of different haemoglobin types and is apparently independent of Hb C production. This assumption is based on the following observations: firstly, the rise in 2,3-DPG was observed in sheep of both Hb A and Hb B types, while the production of Hb C was confined to Hb A sheep only; secondly, the rise in 2,3-DPG was at a maximum at the time when Hb C was just beginning to appear in the circulation; and thirdly, 2,3-DPG level returned to pre-experimental values by day 50, while Hb C was still present in the circulation after day 100.

As suggested by Brewer and Eaton[13], changes in the concentration of a glycolytic intermediate such as 2,3-DPG may be brought about in non-nucleated red cells in 2 general ways; first, by changes in the overall rate of glycolysis and, secondly, by changes within the glycolytic pathway, for example, by increasing production or decreasing degradation of 2,3-DPG. Of these 2 possibilities an overall change in glycolytic rate seems to be more likely as there is no evidence of a major decrease in the levels of other intermediates (such as ATP) when 2,3-DPG levels are increased as a result of various hypoxic conditions[13]. In the present study we have observed a rise in the number of reticulocytes (to approximately 17%) during the period of maximum rise in the level of red cell 2,3-DPG. It is possible, therefore, that the rise in 2,3-DPG was related to increased glycolytic activity in

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these cells. This assumption is, however, contrary to a previous suggestion that reticulocytes have 2, 3-DPG concentrations lower than those of mature red blood cells[13].

What is the significance of the 6- to 8fold rise in 2, 3-DPG in the red blood cells of sheep during anaemia? The answer to this question is obscure, although Professor G. J. Brewer (personal communication) argues that while 2, 3-DPG levels may be low and the affinity of the haemoglobin for 2, 3-DPG be reduced in animals such as sheep, the affinity is *not* zero and an increase in 2, 3-DPG will have some effect on oxygen affinity. His argument seems to be strengthened by the in vitro observations of Bunn et al.[7]. Despite a multitude of studies there is, as yet, no complete understanding of the importance of high concentration of 2, 3-DPG in the red blood cells of some species. It is known to affect pH and the Donnan equilibrium between the red cell and plasma[4,5]. Some workers have postulated that red cell 2, 3-DPG plays a role in potassium transport, possibly by way of 2, 3-diphosphoglycerate phosphatase[14,15]. It is of interest to note that the immature red cells in the blood of anaemic sheep have many times higher concentrations of potassium and a much greater activity of sodium potassium activated adenosine triphosphatase enzyme than the mature (low potassium) red cells[9]. It is apparent that further evaluation of the possible mechanisms relating hypoxia, red blood cell glycolytic rate and concentration of 2, 3-DPG is required.

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Testicular Leydig cells and Δ^5-3β-hydroxysteroid dehydrogenase in cadmium-treated toads (Bufo melanostictus)

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Summary. Injection of cadmium chloride in toad increased Leydig cell size (area) and Δ^5-3β-hydroxysteroid dehydrogenase activity in the testis.

Spermatogenic arrest due to cadmium injection in rat[2], mice[3], bird[4] and toad[5] has been noted by many investigators. Gunn et al[6,7] have reported that cadmium administration in rats and mice developes interstitial cell tumor (ICT) in testis. A single subcutaneous injection of cadmium salt also causes hypertrophy of the Leydig cells in pigeon[8]. To the best of our knowledge, the effect of cadmium on the Leydig cells of hibernating amphibians has not yet been studied.

The present work has been taken up to study the histology of the Leydig cells and the activity of enzyme Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) in cadmium-treated toad testis.

15 male toads (Bufo melanostictus) of average weights from 55 to 60 g were used in the present experiments

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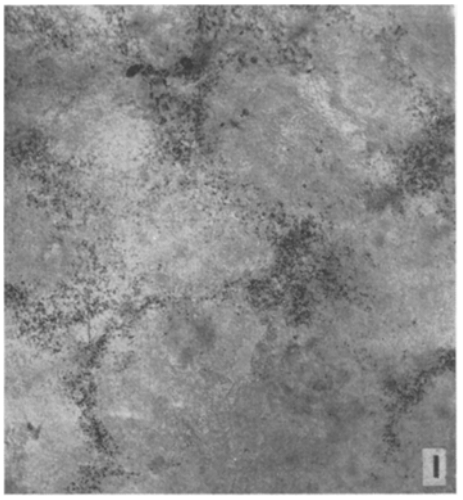

Fig. 1. Δ^5-3β-hydroxysteroid dehydrogenase in the testis of normal toad. × 96.

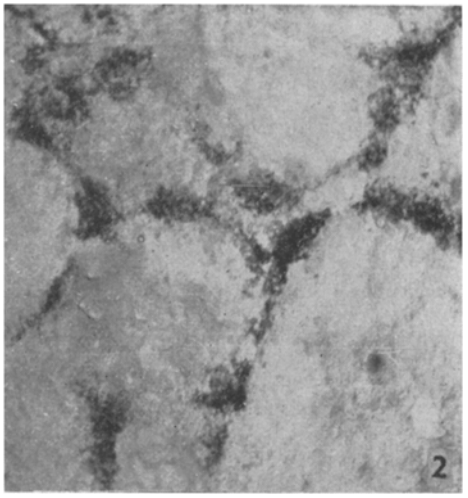

Fig. 2. Δ^5-3β-hydroxysteroid dehydrogenase in the testis of cadmium-treated toad. × 96.