DCMU). Thus, the decrease in boron in the leaf tissue of *Halophila* is considered the result of boron efflux from the leaves to the water.

Bowen (*7*) has shown that inorganic boron, probably borate, is accumulated by excised sugar cane leaves and forms complexes with sugars and other metabolites in the cytoplasm. In view of this result, it seems likely that borate is absorbed by the sea grasses from seawater in darkness. However, boron efflux could represent either excretion of borate, after dissociation from organic compounds within the plant, or excretion of organically complexed boron. The kinetics of the efflux process (Fig. 1A; rapid decrease after a 30-minute lag) suggest that a specific organoboron complex may be produced after illumination begins and then it decreases within the cells under continued illumination. The identification of such an organic material, possibly by chromatographic techniques, would help answer the question of the chemical form of boron in plants, a central issue in the overall problem of boron function in plants. Whether this form is, as others have suggested (*1*, *2*), a polyhydroxy compound with *cis*-hydroxyl groups (for example, a carbohydrate or polyphenol) or some other chemical species is not known.

In order to examine the relation between boron efflux and photosynthetic activity, excised shoots were treated with the inhibitor, DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], which prevents $CO_2$ fixation by interfering with photosystem 2 activity ($O_2$ evolution) (*8*). Metabolic energy (adenosine triphosphate) can still be produced by the processes of cyclic photophosphorylation or respiration. When dissolved in seawater at an external concentration of 6 $\mu M$, DCMU inhibited boron efflux, compared to the control, by 24 percent, while photosynthetic carbon fixation was reduced by 77 percent (Fig. 2). Progressively more inhibition of boron efflux, concomitant with inhibition of $^{14}CO_2$ fixation, was observed with increasing DCMU concentrations, such that 24 $\mu M$ DCMU produced essentially complete inhibition of both processes within an hour. These results would occur if some newly formed organic compound (photosynthate) were the source of organic carbon which formed complexes with boron.

Sea grasses, as demonstrated by *Halophila* and *Halodule*, would seem to offer advantages to elucidating the primary function of boron in higher plants. Since leaves of most other higher plants are not bathed in water, light-induced fluxes of boron, necessarily limited to within these plants, would be much more difficult to observe. However, in such plants, compartmentalization of boron in specific leaf tissues, or translocation to and from various plant parts, may be mechanisms under photocontrol, which regulate boron metabolism.

This information may also have a bearing on the restriction of sea grasses to growth in salt water. Boron, at 4.5 $\mu g$/ml, is about 500 times more abundant in salt water than in freshwater; and it could represent an exaggerated nutritional requirement for these salt-tolerant angiosperms.

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3. A comparative survey of boron contents of aquatic plants revealed that five species of southern Texas sea grasses (families Hydrocharitaceae and Potamogetonaceae) contained much higher levels of boron (dry weight) than seven marine macroalgae (*Enteromorpha, Ulva, Padina, Dictyota, Gracilaria, Centroceras*, and *Rhodymenia*), three salt-marsh halophytes (*Spartina alterniflora, Sporobolus virginicus*, and *Salicornia bigeloveii*), and four freshwater macrophytes (*Elodea, Vallisneria, Ceratophyllum*, and *Potamogeton*). The boron content of sea grasses ranged from 350 to 1000 $\mu g$/g (average, 625), whereas the organisms with the next highest concentration of boron were marine algae, showing 50 to 270 $\mu g$/g (average, 175). Boron in the three halophytes averaged 36 $\mu g$/g, and the freshwater plants averaged 40 $\mu g$/g. On the basis that the dry weight was equal to 15 to 20 percent of the fresh weight, the boron content of sea grasses represents a 20- to 25-fold concentration of boron over the 4.5 mg/liter usually found in seawater.
4. For analysis, single shoots, each consisting of a portion of the stem and six to seven leaves, were harvested at random from one rhizome and dried at 90°C. Boron was assayed by a colorimetric method [W. T. Dible, E. Truog, K. C. Berger, *Anal. Chem.* **26**, 418 (1954)] with the use of the curcumin reagent. Plant material was dry-ashed in the presence of $Ca(OH)_2$ at 550°C. Interference by $NO_3^-$ and Fe was checked and was insignificant.
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## Rejection of Male Skin Grafts by Splenectomized Female Mice

Abstract. *Female mice of the C3H strain normally do not reject skin grafts from males of the same strain; however, 40 percent of splenectomized C3H female mice completely rejected C3H male skin grafts applied 2 weeks later. All splenectomized females showed at least transitory signs of graft rejection.*

In some inbred strains of mice, females reject skin grafts from males of the same strain (*1*). In other strains, including C3H, male to female grafts survive indefinitely (*2*). Nevertheless, C3H/An females that have accepted C3H male skin grafts produce antibody to the H-Y antigen (anti–H-Y), that is demonstrable by reaction with male but not female cells. Furthermore, some C3H/An females that have accepted one male skin graft may reject a second applied 3 to 6 weeks after the first (*3*). Thus, the survival of C3H/An male skin on C3H/An females is not due to failure of the grafts to elicit a response to H-Y. It has been suggested that anti–H-Y might actually protect the grafts (*3*), as in immunological enhancement of tumor allografts (*4*, *5*).

Prehn (*6*) has reported that the successful transplantation of tumor allografts was reduced when the recipients had been splenectomized. This might be construed as immunological enhancement, and suggested that splenectomy might facilitate the rejection of male skin grafts by C3H/An females. Although there were no outright rejections, C3H male skin grafts underwent a transitory crisis in splenectomized females that was

Table 1. Rejection of C3H male skin grafts by splenectomized C3H females.

| Treatment of C3H female recipients | Mice grafted (No.) | Crisis* | Rejected† | Day of rejection |
|---|---|---|---|---|
| Splenectomy‡ | 15 | 9 | 6 | 45,61, 70,91, 91,100 |
| Sham-splenectomy | 10 | 0 | 0 | |
| None | 30 | 0 | 0 | |

*Grafts underwent transitory signs of apparent rejection during which scabs appeared. These grafts ultimately returned to a healthy state with a full crop of tail hair, although they were diminished in size. †Day when no sign of the graft remained. ‡Female recipients were either splenectomized or sham-splenectomized 2 weeks prior to receiving a male skin graft.

never seen in untreated recipients. This crisis seemed to indicate an abortive homograft response (*3*).

We have since performed similar tests with C3H/HeJ mice, and in this case 6 of 15 splenectomized females rejected C3H/HeJ male skin grafts. Male grafts on the remaining 9 splenectomized females underwent a crisis before being finally accepted (Table 1). All the controls, whether untreated or sham-splenectomized, retained their grafts throughout the entire period of observation, more than 300 days. The difference between these results and those described earlier is probably attributed to immunological differences between the two C3H sublines.

It is not clear why splenectomy converts an ineffective or abortive homograft reaction to a fully implemented rejection. Splenectomy did not dramatically reduce the titers of demonstrable anti-H-Y in the serums of grafted females (*3*). The work by Streilein and Wiesner (*7*) on splenectomy in strains of mice that reject male skin showed that removal of the spleen caused accelerated rejection of male grafts. Furthermore, the reintroduction of spleen cells into such mice abrogated the effects of splenectomy on graft survival. Because the spleen is an abundant source of certain types of suppressor cells, cell-mediated suppression offers a likely hypothesis for further study in the H-Y homograft system.

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## Avian Eggs: Thermoregulatory Value of Very High Near-Infrared Reflectance

Abstract. *Studies of the spectral reflectance of the eggs of 25 species of birds from nine families disclosed uniformly high reflectance (often above 90 percent) in the near infrared. This property is associated with the presence of the eggshell pigments protoporphyrin and the bilins. These pigments allow coloration for cryptic or other purposes with minimum solar heating, a combination not possible with the melanin pigments typical of vertebrates.*

Most ground-nesting birds utilize exposed nest sites. If they leave their eggs unattended, the clutch may be lost either to visual predators or to overheating by solar radiation. These two causes of death exert opposing selective pressures on eggshell pigmentation. A disruptive pigment pattern of brown, gray, and olive spots appears to provide cryptic protection against visual predators (*1*) for many species (*2*). However, heating by absorbed solar radiation is increased if the highly reflective (70 to 95 percent, depending on wavelength) shell is covered with dark pigment (*3*). The avian embryo is injured or killed by temperatures more than a few degrees above normal incubation values (*4, 5*), and so any increase in solar heating can be significant. Field investigators have found that overheating by solar radiation is a serious threat to the survival of unattended eggs (*5, 6*). Thus, selection due to predation appears to favor pigmentation, whereas selection resulting from solar heating appears to oppose pigmentation.

We have found that this conflict is minimized by eggshell pigments that absorb only visible radiation. The cryptic pigmentation thus does not significantly reduce the extremely high intrinsic near-infrared (near-IR) reflectance of eggshell (> 90 percent). Solar heating is thus minimized, because nearly half of the incident solar energy is in the near-IR. This result is not a unique freature of the cryptic eggs of ground-nesting birds; the bright blue eggs of some tree-nesting species also show high reflectance in the near-IR. Our absolute spectral reflectance (*7*) measurements, together with a sea-level solar spectrum (*8*) for comparison, are shown in Fig. 1.

The contrast between eggshell pigmentation and the typical gray to brown melanin pigmentation of vertebrates is illustrated in Fig. 1A. Note the spectacular enhancement of the near-IR reflectance of the eggs of Heermann's gull, *Larus heermanni* (*9*), compared to the melanin-pigmented adult feathers. The near-IR reflectance of the eggs exceeds 90 percent, even for the dark brown spots. The difference is entirely due to the absorption characteristics of melanin (*10*) versus the absorption characteristics of protoporphyrin and the biliverdin egg pigments (*11*). Figure 1B shows that the structure and index of refraction of unpigmented eggshell and feathers result in comparable near-IR reflectance.

The details of the spectral absorptance of an egg can significantly influence its cryptic value and the amount of solar radiation absorbed. Spectral absorptance can be affected by the amounts of the three principal egg pigments present [brown protoporphyrin, blue biliverdin IXa, and its green zinc chelate (*11*)] and possibly by the structure of the eggshell. Accordingly, we surveyed the eggs of 24 additional species of birds (*12*) to (i) determine the spectral reflectance of eggshell pigments in situ and (ii) determine whether high near-IR reflectance is a general property of avian eggs.

The curves for eggs of Heermann's gull (Fig. 1A) appear to be representative of protoporphyrin in situ. The curve for eggs of the tree-nesting American robin, *Turdus migratorius* (Fig. 1C), is representative of pure biliverdin IXa (*13*). The spectral reflectance of the brown-spotted blue eggs of the tree-nesting common crow, *Corvus brachyrhynchos* (Fig. 1C), shows the additive effect of combined pigments. No suitable specimens of blue-green eggs containing primarily the zinc chelate of biliverdin were available. Blue eggs show a transition to near-IR reflectance that is more rapid and occurs at a shorter wavelength than in reddish-brown eggs. This is the opposite of the published absorption curves of biliverdin and protoporphyrin in chloroform (*11*). The difference is apparently due to solvent effects and the great thickness of protoporphyrin deposits.

We examined cryptic eggs of 12 ground- or cliff-nesting larid species (including *L. heermanni*) and seven nonlarid ground-nesting species to determine the generality of high near-IR reflectance (the species are listed in the legend to Fig. 1). The reflectance curves of normal brown-spotted eggs of most species fall within the stippled region in Fig. 1C. Little variation is evident in the near-IR. No obvious trends with latitude are evident. There are, however, consistent differences between some taxa. For example, the eggs of the ring-billed gull, *L. delawarensis*, are consistently light (reflectance, 90 to 92 percent in the near-IR), whereas the eggs of the herring gull, *L. argentatus*, nesting in the same colony, are consistently darker (reflectance, 82 to 85 percent). By far the lowest near-


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