minimum yield stress (*4*). In the present case the extensive force may be considered as the sum of forces due to turgor and the weight of the core. The turgor force was eliminated by cutting off the root system, and hence, the water supply. Elongation accelerated slightly upon cutting, probably from the release of xylem tension and water. After 10 minutes, elongation stopped and the leaf started to shrink, although no obvious wilting was observed even after 30 minutes. Thus, the core weight alone was not sufficient to sustain any prolonged elongation, which indicated that the force due to the core weight did not exceed the minimum yield stress.

Another potential source of error was temperature fluctuation, which may cause differential thermal expansion. Attaching the core to a fully grown leaf and varying the temperature of the environment revealed that the measured length increased 45 $\mu$m for each degree of increase in temperature. However, since the rate of elongation of a growing leaf was around 60 $\mu$m per minute, error due to temperature fluctuation is insignificant when growth is measured for many minutes. For very brief growth measurements, it would be important to control the temperature.

Slight water deficits greatly reduced elongation. Drying of the soil, reducing leaf water potential from $-3$ bars (well-watered plants) to $-4$ bars, reduced elongation about 10 percent under the conditions used. Elongation stopped completely when leaf water potential was reduced to $-6.5$ bars, even though wilting was still not obvious. These results generally agree with those of Boyer (*2*).

Growth recovery was virtually instant (*5*) when plants were watered if the prior water deficits were not severe (Fig. 2B). It is improbable that the added water moved so rapidly to the expanding zone in the leaf. The extremely rapid response was more likely the result of the water continuum in the xylem acting as a cohesive and incompressible liquid transmitting the change in pressure and the associated small change in volume from roots to leaves.

There was a transitory period of fast elongation upon watering, which changed to a slower steady rate several minutes later. If we assume that growth rate is the product of gross extensibility and turgor pressure (*1*), the fast rate in the transitory period suggests that the gross extensibility was increased by water deficits. Turgor pressure during this period should be lower than that associated with steady-state growth. Therefore, extensibility must be higher to account for the faster rate.

The rapidity of the response in elongation points to the essential role of water in providing the driving force for cell enlargement. The transitory fast elongation following watering suggests that the main factor in reducing growth during mild water deficits is the reduced driving force, not alterations in metabolism. Apparently, accumulation of metabolites necessary for growth during the period of water deficiency permitted the transitory fast elongation when turgor pressure suddenly began to increase.

With more severe water deficits, however, plant response was different (Fig. 2C). There was a lag after watering before elongation gradually resumed. It is expected that with greater water deficits in the cells more time would be needed for the turgor pressure to increase to the minimum yield stress. After the initial lag, the transitory rapid elongation was again observed (Fig. 2C).

The detail in elongation that this method is capable of resolving is demonstrated in another experiment that determined the effect of varying light intensity (Fig. 2D). Elongation was greatly reduced within a few minutes when light was increased from about 550 to 44,000 lu/m$^2$, conditions that are expected to increase leaf temperature, stomatal aperture, and transpiration. Upon return to the low light, elongation not only resumed in a few minutes but also showed a transitory fast period similar to the response to watering. Although the results can be best explained in terms of changes in transpiration and resultant changes in leaf water balance and turgor, the possibility is not ruled out that growth was affected through other effects of light.

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3. After completion of this study, we were told that G. Meijer [*Acta Bot. Neer.* **17**, 9 (1968)] monitored the inhibition of elongation of dark-grown gherkin hypocotyl by blue light, also with an LVDT.
4. Minimum yield stress is the threshold force above which the plant tissue begins to deform irreversibly. J. A. Lockhart, in *Plant Biochemistry*, J. Bonner and J. E. Varner, Eds. (Academic Press, New York, 1965), p. 826.
5. The lag of several or more seconds, not obvious in Fig. 2B because of the scale, is accounted for, at least in part, by the time required for water to permeate the soil to the root zone.
6. Supported in part by grant B-029-CAL from the Office of Water Resources Research, United States Department of the Interior, by a grant from the Water Resources Center, University of California, and by NSF grant GB 5658.

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## Vitamin $B_{12}$ Binders of Chicken Serum and Chicken Proventriculus Are Immunologically Similar

Abstract. *Two substances that bind vitamin $B_{12}$ are found in chickens, one in the serum and another in the proventriculus. Their molecular weights, as estimated from gel filtration on Sephadex, are approximately 113,000 and 96,000, respectively. Antibody elicited in rats against the proventriculus binder reacts against both binders.*

Two immunologically different substances that bind vitamin $B_{12}$ are found in rats and mice; these are intrinsic factor (IF) and transcobalamin II (TC-II). They are found in the gastric mucosa and serum and are synthesized in the gastric mucosa and liver, respectively (*1*). Another binding substance of higher molecular weight (110,000 as compared to 55,000 for IF and 38,000 for TC-II) is found in human serum, saliva, and gastric mucosa. This has been termed R binder or transcobalamin I (*1*). These three immunologically distinct $B_{12}$ binders probably arose from a single ancestral $B_{12}$ binder during evolution, but little is known about the types of $B_{12}$ binders found in nonmammalian vertebrates.

Chicken serum has a high concentration of substances that bind $B_{12}$ (*2*). Our experiments show that this binder is similar in size to R binder, immunologically similar to the binder found in chicken proventriculus, and immunologically distinct from the R binder.

The extent of $B_{12}$ binding in serum

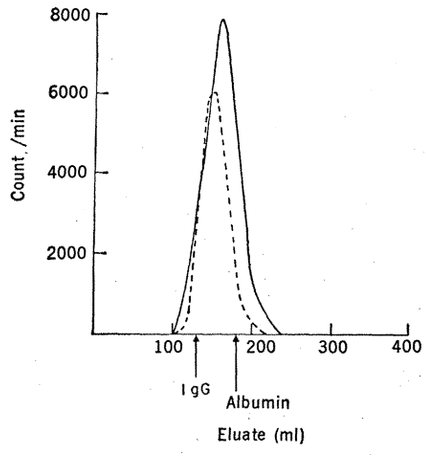


Fig. 1. Cochromatography of the substances which bind vitamin $B_{12}$. The dashed line is the serum binder labeled with [$^{60}$Co]-$B_{12}$. The solid line is the proventriculus binder labeled with [$^{57}$Co]$B_{12}$.

and in a supernatant made from a crude homogenate of proventriculus (1:5, weight to volume in saline; ground in the Sorvall Omni-Mixer at full speed for 5 minutes; centrifuged 3000*g* for 15 minutes; 4°C) was estimated by the zirconyl phosphate gel (z-gel) technique (*3*). The homogenate was purified by gel filtration on Sephadex G-100 columns (2.5 by 100 cm) eluted with *p*H 7.0 tris-HCl buffer (0.1*M* tris) made in 0.9 percent saline, with 0.02 percent azide added as preservative.

Antibody was elicited in rats by injection of a crude perchloric acid extract of binder saturated with vitamin $B_{12}$ prepared in the following manner. Supernatant from five proventriculi was prepared as above. An equal volume of 1*M* perchloric acid was added. The supernatant, after centrifugation, was neutralized with $NH_4OH$, reduced to 5 ml in a Diaflo ultrafiltration cell (No. 50) fitted with an XM 10 membrane and placed on a Sepharose 6B column. The peak eluted with the above buffer was pooled and dialyzed to 5 ml against Carbowax; 90 percent of the binder was recovered.

The partially purified complex of proventriculus binder and $B_{12}$ (saturated binder) was incorporated in an equal volume of Freund's complete adjuvant, and 0.1 ml of this was injected in each rear foot pad of two rats. Two weeks later, these rats were again injected in this manner, and 3 weeks after this, there was a third injection of 0.25 ml in the foot pads. Blood was collected by heart puncture 1 week after the last injection. Antibody that reacts with the $B_{12}$-binding site of the binders (CSAB) and antibody which reacts with $B_{12}$ saturated binder (CAB) were assayed as described (*4*). Titers were determined at 50 percent of total binder neutralization.

Cobalt-labeled vitamin $B_{12}$ was obtained in two forms: (i) a very high specific activity [$^{57}$Co]$B_{12}$ (71.4 to 173 mc/mg; Philips-Duphar, Holland) and (ii) [$^{60}$Co]$B_{12}$ (1 $\mu$c/1.484 $\mu$g; Squibb, New York). Radioactivity was counted in a Packard (gamma) scintillation spectrometer.

Figure 1 is the elution pattern of the binders in serum and proventriculus, with human serum marker. The serum binder was labeled with [$^{60}$Co]$B_{12}$ and the proventriculus binder with [$^{57}$Co]$B_{12}$. The size of these binders is approximately 113,000 and 96,000, respectively. Extraction with perchloric acid did not alter the size of either binder. This has been further confirmed by chromatography of these binders with R binder from human gastric juice. As estimated by the gel filtration method, R binder has a molecular weight of 110,000. On chromatography the chicken serum binder moves with or slightly ahead of R binder, while the proventriculus binder migrates slightly behind R binder.

The CSAB elicited against proventriculus binder reacted equally with both binders [907 and 903 neutralized units (1 unit = 1 ng of $B_{12}$ bound)], whereas CAB cross-reacts to a slightly lesser extent with serum binder as compared with proventriculus binder (853 and 1047 neutralized units per milliliter). The binding sites are probably identical. The different CAB titers might be expected since the difference in size of the binders indicates some structural difference. The R binder did not cross-react with this antiserum.

The serum binder may be a proventriculus binder with a secretory piece added or these binders may be made on similar genes in different organs. If $B_{12}$ binding proteins evolved from a common ancestral protein, as amino acid sequence analysis has suggested for a variety of other proteins (*5*), then chickens with immunologically similar $B_{12}$ binders in the serum and gastrointestinal tract may represent an intermediate evolutionary stage between an organism with a single binder and mammals which have evolved immunologically distinct binders in the serum and gastrointestinal tract.

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## p,p′-DDT: Effect on Calcium Metabolism and Concentration of Estradiol in the Blood

Abstract. *Ringdoves given 10 parts per million* p,p′-*DDT showed a decrease of estradiol in the blood early in the breeding cycle and egg-laying was delayed. There was also a decrease in deposition of medullary calcium and in eggshell weight. Injection of* p,p′-*DDE (150 milligrams per kilogram of body weight) caused reduction of eggshell weight and inhibition of carbonic anhydrase in the oviduct.*

The decline of several species of raptorial birds in Europe and North America has been linked to the use of chlorinated hydrocarbon pesticides (*1*). The symptoms noted during the decline include some or all of the following: (i) abnormally late breeding; (ii) failure to lay eggs (in some cases, laying was followed by egg-eating); (iii) reduced clutch size; (iv) failure to lay again after early loss of eggs; (v) thinner eggshells and increased egg breakage; and (vi) increased embryonic mortality. The total picture can be termed the "raptor-pesticide syndrome" because it was first observed in the falconiformes, although it has now been found in a number of fish-eating birds. I examined the possible underlying pathophysiologic changes.

All experiments were performed on paired ringdoves (*Streptopelia risoria*)