geneous during the whole second reaction stage. This means that the biosynthesis of the secondary wall cellulose reacts by a structure-controlled mechanism (template mechanism).

With regard to the primary-wall cellulose I found no results which point to a similar template mechanism. The corresponding $DP_w$ is much smaller and its distribution very broad. In this period, a high quantity of materials other than cellulose is also produced.

On the basis of these results I have formulated the following explanation for the findings of Hassid: the cell-free synthesis of cellulose by means of enzyme preparations from cotton bolls corresponds to the first reaction period leading to the primary cellulose. Since *in vivo* during this stage other compounds than cellulose are also formed, it is probable that this also happens *in vitro*. The enzyme seems to lose its activity with an increase in the age of bolls, since I have found that the synthesis of the primary cell wall is finished only a short time after the beginning of the secondary wall synthesis[6]. The enzyme responsible for the syntheses of the secondary wall cellulose may be not the same and may react only in presence of a template. Assuming that the template was not yet formed or was destroyed during preparation of the enzyme for the cell-free synthesis, one could explain the great decrease in the amount of formed cellulose using the 21-day-old cotton bolls.

Fig. 1 shows the values found by Hassid with my kinetic results. Although the conditions of growth of the cotton plants were different, the coincidence of the age of the bolls at which the cell-free synthesis loses its activity, and the biosynthesis of the primary cellulose in the cotton bolls finishes, is remarkable.

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## Kinetics of the Biosynthesis of Cellulose in Cotton Bolls by Different Light Intensities

PREVIOUS kinetic investigations of the biosynthesis of cellulose in higher plants suggested that the secondary wall cellulose is synthesized by a structure-controlled mechanism (template mechanism)[1-5]. In order to confirm my assumption I changed the reaction rate by varying the intensity of illumination of the cotton plants. Cotton plants (*Gossypium herbaceum*) were grown in a glass-house at a mean temperature of 25° C (the night temperatures never were less than 18° C). The intensity of illumination amounted to 4,000, 15,000 and 60,000 lux respectively (mean values). The time of maturity was calculated from the time the flowers were fertilized. Bolls of different maturities were picked, immediately opened and the seedhairs quantitatively removed from the seeds. The methods used to determine the conversion of cellulose and the degree of polymerization have already been described[1,2].

If the secondary wall cellulose is synthesized by a template mechanism, then the degree of polymerization must be independent of any variation in the kinetics. The results in Fig. 1 show that this is the case. In spite of the considerable variation of the rate of synthesis with light intensity, the degree of polymerization remains

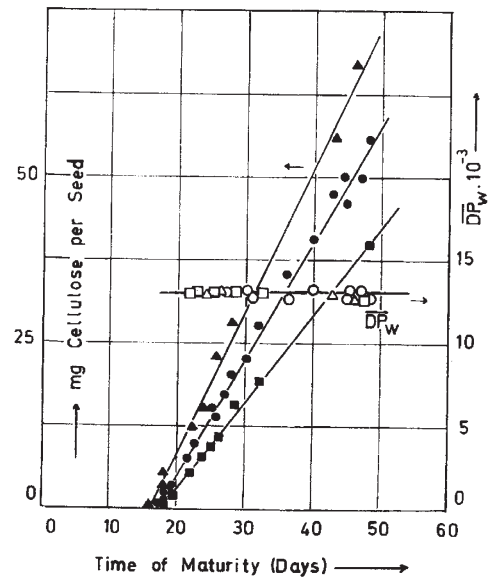


Fig. 1. mg Cellulose per seed and corresponding degree of polymerization (weight average) with time of maturity at different intensity of illumination. ▲, 60,000 lux; ●, 15,000 lux; ■, 4,000 lux; △, 60,000 lux; ○, 15,000 lux; □, 4,000 lux

constant at approximately $DP_w = 13{,}000$, independent of the conversion and the rate of synthesis.

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## Bromination of Fluoresceins by the Dogfish, *Squalus acanthias*

THE natural occurrence of bromo-compounds in the coral fan *Gorgonia verrucosa* and in certain species of shellfish, especially *Murex brandaris* and *M. tranculus*, is well known[1,2]. Bromination of phenol red by an enzyme system isolated from the mould *Caldariomyces fumago* has been observed[3]. *In vitro* bromination of phenol red by uterine slices and bromination of phenol red, chlorophenol red, metacresol purple and fluorescein after intra-uterine administration of these dyes to the pregnant spiny dogfish, *Squalus acanthias*, have been reported[4,5]. The work recorded here is an extension of the latter observations.

Pregnant spiny dogfish were kept in a wooden enclosure with free access to sea water at 14°–15° C. The uterine fluid was withdrawn by pipetting through the uterine pores which open into the cloaca and was replaced with a saturated solution of the dye in sea water. Various dilutions of the dyes were also used. At the end of the experiment, generally 48 h after instillation of the dye, the uterine fluid and resultant dye were easily collected by pipetting. The spectra of the dyes in the visible region before and after instillation were recorded and compared with authentic samples. Paper chromatography was performed on Whatman 3 *MM* paper in systems previously reported[4,6].

Forty-eight hours after intra-uterine administration of fluorescein, the resultant product was found to be eosin (tetrabromofluorescein). Thus, the sulphonic acid moiety present in the phenosulphone phthalein dyes, which are brominated, could be replaced by the carboxylic group