

nine tyrosyl⁹ residues. Therefore, any conformational change induced by the phosphorylation of the active site of the enzyme would be reflected more easily by changes of the tryptophyl spectra in chymotrypsin and by changes of the tyrosyl spectra in trypsin. It should be noted that the DIP-trypsin versus trypsin difference spectrum exhibits a maximum at 285–287 m μ (Fig. 1). From the position of this peak, it is probable that tyrosyl residues are responsible for the difference spectrum^{8,10}.

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Intensification of Absorption Spectra

Keilin and Hartree¹ have described the sharpening and intensification of absorption spectra of haem compounds which occurred on making the observation at liquid air temperatures. They observed that the intensification was due to light scattering by the ice crystals and could be duplicated by placing the cytochrome in a kaolin suspension. They also described a procedure for freezing the cytochrome in a glycerol–water mixture to a glass followed by devitrification by warming slightly and then refreezing the translucent material to give ten-fold intensification.

Usually a five-fold intensification of absorption occurs on quick freezing a cytochrome in aqueous solution. A technique has been developed that permits approximately forty-fold intensification without additives.

Small ice pellets were prepared from aqueous solution of reduced cytochromes by forcing the solution from a syringe through a fine needle (22 gauge) into a container of liquid nitrogen. Care was taken to keep the syringe in motion so that the droplets did not coalesce as they froze. The pellets formed ranged from 2 mm to a small fraction of a millimetre.

The pellets settled to the bottom of the container and were collected and placed in a Potter–Elvehjem homogenizer² that had been pre-cooled in liquid nitrogen. Homogenization by hand operation of the pestle gave an extremely fine ‘snow’. The ‘snow’ was firmly tamped into a cuvette with plastic windows and placed in a low-temperature attachment for the Cary model 11 spectrophotometer³.

Fig. 1 shows the intensification of absorption that occurs at various stages of the process already described. The 25° spectrum was made in the same 5-mm light path cuvette that was used for the low-temperature spectra and with a water blank in the reference beam. The low-temperature spectra were made with three layers of thin cellulose tissues in the reference beam. Direct comparison gives a twenty-fold intensification. However, when the packed snow melted, the solution about half filled the cuvette; thus the total intensification achieved was more than forty-fold.

Since exact reproduction of the homogenization and tamping is difficult to achieve, we believe that this pro-

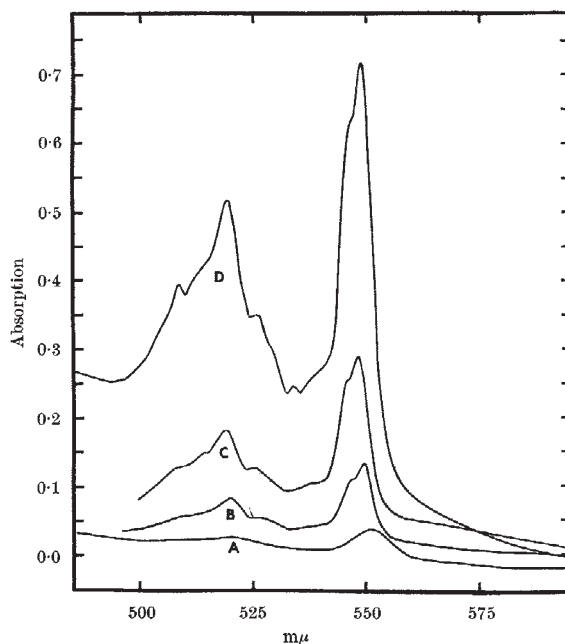


Fig. 1. Absorption spectra of cytochrome *c* reduced by cysteine. *A*, At 25° C; *B*, frozen pellets as collected at 81° K; *C*, less than 1 mm pellets; *D*, tamped ‘snow’ from homogenization. All spectra were made on aliquots of the same solution and in the same cuvette.

cedure would be most useful in qualitative identification of pigments in very dilute solutions, or in cases where only minute amounts of a pigment could be obtained. In many cases it would be possible to make this type of observation without contamination of the pigment solution and with negligible loss.

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PHYSIOLOGY

Failure of Progestogens to prolong Pregnancy in the Guinea Pig

PROGESTERONE treatment in cases of threatened abortion and premature labour has been of little therapeutic value^{1–3}. These rather unexpected results, in view of the now classical work of Corner and Allen^{4,5}, have brought into question the plausibility of the ‘progesterone theory for the maintenance of pregnancy’ when applied to the human being⁶. Recent development of the concept of the local action of progesterone^{7–10}, however, has provided grounds for considering pregnancy maintenance in terms of a ‘basic mechanism’¹¹, which allows for species variation with regard to ovariectomy and response to oxytocin and progesterone treatment. This theory states