

of the hypnotics by the brain. Animals killed at various times after injection of the hypnotics showed no significant difference in the concentration of barbiturate or alcohol in the brain, regardless of whether or not the animals had also received chlorpromazine. This indicated that the effects of the potentiator did not result from an increased concentration of the hypnotics in the brain.

Reserpine, which exerts tranquillizing effects similar to those observed with chlorpromazine, was also found to potentiate markedly the effects of hexobarbitone and ethanol. Its effect on the latter compound was especially striking (Table 2). Like chlorpromazine, reserpine did not affect the rate of bio-transformation of the two hypnotics (Table 2) or alter their uptake by the brain.

The mode of potentiation induced by chlorpromazine and reserpine is obviously different from that of *SKF 525-A*. This difference was shown in another way. When *SKF 525-A* was given intravenously to mice or dogs which had just recovered from hypnosis induced by hexobarbitone, the animals were not visibly affected; but if chlorpromazine was given, the animals reverted almost immediately to a deep hypnosis. This shows that chlorpromazine causes subhypnotic amounts of hexobarbitone to become hypnotic, and is therefore a true potentiator, in contrast to *SKF 525-A*, which acts merely as a prolonging agent.

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### Phosphorylase Activity in Relation to the Layering of Starch Granules

ARTHUR MEYER firmly believed that the layering of starch granules corresponded to the alternation of day and night, and this belief is still shared by the authors of modern text-books. If this were true, then layers should disappear when starch granules develop under constant outer conditions of illumination, temperature and moisture. The only positive result of such an experiment was reported by van de Sande-Bakhuyzen for wheat. All other results appear to be negative. Salter found layers when starch granules grew in darkness, Fischer observed them after constant illumination, and according to Küster more than two layers could be deposited during one day.

Recently, similar results have been obtained for potato starch with more accurate methods<sup>1</sup>. Therefore, the layers must be deposited as the result of an inner rhythmic process, which, of course, might accidentally coincide with an outer rhythm. One possibility would be a fluctuation in enzymatic activity, as suggested by Bünning and Hess<sup>1</sup>. A

day-night periodicity has been reported by Russian investigators for the phosphorylase activity of potatoes, and Shaw<sup>2</sup> mentions the possibility of a seasonal fluctuation in wheat under the influence of light.

We have measured phosphorylase activity in young actively growing potatoes of about 1.5 cm. diameter during 24-hr. periods at 6-hr. intervals. Stem cuttings of potato plants were planted in washed river sand in a greenhouse and allowed to root and form small tubers. All cuttings were grown in one container and received the same treatment. 20–30 gm. sliced tubers were treated with cold sodium hydrosulphite, extracted with 0.05 M cold citrate buffer pH 6.5 in a blender for 3 min., the extract filtered and centrifuged. 1 ml. of the extract was allowed to react with 1 ml. of substrate containing sodium fluoride, mercuric chloride, soluble starch and glucose-1-phosphate in quantities indicated by the work of Porter<sup>3</sup>. The pH was kept at 6.5 with citrate buffer and the temperature at 30° C. After 5 min., the reaction was stopped with cold trichloroacetic acid, the pH of the mixture brought down to 4 with sodium acetate, and inorganic phosphate estimated by the method of Waygood<sup>4</sup>. All determinations were done in duplicate. The results of two series, one starting at 6 a.m., the other starting at 10 a.m., are given in Table 1.

Table 1

Time of sampling	Phos./min./gm. fresh weight	
	I	II
0	36.6	
4		31.2
6	34.3	
10		33.0
12	32.4	
16		33.8
18	37.4	
22		31.5

The results do not show an appreciable diurnal variation of phosphorylase activity, and therefore it is more likely that the shells of a starch granule find their origin in rhythmic depositions conditioned, for example, by periodically occurring diffusion gradients.

As it has now been well established that phosphorylase is located in the plastids, but that it escapes from these structures as soon as the cells are damaged<sup>5</sup>, we can assume that determinations in extracts from comminuted materials give the total amount of phosphorylase available. However, as Russian investigators (cited by Weier and Stocking<sup>6</sup>) noticed stronger absorption of invertase by plastids in the light than in the dark, the possibility of similar rhythmic processes in the plastids of the potato cannot be entirely excluded. It is also possible that, as indicated by recent work<sup>6</sup>, phosphorylase is less involved in starch synthesis than we hitherto thought.

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