

From these results we assume that carbonic anhydrase catalyses the absorption of carbon dioxide in the presence of chlorophyll b in the plant body. The combination of carbon dioxide with chlorophyll b seems to occur in the two -NH radicals of tetra-pyrrole forming carbamate.

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## 68. Studies on Biocatalyses. (XII)

On the Trace Metals in Chloroplasts.

*Kinsuke Kondo, Shigeki Mori and Morikazu Kajima.*

The green leaves (Plant, Pepper, *Capsicum anuum* var *grossum*), previously soaked thoroughly in ice-cold water for one hour, were ground, pressed and filtered through thick cloth.

The filtered juice were kept for 10 hours in a refrigerator at 0~2°C. By centrifuge, the chloroplastic matter was separated at the bottom and cytoplasmic matter as well as water soluble substances on the upper side.

On the two fractions, the trace metals, that is, iron, copper, zinc and manganese were analysed, and as the result we found that the greater part of iron (60% of total Iron) was accumulated in chloroplastic matter and 11.7% Cu, 15.1% Zn and 12.6% Mn, each of their total amount, respectively were localized in this part. But it is uncertain, whether they are actually the essential constituents of chloroplasts or not.

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## 69. Polarographic Studies of Serum Protein. (I)

On the Brdička Denaturation Test.

*Tokio Sasai and Masao Egawa.*

We have studied the digest reaction of polarographic cancer tests (Brdicka's), regarding its fundamental conditions of serum denaturation. Measuring the heights of protein double-wave obtained in cobaltous ammonium buffer solution, we called them  $H_1$  and  $H_2$  respectively. Since the  $H_1$  and  $H_2$  changed always correlatively and the change of  $H_2$  was greater than that of  $H_1$ , we adopted the change of  $H_2$  as an indicator, and called the higher one "activated" and the lower one "inactivated". Although the change of the height was almost negligible when serum was left at room temperature for a week, the height in 60°C water-bath increased about 50% in 2 minutes and then decreased. In case of the alkaline denaturation ( $\frac{1}{2}$  N. KOH) at room temperature, the height was at its maximum

in 5 minutes, about 2 times as the native, and then gradually decreased. After 3 hours, the height became lower than that of the control. In 60°C water-bath this alkaline effect was so rapid that the height in 10 minutes corresponded to that in 24 hrs. Besides the temperature, the slope of this denaturation-time curve was found to be influenced regularly by KOH concentration; the higher the concentration, the steeper the slope.

The quantity of serum protein acted merely as a deciding factor of magnitude of this curve; but a protein-corrected test on a case of hypoproteinemia showed lower wave height. Therefore, Brdicka reaction is considered to be that of qualitative change after all. Furthermore, we found some interesting facts that the curve flattened and the peak (maximum activation) lagged in the pathologic serum such as cancer or portal cirrhosis. From the above results, it may be said that the denaturation, especially alkaline one, might be a combination of two phases, i. e. phase of activation and that of inactivation, which will be characterized by temperature, agent and the nature of serum itself.

#### References:

- 1) Brdicka, R.; Klin. Wochenschr, 18, 305 (1939).
- 2) Müller, O. and Davis, J. S.; J. Biol. Chem. 159, 667 (1945).
- 3) „ „ „ ; Arch. of Biochemistry, 15, 39 (1947).

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## 70. Studies on the Mechanism of the Formation of Polysaccharides. (XIII)

On the Carbohydrate Activator of Starch Formation by Potato Phosphorylase. (1).

*Yoshiyuki Inouye, Konoshin Onodera, Hiromu Kisaki  
and Keizo Hayashiya.*

It has been recently reported (Cori and Cori, J. Biol. Chem., 137, 773 (1940); Hanes, Proc. Roy. Soc., B, 119, 174 (1940); Green and Stumpf, J. Biol. Chem., 142, 335 (1942); Hidy and Day, J. Biol. Chem., 160, 273 (1945); Swanson and Cori, J. Biol. Chem., 172, 815 (1948)) that potato phosphorylase needs some amount of polysaccharide as an activator in the process of starch formation and that the activity of the activator depends on the chain length of the polysaccharide. No detailed description is, however, found about the methods of preparing the activator and the determination of the chain length.

The experiments reported in this paper are concerned with the ability, as activator, of polysaccharides prepared from potato starch which had been hydrolyzed under mild condition, and fractionated successively with ethanol of different