X. THE EFFECT OF CERTAIN BASES ON THE ACTION OF ENZYMES

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The forces controlling cell division, their nature and mode of action have called forth a great amount of investigation and comment within the last few years. The problem is a very complex and perplexing one and a great amount of varied evidence must be considered before any definite conclusions can be drawn.

Laughlin has pointed out that the activity of dividing cells is controlled by definite physical, electrical, and chemical factors and that before this process can be understood, these factors must be measured. A great many of these factors have

been found to exert great influence upon the rate and position of the spindle in cell division.

The evidence indicates that the underlying controlling principle of mitosis is a delicately adjusted chemical reaction within the cell. As the cell is living substance, this chemical reaction must be primiarly organic. That the autolytic enzymes must therefore play a great role in the process of cell division has been suggested by Mathews, Packard, and others. If the process is dependent upon the enzymes present within the cell, then any substance or factor that will change the rate of activity of enzymes would give a similar change in the division rate when applied to living cells. Or, any factor that would accelerate cell division should therefore give a corresponding acceleration, when applied to enzymes.

The background of the present work is briefly as follows— Dr. A. Richards working with Planorbis and a little later with Cummingia eggs demonstrated that the division rate could be accelerated and retarded by the use of X-rays. The effect of X-radiation was then tried on isolated enzymes and quite surprisingly the acceleration and retardation curves secured for the digestive action of the enzymes were similar to those obtained for the division rate in these eggs. The evidence obtained from these experiments pointed strongly to a direct correlation between the action of X-rays on enzymes and their effect on the rate of cell division.

Still later, an acceleration in the eggs of Haminea virescens was secured by very small amounts of bases; 0.004 to 0.009 percent sodium hydroxide, 0.006 to 0.009 percent ammonium hydroxide and 0.006 to 0.009 percent potassium hydroxide, also by thyroid extract and pilocarpine in weak concentrations.

If the agents, which had been employed to accelerate the division rate in Haminea, could be found to exert a similar effect on isolated enzymes, a more definite, if not positive proof, that the acceleration was due to the enzymes contained in the cell, would be established.

The literature on the subject of optimum hydrogen-ion concentration for enzyme action, is in a state of great confusion. There may be many reasons for this confusion, such as nonconformity of criteria for neutrality, impure enzymes, and impurities in the materials used. Again the presence of electrolytes is necessary for enzyme action and their neglect has often been responsible for conflicting results. Baswity, Kjeldahl, Detemers, Duggan, and others, and more recently H. C. Sherman and his

co-workers have described the optimum condition for enzyme activity as alkalinity, neutrality, and acidity.

In our own experiments three types of enzymes were employed, namely, those represented by pepsin, pancreatin, and taka-diastase. The pepsin was the flake pepsin prepared by Merck and was used in a slightly acid medium, to test the action of alcohol and atropine. The pancreatic amylase was Mercks pancreatin and made up into a neutral solution with distilled water. The taka-diastase was that prepared by Parke-Davis in the liquid form diluted one to three with distilled water. In each case only one sample was the source of supply so that the differences in preparation could have no influence. A control was run with every set of experiments.

As a means of measuring the activity of the enzymes the process developed by Mett was employed with egg white, starch paste, and gelatin. The lengths of the tubes were measured by means of a microscope and a mechanical stage, the lengths being accurately read on the vernier. After this had given definite results, a check by titration with Fehling's solution was resorted to, which proved the more accurate of the two and was used throughout the remainder of the work. The starch used was Merck's soluble starch and was heated under the reflux with distilled water until a clear solution was obtained.

In order to be sure that the results obtained were due to the basic properties of the agents employed and not to their electrolytic power, the necessary electrolyte was secured by the addition of an optimum amount of sodium chloride.

To facilitate the comparing of the present results with the former work, the same standard of measurements was employed and no attempt was made to find the final hydrogen-ion or hydroxl-ion concentration of the solution.

The results of these experiments are briefly—

The hydroxides of sodium, potassium, and calcium gave definite acceleration and retardation curves, but a great deal of irregularity was encountered with the hydroxides of strontium and barium. This was possibly due to their insolubility in water.

In the case of these insoluable hydroxides a large excess of the agent was ground up under water and the amount in solution calculated from the solubility at room temperature. This solution was then diluted to secure any desired percentage. Very dilute solutions were found to exert no influence upon the action of the enzyme, even when sufficient quantity was added so that the total percentage exceeded that in the experiments where

the more concentrated solutions were employed. (This phenomenon could not be accounted for, and should be worked out more fully. Concentrated solutions of each of these however had a retarding effect. Calcium gave a very slight but definite acceleration. Barium however gave irregular results, at times showing regular curves and at other times, with no apparent reason, the most irregular behavior. Strontium acted very much like barium but the retarding effect was greater.

These results are in full harmony with the results obtained by the more recent workers such as Effront and Sherman together with their students.

In the case of ethyl alcohol, no retarding effect appeared and in a good many cases no acceleration. The cases where acceleration did occur, might be accounted for by assuming that the optimum concentration of the added electrolytes had not been reached and that the alcohol added, partly ionized and furnished this electrolyte. However, considering the fact that the electrolyte was furnished by a comparatively large amount of sodium chloride, the alcohol, in itself, did have some slight accelerating effect on the action of the enzymes. A few cases were secured where a slight retardation was observed but this is believed to be due to the salting out effect of the alcohol and not to any direct effect. The fact that the alcohol had no retarding effect on the enzymes is in full accord with other workers and with the methods of preparing enzymes. In almost every case, some time during the preparation of an enzyme, alcohol is used as an extractive or as a precipitating agent. Considering these facts its seems safe to conclude that alcohol has little if any retarding effect (proper) upon enzymes.

A considerable amount of work has been done on the action of organic substances upon enzymes, and especially the effect of adding organic bases to the substrate. As these organic substances were not at hand, and could not be secured and utilized in the time alloted to these experiments their action could not be tried. The only organic base, outside of alcohol, tried was atropine and with it definite acceleration was secured. It appears that it's action was due to the basic properties, a conclusion which is in full agreement with Sherman. Although even in acid medium it seemed to have a slight effect.

With such seeming confliting results the action of enzymes appears, at first thought, to have no relation to the activity of cells. But when the acceleration of enzymes, that have the same action or are very similar to the autolytically active proteolytic

types found in various cells, is compared with the acceleration in the divisionn rate of cells, the similarity is striking. Again when the importance of the enzymes found in cells during pathological conditions is considered with relation to the alkalinity or acidity of the cell fluids, such as the lymph and blood that nourish such areas, the evidence is all the more clear. The changing of proenzymes to enzymes and the action of antienzymes has been known for a long time and therefore will not be discussed here. The normal alkaline reaction of the blood and tissue fluids, which changes to an acid condition in pathological conditions and in death, is equally well known.

Judging from the above evidence it is suggested that in all possibility the alkaline reaction needed for living tissue has an accelerating effect upon the anabolic enzymes and a retarding effect upon the catabolic enzymes, which work best in an acid medium.

Thus we may say with security that in normal cell life the enzymes play a very important role, and we tend more and more toward the view that the chemical transformations in the living cells are brought about by enzymes, and that these enzymes are to be considered as the chemical tools of the cell. These tools as in the case of any tool can be sharpened or dulled by physical and chemical agents.