Report of Dr. Avery (assisted by Dr. McCarty)

Studies on pneumococcus. The results of earlier work on the chemical nature of the substance inducing transformation of pneumococcal types were briefly summarized in the last report to the Board. This year a paper dealing in more detail with the chemical properties and biological specificity of the active principle was published in the February issue of the Journal of Experimental Medicine. Further work is now in progress on certain aspects of the problem, the elucidation of which, it is hoped, may provide additional evidence in support of the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming agent. In order to establish the validity of this concept and to acquire a fuller knowledge of the biochemical factors involved in the phenomenon of transformation in vitro, the following problems are under investigation at the present time: (1) the isolation and purification from animal tissues of the enzyme capable of depolymerizing desoxyribonucleic acid; (2) the reversible inactivation of the transforming principle by known chemical substances.

1. Desoxyribonucleodepolymerase. In the paper referred to above it was pointed out that among various crude preparations of enzymes tested only those that proved capable of depolymerizing authentic samples of desoxyribonucleic acid were found to destroy the biological activity of the transforming substance. This was true irrespective of the presence of other enzymes such as proteases, phosphatases, or esterases and regardless of whether the preparations were obtained from pneumococcal cells, from the blood serum or organs of different mammalian species. The fact that enzyme preparations which depolymerize desoxyribonucleic acid of animal origin also inactivate the transforming substance and the additional fact that

the enzymes involved in both of those reactions are inactivated at the same temperature and are inhibited by sodium fluoride are presumptive evidence that the transforming principle itself is a nucleic acid of the desoxyribose type. Consequently, it seemed of prime importance to isolate this enzyme in pure form, free from other enzymes, in order to prove that the loss of transforming activity is actually due to a single enzyme acting specifically upon the bacterial nucleate which is believed to be identical with the transforming substance. If under these conditions depolymerization resulted in loss of biological activity, it would prove beyond reasonable doubt that the nucleic acid and the transforming agent are one and the same substance.

Preparations of desoxyribonucleodepolymerase extracted from commercial pancreatin and purified by fractional precipitation with $MgSO_4$ have been found to possess high activity in concentrations as low as 1 µgm./cc. The activity of the enzyme is measured by its capacity to reduce the viscosity of highly polymerized desoxyribonucleic acid isolated from calf thymus. Similarly, minute amounts of enzyme protein have been shown to bring about rapid and complete inactivation of the transforming substance. Dr. McCarty has developed quantitative methods for the measurement of enzyme activity.

Magnesium and manganese have been found to activate the depolymerase. The fact that Mg serves as activator is in accord with the finding that fluoride inhibits the action of this enzyme. In the case of other enzymes which are activated by Mg, the inhibition by fluoride is attributed to a combination of magnesium, fluoride and phosphate which removes the magnesium ions from participation in the enzyme action. Because of the fact that citrate is known to form a soluble complex with calcium, it

seemed possible that a similar reaction might occur with magnesium and that consequently citrate might serve to inhibit the action of the enzyme. This was found to be the case, and marked inhibition of depolymerase activity can be effected in the presence of citrate. Similarly, the inactivating effect of depolymerase on the transforming substance is inhibited by citrate.

In the present attempts to purify the depolymerase it has not as yet been possible to free the preparation completely of all traces of a trypsin-like enzyme. The proteolytic action of the latter results in considerable loss in activity of the depolymerase during the preparative procedures. Methods for overcoming this difficulty and selective means for separating the depolymerase in pure form are under investigation. Although tryptic enzymes are present, there is ample evidence that they are not responsible for inactivation of the transforming principle, since it has been shown that neither crystalline trypsin nor chymotrypsin has any demonstrable effect on transforming activity. Moreover, these proteases are not inhibited by fluoride or by citrate.

2. Reversible inactivation of the transforming substance. In the course of isolation and purification of the transforming agent it was found that pneumococcal extracts treated with ascorbic acid completely lost their transforming activity. A more detailed study of the nature of this inactivation is now being carried out using highly purified preparations of the transforming principle. A solution containing 3 mg./cc. of active substance, of which 0.02 µgm. suffices to induce transformation, is completely inactivated after contact for 5 minutes with ascorbic acid in 0.01 M concentration. Traces of copper which are known to catalyze the auto-oxidation of ascorbic acid have likewise been found to catalyze its action on the transforming agent. A concentration of 0.001 M ascorbic

acid, which by itself brings about only partial inactivation, causes complete loss of activity in the presence of minute traces of copper.

Hopkins and Morgan have shown that reduced glutathione completely prevents the oxidation of ascorbic acid even when the latter is catalyzed by copper ions. As little as 0.001 M concentration of glutathione has been found to protect the transforming principle from inactivation in the presence of 0.01 M ascorbic acid. Thus, it appears that the oxidation of the ascorbic acid is a necessary step in the inactivation of the transforming agent. More significant is the fact that transforming substance which has been completely inactivated by ascorbic acid can be quantitatively reactivated by subsequent treatment with glutathione. Thus, the reaction is a reversible one apparently dependent on the oxidation and reduction of biologically important groups in the nucleic acid molecule.

A variety of other oxidants, such as ferricyanide, cytochrome C, flavine phosphate, and α-tocopherol phosphate, tested under similar conditions, do not affect the activity of the transforming substance. On the other hand, iso- and glucoascorbic acid, which possess little or no vitamin C activity, are just as effective in bringing about the inactivation of the transforming substance as is ascorbic acid itself. The inactivation induced by these chemical analogues can be prevented when the reaction is carried out in the presence of glutathione. Among other substances tested, catechol has been found to behave like ascorbic acid in producing reversible inactivation of the transforming substance. It is noteworthy that catechol, ascorbic acid and its derivatives all possess in common dienol groups, that their action is catalyzed by copper ions, and that their effects can be reversed by glutathione. The loss of activity induced by these agents can be restored by the action of other sulfhydryl compounds such as cysteine and thioglycolic acid, although

under these conditions reactivation is quantitatively not so complete as in the case of glutathione.

It is hoped that a study of the reversible inactivation of the transforming substance will afford a clue to the nature of the chemical groupings essential to its biological activity. A survey of the agents capable of causing the inactivation and reactivation, definition of conditions under which the reactions occur, and a search for manifestations of the change in the molecule other than that of loss of biological activity (e.g. change in ultraviolet absorption spectrum) may lead to identification of the particular groups involved and their relation to the biological activity of the molecule as a whole.

Publication

Avery, O. T., MacLeod, C. M., and McCarty, M., Transformation of pneumococcal types induced by a desoxyribonucleic acid fraction isolated from Pneumococcus Type III. J. Exp. Med., 1944, 79, 137.