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February 5, 1957

On the Possibility of Detecting "Transformation" of Somatic Cells of Mammals or Birds.

By Leo Szilard

If skin is transplanted from rabbit A to rabbit B, the transplanted skin is sloughed off after a period of apparent healing. If subsequently another skin transplant is made from rabbit A to rabbit B, this second skin transplant does not survive as long as does the first transplant. We may express this fact by saying that the first transplant has induced "intolerance" in rabbit B against some genetically determined specific substances of rabbit A, to which we may refer, somewhat sloppily, as "antigens" - in quotes. What is the nature of these "antigens"?

It has been recently shown by Billingham, Brent and Medawar[1] that intolerance against skin of a strain A of mice can be induced in mice of strain CAB by injecting into CAB mice extract made from nuclei of spleen cells of A mice, and they have further shown that the active agent in these cell extracts is destroyed by desoxyribonuclease. The authors interpret this result by assuming that, if skin is transplanted from A mice

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to CAB mice and induces intolerance against a subsequent transplantation, the "antigens" of A mice which are responsible for producing this intolerance are substances that are destroyed by desoxyribonuclease, and are therefore presumably nucleo-proteins or nucleic acids. They write:

"So far as we are aware, only one hypothesis can accommodate these findings: that the antigenic substances responsible for skin transplantation immunity are desoxyribonucleo-proteins endowed with antigenic and therefore with genetic specificity. This hypothesis is made likely by our evidence, but the evidence falls short of proof."

We wish to point out here the possibility of another hypothesis which would appear to be <u>even more likely</u> and which is as follows:

The extract prepared from nuclei of spleen cells of A mice (in which the active agent can be destroyed by the addition of desoxyribonuclease) induces intolerance in CAB mice against a subsequent skin transplant from A mice not because this extract contains the "antigens" of A mice but rather because this extract - if injected into CAB mice - is capable of causing a certain number of cells of injected CAB mice to produce the relevant "antigens" of A mice. If this hypothesis is correct, then we would deal here with a phenomenon strictly analogous to that known as bacterial transformation. In bacterial transformation

nucleic acid extracted from a strain of bacterium, A, is taken up by a different strain of bacterium, B, and this nucleic acid induces a certain fraction of the bacteria to produce specific antigens of strain A.

In the circumstances one feels impelled to devise a different sort of experiment that might be adequate to detect whether transformation of somatic cells of mammals or birds can, in fact, be accomplished by injecting nucleic acids of one individual into another individual. The principle of an experiment that might accomplish this purpose is as follows:

We shall assume that rabbit B and rabbit A have different blood groups and that rabbit B carries no natural isoantibodies against the red cell antigens of rabbit A. An extract may then be prepared from spleen cell nuclei of rabbit A which contains the nucleic acids and nucleo-proteins but as far as possible very little else. We would regard it as evidence for having accomplished "transformation" if we can show the following:

(a) The purified desoxyribonucleic-acid-containing fraction which is prepared from cell nuclei of rabbit A is treated with desoxyribonuclease and injected into rabbit B. There appear no antibodies against the red cell antigens of rabbit A in the serum of rabbit B.

(b) When the treatment with desoxyribonuclease is omitted, the injection of the extract is followed by the appearance of antibodies in the serum of rabbit B against the red cell antigens of rabbit A.

(c) The purified desoxyribonucleic-acid containing fraction prepared from cell nuclei of rabbit A is treated with desoxyribonuclease. Subsequently, the desoxyribonuclease is destroyed and a purified desoxyribonuclease acid containing fraction, prepared from cell nuclei of rabbit B, is added -- to serve as an adjuvant in lieu of the destroyed cell nuclei of rabbit A. This mixture is then injected into rabbit B. No antibodies against red cell antigens of rabbit A appear in the serum of rabbit B.

If an extract prepared from spleen cell nuclei of rabbit A is indeed capable of forcing a small but appreciable fraction of the cells of rabbit B (say, a total of about one million cells) to produce red cell antigens of rabbit A, then rabbit B could be expected to respond by the production of antibodies specific for these antigens. Such circulating antibodies, if present, can be demonstrated by modern, sensitive, methods that permit the detection of very small quantities of type specific antibodies.

Arrangements are now being made for carrying out experiments of this type.

Should it turn out that transformation can, in fact, be effected in mammals (and the technique discussed above could also show whether transformation can be effected in birds), then there is a remote possibility that transformation might provide the basis for a "cure" for a class of rare hereditary diseases. In these diseases -- galactosemia, phenolpyruvic oligophrenia, hemophilia, etc. -- a defective gene is responsible for the absence of a specific protein in its functional form. Conceivably injecting into the patient DNA taken from nuclei of the spleen of a healthy individual repeatedly and in sufficiently large quantities might transform a sufficient fraction of the cells of the patient to remedy the disturbing manifestations of the defect.

February 1, 1957

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If this hypothesis is correct, then we would deal here with a phenomenon strictly analogous to that known as bacterial transformation. In bacterial transformation nucleic acid extracted from a strain of bacterium, A, is taken up by a different strain of bacterium, B, and this nucleic acid induces a certain fraction of the bacteria ~~bacterium of strain B~~ to produce ~~certain~~ specific antigens of strain A.

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make Stencil
15 cc

1) galactosemia ✓

2) phenylpyruvic oligophrenia
congenital cystinuria

3) hemophilia
afibrinogenica

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