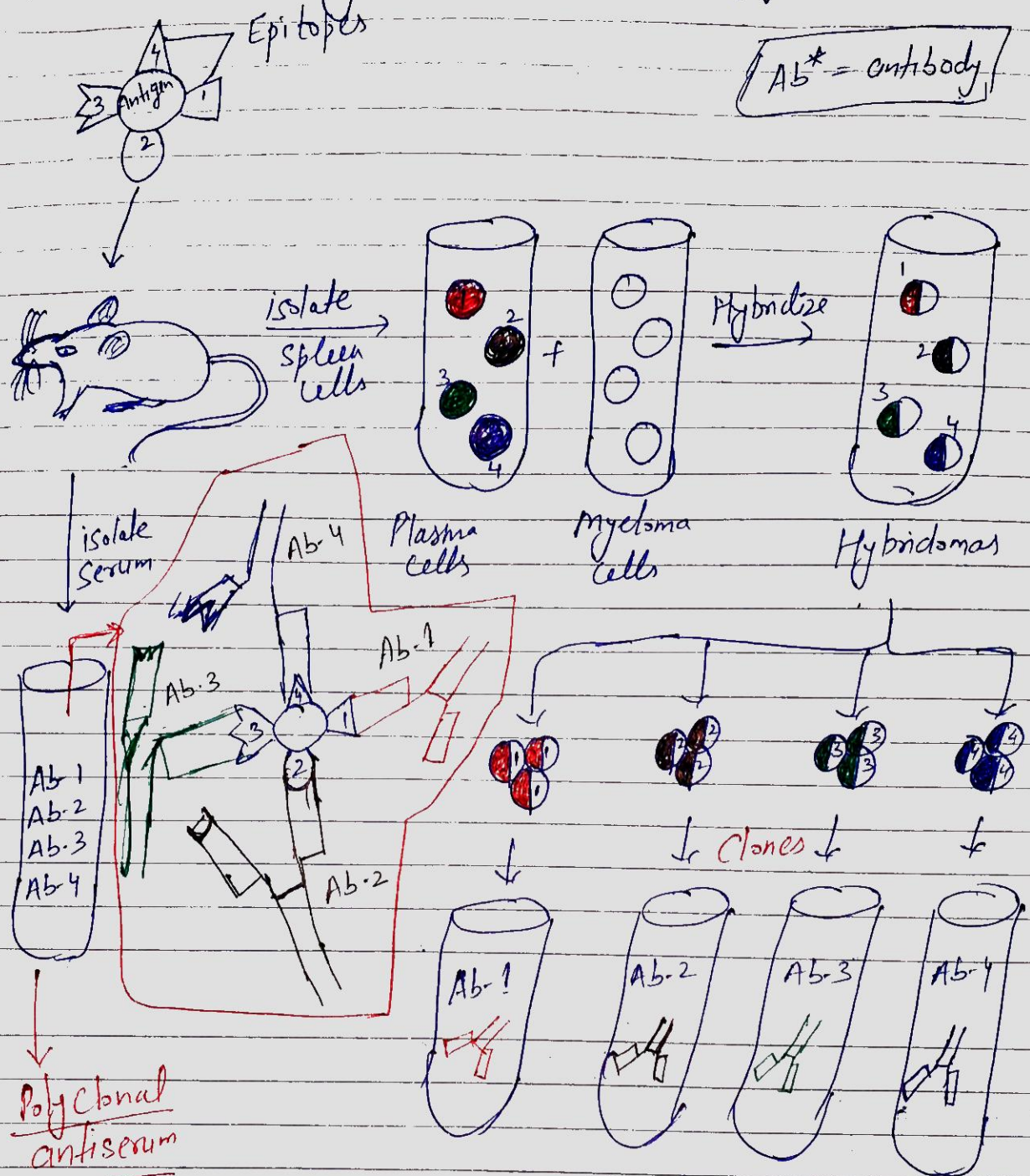
Monoclonal Antibody production using Hybridoma technology

Hybridoma - Technology



The conventional method for the production of polyclonal antiserum and in contrast to monoclonal antibody

As we know most antigen have multiple epitopes and they induce proliferation and differentiation of a variety of B-cell clones, each derived from a B-cell that recognizes a particular epitope. The serum antibodies are Heterogeneous, which comprising a mixture of antibodies and each antibodies specific for one epitope.

→ Polyclonal antibody response facilitates the localization, phagocytosis and complement-mediated lysis of antigen; it thus has clear advantages for the organisms in vivo.

→ Unfortunately, the antibody heterogeneity that increases immune protection in vivo and reduces the efficacy of an antiserum for various in vitro uses.

→ For doing research, diagnostic and therapeutic purposes, monoclonal antibodies, derived from a single-clone and thus specific for a single epitope are preferable.

→ Direct biochemical purification of a monoclonal antibody from polyclonal antibody preparation is not feasible.

→ In 1975, Georges Kohler and Cesar Milstein found a method for preparing monoclonal antibody, which quickly became one of immunology's key techniques.

→ By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to

generate a Hybrid cell, called a Hybridoma, that possessed the immortal-growth properties of the myeloma cell and secreted the antibody produced by the B cell.

→ The resulting clones of Hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely.

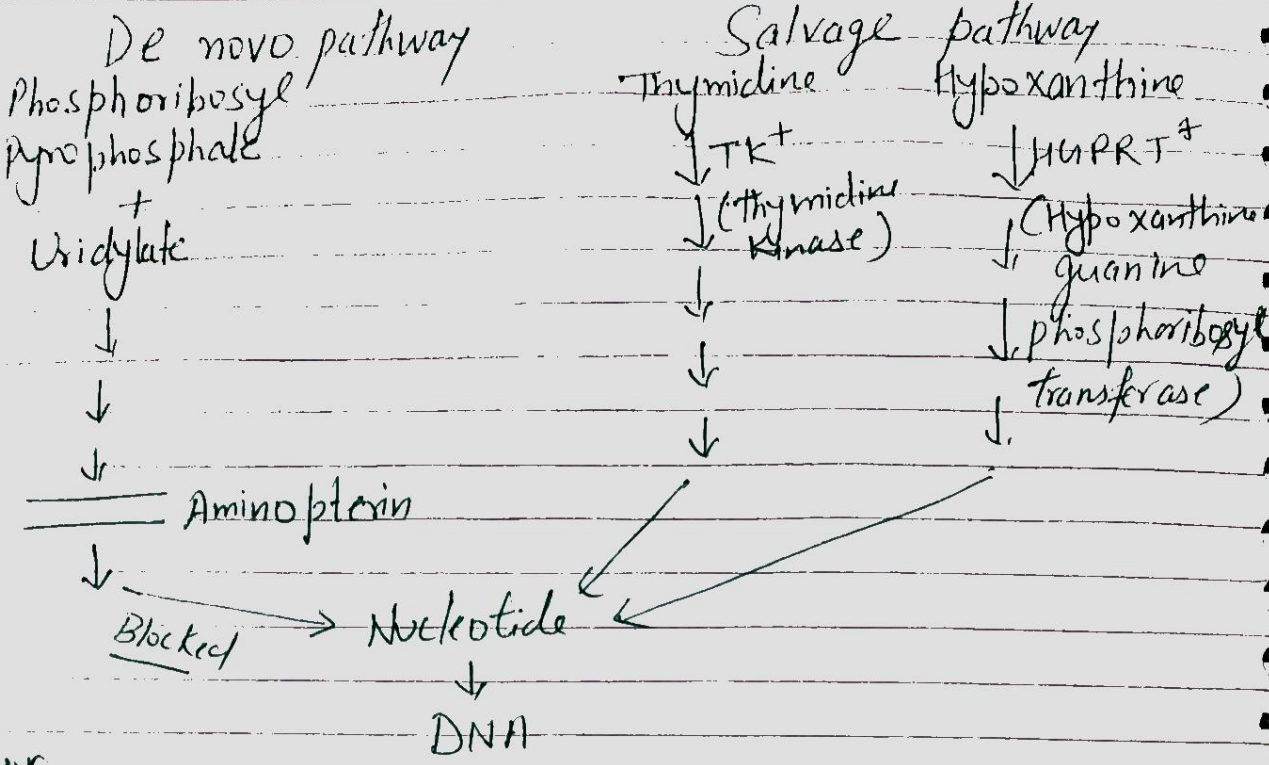
→ The significance of the work by Kohler and milstein was acknowledged when Each was awarded a Nobel Prize in 1984.

Formation and Selection of Hybrid Cells :-

- B-cell Hybridomas are produced by using polyethylene glycol to fuse myeloma cells with B cells from animals that have been immunized with the antigen against which one wants to make antibodies.

- The myeloma cells contribute the immortal growth properties to the fused cells and the B-cells, which are capable of limited growth in vitro, contribute the genetic information for synthesis of the specific antibody of interest.

- Not all of the cells fuse and the procedure gives a complex mixtures of unfused myeloma and spleen cells as well as numbers of fused cells.



* Aminopterin blocks DNA synthesis by the de novo pathway. It acts as an analog of dihydrofolic acid and binds with a high affinity to dihydrofolate reductase, inhibiting purine synthesis. In the presence of aminopterin, cells must use the salvage pathway to produce DNA

→ Among the fused cells, there are three different combinations

1. unwanted fusions of a B-cell with a B-cell
2. myeloma cell with a myeloma cell.
3. The desired fusion of a B cell with a myeloma cell

Thus, the conditions of the procedure must selectively allow the survival and growth of the B-cell and myeloma Hybridomas only.

- One common method requires the use of myeloma cells that are deficient (because of the previous selected mutations) for one of nucleotide salvage pathway, making them unable to grow in HAT medium (Hypoxanthine, aminopterin and thymidine).

- If the mixture of Hybridomas and unfused parental cells is placed in this medium, the parental myeloma cells can not survive.

- The B-cell and (and) myeloma cells can survive because the B-cell contributes the missing enzyme ~~for the synthesis~~ for the salvage pathway.

- Although unfused B cells are able to survive in HAT medium, these cells do not live for long periods in vitro and thus die out.

*HAT medium is used for the selection :-

- HAT selection depends on the fact that mammalian cells can synthesize nucleotides by two different pathways: (1) The de novo pathway
(2) Salvage pathway.

(1) The denovo pathway :- In de novo pathway in which a methyl or formyl group is transferred from an activated form of tetrahydrofolate is blocked by aminopterin, a folic acid analog.

When de novo pathway is blocked, cells utilize the salvage pathway, which bypasses the aminopterin block by converting purines and pyrimidines directly into nucleotides for synthesis of DNA and RNA.

- The enzymes catalyzing the salvage pathway include Hypoxanthine guanine phosphoribosyl transferase (HGPRT) and thymidine kinase (TK).
- A mutation in either of these two enzymes blocks the ability of the cell to use the salvage pathway.
- HAT medium contains aminopterin to block the denovo pathway and Hypoxanthine and thymidine to allow the growth by the salvage pathway.
- Therefore the cells that lacks either HGPRT OR TK will die in HAT medium, because they lack the ability to use the salvage pathway to acquire essential intermediates for the synthesis of nucleic acids

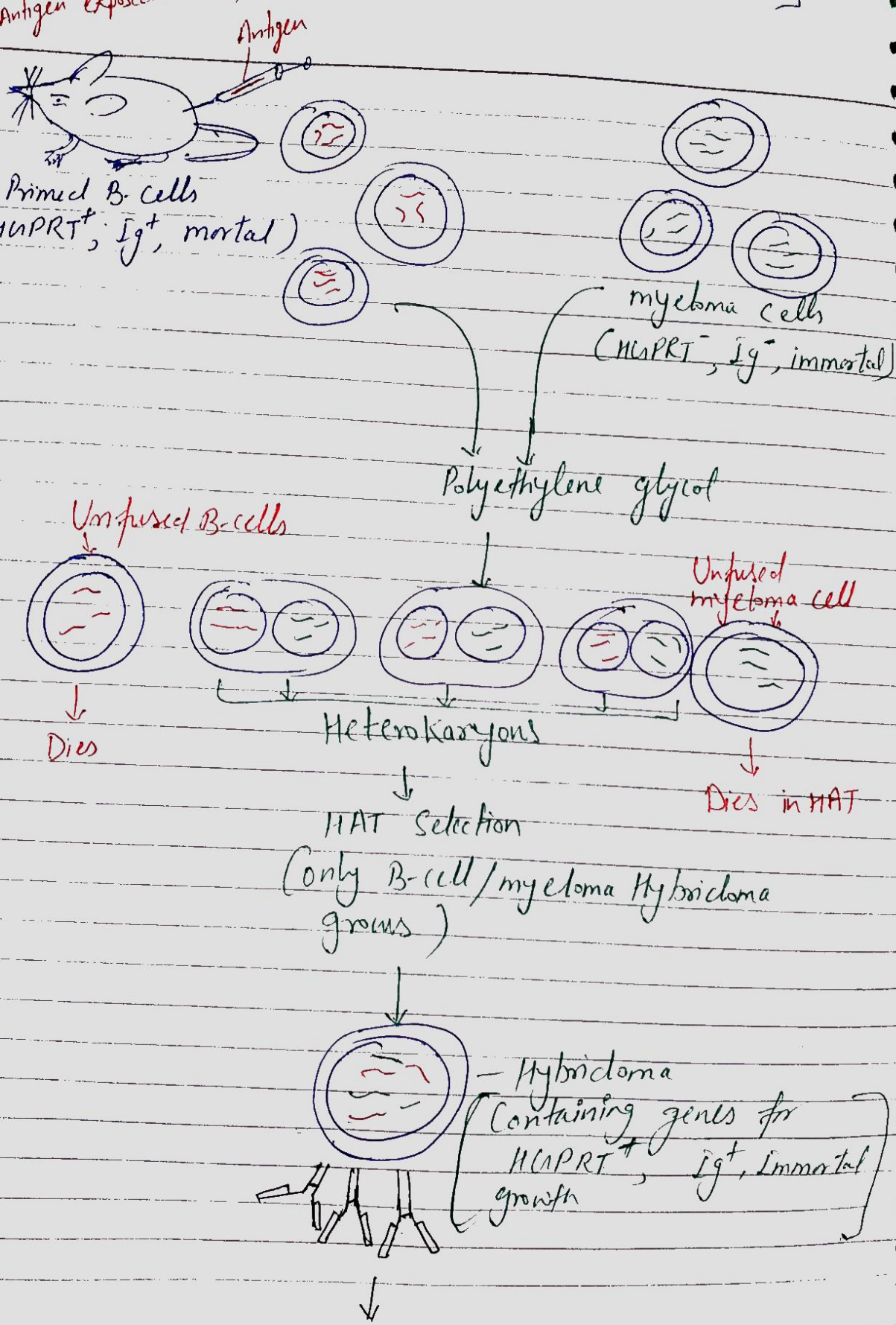
- In Hybridoma technology, the myeloma cells used are actually double mutants. As discussed above, they lack the enzyme HGPRTase and therefore they are deselected on HAT medium.

- They have lost the ability to produce immunoglobulin (Ig⁻ mutant). By using Ig⁻ mutants that the antibodies produced by the Hybridoma are encoded by the spleen cell partner and the myeloma cells only contribute immortal growth properties to the fused cells.

- The other fusion partner is usually a population of spleen cells containing antigen activated HGPRT⁺ B-cells.

- These cells contribute the capacity to utilize the salvage pathway for Hypoxanthine to the Hybridoma thereby enabling their survival in HAT medium.

[The production of Mono clonal Ab]



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Assay for desired antibody in culture ~~supernatant~~

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Reclone $A5^+$ Hybridoma

Exposed positive clones

Culture

↓

monoclonal antibodies

Ascites fluid

↓

monoclonal antibodies

The procedure for producing monoclonal antibodies specific for a given antigen developed by Kohler and Milstein. Spleen cells ($HGPRT^+$ and Ig^+) from an antigen-primed mouse are fused with mouse myeloma cells ($HGPRT^-$ and Ig^-). The spleen cell provides the necessary enzymes for growth on HAT medium, while the myeloma cell provides immortal growth properties. Unfused myeloma cells or myeloma/myeloma fusion fail to grow because of their lack of HGPRT. Unfused spleen cells have limited growth capabilities in vitro and will die within a few days