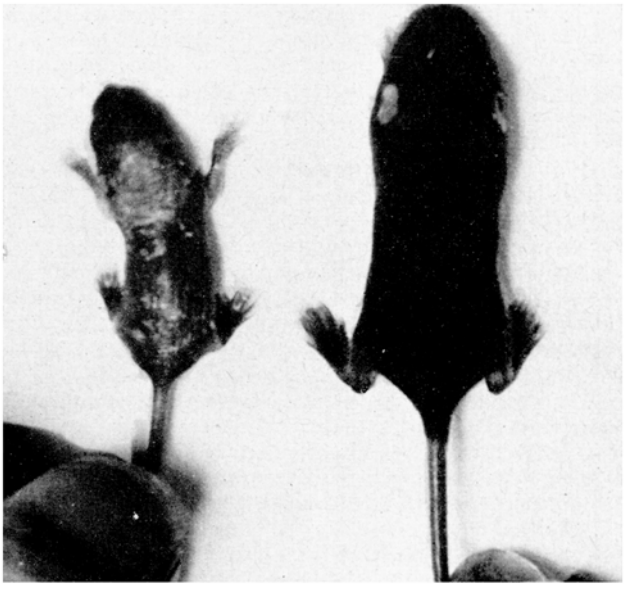

Fig. 3. Maladie homologue chez une $C_{57}Bl_6$ après injection de cellules hématopoïétiques embryonnaires de DBA_2 à la naissance. A droite: souris témoin de 13 jours; à gauche: souris rabougrie du même âge. La souris rabougrie est morte 6 h après.

Tab. I. Souris $C_{57}Bl_6$ recevant à la naissance des cellules hématopoïétiques de donneurs adultes DBA_2

Souris négatives au 1er et au 2e tests	Souris négatives au 1er test et positives au 2e test	Souris positives au 1er test et négatives au 2e test	Souris positives au 1er et au 2e tests
40	4	1	2

Tab. II. Souris $C_{57}Bl_6$ recevant à la naissance des cellules hématopoïétiques de donneurs embryonnaires DBA_2

Souris négatives au 1er et au 2e tests	Souris négatives au 1er test et positives au 2e test	Souris positives au 1er test et négatives au 2e test	Souris positives au 1er et au 2e tests
41	1	8	2

une maladie cachectisante, prouvant, dans les deux cas, la greffe de cellules immunologiquement compétentes homologues.

Il n'y a pas de relation obligatoire entre les succès et les échecs des greffes de ces deux lignées cellulaires, érythropoïétiques, et immunologiquement compétentes.

La possibilité d'induire chez la souris immunologiquement immature une maladie homologue par des cellules prélevées sur foie embryonnaire doit être soulignée. On a déjà observé des maladies secondaires chez des souris adultes irradiées et restaurées par des cellules érythropoïétiques homologues prélevées sur foies d'embryons[18,19]. La possibilité d'une action homologue de cellules embryonnaires même chez un receveur nouveau-né n'avait pas encore été démontrée à notre connaissance.

Le petit nombre d'animaux examinés histologiquement (6 en tout) ne permet aucune conclusion sur l'aspect histologique du syndrôme cachectisant induit par cellules médullaires ou de foies embryonnaires. On peut cependant noter que l'aplasie lymphoïde n'est observée que chez la souris morte au 46e jour. Les souris mortes les 20 premiers jours présentaient, au contraire, une hypertrophie splénique avec prolifération cellulaire.

S'il existe bien une évolution en deux temps, l'aplasie lymphoïde pourrait s'interpréter comme le signe d'un réveil d'une activité immunologique de l'hôte, éliminant après une tolérance transitoire les cellules homologues greffées. Nos résultats montrant le passage d'une prolifération cellulaire lymphoïde à une aplasie totale chez des souris F_7 irradiées restaurées par de la moelle et des cellules ganglionnaires d'une lignée parentale[17] nous conduisent à formuler l'hypothèse d'une action nocive spécifique des antigènes tissulaires de l'hôte tolérant sur les cellules lymphoïdes homologues immunologiquement compétentes du greffon, responsables de l'hypertrophie splénique primitive du receveur.

J. L. Amiel et G. Mathé

Centre de Recherche sur les Leucémies et les Maladies du Sang, Association Claude-Bernard, Service Pr Jean Bernard, Hopital Saint-Louis, Paris, le 24 juin 1960.

Summary

The endoveinous injection to newborn mice of homologous hematopoietic cells either adult or embryonnic, may result in a lasting and functional graft.

The former statement can be demonstrated by use of starch gel electrophoresis which shows the specific hemoglobin of the donors.

In both uses this graft can induce a runt disease.

[18] G. Mathé et J. Bernard, Rev. Fr. Et. Clin. Biol. *4*, 442 (1959).
[19] J. J. Trentin et T. M. du Wayne, Proc. Amer. Ass. Res. *3*, 70 (1959).

PRO EXPERIMENTIS

A Simple Device for Steady State Cultures of Microbes

In the course of investigations on yeast metabolism, the need arose for a simple apparatus for growing yeast cultures in a steady state. The following device has served us satisfactorily.

The feeding system (Fig. 1) is based on the principle of maintaining a constant head over a constant resistance. The constant head results from a constant level of the culture liquid in a small vessel (A) which is provided with an overflow outlet (B). The culture liquid is pumped up into this leveling vessel out of a storage tank (C), and the overflow spills down back into the storage tank. The resistance is determined by a glass capillary (D). A 1-mm bore glass capillary of 4 m length will give a resistance resulting in a flow rate of about 1 ml culture liquid/h/cm head. It is convenient to have the capillary in the form of a helix.

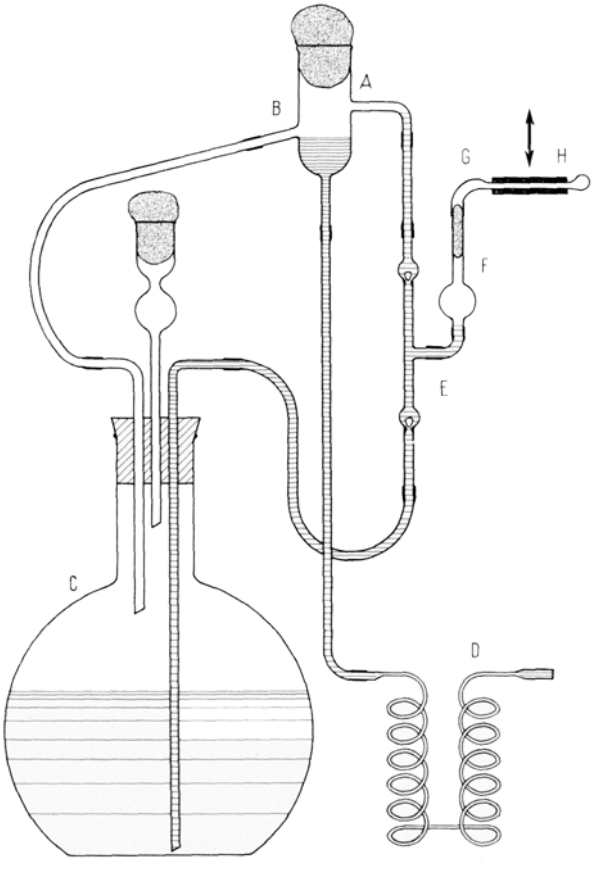

Fig. 1. C. J. E. A. Bulder: A Simple Device for Steady State Cultures of Microbes.

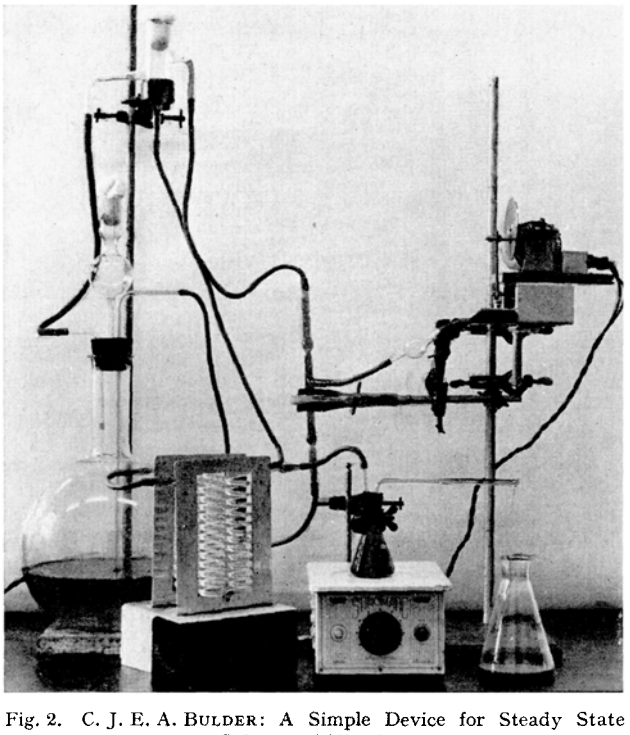

Fig. 2. C. J. E. A. Bulder: A Simple Device for Steady State Cultures of Microbes.

The pump consists essentially of a T-shaped glass tube with two glass non-return valves (E); the third leg of this pump is connected via an air filter (F) to a piece of rubber tubing (G) which is closed at the other end. Alternate squeezing of this rubber tubing results in a pumping action. This is done by a hinge-like device (H) connected to a small (e. g. 40 watt) electromotor, geared down to ca. 60 rpm.

Pumping velocity can easily be changed by changing the position of the rubber tubing in the hinge, by changing the amplitude of the reciprocating movement of the hinge, or by changing the volume of that part of the rubber tubing which is not compressed by the hinge. The only requirement for the pumping velocity is that it should be not lower than the feeding rate of the culture.

The feeding system described has several advantages over other systems used. It can be sterilized as a whole in an autoclave; it is advisable to take care that all tubings shall be in contact with saturated steam. The flow rate is easily changed by altering the height of the leveling vessel (A).

The rubber compression tube may eventually crack in the hinge, but it can be replaced without affecting the sterile parts of the system. Breaks of the electrical current will not immediately spoil a run, since the constant head will only drop rather slowly, depending upon the ratio between the feeding rate and the volume of the leveling vessel. Changes in barometric pressure are without any influence.

As a disadvantage, it may be mentioned that some media produce a precipitate in the capillary, thereby changing its resistance; daily checking of the flow rate is therefore advisable.

In Figure 2, the arrangement for experiments without extra aeration is shown. The culture vessel consists simply of a 50-ml Erlenmeyer flask, closed with a rubber stopper provided with an inlet tube and an outlet tube. The inlet tube reaches about half way into the flask and the outlet tube only to the lower side of the stopper. Thus the culture vessel is always completely filled with liquid, and no foam can collect under the stopper. The liquid is stirred by a magnetic stirrer.

No more oxygen is supplied than that dissolved in the fresh medium. This quantity can be calculated rather exactly, assuming that the gases dissolved are in equilibrium with the air. In the case of a vigorous fermentation, e. g. in culturing a yeast in a sugar-rich medium, the outflow entirely consists of foam. After calibration of the outflow tube, the flow rate of the foam, leaving the culture vessel, can be measured easily. Subtracting the flow rate of the liquid gives, after correction for dissolved carbon dioxide, the amount of this gas evolved per unit of time. This is a convenient way to check the metabolic activity of the culture.

As an example it may be mentioned that, in the device described, we have cultivated a strain of *Saccharomyces cerevisiae* for several weeks in a 50-ml flask at a dilution rate of ca. 0.25/h. The cell density was about 0.3×10^8 cells per ml; the carbon dioxide production 20–25 ml/h; the inflow of dissolved oxygen ca. 0.07 ml/h.

C. J. E. A. Bulder

Laboratory of Microbiology, Technological University, Delft (Netherlands), August 10, 1960.

Résumé

Description d'un appareil simple permettant la culture continue des microbes et qui a été appliqué avec des résultats satisfaisants pour la culture des levures.