AD NUMBER

AD843937

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BDRL ltr, 13 Sep 1971

(1)

TRANSLATION NO. 229

DATE: July 1968

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Methods and utilization of freeze-drying.

by K. Neumann and G. Matz.

Chemie-Ingenieur-Technik, 27: 9-10 (1955). (Only designated portions have been translated).

Freeze-drying of microorganisms, cells and tissues.

The anatomist Altmann (15) of Leipzig in 1890 probably was the first to utilize freeze-drying in the solution of biological problems by desiccating tissues for microscopic examination. He took advantage of the fact that tissue particles retain their structure in nearly unaltered form upon rapid freezing and drying, so that fixation in liquid chemicals, which causes artifacts, could be circumvented. This method of preparing tissue for microscopic or electron-microscopic examination currently has found acceptance in numerous institutes. It may not replace the older methods everywhere. However, if decomposition or destruction of certain tissue components, or their displacement owing to treatment with liquids are to be prevented, or if uncontrollable contraction of individual tissue elements must be eliminated, this method is preferable (26,27). Such a decomposition of tissue components may be expected even in the case of compounds which as a rule are thought to be deeply anchored in the tissue, such as nucleic acids.

In recent times, freeze-drying of tissues or tissue extracts for therapeutic purposes has increased in scope; hormones, vitamins and ferments, chorion and hypophysis gonadotropine, vitamin B complex and hyaluronidase may be mentioned. The conservation of whole extracts and injectable organ suspensions, known in connection with so-called "cellular therapy" (Niehans, 28), is added thereto at an ever increasing rate.

In addition, material for transplanting is conserved by freeze-drying, since long periods of time frequently elapse between the removal of the preparation and its implanting. For example, many diseases lead to the closure or rupture of important blood vessels. The patient may frequently be saved by their operative replacement. Transplantations with animals, conducted in American clinics (29,30) have revealed the superiority of vascular preparations treated in this manner over fresh material (see table 1). Transplantation of bone and skin was also facilitated in this manner, so that the next few years may bring the possibility of selecting freeze-dried tissue preparations of suitable form and origin from the tissue bank, and desiccated blood preparations from the blood bank.

The conservation of viable microorganisms or vaccines today represents one of the most important medical and industrial applications of freeze-drying. For example, the control of large epizootics in tropical regions is hardly possible with non-desiccated vaccines, since these lose their efficacy within a short time. These conditions have been changed considerably by the introduction of heat-resistant and durable dry vaccines (31). This development is also highly important in human medicine. In addition,

important strain cultures of microorganisms used in research may be stored for years without further measures after a single subjection to freeze-drying. Here the composition of the suspension's medium is significant, especially the addition of suitable protective solutions. The number of effective media is very large. Their selection depends on the type of germ or virus that is to be dried. The prediction of an optimal protective solution requires extensive experience. For instance, glycerol serves as protective solution in the freeze-drying of red blood cells (32).

Generally a considerably longer survival rate of dried germs is obtained if the ampullae containing the dry material are sealed in vacuo. At least no atmospheric humidity should enter the material. The well-known method of sealing bottles of penicillin seems to meet most requirements of practical interest. When these precautionary measures are observed, germs may remain capable of reproduction as much as 12 years later.

A clear borderline of the organisms' level of differentiation, up to which the "conservation of viability" may be achieved with the aid of freeze-drying, cannot be set at this time. The drying of yeast is possible, that of amoebae doubtful; the freeze-drying of red blood cells has not succeeded so far. Recently, reports of sub-cooling of sperms far below the freezing point have become more frequent. The renewed growth of dried cancer cells of mammalian organisms has also been described by several investigators (33,34). Re-examinations show that the conditions for drying become quite complicated and that results fluctuate. Higher organisms so far have not been kept alive by freezing.

Table 1.
Arterial transplantations in the dog.
(after Pate and Sawyer, 1953)

	Fresh transplants	Transplants after freeze-drying
Number of cases	22	48
Excellent result	12 (55%)	46 (96%)
negative result	10 (45%)	2 (4%)
death of the animal	3 (14%)	1 (2%) *
thrombosis	5 (23%)	1 (2%)
hemmorhage	2 (9%)	1 (2%) *

* the same case: The preparation had not been stored in vacuo.

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