Methods of Neurochemistry

volume 4

edited by Rainer Fried

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Edited by RAINER FRIED

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Chapter 1

CULTURE OF NERVE TISSUE

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I. HISTORY

Although Wilhelm Roux (1) was successful in maintaining the medullary plate of a chick embryo in normal saline for a few days in 1885, Ross Harrison's publication (2) in 1907 is generally regarded as the beginning of nerve tissue culture.

Harrison explanted fragments of neural tube from an early frog embryo and observed, over a period of a few weeks, the development of axons. These pieces were cultured in frog lymph, could be kept alive under aseptic conditions for weeks, and offered a reproducible technique. A few years later Burrows (3) introduced the use of plasma instead of lymph clot and Carrel (4) suggested the use of embryo extract as a strong growth promoting substance. Essentially, this method with a certain modification in the composition of the feeding medium, was used by a number of investigators during the subsequent years who were successful in culturing neurons and neuroglia from nerve tissue of chick embryo (5-8) and mammals (9-14). Later this method was successfully applied in growing adult human sympathetic ganglion cells (15) and cells from fetal (16-18) and adult human brain (19, 20).

The use of reconstituted rat tail collagen was suggested by Bornstein (21) in 1958, and with the improvement of culture techniques, the explanted nerve tissue fragments could be kept alive for months (7, 23). The method proved to be very useful for morphological studies (24-27), for enzyme histochemistry (28-31), and for study of demyelinating processes (32, 33) but was not used primarily for biochemical studies.

In the past few years, however, and with increasing frequency, the potentials of nerve tissue culture methods are being utilized in neurochemistry (34-41).

Another interesting development which took place in the past decade is the introduction and establishment of cell culture from central nervous system tissue. During its first 50 years of existence, for all practical purposes, nerve culture methods were limited to tissue or organ culture. Fragments of various parts of the nervous system were dissected and kept alive under aseptic conditions for a certain period of time. No successful attempt was made to dissociate the cells of the dissected nerve tissue fragments and grow them as cell culture. Lately, however, a number of investigators succeeded culturing cells of nerve tissue, dissociated by various means, either in a static or a suspension system (42-46).

II. INTRODUCTION

A. Role in Neurochemistry

Nerve tissue or cell culture is a valuable addition to the investigative tools of neurochemistry. Its main value is that it provides a system where nerve tissue is grown in a controlled environment and is also available for visual observation. Thus, it is eminently suitable for simultaneous interpretation of structure and function, and for correlation between morphological and biochemical changes. Since nerve cell culture can be maintained in quantity, it lends itself to microchemical methods and also for cytochemistry.

As is true for every method, tissue culture also has its advantages and limitations. Careful consideration of these factors will decide whether this method is applicable to the investigation of a particular problem.

B. The Advantages

1. The tissue or cells can be visually observed throughout the entire duration of the culture.

2. The physical factors of the environment (temperature, light intensity, etc.), and the composition of the feeding solution can be controlled within a wide range.

3. The interaction with other organs, which are necessarily present in an experimental animal and potentially participate in the metabolism of substances to be studied, is eliminated.

4. The blood-brain barrier is absent, thus the direct action of various agents on the nerve cells can be observed and studied.

5. The harvested feeding solution is available for biochemical studies.

6. The tissue, as a substrate, can be used for biochemical, histochemical, or morphological studies.

7. Cultures from various structures of the nervous system of the same animal can be prepared and the action of various agents can be studied without interaction from other structures.

8. The system is isolated, and thus the action of toxic agents on the nervous system can be studied far beyond the lethal dose for an experimental animal.

C. The Limitations

1. The initial cost of the laboratory, particularly the water-deionizing and air-filtering equipment, is very high.

2. The tissue survives only for a few weeks or months at the most and thus, it is not suitable for long-term experiments.

3. No purified feeding solution is available yet for nerve tissue which would eliminate the use of a natural medium (serum, tissue extract). This introduces a certain number of unknown factors.

4. The morphological integrity of the cellular elements within the tissue is well preserved and is maintained over a long period. However, the anatomical correlation between cell groups, tracts, etc., is less well maintained.

5. The morphological parameters of nerve tissue in culture of various species are relatively well known, but the biochemical characteristics of this system are not well studied yet.

6. The tissue dissection, feeding, and other manipulations are time-consuming, since aseptic techniques are required.

The scope of this chapter is to describe the currently available nerve tissue culture methods, to evaluate them, to give a detailed description of a laboratory and also of the most important techniques.

Its aim is to provide helpful advice for those who intend to establish this method, who want to extend their investigative work in this interesting and potentially rewarding field.

In describing the various methods and techniques, a certain knowledge of tissue culture work is expected; the reader is referred to a number of excellent modern publications (47 - 50).

III. BASIC REQUIREMENTS

A. Laboratory

There are certain basic facilities which are needed in every nerve tissue culture laboratory regardless of the research objective or volume of the work. These are: 1. dishwashing, wrapping, and sterilizing area. 2. preparatory area. 3. sterile room. 4. incubator.

1. Preparation of glassware, cleaning of the dissecting instruments, wrapping of various items before sterilizing, and sterilizing itself takes place in this area. It contains: a. large sink with numerous faucets; b. electric or gas stove; c. dishwashing machine; d. autoclave; e. oven; f. hood with negative pressure for acids used in glassware cleaning; g. large table surface.

Figure 1 is a sketch of the dishwashing, sterilizing, and wrapping area of the nerve tissue culture laboratory at the New York State Institute for Research in Mental Retardation and gives good information about the layout and dimensions.

a. The sink is made of stainless steel, is elongated, and is supplied by 14 faucets which are arranged in two rows. The faucets in the front row deliver deionized water from the storage tank; those in the back, supply distilled water. Tap water is provided by two faucets at each end of the sink. The sink is not uniform in depth but its front part is deeper to accommodate pails and also pipet washers.

b. The stove should be large enough to accommodate long stainless-steel dishes for pipets and preferably should have four to six burners. It can either be electric or gas; the gas, however, has to be filtered.

c. The dishwashing machine is not essential for a laboratory with small volume of glassware, although its use speeds work and reduces labor expenses. The machine in our laboratory has five washing cycles; four of which use deionized and the fifth, distilled water.

d. The autoclave is essential for sterilizing those items which cannot be exposed to high temperature. Preferably, there should be two autoclaves: one operated with household steam for items which are not sensitive to ionic contamination (gowns, etc.). The other is operated with steam generated from twice-distilled water and is used for items which come in contact with nerve tissue, feeding solution, and other reagents where the absence of toxic or potentially harmful ions is important.

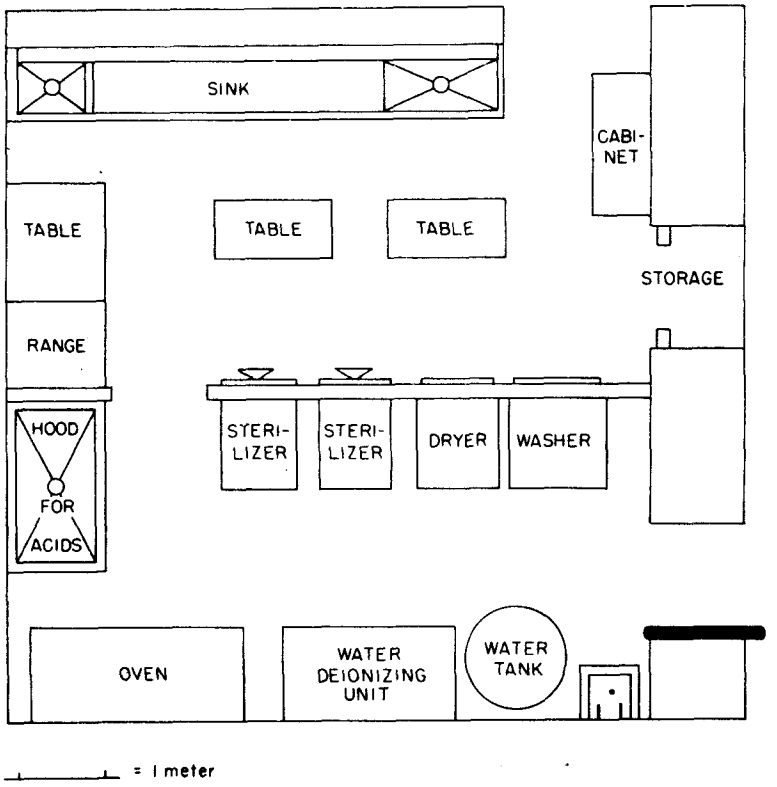

FIG. 1. Dishwashing and sterilizing area.

e. Sterilizing of glassware and instruments with dry air takes place in the oven at a temperature of 170° C or higher. If space and budget permit, it is advisable to have two ovens: one for drying the glassware after washing, the other for sterilizing only.

f. The preparation of coverslips involves soaking in concentrated nitric acid. This procedure is preferably done under a hood with negative pressure in order to eliminate corrosive fumes from the dishwashing area.

2. Preparation of the animals before dissection, opening the double coverslip assemblies, sealing them after feeding, scrubbing before entering the sterile room, etc., is performed in this area. It contains a table, double boiler with paraffin and wax mixture for sealing, a sink with soap and disinfectant dispenser (e.g., Phisohex), a shelf for sterile gowns

and caps, a hamper for used gowns. Careful handwashing, not unlike a surgical hand toilet, before any work in the sterile room which involves handling of tissue or feeding, is important. Repeated rinsing of the hands with a small amount of 70% ethyl alcohol in the sterile room, while dissecting or feeding the cultures, is recommended.

3. The sterile room is one of the most important areas of tissue culture work, and the purity of the air of the room is of great importance. Figure 2 gives an outline of the sterile room in our laboratory. It contains:
a. base cabinet and table; b. hood; c. refrigerator; d. centrifuge;
e. dissecting microscope.

a. The base cabinet and table is preferably made of stainless steel. The drawers of the cabinet serve for storage of glassware and instruments.

b. The hood proyides a certain amount of protection for the tissue during dissection and feeding, and also serves as support for glassware during these procedures. It should be constructed of a material which is resistant to ethyl alcohol and routinely used disinfectants. Its top is made of thick glass and light is supplied by two fluorescent light fixtures at the sides.

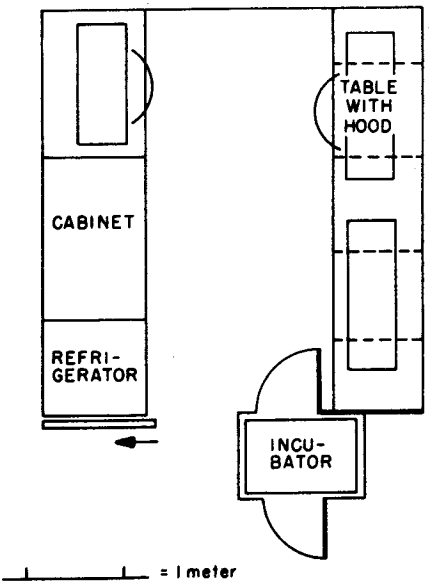

FIG. 2. Sterile room.

c. The refrigerator serves for storage of feeding solutions, balanced salt solution, collagen, etc. An ordinary household refrigerator with a freezing compartment serves this purpose well.

d. A low-speed, table-top centrifuge is used in this room for centrifuging cells, serum, etc.

e. The dissecting microscope is a stereo microscope providing a magnification range of 10 - 25 x. Its light source should preferably be shielded by a heat absorbing filter in order to protect the tissue from the heat of the light bulb.

The sterile room preferably should have its separate air supply, under positive pressure, the air should be cleaned either through filters or by means of an electric air precipitator. In our sterile rooms the air enters the space with a positive pressure and is filtered through three different filters of various pore size. The last one has the smallest pore size, 0.3 μ. These filters are changed at regular intervals. The airspace in the sterile room is continuously sterilized, when not in use, by means of ultraviolet lights. Two types are preferred. One is ceiling mounted and fixed; the other is a movable floor lamp and radiates those areas which are not accessible to the ceiling lamps. Our lights emit strong enough radiation to sterilized the airspace in 20 minutes.

The paint on the walls should be washable and resistant to ethyl alcohol and disinfectants. Our sterile room walls are epoxy coated and are washed once a week with 70% ethyl alcohol, or more frequently if the room is heavily used. In addition, the airspace is regularly sprayed with bactericidal and fungicidal agents at the end of the workday. The purity of the airspace in the sterile room is regularly checked by means of blood plates. Commercially available blood agar plates are exposed to the air when the room is not in use and with the uv lights off. The number of colonies is determined after a 24-48 hr incubation. Preferably not more than two or three should be present. If the number of colonies is greater, the sterile room techniques have to be reviewed and cleaning procedures tightened.

4. The size of the incubator depends on the number of cultures. It should be placed close to the sterile room and be supplied with an alarm system, should the temperature regulator fail. The optimal temperature for nerve tissue culture is in the range of 34° - 37° C. As a rule lower temperature, although not optimal, is far better tolerated than higher one. A temperature of 42°C causes death of the cells in a few hours, but cultures can be kept at room temperature for a much longer period without any adverse reaction.