of the slices was carried out by sealing them inside polythene bags and placing their surfaces in contact with X-ray films (Kodirex).

Samples of guinea-pig, rabbit, and human serum and of human γ- and β-globulins were labelled with ^{131}I (McFarlane[3]) and were separated either by albumen-gel or by starch-gel electrophoresis (Poulik[4]). The autoradiographic patterns are shown in Figure 2. As a high electro-osmotic flow was present, the origin was set at 7–8 cm from the anodal end of the slab. The albumin was visible because of binding bromo-phenol blue and entered the gel on both sides although it moved very little from the origin. However, rabbit albumin was all on the anodal side. The γ-globulins moved far from the origin towards the cathode and showed a large range of electrophoretic mobilities. Transferrin was localized next to γ-globulin as a narrower band of characteristic salmon-pink colour. α-globulins were always on the cathodal side between albumin and β-globulin. Rabbit haemoglobin, without showing interactions with the stabilizing medium, could be located at about 4 cm from the origin towards the cathode. Results were easily reproducible. This investigation presents evidence that autoradiographic patterns of serum proteins obtained after separation on a proteic gel medium resembled those obtained in a starch-gel, the latter medium having, however, higher resolving power. The strong electro-osmotic flow did not diminish with the use of cellulose membrane barriers or by increasing the ionic strength of the buffer. Attempts to modify the pore size of the gel either by increasing the concentration of albumen proteins, or by separating the serum proteins in a gel slab previously having undergone a blank electrophoresis, did not change the electrophoretic pattern[5].

Riassunto. Un gel composto dalle numerose frazioni proteiche dell'albumina d'uovo fu preparato ed usato quale mezzo di supporto per la separazione elettroforetica di siero-proteine marcate con ^{131}I.

A causa di un notevole flusso elettro-osmotico la separazione proteica, dimostrabile mediante autoradiografia, si spostò verso la regione catodica mostrando tuttavia analogie con quella ottenibile su gel di amido.

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[3] A. S. McFarlane, Nature *182*, 53 (1958).
[4] M. D. Poulik, Nature *180*, 1477 (1957).
[5] Supported by a grant from the Consiglio Nazionale delle Ricerche, Roma.

PRO LABORATORIO

Laboratory Deep Culture of Micro-Organisms Using a Vibrating Stirrer

Deep-culture methods with forced aeration are frequently required in the laboratory in order to obtain the required amount of cell mass or metabolic products of aerobic organisms in one single batch, or to increase the rate of growth or metabolism, or to carry out scaling-up operations. A homogeneous culture (i.e. absence of cell clumps) and a reliable mechanical set-up for aeration and agitation will undoubtedly increase the reproducibility of the results.

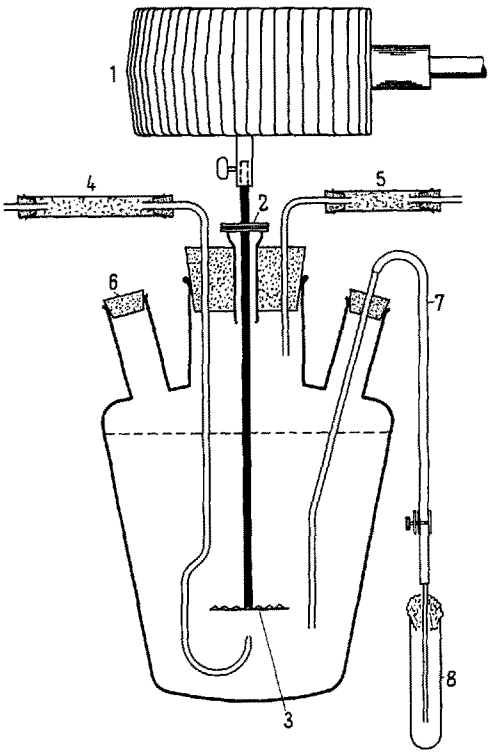
The earlier methods for mass cultivation of aerobic micro-organisms consisted in forcing air through sintered glass into the substrate without using mechanical agitation. Satisfactory results on the laboratory scale could then be obtained for propagation of yeasts and bacteria where it is easy to obtain a high degree of homogeneity of the culture. Filamentous fungi, however, tend to agglomerate, and can form pellets of variable and not always reproducible size; furthermore, they can increase the viscosity of the culture very strongly and thus make a uniform oxygen transfer to the fungal hyphae impossible; clogging of the sintered glass by fungal growth is also a common observation. Using more or less strong agitation the first two disadvantages can generally be avoided, thus restoring homogeneity and reproducibility and maintaining the growth rate at a sufficiently high level. Agitation is generally done by a rotating stirrer fitted into the culture vessel, alternatively the vessel itself may be rotated. In a recent review[1] various types of apparatus have been described. If contaminations are completely to be avoided, technical complications arise which cannot be ignored even if a positive pressure is maintained inside the vessel.

In the present article a vibrating stirrer (Vibro Mixer) is described, of which the major advantages are the omission of rotating parts, a very fine distribution of air without using sintered glass, and easily adjustable degree of agitation. The apparatus has now been used for many years and has given interesting results in the study of growth kinetics of moulds, in the production of amylase by *Aspergillus oryzae* and for the oxidation of long-chain hydrocarbons by micro-organisms.

Description and Operation of the Apparatus[2]. Vertical vibrations are generated at the frequency of the alternating current of the mains and are of gradually variable amplitude with a maximum of about 3 mm. The stirrer consists generally of a circular plate fitted firmly to a shaft which is inserted into the holder of the vibration generator. A considerable degree of agitation can be imparted to the liquid with a large amplitude when position and size of the stirrer plate in relation to the vessel are suitably chosen (by trial and error). For aseptic work the stirrer is fitted through a tight sealing membrane (preferably of neoprene) mounted in a holder which can be inserted into a rubber bung in the neck of the culture vessel. The whole vessel can be sterilized with the stirrer *in situ*, disconnected from the vibrator.

[1] R. Elsworth, in *Progress in Industrial Microbiology* (Hockenhull, ed., 1960), vol. 3, p. 103.
[2] Full technical details of the presently described apparatus are given in the descriptions of the manufacturer: Chemie Apparatebau AG., Männedorf, Zürich (Switzerland).

Another distinctive advantage of the apparatus is that air is divided up in very small bubbles if the air inlet is centred underneath the stirrer plate; thus a highly efficient transfer of oxygen is ensured. All other fittings in the culture vessel can be seen in the Figure. For most experiments a 6-l flask with 5-l working capacity and stirrer plates of glass or metal up to 65 mm diameter have been used. Larger volumes of culture (up to 10 l) can be satisfactorily stirred with the smallest vibrator supplied. Larger models are made by the manufacturer.



Assembly of the Vibro Mix Apparatus. (1) Vibrator, (2) sealing gland, (3) stirrer disc, (4) filter for air coming in, (5) filter for used air let out, (6) inoculation orifice, (7) siphon for sampling, (8) sterile tube to prevent contamination of the siphon.

For most purposes it is satisfactory to estimate the amplitude by eye and adjust accordingly. If the growth and fermentation characteristics depend very strongly upon the mechanical stress imposed, stroboscope and telescope can be used as aids for an accurate adjustment of the amplitude. There are generally no variations of the amplitude over a period of about two days.

A suitable criterion for a method of cultivation is the behaviour of the culture itself. With the described apparatus exponential growth (i.e. unrestricted growth with constant rate of multiplication) can be obtained over a wide range of the growth curve. In substrate A or A_4[3,4] the average rate of multiplication of *Aspergillus oryzae* was 0.28 to 0.3 duplications of dry weight mycelium/h, this rate remaining constant up to about 160 mg dry weight mycelium/100 ml. At 37°C the rate of multiplication is considerably higher although the exact figure has not been estimated. The results compare favourably with those obtained by other authors: 0.109 to 0.2 duplications/h for *Penicillium chrysogenum*[5]; 0.264 duplications/h for *Aspergillus ochraceus*[6].

Zusammenfassung. Ein Apparat zum Mischen und Umwälzen von Flüssigkeiten durch Vibrationen (Vibro-Mischer), besonders zur submersen Kultivierung von Mikroorganismen wurde entwickelt. Eine sehr leistungsfähige Belüftung konnte ohne poröses Material zur Feinverteilung der Luft erzielt werden. Mit dieser Methode wurde bei *Aspergillus oryzae* eine exponentielle Vermehrungsphase mit etwa 0,3 Gewichtsverdoppelungen pro h bei 25°C über einen weiten Bereich der Wachstumskurve festgestellt.

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STUDIORUM PROGRESSUS

Further Morphological and Biochemical Studies on Normal and Hybrid Embryos of Sea Urchins[1]

The present authors have studied in previous experiments the synthesis of desoxyribonucleic acid (DNA) during normal and hybrid development of the sea urchin embryos[2–6]. In addition, the metabolic activity of ribonucleic acid (RNA) has been analysed by using the technique of isotopic labelling[7]. The present paper deals with further results of our RNA experiments which have been extended to other hybrid combinations. At the same time it appeared very desirable to have detailed data about the numbers of nuclei in different developmental stages of both the pure species and the hybrids. In the following, we shall summarize the results of the nuclear countings in the first section; the biochemical findings will be presented in the second section.

I. Counting of Nuclei (BALTZER)

Technique. For counting the nuclei squashed preparations were used. The embryos were fixed for $^1/_2$ to $1^1/_2$ h in picro-acetic acid (Boveri), washed several times in distilled water and stained for $^1/_4$ h in Mayer's Haemalum. Then they were dehydrated in alcohol and squashed under a small cover-glass in a minimum of xylol and Canada balsam. By squashing, the nuclei were distributed in a