METHOD OF ISOLATION OF ALVEOLAR MACROPHAGES AND STUDY OF THEIR FUNCTION

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With the increasing interest nowadays in reactions of alveolar macrophages (AM) in disease, questions concerning the techniques used to obtain and study them are assuming ever-increasing urgency. Meanwhile there is as yet no generally accepted method of isolating these cells and evaluating their functional state. The most widely used method is that of Myrvik et al. [4]. Using this as the basis, we have introduced a number of improvements to facilitate the isolation of AM and working with them, and we have also suggested our own modification for estimating their phagocytic function.

EXPERIMENTAL METHOD

Experiments were carried out on 25 adult Chinchilla rabbits weighing 3000–3500 g. To obtain AM the animals were killed by air embolism, which is preferable to killing the animals by inhalation of ether or by high decapitation [3]. These last methods have serious disadvantages: Ether, which acts directly on AM, affects the function of the cells and changes their enzymic activity, whereas decapitation is accompanied by leaking of blood into the trachea, and this is a very undesirable factor which distorts the experimental results.

After thoracotomy, the heart–lung complex was not removed from the chest but left in situ; first, this prevents the possibility of very undesirable trauma to the lungs and, second, it enables the adequacy of stretching of the lungs during filling with fluid to be judged later. After compression of the trachea, 50–60 ml of sterile Hanks' solution at a temperature of 4–6°C and pH 7.2–7.4 was injected into its distal part from a syringe. The criterion that an adequate volume of liquid has been injected was enlargement of the lungs until they occupied the whole thoracic cavity, so that overstretching of alveoli, leading to rupture and the appearance of erythrocytes in the washings could be avoided. The washings were withdrawn 2–3 min after injection by means of the same syringe. If necessary this manipulation can be repeated. Withdrawal of fluid was accompanied by careful massage of the filled lungs. In this way, without effort, it was possible to obtain about two-thirds of the volume of fluid injected into the lungs.

The fluid obtained by pulmonary lavage was centrifuged at 1500 rpm for 10 min; the supernatant was poured off and the cell residue resuspended in sterile Hanks' solution with the addition of 10% native bovine serum. The AM thus obtained were studied both in suspension and on coverslips. The yield of cells, of which the great majority were AM was 10^5–10^7. To obtain cytological preparations the AM suspension was poured into flasks with fragments of coverslips, and the flasks were incubated in a tilted position.

In descriptions of investigations of AM no technical details of this stage were given. Yet there are definite difficulties due to the fact that fragments of coverslips in the flasks adhere to each other and are separated with great difficulty, and on their subsequent removal from the flasks with forceps, they often break. To prevent adhesion of the coverslips, split matchsticks have been used, but these hold the glass insecurely and also break. For this purpose we suggest special coverslip holders made of stainless alloy and consisting of short spring clips with permanently closed jaws which firmly fix the coverslip held in them. The clips with coverslips held by them are sterilized in flasks closed with rubber stoppers. During subsequent work the coverslips are taken from the flasks together with the clips by means of forceps.

Furthermore, to hold the flasks in the tilted position, we have suggested a stand consisting of a metal support with sockets and with a bracket to allow the stand to be tilted through the necessary angle (Fig. 1).

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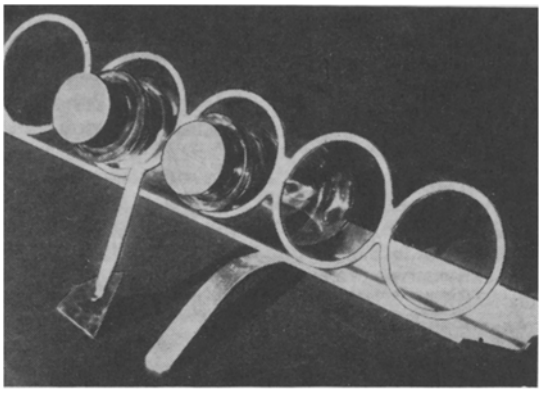

Fig. 1. Stand holding flasks in tilted position, and clip holding coverslip.

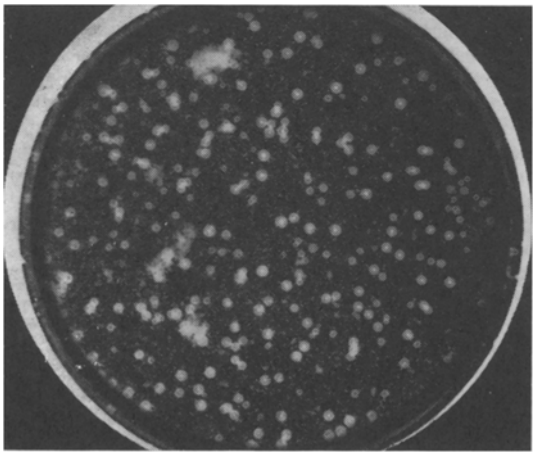

Fig. 2

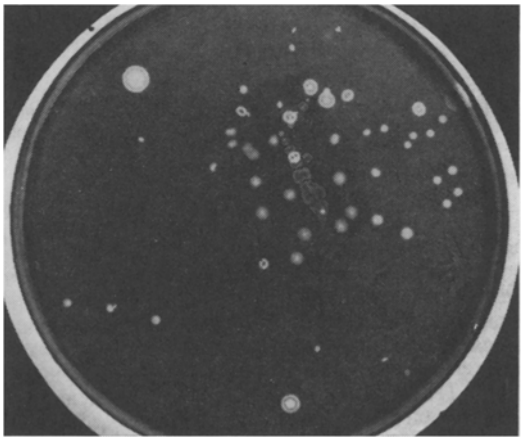

Fig. 3

Fig. 2. Growth of staphylococci from original suspension of macrophages and bacteria.

Fig. 3. Growth of staphylococci from suspension of macrophages and bacteria after incubation for 3 h.

The phagocytic activity of AM was studied against the staphylococcus (standard strain No. 600) and micrococcus. We used Hanks' solution which differed from the standard in not containing antibiotics, which affect the viability of the test microorganisms. The ingestive power of AM was studied microscopically by the usual method [1, 5]. After incubation of the suspension of macrophages and bacteria for 30 min two parameters were estimated: the phagocytic activity of the macrophages (PAM), expressed as the number of macrophages, in percent, undertaking phagocytosis of the microorganisms, and the phagocytic number (PN), characterized by the number of microorganisms ingested by one macrophage. The digestive activity of the macrophages was studied by two parallel methods, the first of which is generally adopted [1, 4, 5], whereas the second is our own modification of a method devised by the writers previously [2], for determining the index of completeness of phagocytosis (ICP) by peripheral blood leukocytes. Having kept to the principle of the method, we modified the proportions of the ingredients used in the test. To 1 ml of a suspension of macrophages in standard dilution (200,000–300,000 cells in 1 ml) a suspension of the test microorganism was added in the proportion of 50 bacterial cells per macrophage, after which one standard bacteriological loop, obtained from the original suspension of macrophages and bacteria, was transferred to 1 ml of sterile physiological saline, which was poured into a Petri dish, and flooded with agar, for subsequent counting of the colonies which grew on the Petri dish after incubation for 24 h. The material was reseeded in the same way after incubation of the suspension of macrophages and bacteria for 3 h at constant temperature. The number of colonies per dish was counted by means of an instrument for counting bacterial colonies. The ratio of the number of colonies growing on the dish from the original suspension of macrophages and bacteria (Fig. 2) to the number of colonies growing from the suspension of macrophages and bacteria after incubation for 3 h (Fig. 3) gives the value of ICP. If ICP exceeds

1.0 this means that phagocytosis is complete. According to our data, ICP of staphylococci by macrophages from intact rabbits was 1.9 ± 0.2 and for micrococci ICP was 9.2 ± 1.1.

Parallel investigations of the function of digestion of the test microorganisms by AM by the two methods (microscopic and bacteriological) showed conclusively that the results were constantly uniform. Each method has it own advantages: Microscopic analysis gives some idea of both the ingestive and the digestive activity of the macrophages, whereas the bacteriological method reflects only the end result of the phagocytic reaction and characterizes the defensive function of the cell as a whole. Meanwhile the bacteriological method of determination of ICP, while completely reliable, is less tedious and laborious and can be recommended for use in research.

The technical improvements described above thus facilitate the work and save time, and the modified bacteriological method of determination of the completeness of phagocytosis can be recommended for assessment of the defensive function of macrophages, both parallel with microscopic investigation, making the results more reliable, and as an independent method.

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A LUMINESCENCE METHOD FOR EXPERIMENTAL HEART RESEARCH

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A promising trend in diagnostic medicine is observation both of the luminescence of luminescent substances (LS) introduced into the body and selectively accumulating in certain organs and tissues, and also of intrinsic luminescence of the tissues. For example, measurement of intrinsic tissue luminescence was used in [1] to study oxidative metabolism of the dog heart in vivo during acute ischemia and hypoxia. Advances in fiberoptic technology, so that exciting luminescent radiation can be introduced inside the body and also led out, has greatly widened the scope for the use of luminescence methods and has stimulated their further development. For instance, in [2] a hematoporphyrin derivative was used as LS, enabling malignant tumors to be diagnosed in situ in the early stage by means of fiberoptic laser fluorescent bronchoscopy. The search for other LS suitable for use in the luminescence diagnosis of diseases of the internal organs or tissues, especially those suitable for investigations by fiberoptic techniques, is of much interest.

Pyrrole derivatives, which are widely used in the manufacture of chemotherapeutic preparations, are evidently noteworthy from this point of view. Compounds with spasmolytic and local-anesthetic properties [3], with an active effect on the central and autonomic nervous system [4] and capable of lowering blood pressure [5], are known. The antiarrhythmic activity of compounds of this type also has been described [6], which encourages the hope that they may accumulate preferentially in corresponding zones of the heart and, if they are in the LS category, that they can be used for luminescence diagnosis of these zones.

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