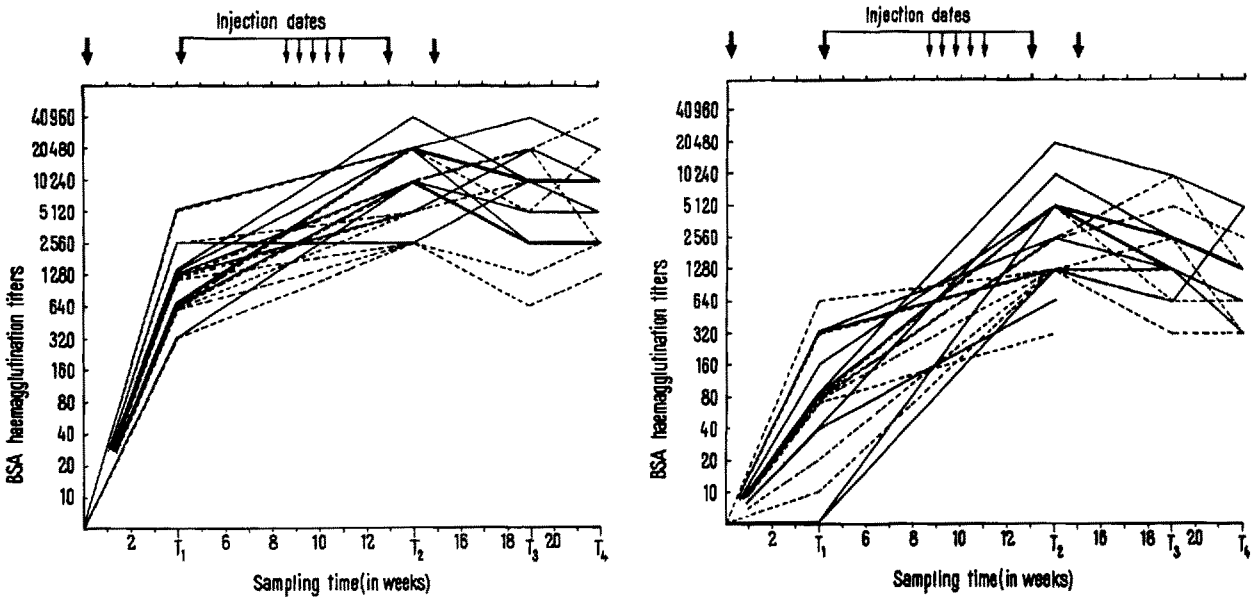

Fig. 2. Diagrams drawn by joining the points corresponding to the BSA haemagglutination titers in each animal. ·····, IFA; ——, CFA. Large arrow: injection of BSA mixed with adjuvant. Thin arrow: injection of BSA without adjuvant. Left: young rats; right: aged rats.

(1) Four weeks after the first injection of BSA mixed with adjuvant (T_1), the production of antibodies in aged animals is definitely lower and the individual values more scattered than in young animals. All the young animals produced antibodies that can be shown by passive haemagglutination; two aged animals have not produced measurable quantities. In both young and aged animals, the antibody production is practically unchanged whether CFA or IFA is used.

(2) After immunization has been continued (T_3), the production of antibodies by aged animals remains lower than in young animals. However, the repetition of the antigenic stimulus noticeably lowers the difference between young and aged rats. In both young and aged animals, the production of antibodies is higher when the animals have been immunized in the presence of CFA.

(3) After the last injection of BSA-CFA (T_3 and T_4), a certain irregularity in antibody production can be found in some animals. But on the whole there appears, in young animals, to be a stabilization in antibody production and, in aged animals, a beginning of decrease of this production.

Under the experimental conditions we used, the production of antibodies in the aged animals group has always been inferior to the production in the young animals group[4].

Résumé. La production d'anticorps antiprotéiques a été étudiée comparativement chez des rats de 3 mois et de 22 mois, par la méthode d'hémagglutination passive. Les résultats montrent et précisent la différence de comportement immunologique entre animaux jeunes et animaux âgés.

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[4] We thank Mrs. C. GAILLARD and Miss O. JEANNEQUIN for their technical assistance.

STUDIORUM PROGRESSUS

Postoperative Hypoxia after Extracorporeal Circulation: A Possible Graft against Host Reaction (Preliminary Communication)

The problem of respiratory insufficiency after extracorporeal perfusion for open heart surgery, particularly in correcting the tetralogy of Fallot, is one which has caused general concern. There are several reasons for the postoperative onset of such respiratory complications and these include pain, respiratory obstruction, and tampon-

ade. Even when these more obvious causes have been excluded there remains still unexplained a postoperative hypoxic state which clinically resembles pneumonia.

In recent months we have turned our attention to this particular type of hypoxia. Initially no auscultatory or radiological signs are present to account for the respiratory impairment which is manifested by a progressive desaturation in spite of positive pressure respiration with pure oxygen. At this stage it is difficult to separate a cardiac cause from a purely respiratory one, but measure-

ments of gas exchange reveal a marked right to left shunt due to venous blood crossing the pulmonary bed without coming into contact with oxygen. (Data supplied by Dr. M. K. SYKES and Dr. B. ROBINSON, Department of Anaesthetics.) This inability to exchange normally oxygen and carbon dioxide is inevitably followed by a progressive acidosis which together with progressive hypoxia can lead to the death of the patient. Such a sequel is rare. More usually patients in whom this condition is recognized make a spontaneous recovery on the third or fourth postoperative day and rapidly regain normal lung function.

Recently, a report by KOUNTZ et al.[1] from this laboratory showed that certain circulating cells – plasma cell precursors – leech on to vascular endothelial cells of the homotransplanted kidney and then rapidly differentiate into plasma cells. In migrating through the walls of the intertubular capillaries they cause disintegration of the endothelial continuity which results in hypoxia of the tubules (TYLER et al.[2]) and finally necrosis. These, in fact, are the physical and biochemical features of rejection of canine homotransplanted kidneys. The exact immunological factors involved still remain undefined (WILLIAMS et al.[3]).

It occurred to us that the role of the plasma cell precursors could be extended to a study of the lungs of patients dying after extracorporeal perfusion because such lungs contain large numbers of plasma cells. The pathology of many of these lungs is complicated by preexisting disease and so we have used laboratory models to develop a possible explanation of respiratory insufficiency after extracorporeal perfusion. We had access to material derived from laboratory models which allowed a more general analysis to be carried out.

Materials and methods. Group 1: The material consisted of sections of the lungs from 18 patients dying in the first five postoperative days following open heart surgery, particularly for the correction of tetralogy of Fallot. We have excluded the lungs of patients dying within 24 h of the operation. Such lungs show gross vascular stagnation and fibrous contraction and death has probably been due to mechanical factors following the correction of the congenital abnormality. This histology was made available to us through the courtesy of Dr. MONICA BISHOP.

Group 2: Lungs were made available from laboratory experiments on dogs subjected to open heart surgery using an extracorporeal circulation with and without priming with homologous blood.

Group 3: The lungs of 24 dogs were made available for histological examination through the courtesy of Dr. J. B. WEST et al., who are currently engaged in a physiological study of the isolated lung perfused by blood cross-circulated from another dog.

Group 4: 6 lungs of the donor pump dogs of Group 3 experiments were also available for study.

Group 5: 6 hearts were homotransplanted by the technique of Downie (1953).

Group 6: Normal canine lungs.

Various experiments in Group 2 are in progress which are designed to study the effect on the lungs of animals perfused with uncrossmatched homologous blood, autologous blood, and rheomacrodex in dextrose alone as primer of the extracorporeal pump.

Histology. The lungs were fixed in normal saline. Sections were cut at about 5 μ and stained routinely with several preparations, including methyl green pyronine. Biopsies of some lungs in Group 3 and hearts from Group 5 were also taken for electron-microscopic study (Dr. P. L. WILLIAMS, Guy's Hospital Medical School).

Results. Normal lungs show occasional plasma cells in the peribronchial tissues. For this reason the periphery of the lungs was examined to exclude this phenomenon.

The lungs of the first three groups showed varying degrees of plasma cell infiltration. The lungs of Group 4 showed no plasma cell infiltration.

It would appear that, in the lungs derived from the first three groups of projects, the same process of cell differentiation into plasma cells is taking place. The plasma cell precursor has also been found in kidney homotransplants in both humans and dogs (GALLE and DE MONTERA[4], KOUNTZ et al.[1], WILLIAMS et al.[3]). Similar cells can also be found invading the endocardium of the canine homotransplanted heart. The lungs of Group 3 were particularly interesting because even after 1 h of perfusion one could detect early plasma cells. This makes the isolated perfused lung a very convenient technique for studying the leeching on of the plasma cell precursors and their further differentiation and migration in strict relation to time. For an adequate assessment of their cytoplasmic status it is essential to use the electron microscope.

In Group 1 and Group 2 the plasma cell precursors may have originated from the fresh blood used to prime the extracorporeal machine. Any disturbance they may cause may be termed a graft against host reaction. In Group 3 the plasma cell precursors originate in the donor dog and so they may constitute a host against graft reaction such as has been described by KOUNTZ et al.[1] and WILLIAMS et al.[3] in the homotransplanted kidney.

The isolated lung preparation is also very convenient because it allows hourly biopsies to be taken over a period of about 5 h perfusion so as to follow the process of differentiation, the nature of the migration through the vascular endothelium and any possible relation to the development of interstitial oedema.

An even better preparation than the isolated lung for a study of the nature of the circulating cells which make contact with foreign endothelium, is the heart homotransplanted by the technique of DOWNIE[5]. The endocardium and subendocardium appear to be the first targets of the invading cells. For electron-microscopic purposes, a section of endocardium provides one with a concentrated collection of cells. An electron-microscopic assessment of the nature of the invading cells is in preparation (Dr. P. L. WILLIAMS).

Discussion. The cell which first leeches on to the capillary endothelium of a homotransplanted kidney is one which has a cytoplasm capable of developing into a plasma cell since it is well endowed with ergastoplasm. It resembles in its nucleus/cytoplasmic ratio a lymphocyte: it has a nucleus of about 6μ under the electron microscope. Its cytoplasm differs markedly from that of any normal lymphocyte in that it has abundant ergastoplasm. A similar cell has been observed in a human transplanted kidney and is referred to as an unidentified cell (GALLE and DE MONTERA[4], Figure 14). It is possible that a similar cell has been observed in human thoracic duct lymph; it has been estimated to constitute 2% of the monocytes

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(ZUCKER-FRANKLIN[6]). MARCHESI and GOWANS[7] have also observed a similar cell in the thoracic duct lymph of rats. Both ZUCKER-FRANKLIN and MARCHESI and GOWANS regard the cell they have observed as a large cell and indeed classify it as a large lymphocyte; ZUCKER-FRANKLIN refers to it as a metabolically more active cell than the usual large lymphocyte; MARCHESI and GOWANS have termed it a Type 3 lymphocyte. We question the wisdom of classifying such a cell as a lymphocyte because of its distinctive cytoplasm, and until further clarification we prefer to use the term 'plasma cell precursor'. Whatever kind of cell it may have originated from it is clear, from electron-microscopic studies, that it can differentiate through all the stages up to a mature plasma cell. It can transform in a few hours into a plasma cell and it can undergo division.

If the cell we have described as the first to leech on to foreign endothelium is the same cell as has been observed in the thoracic duct lymph, it would suggest that there is evidence for natural immunity against homografts. It is suggestive that this circulating cell can, on its own initiative, make contact with foreign tissue and differentiate into a plasma cell. We have no evidence that an antigen-antibody reaction is involved. We have evidence from several homotransplanted organs that capillaries are destroyed when this cell starts to migrate into the interstitial tissue.

The lungs of human subjects who die in the first few days following an extracorporeal operation for the correction of tetralogy of Fallot show a plasma cell infiltration, interstitial oedema and a red-cell extravasation in addition to stagnation due to inadequate respiration. Clinically, this presents as a pneumonic state and as a result of the hypoxia a lactic acidosis develops which can cause the death of the patient. If death does not ensue a spontaneous remission occurs some days later which is probably due to the natural defences of the patient reacting against the cells and so causing their disintegration.

It would be difficult, if not impossible, to build a graft against host theory on the evidence of the lungs in Group 1 alone. The histology of the lungs in this group is complicated by: (1) The usual and temporary histological disturbance caused by extracorporeal circulation. The correct histological basis for this study derives from the lungs of Group 4 and Group 2 when homologous blood is not used. (2) The histological lesions caused by the migrating cells. The correct histological basis for this study derives from the lungs in Group 2 and 3. (3) The effects of impaired respiration for several days resulting in stagnation of secretions. And (4) the preoperative pathology in the lungs. Thus it is necessary to resort to the laboratory models described in this communication in order to attempt to reconstruct the whole history.

Fresh blood is usually used for priming extracorporeal machines. This means that a truly massive infusion of living white cells is inevitable. That these infused white cells appear in the lung of the individual being perfused is well established. What is more difficult to establish, so far as the lung is concerned, is whether the cells are filtered off by the natural function of the lung (WEISBERGER et al.[8]) or whether the cells actively make contact with the endothelial cells of the lung.

The next point is to decide whether all the physiological changes in the perfused lungs can be attributed to the activity of the foreign white cells in causing capillary damage. This possibility could be included in the category of 'graft against host reactions' with the very definite proviso that we have no evidence of an antigen-antibody reaction; the physical results of migration (i.e. increased capillary permeability, perivascular oedema and altered lung blood flow) can sufficiently explain the physiological effects. One of us has already claimed that in other reports of graft against host reactions, any reference to antigen-antibody reactions is usually no more than a reasonable assumption and that the secondary syndrome can be explained by graft lymphoid aplasia resulting in infection (DEMPSTER[9]); the wasting disease after thymectomy would support this view (SHERMAN et al.[10]).

The question whether the physiological changes are wholly due to the filtered white cells was studied by means of some experiments in which fresh homologous blood was excluded from the extracorporeal machine and substituted by dextrose, saline and Rheomacrodex. Physiological tests 2 h after a 2-h perfusion show that the lungs of perfused animals are capable of normal gas exchange, although histologically the lungs show the usual alarming haemorrhagic state following extracorporeal circulation but without plasma cells. The lungs of Group 4 show similar vascular extravasation and increased cellularity – features already well documented by SCHRAMEL et al.[11]. Factors other than white cells, therefore, appear to be involved but which do not lead to progressive impairment of lung function some hours after operation. The general effects of cross-circulating blood are well-known and have to be taken into consideration. It is probable that the usual basic 'perfusion lung' damage enhances the capillary damage brought about by the migrating plasma cells. The clinical implications of this concept, if it be confirmed, must discourage the use of fresh homologous blood as a priming fluid in extracorporeal machines.

The search for the nature of the first cell to leech on to the vascular endothelium can only be done by electron microscopic methods. For this particular purpose the perfused lung is an easier tissue to examine than a homotransplanted kidney. Foreign circulating cells can settle in a perfused lung and can be recognized as early plasma cells within an hour. Masses of plasma cells can be demonstrated after 6 h perfusion. Easier still than the perfused lung is the homotransplanted heart. Within 24 h the endocardium and subendocardium are heavily infiltrated with early plasma cells. A section of these areas provides one with a massive concentration of migrating plasma cells in various stages of differentiation. This greatly facilitates the problem of tracing the origin of the plasma cell precursors and the stages of differentiation into plasma cells. These cells, which have been identified in the thoracic duct lymph, may be cells which have already been committed to another antigenic stimulus and are in transit; this is a view held by our colleague P. L. WILLIAMS. We have not sufficient evidence to rule out that the plasma cell precursors do not, in fact, transform from small lymphocytes. It is possible that the plasma cell precursors of the dog are more antagonistic to foreign tissue than are the human cells. However, the plasma cell infiltration in human lungs after prolonged periods of extracorporeal circulation consistently correlate with a severe clinical state of hypoxia.

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There would appear to be no clear correlation between the severity of the hypoxic state and the preoperative state of the lungs and the nature of the cardiac lesion. Thus we have been forced to consider factors other than pre-existing mechanical damage.

The severity of the postoperative hypoxic state could vary with such factors as: (1) the histocompatibility of white blood cells and the lungs of the perfused patient; (2) the age of blood used to prime the pumps; (3) the duration of the perfusion by the extracorporeal pumps; (4) the preoperative state of the lungs; (5) the preoperative state of the coronary circulation.

The spontaneous recovery, we believe, is due to the destruction of the invading plasma cells by the defences of the host and also to the resolution of the oedema caused partly by the plasma cells and partly by the general effect of the extracorporeal perfusion[12].

Zusammenfassung. Bei Patienten, bei welchen ein extrakorporaler Blutkreislauf durchgeführt wurde, fanden sich in den Lungen grosse Mengen von Plasmazellen. Bei Benützung von frischem homologem Blut für die Ingangsetzung des extrakorporalen Kreislaufes scheinen die Plasmazellen des Spenderblutes in den Lungen schädigend zu wirken. Sie können möglicherweise für Kapillarschädigungen und Störungen des normalen Gasaustausches verantwortlich gemacht werden.

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[12] Acknowledgments: The expenses involved in these experiments were defrayed by a grant from the Nuffield Trust (D.G.M.) and by a grant from the Wellcome Trust and by a generous personal gift from Sir BILLY BUTLIN (W.J.D.). We are grateful for technical assistance from Miss BEATON, Miss BROWN, Miss HANLY and Mr. SPARKS, Mr. WILLIAMS and Mr. ADAMS.

PRO EXPERIMENTIS

Polyzonale Dünnschichtchromatographie. Chromatographische Fliessmittelentmischung und ihre Anwendung zur Trennung von Substanzgemischen

I. Teil: *Duozonale Dünnschichtchromatographie, Theorie und Praxis*

Dünnschicht- (DC) und Papierchromatographie (PC) unterscheiden sich von anderen Chromatographierverfahren wesentlich dadurch, dass das trockene Sorptionsmittel erst während des Trennprozesses mit dem Fliessmittel in Berührung kommt. Es besteht also anfangs kein Gleichgewicht zwischen stationärer und mobiler Phase, und deshalb erleidet ein mehrkomponentiges Fliessmittel beim Eindringen in die Schicht zwangsläufig eine teilweise Entmischung. Diese Erscheinung ist von der PC her wohlbekannt[1–9]. Ein entsprechender Vorgang bildet die Basis der Frontalanalyse[10] nach TISELIUS[11–15].

Die Entmischung macht sich besonders deutlich bemerkbar, wenn das Fliessmittel – z. B. ein binäres Gemisch – Komponenten von deutlich verschiedener Polarität enthält. Das Sorptionsmittel nimmt vom einströmenden Fliessmittel beide Komponenten bis zur Einstellung des jeweiligen lokalen Gleichgewichts zwischen mobiler und stationärer Phase auf. Weil dabei die polarere Komponente bevorzugt abgefangen wird, entsteht allmählich hinter der Fliessmittelfront (α-Front) ein sich stetig erweiternder Bereich (α-Zone), der nur noch die weniger polare Fliessmittelkomponente enthält. Nach hinten ist die α-Zone durch eine β-Front gegen eine β-Zone abgegrenzt, welche das Fliessmittel in seiner ursprünglichen Zusammensetzung enthält und welche bis zum Eintauchspiegel zurückreicht. Da aus dem Fliessmittelbehälter ständig frisches Fliessmittel nachströmt, erweitert sich auch die β-Zone. Die Wanderungsgeschwindigkeit der β-Front ist gegenüber derjenigen der α-Front um einen konstanten Verzögerungsfaktor k_β[16] kleiner. Es ist deshalb in jedem Zeitpunkt

$$\frac{\text{Distanz Eintauchspiegel–β-Front}}{\text{Distanz Eintauchspiegel–α-Front}} = k_\beta . \qquad (1)$$

Die durch k_β gekennzeichnete Lage der β-Front ist für ein gegebenes Sorptionsmittel und eine gegebene qualitative Fliessmittelzusammensetzung eine Funktion des

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[10] Bei der Frontalanalyse bringt man zunächst die Chromatographiersäule ins Gleichgewicht mit dem Lösungsmittel und lässt dann eine verdünnte Lösung des Substanzgemisches solange hindurchlaufen, bis die Substanzkonzentration (Brechungsindex) im Säuleneinlauf und Säulenauslauf identisch wird. Jede Komponente des Substanzgemisches verursacht eine *Stufe* im Diagramm «Brechungsindex gegen ausgeflossenes Volumen».
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