ORGAN AND TISSUE TRANSPLANTATION

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CONTENTS

The recent interest in transplantation of organs is largely due to the technical advances in surgical procedures making it possible to replace organs in the human, as well as to an increased understanding of the basic biological problems underlying the rejection of such grafts. Moreover, the therapeutic armamentarium which can to a certain extent suppress such immunological reaction has been greatly increased in recent years.

At this first International Congress widely ranging topics were discussed, including organ transplantation, mechanism of graft rejection, methods of immuno-suppression, genetics of transplantation, bone marrow transplantation, and finally, cancer as homograft. It would be quite impossible in this brief communication to do more than give an outline of some of the papers presented. Further details can be obtained from the compendium of abstracts of paper*, and from the more com-

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* A copy of these Astracts can be obtained from the Medical Library, St. Luke's Hospital.

prehensive book giving the actual papers, to be published in the near future.

A. Organ Transplantation

1. Kidney Transplantation

It is only natural to expect that kidney transplantation would receive a lot of attention. Thanks to the Human Kidney Transplant Registry (Murray et al. Boston, USA) more than 1200 kidney transplantations have been recorded, and the various factors which influence survival can be compared. It was shown that 75% of kidneys from parents or siblings, and 60% of grafts from living donors or cadavers (unrelated) were still functional one year after transplantat. This represents a considerable improvement over previous reports. (A more detailed study appears in the July issue of "Transplantation"). In a personal communication J. E. Murray stated that survival of parental kidney grafts at 18 months is 49% and at 24 months is 43%.

Immunosuppressive therapy plays an important part in preventing rejections of homografts. Sheil et al. (Boston, USA) reported that in dogs the expected life of a grafted kidney can be increased threefold by such means. Kountz & Cohn (Palo Alto, USA) indicated that injection of these drugs into the graft was a better way of preventing graft rejection than either oral or parenteral administration.

The development of micro-surgical techniques enabled the transplantation of kidneys in small laboratory animals such as the rat. This is important because the rat is the largest animal which is readily available in highly inbred strains, so that comparison between isografts and homografts could be made. Using such a technique Lindquist et al. (Boston, USA) found that two days after transplantation mononuclear cells were present in the peritubular capillaries and some adhered to the vascular epithelium. IgG was localised on the walls of many peritubular capillaries and in vein walls. Beta 1c globulin, but not fibrinogen or alpha 2 macroglobulin was present. IgM was not found.

Changes in the glomerular architecture included endothelial cell swelling and proliferation, mesangial swelling and some basement membrane thickening. Perivascular infiltration of mononuclear cells with large vesicular nuclei, prominent nucleoli and pyroninophilic cytoplasm appeared 2-3 days after allografting and infiltrated the cortex diffusely by the 5th day.

It is essential to be able to detect rejection as early as possible so that suitable therapy can be instituted. Spencer and Posborg-Petersen (Aarhus, Denmark) suggested that the quantitative determination of urinary lymphocytes excretion was a valuable aid in the management of patients with renal transplants. With clear-cut rejection episodes the number of lymphocytes was more than 25,000/hour, and these lymphocytes disappeared from the urine on initiation and maintenance of adequate steroid therapy. Likewise 131I hippuran renography performed daily in dogs subjected to renal allografts showed after 2-7 days a continuous decrease of excretory capacity which finally resulted in a renogram of the obstructive type. This was shown by 125I hippuran auto-radiography to be due to ischæmia and not to obstruction by œdema (Lundgren et at. Stockholm, Sweden).

The problem of glomerulonephritis in the transplanted kidney was discussed by Feldman et al. (Boston, USA) and Hamburger et al. (Paris), and the nephrotic syndrome was described by Williams et al. (Richmond, USA). As pointed out by Merrill (Boston, USA) these incidents provide rare examples of disordered physiology that could only be studied in the setting of human transplantation, and could provide answers to problems about the relationship between the kidney and hypertension, erythropoiesis, etc.

2. Liver Transplantation

The problems of liver transplantation, although technically more formidable than renal grafting, are not insurmountable, and this procedure was described in pigs, dogs, and rats. Liver homotransplantation was described in pigs by Riddell et al. (Bristol, Eng.). The histological picture of

rejection of the transplant was described. The use of antilymphotic serum (ALS) was mentioned as of possible therapeutic importance. A whole session was devoted to the mode of action and use of ALS and will be described later. Cockburn *et al.* (Boston, USA) described a method of transplanting fresh or preserved liver heterotopically in the pelvis of the rat. About 30% of a donor rat liver was transplanted. Shorter *et al.* (Rochester USA) in transplanting liver in dogs found that urinary uric acid levels characteristic of the donor animal were found in the recipient dog, thus demonstrating again the use of transplantation techniques in the study of the physiology of body systems.

3. Heart Transplantaion

Shumway (Palo Alto, USA) introduced this session by considering the possible consequences of heart transplantation. The immediate causes for concern he suggested, were (a) disorders of rhythm — these became normal again by 4-5 days, and (b) disorders of cardiac output — output was normal by 3-4 weeks. Late consequences could be ascribed to disorders of sympathetic and parasympathetic innervation. These were usually normal by 3-4 months, but disorders could occur if sympathetic nerves innervated parasympathetic nerve endings, with obvious malfunction of the heart itself as well as altered drug action (e.g. sympathetic drugs will have a parasympathetic action). The technique was beautifully illustrated by a ten-minute film which, it seemed, was necessary to make the incredulous believe that heart transplantation is actually possible.

This technique enabled various physiological studies to be undertaken. Lower and Celeveland (Richmond, USA) demonstrated that the denervated heart graft had the capacity to increase the cardiac output under a variety of physiological conditions, including hypoxia and exercise. Cardiac output was still normal $5\frac{1}{2}$ years after autograft of the heart. Re-innervation was unequivocally demonstrated in the majority of animals 3-5 months after autotransplantation. Similar studies were performed by Willman *et al.* (St. Louis,

USA) in dogs and baboons. ECG changes after transplantation were studied by Dureau *et al.* (Brooklyn, USA).

The fact that current surgical techniques were successful in 80-90% of experiments (Lower and Cleveland, Richmond, USA), and that both canine and human cadaver hearts have been successfully resuscitated after 30 minutes of clinical "death", with, in the dog, successful subsequent transplantation after 4-7 hours of storage under hypothermia, renders this a practical proposition. The fact that heart grafts are less easily rejected than kidney grafts renders the control of rejection crises easier to deal with (Lower and Cleveland, USA; Cacher *et al.* Paris, France).

4. Lung Transplantation

The problem of lung transplantation was discussed by Blumenstock (Cooperstown, USA), Hardy *et al.* (Jackson, USA), and Neveux *et al.* (Paris, France). Lung transplantation in 1000 dogs enabled various physiologic and immunologic studies to be undertaken. There was an immediate fall in O_2 uptake, blood gas values, pH, and pulmonary arterial pressures. These gradually improved but there was no return of the cough or of the Hering Breuer reflex up to 18 months after transplantation. Lymphatic regeneration could be demonstrated within two weeks. With homotransplantation, the average survival of the allograft was 40 days, with the use of mediastinal irradiation, azathioprine and steroids, but survival for 2-5 years with methotrexate was described by Blumenstock.

5. Pancreas Transplantation

Two papers by Kelly *et al.* (Minneapolis, USA) and Bergen and Teixera (Chicago, USA) described transplantation of the pancreas in dogs and in patients suffering from diabetes mellitus.

6. Other problems

A number of other related problems were discussed, including the ethics and legal aspects of human organ transplan-

tation (Woodruff, Edinburgh, Scotland; Couch, Boston, USA); the use of local X-rays in the treatment of kidney transplantation (Kirpatovsky et al., Moscow, USSR); the irradiation of lymph nodes (Shikata et al., Kyoto, Japan); the use of preserved organs (Ackerman et al., London, England); the selection of donors for kidney transplantation (Dausset et al. Paris, France); the presence of hæmagglutinins in human kidney transplant recipients (Rapaport et al. New York, USA); the detection of onset of graft rejection by immunological methods (Kaskova, Praha, Czech.); and the lung complications associated with kidney transplantation — a condition known as "transplant lung" (Slapak et al. Montreal, Canada).

An interesting and unexpected result was the transfer of secondary epidermoid carcinoma from a donor who died of this condition without evidence of metastasis to the kidney (Wilson et al. Boston, USA) — a finding which seems to rule out cancer patients as prospective donors of organs for transplantation.

It can be seen from the above summary that surgical techniques are no longer the main factor responsible for the success of an organ transplant. Much more important is a knowledge of the various factors which make the graft unacceptable to the host, and ways and means of suppressing the immune reaction. These basic considerations will be dealt with briefly below.

B. Mechanism of Graft Rejection

Although Nossal (Melbourne, Australia) described this as "a golden age of immunology" in view of the rapid and important advances in this field, we still do not fully understand the mechanism by which the body recognizes a graft as foreign, and then mounts an immunological attack on it with subsequent rejection. It is known that recognition depends on the strength of the transplantation antigens themselves, as well as on the presence of an intact immune system which involves recognition (afferent) and effector (efferent) mechanisms.

That the graft is immunologically immune as long as it has no lymphatic contact with the host was further emphasised by Barker and Billingham (Philadelphia, USA) who described a technique of isolating a flap of skin in guinea pigs so that the blood supply was intact, but the lymphatics were destroyed. This flap could retain grafts as long as the lymphatics were absent, indicating that an intact lymphatic drainage is essential for rejection of skin homografts.

The production of tolerance of homografts is a well authenticated phenomenon in immunobiology, and can be produced in suitable animals by the injection of antigenic substances in adequate amounts. This can result in grafts surviving for prolonged periods. Hasek et al. (Prague, Czechoslovakia) produced evidence that serum antibody can abolish tolerance and produce destruction of tolerated skin grafts, while Wright (Palo Alto, USA) demonstrated specific tolerance to H-2 iso-antigens induced by enhancing antibody. Sabbadini and Sehon (Montreal, Canada) showed that RNA extracted from lymph nodes of immunised mice can confer immunity. Of major significance is the finding of Nossal (Melbourne, Australia) that minute quantities of antigen can induce tolerance whereas larger quantities produce immunization — an extension of the work of Mitchison (London, England).

The recent enormous interest in the thymus and its role in the immunological system was reflected by the number of papers presented. Miller and Mitchell (Melbourne, Australia) studied the influence of the thymus on antigen-reactive cells and their precursors. They found that the proliferation and differentiation of such cells are not thymus dependent, but require contact with antigen. Tyan et al (San Francisco, USA) found that lymphoid "stem" cells in mouse fœtal liver were thymus dependent by 14 days of gestation. Trainin et al. (Rehovot, Israel) showed that although thymectomised mice fail to reject homografts, and lymphoid cells from such animals fail to produce a graft-versus-host reaction in F_1 hybrid recipients, however, lymphoid cells from such animals plus

thymus extracts were able to produce a graft-versus-host reaction. Davies *et al.* (London, England) discussed the role of thymus-derived cells in an immune response.

This obviously sketchy account of this section is indicative of the intricate nature of the problem of the mechanism of graft rejection and and of the relation of lymphoid tissue, and especially the thymus, to this process.

C. Methods of Immuno-depression

1. Antilymphocytic Serum (ALS)

The production of serum active against lymphocyte is an important development in the treatment of immune rejection phenomena. Woodruff and his group (Edinburgh, Scotland) who pioneered this form of treatment gave evidence that ALS inhibited skin homograft rejection, whereas antibody fragments (Fab')² prepared by pepsin digestion of anti-lymphocytic immunoglobin did not. Lance (Mill Hill, England) observed that continuous steady levels of ALS prolonged graft survival whereas pulsed doses were not so effective. Prent *et al.* (Southampton, England) observed that while newborn mice injected with allogeneic lymphoid cells died of graft-versus-host disease, ALS prevented this if given 6 days after the production of GVH. *In vitro* treatment for one hour was highly effective in suppressing runting. Similar results were reported by van der Werf *et al.* (Boston, USA). Boak *et al.* (Boston, USA) gave ALS to donor animals and found that cells from such animals had lost the ability to initiate a GVH reaction when transferred to a suitable recipient. The survival of renal homotransplants in dogs was also increased after treatment with ALS (Pilchmayr *et al* Munich, Germany).

Although toxic manifestations following ALS treatment included anæmia, evidence of glomerulophritis, anaphylactic shock, as well as the expected lymphopœnia and lymphocytic depletion of the spleen and lymphoid orgfians, (Lance, Mill Hill, England), these drawbacks can be overcome by purification of the crude ALS and the use of the specific immunoglobulin fraction.

2. Chemical Immuno-depression

Various agents have been used to suppress the immunological reaction. Cytotoxic and antiproliferative agents include radiation, purine and pyrimidine analogues, alkylating agents, folic acid antagonists, methylhydrazine derivatives, and antibiotics (actinomycin, mitomycin, and puromycin). Steroids of the hydrocortisone family probably act in two ways (Medawar, London, England): i) the acute effect following high doses is largely peripheral, while ii) chronic doses probably prolong immunosuppressive action by slowing down lymphocyte turnover. Boylston *et al.* (London, England) found ribonucleases effective in suppressing a number of immune responses including rejection of mouse skin allografts and canine renal allografts, if given two days before grafting. Changes in the rate of synthesis of IgG, IgM and specific antibody were always found.

A knowledge of the mode of action of these agents in relation to the cell cycle may be important as shown by Amiel and Dore (Villejuif, France), who found that agents which interfere with DNA synthesis (e.g. mitomycin C) exert maximal immunodepressive activity when given before the antigenic stimulus, whereas alkylating agents and methylhydrazines which can affect cells not in DNA synthesis suppress the immune reaction when given after the antigenic stimulus. The cytotoxic action of lymphocytes *in vitro* — which is independent of DNA synthesis or of mitosis — was abolished by antimycin A, iodoacetate, 2,4-dinitrophenol, while immunosuppressive agents like imuran and cyclophosphamide had only weak effect. (Holm and Perlman, Stockholm, Sweden).

Immunosuppression is of great importance in preventing rejection of grafts. The obvious blunderbuss therapy with ALS, although effective, will certainly be refined to produce fractions which are more active and less toxic. It is also possible, as indicated above, that the use of these and similar pharmacological agents

will provide further insight into the mechanism of graft rejection.

D. Genetics of Transplantation

The importance of basic knowledge of the factors which make the host different from the graft, and the necessity of identifying the most likely donor for prospective graft transplantation, made it imperative for techniques to be developed to identify as many antigenic sites as possible. This work, pioneered by Van Rood, Ceppelini, Batchelor, Terasaki and others, has become of major importance in the study of transplantation in general.

Ceppelini (Turin, Italy) introduced this meeting by summarising the recent advance in leucocyte typing, indicating that "finger-printing" of these antigens is now possible. This has become so complex in recent years that computer methods have been developed to analyse the available data. Studies of the genetic linkage and inheritance of the major loci responsible for histo-compatibility have been worked out. These results were discussed in a recent "workshop on histo-compatibility testing" which took place in Turin in June 1967.

Amos et al. (Durham, USA) described the Hu-1 system in man as the strongest transplantation system, equivalent to the H2 system in mice. Chromosomal inheritance patterns could be easily recognised. Van Rood et al. (Leiden, Netherlands) described the "Group FOUR system" of transplantation antigens in man, and showed that for patients who receive a kidney transplant from a sib or a parent, prior matching of the leucocyte antigens was extremely important for graft survival.

Rapid methods of leucocyte typing were described by Payne et al. (San Francisco, USA), making use of micro-agglutination technique. Bach (Madison, USA) described a method for assay of the major histo-compatibility locus in man (Hu-1), using a mixed leucocyte culture technique.

The obvious importance of this section of research cannot be adequately dealt with in this short summary. Further details can be obtained from the book of abstracts.

E. Bone Marrow Transplantation

Bone marrow transplantation has been of great theoretical importance in biology because of the insight it gives us on the kinetics of stem cells in general and of erythroid cells in particular. It has lately also assumed considerable practical importance in the treatment of aplastic anæmias, leukæmia, and other states associated with marrow aplasia (Mathe, Villejuif, France; van Bekkum, Rijswijk, Netherlands). The papers presented at this Congress dealt with some aspects of the kinetics of bone marrow transplantation, and with the control of the secondary syndrome.

1. Kinetics

The ability of the bone marrow to produce colonies of cells in the spleen of whole body irradiated recipient mice (as described originally by Till and McCulloch, 1961) has been extensively used in animal experimentation. The identity of the colony forming unit (CFU) is unknown. Barnes et al. (Harwell, England) studied the fate of the fœtal liver cells injected into irradiated recipients and found that a percentage of small lymphocytes do contribute temporarily to the marrow population by 14-21 days. It was suggested that the proliferation of bone marrow was proportional to the lymphocyte content. Micklem and Ford (Harwell, England) found that fœtal hæmopoietic cells proliferated faster than adult cells when injected into suitable recipients.

2. Control of Secondary Syndrome

Animals that are given doses of whole body radiation that are otherwise lethal, can survive if given bone marrow cells. Although recovery from radiation occurs, the animals can die from what is called "secondary syndrome" if allogeneic or homologous bone marrow had been used. This is a disease associated with aplasia of lymphoid tissue, depletion of rapidly dividing tissues, e.g. bone marrow, intestine and skin, severe wasting and finally death in a few weeks. This syndrome can

occur whenever immunologically competent cells (ICC) from lymphoid organs, bone marrow, peripheral blood, etc. are injected in sufficient numbers into suitable recipients, and it is equivalent to the graft-versus-host disease. It was calculated by van Bekkum (Rijswijk, Netherlands) that in the human, secondary syndrome can occur by the intra-venous injection of as little as 500 ml of fresh blood in the adult (and only 25 ml of blood in the newborn) or 3×10^7 bone marrow cells/kg body weight, or 3×10^8 liver cells. It is important to realise that normally such allogeneic cells are rejected and only in special cases, when the immune response of these recipients is depressed (e.g. after relative high doses of whole body radiation, alkylating or other cytotoxic agents ,or in certain rare disorders of the immune system such as the Swiss type of alpha-gamma globulinæmia, and hereditary ataxia telengiectasia), can these cells "take" and produce secondary syndrome.

The experiments described at this Congress dealt with the control of Secondary syndrome in animals other than man. Congdon et al. (Chicago, USA) observed that hypoxic animals given bone marrow survived better than normal animals (after irradiation), indicating that stem cells in the bone marrow can differentiate into erythroid precursors rather than into immunologically competent cells under the hypoxic stimulus. Ledney and van Bekkum (Rijswijk, Netherlands) observed that anti lymphocytic serum (ALS) was of little value in preventing graft-versus-host disease in the mouse if it is given after the immunocompetent cells. A striking suppression of the secondary syndrome was however observed by treating donor mice with ALS prior to innoculating their spleen cells into irradiated recipients. In the monkey (Balner et al., Rijswijk, Netherlands) ALS was proved capable of prolonging skin grafts. In some respects therefore, the effect of ALS is dependent on the species used: it is very effective in mice if given to the donors, while in the monkey, it is more effective if given to the recipients.

The control of the secondary syndrome, at present only a laboratory disease can be expected to play a much more important role in the future when the agents mentioned above are used more extensively in the treatment of malignant conditions as well as in transplantation procedures.

F. Cancer as Homograft

The search for antigens specific to cancer was discussed by Sjogren (Stockholm, Sweden). A colony inhibition in vitro technique was described and he concluded that tumours of viral origin in different species had specific cross-reacting transplantation antigens. These results suggest that the specific transplantation antigens are produced under the direction of the virus genome — a conclusion supported by the findings of Bonneau et al. (Marseille, France). McKhann and Harder (Boston, USA) used an indirect isotope labelling antibody technique to demonstrate the presence of tumour specific antigens on cells of virus-induced lymphomas, polyoma-induced sarcoma and methylcholanthrene induced sarcoma in isologous mice.

The curious relationship between normal and malignant trophoplast and the host was discussed by Currie and Bagshawe (London, England) who found that both normal and malignant trophoblast are antigenic to their respective host lymphocytes. Failure to reject either pregnancy or choriocarcinoma (both being antigen-containing allografts) suggests that there is some mechanism preventing their recognition by lymphocytes. It was suggested that "fibrinoid" material (consisting of muco-protein and sialic acid) might produce an electro-chemical barrier which prevents close contact between trophoblast and host lymphocytes.

That the host can at times reject a tumour was indicated from the studies of Hall et al. (London, England). Lymphocytes obtained from lymp nodes of sheep draining the area of transplatation of rat fibrosarcoma, as well as RNA derived from these cells produced a "dramatic albeit temporary regression of the tumour" when injected back into the host — an action

which was found to be specific.

That these experimental studies have some bearing on the clinical problem was demonstrated by Schwarzenberg and Mathe (Villejuif, France). They showed that allogeneic bone marrow transferred to whole body irradiated patients suffering from acute leukæmia produced complete remission in four patients. Transfer of white blood cells resulted in remission in 10/21 patients with acute leukæmia. Regression of pleural and ascitic fluid was obtained after the local injection of heterologous lymphocytes immunised against the tumour.

Although it is too early to be optimistic about these new lines of treatment, it can be seen that an immunological attack against cancer is possible, and some progress has been made in recognising antigenic sites on the tumour which are lacking in the host; in the preparation of lymphoid cells or fractions thereof (e.g. RNA) that are capable of attacking the tumour cells and thus help the host to reject the tumour; and finally, in the use of graft-versus-host reaction employing allogeneic lymphoid cells to enhance rejection of tumour. These and similar methods can be useful adjuncts to radio- or chemo-therapy.

G. Conclusion

The rapid rate of expansion in the field of transplantation witnessed in the last few years can be confidently expected to continue. New techniques of organ transplantation, new concepts in the antigenic structure of cells, new methods of purifying subcellular fraction of anti-lymphocytic serum, new drugs to combat rejection phenomena, and above all a fresh outlook on the mode of action of these drugs at a molecular level, will doubtless render this an exciting new approach to biology in general and to clinical problems in particular.