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THE AUSTRALIAN NATIONAL UNIVERSITY

John Curtin School of Medical Research

Annual Report 1966

Department of Experimental Pathology

STAFF

Professor:

F.C. Courtice, M.A., D.Phil.(Oxon.), D.Sc.(Syd.), L.R.C.P.(Lond.), M.R.C.S.(Eng.), M.C.P.A., Hon. F.R.A.C.S., F.R.A.C.P., F.A.A.

Professorial Fellow:

B. Morris, B.V.Sc.(Syd.), D.Phil.(Oxon.).

Senior Fellow:

W.E. Stehbens, M.D., B.S.(Syd.), D.Phil.(Oxon.), M.C.P.A., M.R.A.C.P., M.C.Path.(Eng.)(from March to July).

Fellows:

K.J. Lafferty, B.Sc.(Melb.), Ph.D; W.J. Cliff, M.A., M.B., B.Chir.(Cantab.), D.Phil.(Oxon.).

Senior Research Fellows:

W.E. Stehbens, M.D., B.S.(Syd.), D.Phil.(Oxon.), M.C.P.A., M.R.A.C.P., M.C.Path.(Eng.)(to March); E.G. Cleary, M.B., B.S.(Syd.), M.D.(Syd.), M.R.A.C.P. (from June); Gutta Schoefl, B.A. (Reed Coll.), M.A., Ph.D.(Harv.)(from September).

Research Fellows:

M.W. Simpson-Morgan, B.V.Sc.(Syd.), Ph.D.(from March); P.J. McCullagh, M.B., B.S.(Melb.), D.Phil. (Oxon.), M.R.C.P.(Lond.)(from September).

Visiting Fellows:

D.B. Zilversmit, B.S., Ph.D.(Calif.)(January to June); B. Osogoe, M.D.(Kyoto)(from August); M.W. Whitehouse, M.A., B.Sc., D.Phil.(Oxon.), F.R.I.C. (from August).

Honorary Fellow:

Margaret Jennings, M.A., D.Phil.(Oxon.)(July to August).

Research Assistants.

R.J. North, B.Sc.(Syd.); Carolyn Podger, B.Sc. (W.Aust.)(to October); Karen Schmidt, B.Sc.(Chic.) (from November); Mary Barton, B.Sc.(Lond.)(from May); Jacqueline Dempsey, B.Sc.(Louisiana)(from Jan. to Oct.).

Head Technician:

J. Harding.

RESEARCH WORK

Introduction

The major activities of the Department concern the origin and development of atherosclerosis, the mechanisms involved in inflammation and in lipid transport and metabolism, the physiology and pathology of the lymphatic system and the phenomenon of immunity.

Walls of large blood vessels (Cliff)

(i) The structure of thoracic aortae from a colony of young growing rats and from a colony of old rats with ages in excess of one year was studied and compared by electron microscopy. Changes attributable to ageing included an increase in fibrous tissue and an accumulation of extracellular debris containing lipid in both the intima and media.

(ii) The walls and the luminal contents of doubly ligated segments of carotid arteries and jugular veins of rabbits were studied by light and electron microscopy. In these experiments colloidal carbon alone or mixed with homologous chylomicrons was introduced into the lumina of the isolated vascular segments at the time of operation, so that a detailed investigation of the cellular reactions of the walls of the segments to these test substances could be made.

Elastin metabolism and arterial wall composition (Cleary)

To study the factors regulating the deposition of the structural proteins, collagen and elastin, in the arterial wall, it is necessary to isolate and characterize the presumed soluble precursor of elastin (tropo-elastin) secreted by the synthesizing cells. A detailed study of the developing bovine nuchal ligament, a rich source of elastin, has been undertaken and the optimal

conditions for protein extraction defined. Extracts of this material are being fractionated by column chromatography and fractional precipitation and monitored for soluble elastin like products.

Elastin hydrolysates contain a number of amino acids which are peculiar to this material and which appear to have a cross-linking role. The pattern of changes in these amino acids during development has been studied in nuchal ligaments of normal cattle and in aortae of normal and lathyritic pigs. Preliminary studies using radioactive lysine have shown that this amino acid is concerned in a very complex manner in the formation of these cross-links in the normal chick aorta. Further studies of the defects in cross-linking in lathyritic and copper deficient animals, in both of which aortic rupture and abnormal elastic tissue are common, are being made.

Elastin has been shown to be weakly antigenic. Rabbits have been given repeated series of injections of purified elastin obtained from bovine nuchal ligaments. After 8-10 months a low titre of haemagglutinating anti-elastin antibody has been formed. Attempts have been made to use this antibody to monitor the fractions from the extracts of bovine ligament, for soluble elastin, but the low titre of antibody is associated with non-specific haemagglutinating and anti-complementary activity. Attempts are being made to improve the titres of antibody. The relationship of circulating anti-elastin antibody to arterial disease is being investigated in rabbits.

Behaviour of platelets (Stehbens)

The behaviour of platelets was investigated in the rabbit ear chamber preparation. Platelets did not adhere to one another or to endothelium at the branchings of vessels nor in eddy currents. Following the intravenous infusion of commercially prepared lipid emulsions, the effects were similar to those produced by intravenous administration of colloidal suspensions and macro-molecular substances.

In conjunction with Dr. Biscoe an electron microscopic investigation was made of trauma-induced thrombo-embolism in the carotid bodies of cats. Initially, platelets were loosely aggregated or adherent to leucocytes and displayed little or no distortion in shape. Amorphous or fibrillary material frequently surrounded or covered the surface of these platelets. Subsequently, in the stage of viscous metamorphosis, the platelets tended to apply themselves to their neighbour with the result that close packing and distortion of shape became evident. Dense granules exhibited localized eccentrically placed densities which often had a cross striation with a periodicity of 170 to 180Å. Displacement of dense granules was not observed and degranulation and fibrin formation were little in evidence.

Structure and function of the carotid body (Stehbens and Biscoe)

The fine structure of the carotid body was examined in normal cats and rabbits. The notable findings were the remarkable vascularity, the close relationship of the Type I and Type II cells, the rich nerve supply with nerve endings closely related to the Type I cells and also near blood vessels. Both Type I and Type II cells possessed cilia. Degenerative changes occurred in nerve endings related to Type I cells following sinus nerve section.

Studies on renal lymph (McIntosh and Morris)

A study of the mechanisms concerned in the production of renal lymph requires the establishment of chronic lymphatic fistulae of kidney lymphatics to enable lymph to be collected for long periods of time in conscious animals. A surgical technique has been developed in sheep in which the lymph draining from the pelvis of the kidney can be collected under essentially physiological conditions. Lymph flow from the sheep's kidney under normal conditions of hydration was found to be about 1-2 ml./hr. A comparison has been made between the biochemical constituents of renal lymph and plasma and this has shown that the protein concentration of kidney lymph is on the average about

30-40% of that of plasma with a somewhat higher albumin globulin ratio. Contrary to results obtained by other workers, it has been found that the urea content and non-protein-nitrogen content of kidney lymph is not significantly different from plasma nor from lymph obtained from other tissues.

Experiments in which large amounts of electrolyte solutions were infused intravenously led to a copious diuresis and an immediate increase in the rate of lymph flow from the kidney. This increase in lymph flow occurred much more promptly and was proportionately much greater than the change in lymph flow occurring in other tissues. These experiments are continuing with the idea of establishing more precisely the exact physiological relationships between renal blood flow, lymph formation and urine production.

Reaction to thermal injury (Roberts and Courtice)

(i) Lymph proteins

Experiments have continued on the effects of thermal injury on a tissue as reflected in the lymph obtained from the injured region. Lymph from the hind paw of the rabbit was examined by various types of electrophoresis before and after thermal injuries of different intensities. Leg lymph was obtained from rabbits both before and after injury and used to produce antisera in guinea pigs. Immuno-electrophoresis using this antisera showed that normal leg lymph contains the same proteins as in plasma, but that after injury, a new protein appears in the β_2 region.

This protein is more prominent in more severe burns, appears immediately after injury, but persists for only a few hours. It has not been shown to be present in the plasma. Further experiments are being performed to ascertain its origin and significance.

(ii) Capillary permeability

The lymph proteins from the leg have been studied

after various degrees of thermal injury and especially during the prolonged recovery phase for periods up to six months. The changes in the permeability of the vessel walls in these conditions will be related to the morphological changes observed with the electron microscope and compared with the action of other injurious agents.

Cholesterol transport in thoracic duct lymph in the rabbit (Zilversmit, Courtice and Fraser)

The rabbit readily develops hypercholesterolaemia and atherosclerosis when cholesterol is added to the diet whereas other animals do not. Experiments were, therefore, performed to see whether cholesterol was absorbed from the intestinal lumen mainly as smaller lipoproteins whose fate in the bloodstream might be different from the larger chylomicrons.

A method was devised whereby thoracic duct lymph was collected for several days in conscious rabbits fed either a high cholesterol-low fat diet or a high cholesterol-high fat diet.

The lipoproteins in the lymph were separated by the preparative ultracentrifuge into three fractions, viz: (i) chylomicrons of Sf > 400; (ii) very low density lipoproteins (VLDL) of Sf12 - 400 and d < 1.019; and (iii) low and high density lipoproteins of d > 1.019.

The results suggest that most the lipoproteins of the first two fractions were derived mainly from the intestine whereas most of the d > 1.019 fraction originated from the plasma by filtration. Most of the cholesterol, 60-80%, was carried in the chylomicron and VLDL fractions and there was relatively more in the VLDL fraction than has been reported in similar experiments in the rat and dog.

Chylomicron size and composition in relation to dietary fat load (Fraser, Cliff and Courtice)

Much attention has been focused in recent years on the effect of dietary fat on the incidence of coronary heart disease, and on the development of the underlying coronary atherosclerosis.

In this broad field of investigation, the formation and ultimate fate of the chylomicron, the form in which absorbed fat is mainly transported from the gut, have been intensively studied. Several investigators have determined the effect of the ingestion of different types of fat, e.g. cream and corn oil, on the size of chylomicrons in the intestinal lymph without controlling the dietary fat load. The results have so far been conflicting.

In the present investigation the size of the chylomicrons in the thoracic duct lymph of both rabbits and rats was determined by electron microscopy after separation in the ultracentrifuge and fixation in osmium tetroxide solution. The triglyceride (TG) and phospholipid (PL) contents of the chylomicron fraction were also determined chemically.

It was shown that with increasing fat load, the chylomicrons increased in size, this increase running parallel with the $\frac{TG}{PL}$ ratio which would be expected if the triglyceride forms the core of the chylomicron and the phospholipid is spread uniformly over the surface. The mean diameter of a chylomicron on a low fat diet was about 1200Å, while that on a high fat diet was about 2000Å. The significance of these results may be related to the findings of other workers that larger chylomicrons are removed from the circulation more rapidly than smaller ones.

Studies on chylomicron metabolism (Simpson-Morgan)

Although the introduction of new analytical techniques has contributed a great deal of information about the metabolism of the various constituents of chylomicrons, the mechanisms by which these particles are removed from the blood stream are poorly understood. The small samples of blood which can be obtained during experiments with small animals requires that adequate micro analytical methods are available for the routine analysis of large numbers of lipid samples. Such techniques have been developed in preparation for studies in which the removal of the different lipid components of chylomicrons from the circulation of the isolated rat heart-lung preparation, and the kinetics of the removal of chylomicron triglyceride from the circulation of intact rats

will be studied.

The lipids from 0.05 ml. samples of plasma can be extracted and quantitatively applied to silicic acid thin layers and separated into phospholipids, monoglycerides, 1, 2 and 1, 3 diglycerides, cholesterol, unesterified fatty acids, triglycerides and cholesterol esters in one chromatographic run. These various lipid classes can then be recovered from the chromatogram and their radioactivity measured.

The simultaneous counting of tritium, radioactive carbon and radioactive phosphorus has been studied and a simple method developed to correct observed counting rates for quenching. A method is under development by which the fatty acids in these various lipid classes can be measured without destruction of the fatty acids which would then be available for radioactive measurement or gas chromatographic analysis. With these methods, it should be possible to quantitate the various lipid classes and measure their radioactivity using only one sample of plasma lipid.

Plasma protein metabolism in intact animals (Simpson-Morgan and Smeaton)

Animals with chronic lymphatic fistulae lose large amounts of plasma proteins, relative to their normal rates of catabolism. It has been found, however, that these animals are able to maintain a constant level of circulating plasma protein which is not very much lower than normal. Studies have been commenced to determine whether the increased synthesis of plasma proteins which occurs in rats with chronic lymphatic fistulae is carried out at the expense of the synthesis of other proteins.

Work to date has been of a preliminary nature and has been concerned with finding ways to deplete animals of specific proteins with defined physiological function with minimal interference to the animals, of measuring the circulating levels of plasma proteins of interest and their radioactivity, and methods of labelling proteins for studying the kinetics of removal of these proteins from the circulation.

The responses of the lymphatic system to antigen (Smith and Morris)

Investigations have continued into the reaction of the popliteal and hepatic lymph nodes to Influenza virus injected intravenously or subcutaneously. These experiments have demonstrated that neither the hepatic nor the popliteal lymph nodes respond to a primary intravenous challenge with virus, either by synthesizing specific antibody or by producing reactive cells. When the primary challenge is given locally into the tissue fluid drained by the lymph nodes a vigorous synthesis of antibody ensues in the node and many reactive basophilic lymphoid cells appear in the efferent lymph.

Experiments involving secondary challenges with Influenza virus gave results which suggested that reactive memory cells were present in lymph nodes and that the presence of these cells was not dependent on antigen having previously been localized in the lymph node. It was shown that a secondary response occurred in the popliteal lymph node after the node of the contralateral leg was challenged primarily by a local injection of virus. Similarly reactive cells capable of initiating a secondary response were present in the popliteal and hepatic lymph nodes of sheep which had received the primary stimulation intravenously.

A study has been made of the cellular components of afferent lymph collected from different tissues of the body. Afferent lymph from tissues such as the lower leg, testis, ovary, kidney and liver is characterised by the presence of a high proportion of macrophages and monocytes. These cells usually comprise 10-20% of the total cell population. There are, however, significant differences in the numbers of cells in afferent lymph. Lymph coming from the ovary, testis and kidney rarely has more than 500-1000 cells/c.mm., whereas afferent lymph from the liver normally contains 1000-3000 cells/c.mm.

The numbers of cells in afferent lymph have been shown

to increase dramatically in certain circumstances. When an antigen was localized beneath the skin of the lower leg, by the establishment of a granuloma there, the cell output in the afferent lymph increased 20 fold. The flow of afferent lymph from the liver has also been found to increase following the injection of particulate material intravenously and the cell numbers and output of cells from the liver increases many times. For a period of several weeks after the injection of colloidal carbon intravenously, macrophage cells containing the injected particulate material can be identified leaving the liver in the afferent lymph. This finding suggested that the Kuppfer cells of the liver are not an entirely residential population of cells but are capable of migrating from the liver in large numbers under certain conditions.

The development of the lymphoid apparatus in newborn animals in relation to immunological reactivity (Cole and Morris)

The life history of the lymphocyte in relation to immunological reactivity is being investigated in newborn animals. Chronic lymphatic fistulae of the popliteal lymph node have been established in newborn lambs and the cell output from the node before and following antigenic challenge has been measured. It has been found that the popliteal lymph node of newborn lambs is capable of producing a cellular reaction to a primary challenge with antigen and very large numbers of basophilic lymphoid cells leave the lymph node in the afferent lymph. It has not been possible, however, to identify any antibody in the efferent lymph from the stimulated node following a primary challenge. When the popliteal node is stimulated by a secondary challenge a cellular reaction with antibody formation ensues.

These experiments have been carried out in lambs deprived of colostrum (agamma-globulinaemic) and in lambs fed colostrum, and no essential difference in the immunological response has been found.

Studies on the biochemistry of lymphocytes (Whitehouse)

The metabolism of lymphocytes from the popliteal lymph of sheep and from the thoracic duct of rats is currently being studied with particular reference to changes in the energy-producing mechanisms and in the protein and nucleic acid metabolism of these cells.

These parameters have been studied following the administration of ACTH to the whole animal; the incubation of these cells in vitro with adrenocorticosteroids and other anti-inflammatory drugs; and the stimulation of the popliteal lymph node with subcutaneously administered antigen.

The reactivity of lymphocytes (Lafferty and Jones)

Two main lines of work have been followed. The first is a study of the normal lymphocyte transfer (NLT) reaction and the second is a study of the behaviour of lymphoid tissue when grafted into the chicken embryo.

The lymphocyte transfer reaction

Earlier studies have shown a close correlation between the NLT reaction and the dermal reaction produced in response to the inoculation of sheep with RNA obtained from allogeneic lymphocytes and it has been suggested that the stimulus responsible for the reaction may be the transfer of RNA between the transferred cells and cells of the host. Further investigation of this problem has shown that whereas the RNA of allogeneic lymphocytes or whole spleens is active in this respect, RNA isolated from the kidney or liver of the same donor animal is inactive.

No reaction results from the intradermal inoculation of xenogeneic (chicken or guinea pig) lymphocytes into the skin of sheep. However, xenogeneic cells are reactive if they come from an animal that is more closely related to the sheep. Thus goat lymphocytes produce a reaction that is very similar to that produced by sheep lymphocytes, whilst bovine lymphocytes give a rather atypical NLT reaction insofar as virtually no reaction is

seen until four days after inoculation.

Experiments with in vitro mixed cultures of allogeneic sheep lymphocytes or mixtures of sheep and goat lymphocytes have been shown to have significantly increased DNA synthesis over control preparations. On the other hand, cultures of sheep lymphocytes mixed with cells from a distantly related animal such as the goose do not show any increased DNA synthesis.

Grafting of lymphoid tissue into the chick embryo

Allogeneic lymphocytes produce a vigorous graft versus host (GVH) reaction when inoculated either onto the chorioallantoic membrane (CAM) or introduced intravenously into the chicken embryo. In contrast, lymphoid cells show the goose, duck, pigeon, sheep or guinea pig produce little or no reaction in the chicken embryo. Xenogeneic lymphoid grafts such as grafts of pigeon spleen or sheep lymph node survive and proliferate in the chicken embryo without any ill effects on the embryo itself. Moreover, it can be shown that xenogeneic cells are potentially capable of reacting against the chicken embryo. Blood leucocytes taken from geese or pigeons that have been immunized with chicken spleen cells emulsified in complete Freund's adjuvant produce a strong GVH reaction when placed on the CAM of the chicken embryo. A similar result is obtained when the lymph node cells obtained from guinea pigs that are hypersensitive to chicken tissues are inoculated on to the CAM of chicken embryos.

The pathological picture that results from the inoculation of the chick embryo with a purified preparation of allogeneic small lymphocytes is not associated with antibody formation by the grafted cells. All attempts to find antibody forming cells either by plaque formation or fluorescent antibody staining were negative. Moreover, the red cells of the embryo did not show a positive antiglobulin reaction at any time up to the death of the embryo.

Adoptive transfer of immunological competence to the chicken embryo.

The transfer of blood leucocytes from adult chickens to isogeneic embryos enables the embryo to reject grafts of allogeneic

embryo bone. As few as 10^5 transferred cells are sufficient to bring about graft rejection. In contrast the inoculation of 100 times as many cells along with sheep red blood cells does not result in the formation of antibody producing cells in the spleen of the embryo. The transfer of 10^6 blood leucocytes consistently results in the rejection of 100% of grafted allogeneic bones. However, embryos treated in this way are considerably less efficient in rejecting xenogeneic bone grafts. If the graft is duck bone, approximately 30% of the bones fail to be affected at all, whilst the remainder show a relatively mild reaction. In the case of pigeon grafts only 10 to 20% of the grafts show any damage, and this is usually very mild. If, on the other hand, the embryo is treated with cells from an adult that has been previously immunized with pigeon tissues, all the pigeon grafts are completely destroyed.

Not all grafts of allogeneic tissue are destroyed by embryos treated with adult isogeneic leucocytes. Grafts of embryonic spleen and liver are destroyed in a similar manner to bone grafts. However, grafts of embryonic heart muscle, that has been carefully freed of blood cells, show no ill effect when grafted into such embryos. Considerable damage is produced if spleen cells from the donor embryo are introduced into the heart muscle before grafting. These data indicate that the graft rejection observed in this system is dependent on the presence of lymphoid cells in the graft. This contention is supported by the finding that the treatment of bone and spleen grafts with γ-irradiation before grafting results in a dramatic reduction in the intensity of the rejection process.

The blood supply of a lymph node and effects of its occlusion

(Oscgoe and Courtice)

The anatomy of the blood supply of the popliteal lymph node in the rabbit has been defined by arterial and venous injections of latex. The effects of cutting off the blood supply, either partially or completely, on the microscopic structure of the node and on the cellular and protein content of the efferent

embryo bone. As few as 10^5 transferred cells are sufficient to bring about graft rejection. In contrast the inoculation of 100 times as many cells along with sheep red blood cells does not result in the formation of antibody producing cells in the spleen of the embryo. The transfer of 10^6 blood leucocytes consistently results in the rejection of 100% of grafted allogeneic bones. However, embryos treated in this way are considerably less efficient in rejecting xenogeneic bone grafts. If the graft is duck bone, approximately 30% of the bones fail to be affected at all, whilst the remainder show a relatively mild reaction. In the case of pigeon grafts only 10 to 20% of the grafts show any damage, and this is usually very mild. If, on the other hand, the embryo is treated with cells from an adult that has been previously immunized with pigeon tissues, all the pigeon grafts are completely destroyed.

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lymph have been studied.

The main artery to the node is a branch of the popliteal artery. Besides, there are two collateral arteries coming from the adjacent muscles, one from the semitendinosus and the other from the biceps femoris. A complete blockage of all these arteries results in an extensive degeneration of cells within the node, particularly lymphocytes, and only a few cells adjacent to the lymphatic sinuses may survive, as long as the lymph flow through the node is maintained at a certain level. In contrast, there occurs no degeneration of cells within the node after partial blockage of the blood supply and the cellular architecture of the node appears almost normal. In such instances, no significant reduction in the cellular content of the efferent lymph was seen.

Similar investigations are being made after blocking either the efferent or afferent lymphatic vessels of the node or both these sets of vessels. When the lymph flow through the node is blocked completely, there occurs a striking accumulation of small lymphocytes within the node, especially in the cortex, and secondary nodules become fewer and less active. Moreover, they often disappear almost completely in some areas, so that the cellular architecture of the cortex resembles that of the thymic cortex. A partial blockage of the lymph flow through the node also produces similar changes but to a lesser extent.

Immune reactions and tissue grafting (Cliff)

Tissue grafting is potentially of great importance in the replacement of diseased organs, two significant examples in the cardiovascular field being blood vessel grafts and kidney grafts. It is usually necessary to use homografts which provoke in the recipient homograft rejection processes.

Rabbit ear chambers are being used to study immune reactions to foreign proteins in vivo with particular reference to the minute blood vessels. The in vivo reactions to kidney homografts established in rabbit ear chambers are being investigated in a similar manner to determine the vascular and cellular responses involved in homograft rejection.

Phagocytic cells (North)

The electron microscope was used to study the distribution of acid phosphatase in polymorphonuclear leucocytes both before and after they had ingested particulate matter. It was found that acid phosphatase was confined to a relatively small population of cytoplasmic granules which differed structurally from the specific granules. The ingestion of particulate matter by these cells resulted in the occurrence of fusions between the granules which contained acid phosphatase and the vacuoles which contained ingested matter. At the same time the specific granules lysed in the cytoplasm. The results of these studies indicate that polymorphonuclear leucocytes contain two types of cytoplasmic granules. One type is concerned with intracellular digestion while the other type is responsible for liberating an oxidase which participates in energy metabolism.

Cytophilic antibody (Gowland and Boyden)

A series of investigations has been carried out to determine whether or not cytophilic antibody acts as the specific mediator of delayed-type hypersensitivity reactions in the guinea pig. It was found that there was a poor correlation between levels of cytophilic antibody and delayed-type hypersensitivity to sheep erythrocyte antigens in animals tested at varying intervals following immunization with antigen in complete Freund's adjuvant. Delayed-type hypersensitivity reactions could be elicited before cytophilic antibody could be detected in the serum or on peritoneal cells. Later after immunization (approximately three months) cytophilic antibody had frequently fallen to undetectable levels, but there was no evidence that delayed-type hypersensitivity had waned. In contrast, high titres of cytophilic antibody were observed in guinea pigs following injection with immune serum or serum-globulins, but in no case was delayed-type hypersensitivity passively induced.

Animals sensitized against hapten-protein conjugates produced high titres of cytophilic antibody reactive with hapten determinants without regard to carrier protein specificity but

none of the animals investigated exhibited a delayed reaction to the specific hapten when it was conjugated with a protein unrelated to that used in the immunizing conjugate.

In view of these findings it appears unlikely that cytophilic antibody is the specific mediator of delayed-type hypersensitivity reactions in the guinea pig.

Macrophages and the immune response (McCullagh)

An investigation of the immune response which was initiated in Oxford has been resumed. At present the evidence accumulated indicates that the presence of macrophages is mandatory for the stimulation of lymphocytes to produce antibody directed against heterologous erythrocytes. Further experiments will be aimed at elucidating in greater detail the role fulfilled by the macrophage during the immune response. An examination of the functional potentialities of macrophages from immunologically tolerant animals is also being undertaken.

Comparative immunology (Tait)

In a study of immunological mechanisms in lower vertebrates, particular interest was centred on the effect of temperature on the production of antibodies by various species of cold-blooded vertebrates. Results of experiments on antibody production in the giant toad (Bufo marinus) and the lizard (Egernia cunninghami) have shown a basic difference in the ability of these two species to produce antibodies over a range of environmental temperatures (20°C-30°C). In the toad, temperature affected the rate of antibody production but the maximum titre of antibody achieved was independent of the environmental temperature. On the other hand, the lizard produced antibodies only over a very restricted temperature range. This range correlated very well with information on the body temperature of this species of lizard in its natural environment. A hypothesis has been put forward, suggesting that with the evolutionary development of some degree of maintenance of a preferred body temperature, reptiles have become restricted to a narrow

temperature range over which physiological functions can operate efficiently.

Studies have also been made in cold-blooded vertebrates on the production of antibodies to soluble antigens. Many authors have had difficulty in inducing the production of antibodies to soluble antigens in various species of cold-blooded vertebrates. Experiments have shown that a possible reason for this difficulty is that a state of immunological paralysis has been produced by injection of an excess of antigen.

Other studies have been concerned with the effect of opsonization of antigen by antibody on the rate of phagocytosis of antigen by leucocytes and the effect of temperature on phagocytosis.

TEACHING AND OTHER ACTIVITIES

There are ten students proceeding to the degree of Doctor of Philosophy. New students who commenced work during 1966 are: Mr. R. Fraser, Mr. G. McIntosh, Mr. G. Cole (Wool Board R.F.) and Mr. T. Smeaton.

Dr. W.E. Stehbens was awarded the R.T. Hall Research Prize of the Cardiac Society of Australia and New Zealand.

During the year, Dr. E.G. Cleary and Dr. Gutta Schoefl took up their appointments as Senior Research Fellows and Dr. M.W. Simpson-Morgan and Dr. P. McCullagh as Research Fellows.

Several visitors have worked in the department during the year: Dr. D.B. Zilversmit from the Department of Physiology in Memphis, Tennessee (and now at Cornell University); Professor B. Osogoe from the Department of Anatomy, Okayama, Japan; Dr. M.W. Whitehouse from the Department of Biochemistry, Oxford and Dr. Margaret Jennings from the Sir William Dunn School of Pathology, Oxford.

Dr. Stehbens resigned in July to take up the position of Pathologist-in-Chief, the Jewish Hospital, St. Louis, Missouri, U.S.A.

Members of the staff and several visitors have given

seminars within the department during the year. Several members of the department read papers at the International Congress of Haematology held in Sydney, August 22nd - 29th and at meetings of the Australian Physiological Society, the College of Pathologists, the Immunological Society. Dr. Stehbens read papers at the 4th European Microcirculatory Conference in Cambridge and the 1st International Conference on Haemorheology in Reykjavik, Iceland.

The work of the department has been partly supported by grants from the National Heart Foundation of Australia.

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