THE ADRENAL CIRCULATION

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PREFACE

FOR some fifteen years R. G. H. has been interested in the functional morphology of the adrenal gland, with particular reference to its blood supply. Only as a result of researches carried out over the last few years, however, has it become apparent how the blood flow within the gland may become modified as a result of direct action upon its intracortical vessels. The mechanism of this action was first investigated in the rabbit, but in the years 1958-59, an extensive study on the vascularization of the adrenal cortex in the rat was undertaken by M. J. H. for the B.Sc. Honours degree in Anatomy in this Department, and by R. G. H. on the rat and monkey. It was considered that these researches, combined together in integrated form, would warrant publication as a monograph, particularly since, if they had been published as separate papers, their individual significance may have been obscure. We are aware that many aspects of this study could warrant further investigation, and research on these lines is still being actively pursued, but in view of the importance of these discoveries, it seemed that publication of the researches effected so far was indicated.

Our thanks are due to the technical staff of this Department for their assistance in much of the experimental work described in this book, and in particular Mrs. C. Morley for preparation of the histological material, and Mr. A. Taunton for the photography. We are also indebted to Miss M. R. Crowther for typing the manuscript, and to Mr. L. G. Cooper for photographing and Mr. D. J. Kidd for drawing some of the illustrations in this book. The researches described herein have been financially assisted by grants from the Medical Research Council, and the Sir Halley Stewart Trust. We also wish to express our gratitude to Damancy & Co. Ltd. (and in particular Mr. P. G. Horlington) for providing facilities for the viscosity measurements described in Table I; Professor Barry J. Anson, Department of Anatomy, Northwestern University Medical School, Chicago, for permission to reproduce Fig. 14; Dr. W. J. Tindall, Organon Laboratories Ltd. for supplying the hormones utilized in these researches; Dr. A. Spinks and Dr. J. S. Lowe of Imperial Chemical Industries Ltd. (Pharmaceuticals Division) for supplying the serotonin, ox kallidin and adrenochrome; Professor A. Wilson, Department of Pharmacology, University of Liverpool, for providing the Levophed and Dibenamine used in these experiments; and to Dr. J. L. Braithwaite, Professor A. Haddow and the Chester Beatty Research Institute, for providing the tumour-bearing rats used in the experiments described on p. 58.

Finally, we wish to thank Mr. Per Saugman, the staff of Blackwell Scientific Publications and the Alden Press for their continued help in the preparation of this book.

RONALD G. HARRISON
M. JUNE HOEY

Liverpool, May 1960

CONTENTS

CHAPTER I

INTRODUCTION

CLAUDE BERNARD (1858) claimed that the state of the venous blood which issues from secretory organs differs according to whether the organ is functioning or resting. Further, that this relationship between blood draining the gland and glandular activity is determined by the activity of the nervous system.

For some time it has been claimed that the level of adrenal cortical secretion is governed in large part, if not entirely, by the anterior lobe of the hypophysis cerebri. Any influence of the nervous system on the physiological state of the adrenal cortex has been considered (Harris, 1955) to be effected through the intermediary of the hypothalamus, the hypophyseal portal circulation, the anterior lobe of the pituitary and thereby its secretion of adrenocorticotrophic hormone (ACTH), which in turn stimulates the secretion of steroid hormones from the cortex of the adrenal gland. Although such a complicated mechanism is called into play in certain circumstances, complete acceptance of it precludes any *direct* effect of hormones or environmental change on adrenal cortical secretion, it does not explain the increasingly close topographical association of adrenal cortex and medulla in non-aquatic vertebrates during the evolutionary process, and has laid so much stress on the importance of the activity of the anterior lobe of the pituitary, that manifestations of the action of adrenal cortical hormones are now frequently interpreted *ipso facto* as an indicator of ACTH secretion.

Many of the investigations on the regulation of the secretion of adrenal cortical steroids by the pituitary have employed adrenaline or some other vasoconstrictor. Yet recent experiments (Harrison, 1957) have demonstrated that adrenaline has a direct effect on the vascularization of the adrenal cortex of the rabbit independent of the hypothalamus and pituitary, since an increase in adrenal cortical vascularization could be obtained in the decerebrate, hypophysectomized rabbit following the injection of adrenaline.

Since 1957 many additional experiments, to be reported here for the first time, have confirmed that adrenaline, other vasoconstrictor agents and the submission of an animal to stress, may have a similar direct effect on the adrenal cortex by increasing its vascularization. It was therefore deemed advisable to collate these observations and publish them as a monograph in conjunction with a description of the gross anatomy of the adrenal circulation, in view of the knowledge of the anatomy of the adrenal gland now made necessary by the increased frequency of adrenalectomy in surgical practice.

CHAPTER II

METHODS AND TECHNIQUE

THE injection of vessels supplying the adrenal gland is essential for a thorough investigation of their course and complexity, since the adrenal arteries are multiple and of fine calibre, and any attempt at their dissection without prior injection is both laborious and difficult. Of the various injection media utilized in this laboratory for observations on the gross anatomy of the circulatory system, the one found of greatest value is a stabilized micro-dispersion of barium sulphate (Micropaque; Damancy & Co. Ltd.). This has the advantage of penetrating into the finest branches of the arterial system and even into capillaries on occasion, but very rarely, if ever, through them into the venous system, when injected into a major artery; the particle size of Micropaque varies from 0.1-1 μ, yet the particles do not pass through capillaries whose diameter is approximately 10 μ, and this may be explained by the high viscosity of 50-100% Micropaque (Table I), which is much greater than that of blood. If, however, arteriovenous anastomoses, or vessels of larger than capillary size linking the arterial with the venous system (e.g. the

TABLE I

THE VISCOSITY OF MICROPAQUE SUSPENSION AT 20° C

Dilutions	*Dynamic viscosity in centipoises*	*Specific gravity*	*Kinematic viscosity in centistokes*
100% Micropaque	290	1.793	162
50% ,,	26	1.387	18.7
30% ,,	10	1.232	8.1
25% ,,	8	1.184	6.8
Pure water	1.005	0.998	1.007

Note: 1. Viscosity will be lower still at body temperature (around 37° C).
2. The viscosity of blood is 3.5-5.4 times that of water.

juxta-medullary glomeruli of the kidney), are present and functioning, Micropaque may readily appear in the venous system draining an organ. Similarly, following retrograde injection into a large vein, this injection medium will penetrate (the absence of valves permitting) into the venous system and its ramifications only. By separate injections it is therefore possible to demonstrate only the arterial or venous supply of an organ to the exclusion of the other. Being radiopaque the vessels injected with Micropaque can also be demonstrated by radiography prior to dissection: when vessels ramify in different planes, this is often of great advantage. Finally, the vessels following injection

stand out clearly as white strands and after formalin fixation they can be dissected more easily, since the Micropaque then remains in the vessels, and does not diffuse into surrounding tissues, a disadvantage possessed by other radiopaque media which are solutions, such as those commonly used in radiography in clinical practice. Since these latter solutions depend for their radiopacity on their content of iodine they are also not as radiopaque as barium sulphate. Other radiopaque media (Harrison, 1951a) have a larger particle size and do not penetrate as far into the finer branches of the vascular system. Thus, at one time bismuth oxychloride suspension was considered ideal, but it was later found that Micropaque is more effective. The Micropaque suspension as provided by the manufacturers is at a strength of 100% w/v but it is usually found more desirable to dilute this 50% with water or saline, thus providing a 50% suspension. Generally, the weaker the suspension, the more effective the filling of the finest vessels (see Brookes and Harrison, 1957), and this may be explained by the lower viscosity of the weaker suspensions (Table I). Preparations obtained by the injection of latex rubber or plastic injection masses have usually been found unsuitable and more tedious to prepare in this laboratory: generally unphysiological injection pressures have to be used in order to fill even small arteries, subsequent corrosion of the tissue is necessary, and radiography prior to dissection is either impossible or unsatisfactory. Similar criticisms may be made against the use of other injection media, such as carmine gelatine or india ink for the visualization of the gross anatomy of the circulatory system.

The gross arterial supply of the right and left adrenal glands has been examined in twenty albino rats, eight rabbits, four cats and four macaque monkeys following injection of Micropaque into the descending thoracic aorta by the method outlined above.

The examination of the distribution of the finer vessels within tissues or organs should preferably be undertaken after elucidation of the gross anatomy, thereby correlating the arterial supply and venous drainage. Some information may already have been obtained from specimens injected with Micropaque, but it is usually also necessary to utilize an injection medium which will flow easily through the capillary bed, such as india ink. The organ or tissue is then immediately fixed in formol saline, cleared and serial frozen sections, 50-100 μ thick, prepared. It may also be desirable to stain the sections lightly, in which case they must be made thinner (10-15 μ). If it is desired to radiograph the sections then a radiopaque medium which will easily fill the capillaries must be utilized: the most desirable substance from this point of view is Thorotrast (Testagar & Co., Inc.), a colloidal preparation of 24-26% thorium dioxide whose particles are of ultra-microscopic size. This medium has the additional advantages that it is non-toxic, freely miscible with blood

and other body fluids, and does not itself appreciably affect blood flow or pressure (the injection of 10 ml. Thorotrast intra-arterially in a mature rabbit produces a rise of blood pressure lasting only 20-30 seconds of the order of 10 mm. mercury), or the calibre of blood vessels. It is also markedly radiopaque. It can only be utilized in relatively acute experiments on animals, however, since it is taken up by the reticulo-endothelial system, particularly in the spleen, and may produce neoplastic growth there by means of its γ-radiation. Since Micropaque is a particulate dispersion it should also never be utilized for the injection of blood vessels in the living human, because of the danger of embolism.

When the gross vascular pattern of an animal or one of its organs is to be examined, it should preferably be injected immediately after the death of the animal or when the organ or tissue is removed immediately after death. In this laboratory the animal is killed by means of an overdose of chloroform or coal gas; in such a case no blood clotting sufficient to obstruct the filling of even finer blood vessels has been observed, but if it is impossible to inject the animal immediately after death, the ante-mortem injection of heparin (e.g. 1000 i.u. intraperitoneally in a 300 g. rat) will mitigate the post-mortem clotting and facilitate injection. The injection of an animal is made either into the descending thoracic aorta, the common carotid artery or the inferior vena cava after the insertion of a needle or polythene cannula, which is secured in place by a thread ligature around the vessel. For injection of the thoracic aorta the left side of the chest wall is first opened, and similarly the right side of the chest wall for the inferior vena cava. Injection can, of course, be made into any vessel, and either along the direction of blood flow or retrogradely. In the case of isolated organs the cannula is inserted directly into the vessel of choice. The largest bore needle or cannula which may be admitted by the vessel to be injected is advisable when using particulate media such as Micropaque, in order that it may not be obstructed. A syringe charged with injection medium may be utilized for the procedure, and injection carried out with a steady and even pressure. Such a pressure can also be achieved by an apparatus working on simple hydrostatic principles, or utilizing positive pressure from a pressure bulb or oxygen cylinder, and it is usually desirable in such cases to fit a manometric device so that injection can be made at known physiological pressures. With media of larger particulate size, a point of resistance is reached beyond which it is ill-advised to attempt further injection, otherwise vessels will burst, owing to the medium being held up at the capillary bed. It is often of great value to make an incision into the plantar aspect of the foot of a rat before injection: this prevents the attainment of high pressures in the vascular system and the occurrence of burst vessels. The vessel injected should be clamped and ligated following injection.

METHODS AND TECHNIQUE

In the investigation of the adrenal circulation an additional requirement was necessary, for an injection medium which could be introduced into the general circulation, and whose course through the adrenal could be followed in the living animal in varying functional states over successive periods of time during the course of an experiment lasting up to 2 hours or more, and whose distribution within the vessels of the gland could be examined at the termination of the experiment. Thorotrast was found to be the most suitable medium, for the reasons mentioned above, since its course and ultimate distribution could be followed radiographically at periods up to 3 hours after injection. It was introduced through a cannula into a common carotid artery of the animals used, in quantities varying from 3-10 ml. depending on the size of the animal. In larger animals (rabbit and monkey) the degree of filling of intraglandular vessels of the adrenal could be detected by the radiopacity of the gland on ordinary radiographic examination, since it was found (Harrison, 1957) that the opacity of the gland to X-rays in these animals increased with increasing filling of adrenal cortical capillaries. If intracortical vessels are not filled with radiopaque medium, the gland is not visible radiographically. In the rat, however, because of the smaller size of the gland, such methods were unsatisfactory. Nevertheless, in all animals, at any time during an experimental procedure, or at the termination of the experiment, it was possible to remove a gland from the anaesthetized animal within seconds and place it in 70-96% ethyl alcohol, which fixed the Thorotrast *in situ* exactly in the location it occupied at the moment of removal. By subsequent frozen sectioning of the gland, the resultant 100-250 μ sections (the thickness depending on the experimental animal) may be examined by radiographic methods.

The radiographic examination of gross specimens (e.g. a whole rat, or an organ from it) is accomplished by routine methods. In this department a Watson portable Mobilix apparatus has been found to be quite satisfactory. For routine radiography, Ilfex (Ilford Ltd.) non-screen radiographic film, envelope packed, is used; an exposure of 0.2 second at 60-67 kV, 53-50 mA being adequate for a 300 g. rat. For a rabbit 0.3-0.4 second, and for a 3-6 kg. monkey 0.5 second, at the same kV and mA are necessary. Such radiographs are not suitable for enlargement: in fact, the maximum enlargement permitted by Ilfex film without the appearance of grain is 2-3 diameters. For the demonstration of finer detail in gross specimens, Kodaline film (Kodak Ltd., R.O. 5) is used: in these circumstances an exposure of 7-8 seconds at 60-67 kV, 53-50 mA is needed for a whole rat, and for its excised organs, such as the testis or spleen, 4 seconds at the same kV and mA. The radiographs obtained on Kodaline film can be enlarged 6-8 diameters with excellent results. For the examination of smaller organs, such as sections of the adrenals of a rat, more specialized methods are necessary, since even after exposure on Kodaline

film subsequent enlargement reveals the grain of the film before details of intraglandular vessels are visible, an enlargement of more than 8 diameters being necessary. Therefore the technique of microradiography must be applied.

The first serious attempt at the visualization of fine vessels by radiographic methods in biological material following the injection of radiopaque media was made by Barclay (see Barclay, 1951). He realized that, in order to observe the details of such vessels, a film grain of sufficiently fine size is necessary in order that the radiograph obtained may be enlarged adequately, and a specialized type of X-ray apparatus is necessary in order to produce soft X-rays and expose the fine-grain emulsions for longer periods than are possible with the conventional type of radiographic machine. Such requirements are met by the various modifications of X-ray diffraction units now available, and the one employed in this laboratory is the Hilger microfocus X-ray unit incorporating the Ehrenberg and Spear tube. This unit has a tube which is continuously evacuated, and an anode which is cooled continuously by means of a very thin oil circulated by a pump. We use it with a Beryllium window in the tube, and Kodak Maximum Resolution plates, extremely fine emulsion plates which are capable of resolving at least 1000 lines per mm.

Since Barclay's pioneer investigations much subsequent research has been performed on the technique of microradiography (see *Kodak Bibliography on Microradiography and Soft-X-ray Radiography*, and in particular Bellman, 1953; Cosslett, Engström and Pattee, 1957). The classical method of micro-radiography (the 'contact method') consists in exposing the object (e.g. a section of adrenal), placed close to the fine-grain emulsion plate, to the X-rays from the tube which is at a distance, and subsequent enlargement of the plate ('secondary magnification'). This is the method we utilized, the tube filament-plate distance being 40 cm., and the exposure made at 40 kV, 300-400 mA for 10-15 minutes. It is also possible to utilize projection microradiography in which the object is placed close to the source of X-rays, using a fine focus tube, so obtaining primary enlargement ('primary magnification') of the object on the plate, but this method was not utilized in our experiments.

In the preliminary investigation of the functional control of adrenal vascularization (see Harrison, 1957) the rabbit was the animal of choice, since its adrenals are large enough to be visualized *in situ* by serial radiography on Ilfex film following the injection of 10 ml. Thorotrast into a common carotid artery, the axillary artery, axillary vein or a jugular vein – usually the first or the last. Eighteen rabbits of both sexes varying in weight from 1.8-3.7 kg. were used in the experiments and anaesthetized by intravenous nembutal and ether. Arterial blood pressure was measured by a mercury manometer attached to a cannula inserted into the other common carotid artery. The

left adrenal was examined by radiography at intervals of 2 seconds to 5 minutes in the earlier experiments, but in later experiments this period was lengthened, often to as much as 30 minutes. When a radiograph was being taken, the spleen, stomach and intestines were removed from over the area of the left adrenal by means of retractors through an abdominal incision parallel to and about 1 in. below the left costal margin.

In three of the rabbit experiments, the control of the adrenal circulation was examined in decerebrate, hypophysectomized animals. This procedure was effected through a trephine hole in the calvarium after ligature of both carotid arteries high in the neck. All brain substance above the level of the pons was removed and the hypophysis cerebri quickly extracted by strong suction effected by a vacuum of 12.5 cm. mercury. Careful examination of the sella turcica after the conclusion of the experiments by means of a binocular stereoscopic microscope at a magnification of $\times 35$ confirmed the effectiveness of this procedure. Such rabbits required artificial respiration through an intratracheal cannula by means of a respiratory pump during the remainder of the experiments.

In order to determine whether the findings in the preliminary experiments on the rabbit are also applicable to another mammal, further similar observations were carried out on five macaque monkeys, one male and four females, varying in weight from 3.5-6.22 kg. The details of the experimental procedure were exactly as for the rabbit.

It was soon realized, however, that more extensive examination of the adrenal circulation in large numbers of animals was necessary, in order that the time relationships and effect of functional variations on adrenal circulation could be examined more thoroughly. The rat was considered ideal since it is available in large numbers, but it has the disadvantage that its adrenals are too small to be examined by conventional radiographic methods during the course of an experiment. However, the adrenals could be removed at any time during the course of an experiment and examined by microradiographic methods by the technique outlined above, so providing an analysis of adrenal vascularization at varying intervals after the introduction of some functional alteration in the animal. Accordingly, in a further series of experiments, 175 male rats, varying in weight from 272-474 g. were used for observation of the effect of several physiological and pharmacological media and experimental procedures.

The rats were anaesthetized with ether, injected intraperitoneally with 1000 i.u. heparin, a cannula inserted into the left common carotid artery and ligatured in position. 3-5 ml. Thorotrast were then injected slowly into the cannula. The experimental procedure (e.g. injection of adrenaline) was then undertaken, and the adrenals removed after a varying time period, fixed in

96% alcohol, 250 μ frozen sections taken and subjected to microradiography. Five rats were hypophysectomized by means of the standard para-pharyngeal approach originally devised by Smith (1930) using an intratracheal cannula 2, 5 and 22 days before subjecting them to the investigation just outlined. As a safeguard against the occurrence of secondary infection, the hypophysectomized rats were injected intraperitoneally immediately post-operatively and again on the two succeeding days with 20 mg. Terramycin hydrochloride in 0.2 ml. pyrogen-free distilled water. The success of the operation was determined post mortem by removing the calvarium from the skull, lifting up the brain and exposing the pituitary, if present, using a binocular microscope, if necessary. In addition, the testes, adrenals and thyroids of such animals were weighed, examined histologically and compared with those of littermate rats. Unilateral (left) adrenalectomy was performed in some rats through a midline dorsal skin incision, and a muscle incision bisecting the left renal angle. In a further experimental series some rats were exposed to the stress of heat shock in a hot room, or to a low temperature in a refrigerator; details of these experiments will be given later.

CHAPTER III

THE ANATOMY OF ADRENAL VASCULARIZATION

THE uniform feature regarding the vascularization of the adrenals of all mammals so far examined is the multiplicity of arteries supplying the gland, whereas there is only one, or at the most three, veins draining each adrenal. The right vein constantly drains into the inferior vena cava and the left into the left renal vein. In man the intraglandular part of the single adrenal vein has only longitudinal smooth muscle in its coat for the greater part of its length (Brunn, 1873; Ferguson, 1906; Bargmann, 1933; Velican, 1948; Heinivaara, 1954). Within the gland there is also a unique feature which has been found to occur in all mammalian adrenals investigated. This is the presence of an arterial supply to the medulla independent of the vascularization of the cortex, accomplished by means of the 'arteriae medullae' of Flint (1900) — medullary arteries which pass radially through the adrenal cortex to the medulla in centripetal fashion without giving off any branches to the cortex.

THE RAT

An arterial stem, taking origin from the ventral aspect of the aorta, is the main source of arterial supply of the left adrenal in the rat; it divides into two main arteries both of which give off branches to the cephalic aspect of the gland (Harrison, 1951b). The anterior of the two branches also supplies the inferior aspect of the diaphragm, and may therefore be considered as homologous with the human inferior phrenic artery. A second arterial stem arising from the ventral aspect of the aorta at a slightly more caudal level, also provides a branch to the medial aspect of the gland (Figs. 1 and 2). The left renal artery provides an inconstant adrenal artery to the inferior pole of the left gland. A similar arterial supply is provided to the right adrenal (Fig. 3), except that almost the whole vascularization is effected through the branches from the inferior phrenic artery. One or two arteries may also be given off from the right renal artery to the caudal aspect of the gland. The venous drainage is by way of a single vein into the posterior vena cava on the right, and the renal vein on the left.

The intraglandular circulation in the rat consists of two types of vessels arising from a poorly defined capsular plexus (Gersh and Grollman, 1941):

1. The arteriae medullae, which pass through the cortex without giving off any branches and empty into the medullary sinusoids.

2. The cortical circulation consisting of capillary sinusoids which first form an efficiently anastomotic network around the cells of the zona glomerulosa. From this network arise longitudinal capillary sinusoids which pass centripetally in between the columns of zona fasciculata cells and anastomose with each other very poorly; these then open into a plexus of sinusoids of larger bore and irregular outline in the zona reticularis, and these in turn empty into the medullary sinusoids which are drained by the central adrenal vein.

The arterial supply to the adrenal cortex is of great interest from the viewpoint of anastomotic efficiency. In a female rat weighing 190 g. one of the main arteries reaching the cephalic aspect of the gland was interrupted (at X in Fig. 1), and the animal killed 3 days later. The resulting histological appearance of the gland demonstrates an area of focal necrosis involving approximately one-third of the zona fasciculata of the cortex (Fig. 4). Over the surface of this necrotic zone the capillaries of the glomerulosal network and a thin outer rim of zona fasciculata are distended with erythrocytes; the remainder of the cortex shows very pronounced hyperaemia and the cortical capillary sinusoids here are so distended with erythrocytes that localized cystic dilatations may be seen in the zona fasciculata (Harrison, 1951b). Individual adrenal arteries are therefore end-arteries to the zona fasciculata of the cortex. Since the medulla has a very efficient blood supply from two sources it is very unlikely that localized ischaemia of the gland would produce focal necrosis of the medulla; the efficient anastomotic

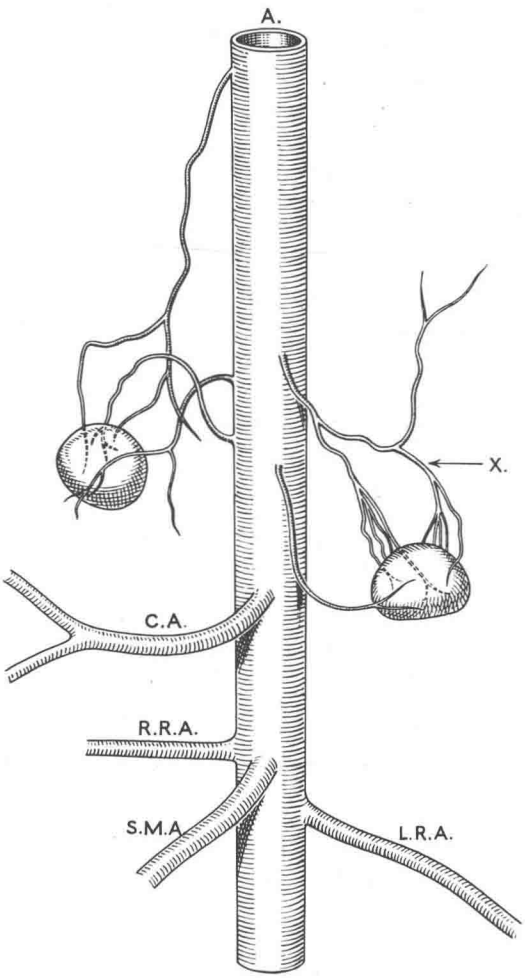

FIG. 1. Diagram of the arterial supply of the adrenals in the rat.

A = Aorta, C.A. = Coeliac artery, L.R.A. = Left renal artery, R.R.A. = Right renal atery, S.M.A. = Superior mesenteric artery.