Recent Advances in the Medical Sciences with emphasis on Anatomy: A Review Article

J. O. Oguntanti

Department of Anatomy, Faculty of Medical Sciences, University of Jos, Nigeria.

ABSTRACT

This article examines newer areas of modern anatomy which have received acceptance amongst scientists but nevertheless widely unknown to some teachers, and students. The traditional division of the subject into gross anatomy, embryology and microanatomy is followed in the discussion. Most of the areas in gross anatomy rely on the use of semi-micro dissection in view of the expanding application to microsurgery. Early embryology and its modern applications are discussed at length and new factors and hormones localized in various tissues highlighted. Molecular biology and recombinant DNA technology are examined to include modern genomics. Anatomy being no longer only purely descriptive, must continue to play a major role in learning in the study of the biomedical sciences.

Keywords: Anatomy, Embryology, *In vitro* fertilization, Implantation.

INTRODUCTION

"Early anatomists were interested in descriptive aspects of the discipline with great regard for function. Modern anatomy has continued this erstwhile tradition. Williams *et al* [1] opined in the 37th edition of the famous text Gray's Anatomy".

It is difficult to imagine investigation of structure without concomittant desire to elucidate function, development or any other aspects of structure.

This sentence summarizes in a few words, modern thinking on the role of anatomy in the biological sciences. When a new structural apparatus is discovered by the anatomist, his rudimentary functional study would provide him an *ab initio* theory on the role of such apparatus in the overall physiology of the human body, in health and disease.

For the purposes of modern studies, a modern anatomist is one who uses knowledge acquired in any area of the biological sciences to elucidate structure for the full understanding of function in health and disease.

This article reviews modern knowledge in the medical sciences with the use of the subdivisions of anatomy – viz – gross anatomy, embryology, and microscopic anatomy (histology).

1. *Gross anatomy*

This will be examined under the following headings - pure and clinical anatomy.

1.1. Pure anatomy

New information on structure, functional topography and parcellation have been obtained in recent times on the anterior abdominal wall, sole and muscles of facial expression to justify a review of these areas. The most profound of such areas have relied on methods akin to semi-

microanatomy (SMA) in which closer and magnified look into certain structures have led to better understanding of the structure and *ipso facto*, the necessity to change old descriptions (2-5).

1.2 Clinical anatomy

Considerable changes have occurred in the modern understanding of the human body which require the attention of clinician and anatomists alike. Some of the areas include the liver, heart, fibrous flexor sheaths of the hand, *nasopharyngeal* pituitary gland and female reproductive tract.

Liver Surgeons performing hepatectomy have noted that the division of the liver by current anatomical descriptions is not satisfactory. Although Hjortso [6] had described the unsatisfactory nature of the anatomical division of the liver into two lobes by the ligamenta venosa and teres, this information persists in many textbooks. Even then segmentation of the actual anatomical lobes (divided by a plane to the left of the old division at the level of the inferior *vena cava* and the gall bladder) are many and varied. It however seems that a considerable number of surgeons have settled, not only for the division of the liver into two hemilivers after the method of Hjortso [6], but have also adopted a segmentation of each lobe (hemiliver) into 8 segments each [7]. This segmentation follows the subdivision of hepatic vasculature. In this particular instance, surgeons are far ahead of anatomists because of dictates of expediency. Thus segmental hepatectomy is much more feasible and viable than the old method of lobar hepatectomy for the removal of masses in the liver [8].

Nasopharyngeal pituitary gland: McGrath [9] has described transphenoidal portal venous system which brings the newly popularized nasopharyngeal adenohypophyseal tissue under the control of the hypothalamus. This has important implications in pathology and medicine such as the development of neoplasia. See also [10] for newer descriptions of fibrous flexors sheaths of the hand.

Female reproductive tract (FRT): This tract has no new structure described but a better understanding of the physiology is underway based on previously recognized anatomy of the vascular system. Thus, it has been shown that there is close proximity between arteries and veins of the tract called venoarterial contacts. This is thought to lead to exchange of substances across the vessel walls along concentration gradients called venoarterial passage by Del Campo and Ginther [11-13]. This has been extensively investigated in animals and has only gradually become extended to the human in view of the similar close proximity in vascular arrangements between artery and vein first shown for rhesus monkey (a primate) by Ginther *et al* [14]. There is now gradual recognition of a functional portal circulation in the FRT involving the vagina which is connected to the utero ovarian anastomoses through the azygos artery of the vagina [5]. Interruption of this anastomosis going to the vagina by ligature in experimental rats led to vaginal acyclicity and experimental infertility. If proven for the woman this has important implications in sex hormone assays in gynecology, including assessment of cyclicity of any reproductive organ in the woman. Without functioning anastomoses of the FRT, any assay is bound to give wrong results.

2. *Embryology*

Embryology has taken significant leaps in the decade, especially in the field of early embryogenesis and oviductal embryology, which have been carried into test tubes and therefore can be watched thoroughly.

2.1. *Early embryo science*

This is the science which studies the embryo at its formation and development before implantation. All the stages have been carried out in tissue culture media artificially and we now have a good picture of what the human egg passes through during early oviductal development [15]. Varying culture conditions, we are now aware of some of the essential aspects of growth requirements of the early embryo. We are now aware that oviductal environment is essential if eggs (or indeed

sperm or early embryos) are to develop well [16-19] Johnson [20] described the cellular rules governing the emergence of two-cell lineage from the single zygote of the embro, viz inner cell mass, and outer cell mass. Willadsen [21] has shown that it is quite possible to grow xenogenous inner cell mass within an outer cell mass with foster mother of the outer cell mass, by growing goat inner cell mass in a sheep outer cell mass in the uterus of sheep foster mother. This may lead to the birth of a pure bred goat or goat-sheep chimera [20-22]. Also Willadsen [20] has mapped out developmental capacities of various early blastomeres in the experiment of embryo splicing or cloning thereby helping to re-write the principles of totipotency and pluripotency in early embryos. Totipotent cells can be isolated in donor zonae and grown in sheep foster uteri but their capacity to develop is dependent on the period of obtaining them. Thus, each of a two-cell embryo blastomere has a capacity to develop into a full embryo (a process referred to in modern parlance as embryo cloning – see below). Sixty to seventy percent of transferred eggs will develop to term when transferred at this stage. At a 4-cell stage, there is a slight reduction of developmental capacity to 50%, while this falls sharply to 6% at 8-cell stage. Simple bisection of a sheep blastocyst will lead to formation of identical twins.

Ovulation: We now know that certain cytokines such as interleukin-1 are necessary for luteinization after ovulation [23]. We also know that ovarian renin-angiotensin system aids ovulation [24].

Fertilization: We can now inject spermatozoa directly into the perivitelline space to effect fertilization under the program of *in vitro* fertilization (IVF) [25] or directly into cytoplasm of the egg [26]. *In vitro* techniques are being perfected and it has been shown that transfer made into the Fallopian tube instead of the uterus increases implantation rates [27] hence the development of the techniques of intrafallopian transfers such as GIFT-gamete intrafallopian transfers [28, 29] ZIFT-zygote intrafallopian transfers [30] SIFT-sperm intrafallopian transfer, PROST-pronuclear stage transfer, MIST-micro-insemination sperm transfer (MIST) [31]. Other methods in the assisted reproductive technologies (ART) in more recent times include intrauterine insemination (IUI), natural cycle oocyte retrieval intravaginal fertilization (NORIF) and Natural proactive technology (NaPro) [32]. There are presently debates on the ethics of human embryo research and the debate is not yet over [33,34]. The oviduct is increasingly being recognized as the leader of the orchestra in ART [31].

Enders [35] discussed anatomical aspects of blastocyst maturation and implantation. At the time of blastocyst formation, the ultrastructure of blastomeres are similar to the primitive early stages of the ovum with little polyribosomes and complex mitochondria and agranular endo-plasmic reticulum. In most species, there is a change in blastocyst morphology from spherical to elongate type at implantation.

Early embryo antigens: Monoclonal antibodies produced against several stage specific antigens have led to the development of differential antigen expression theory [36]. This theory suggests that it is the surface associated antigen in embryonic differentiation of embryonic cells, that is most useful in determining differentiation of embryonic cells. Stage specific antigens are known to be expressed in large amounts in teratocarcinoma cells. These are cells that have been removed from primordial germ tissue and grown in culture for the characterization of their antigens. The antigens are also expressed in tumors and are therefore called oncofetal or onco-developmental antigens. Amongst the best known are 402AXI, 402AXII, 402AXIII, A-NI, F9, MI/22, 25, SSEA-1, and TER. The best studied is F9 [37,38].

Certain developmental antigens have been identified as being responsible for the ubiquitous embryological induction and organization. Thus H-Y antigen described by Nagai et al [39] seems responsible for organizing testicular tissue.

2.2. *Genetics of early embryos.*

Several investigators have focused attention on the cytogenetics of blastomeres of early embryos in the hope of understanding the genetic mechanisms underlying cleavage divisions. Unfortunately, most of the cytogenetic studies have been on IVF eggs which have failed to be fertilized. Angell *et al* [40] called attention to a rate of 23-31% of cytogenetic abnormalities in IVF eggs. They found that the most common cytogenetic abnormality in IVF eggs is aneuploidy which is about 10%. Other investigators have described hypohaploid, hyperhaploid, polyploid and diploid oocytes failing to fertilize *in vitro* [41].

2.3. New concepts in mechanistic embryology.

We have mentioned above the discovery of antigens involved in induction mechanisms. concepts in pattern formation, and positional information mechanisms in embryology.

2.4. Growth factors in Development.

Gluckman [42] in his extensive review, reported that classical hormones such as growth hormone, thyroid hormones and some growth factors such as nerve growth factor (NGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) had little or no effect on progression of cells from growth phase to the phase of synthesis during cell division. Growth factors and hormones which are mitogenic are divided into two broad categories – a) competence factors which assist cells to move from a quiescent state termed G_0 to a beginning of mitosis. They include PDGF and FGF. Other factors are those that move the cell from G_1 to stage of synthesis of DNA termed the S phase. They include insulin and insulin-like growth factors I & II (IGF – 1, IGF -2). There is very little evidence that growth hormone affects fetal development. But other hormones enumerated above seem to have effect on fetal growth. Indeed it is because insulin acts like a growth factor that leads to increase in weight of infants of diabetic mothers who have to produce large amounts of their own insulin to cope with the transfusion of maternal hyperglycemia [42]. The fnctions of growth factors have been expanded to include the following endocrine, autocrine, paracrine, juxtacrine, intracrine and matricrine [5].

2.5. Molecular Embryology

This field is gaining ground worldwide as a field of the 21st centrury. It involves the study and use of genes in understanding development processes using methods like injection of genes into early embryos for their somatic incorporation and also the study of genes which aid developmental processes such as oncogenes. Molecular biology in general, studies genes (known as genomics) and their products (known as proteomics since they express protein). A gene is a segment of a chromosome containing polynucleotides. It has a sequence of nucleotide bases which are specific to it and these are measured in kilobases (kb). 1kb is 1000 bases. A gene has from 1 to 300 kbs. Techniques have developed to open up a gene and determine its arrangement in order to synthesise it *in vitro*. Nucleotide sequences have been put together to synthesise genes *in vitro* in order to use them for transcriptional and translational purposes. Sanger and co-workers in 1977 [43] together with Maxam and Gilbert [44] reported methods for sequencing genes, and these methods have been used extensively now to produce the sequence of all genes in the human genome [45, 46]. Southern [47] reported his method of identification of DNA in tissue with the use of what are called gene probes. The development of recombinant DNA, which is a logical step forward in molecular biology has led to the development of methods for the synthesis of proteins *in vitro* by the use of genes which have been previously amplified by a new technology called polymerase chain reaction(PCR) – [48]. Such genes are inserted into prokaryotic (mostly) vectors such as *Escherichia coli* which then translate them into their protein product(s). The technique has been used to synthesize human insulin, somatostatin and growth factors among others [48-50].

2.6 Molecular early embryology

We now know that there are factors in the human oviduct which assist genomic transformation of early oviduct-commuting embryos [51,52]. Artley and Braude [52] concluded from their study of human embryos in culture that human embryo gene expression is associated with an α-amanitin sensitive qualitative change in protein synthesis. Zygotic genome is activated and maternal genomic control suppressed during certain cleavage stages in early embryogenesis. Such changes are controlled by oviductal factors which have not been properly elucidated. When such factors are unavailable, as *in vitro* culture, block to cleavage at specific stages of development ensues.

2.7. *Developmental genes*

Rapid understanding of the genes which control development has been made in the last two decades. By studying homeobox genes of mouse we have better understanding of human genes which control development. There are 8 homeobox genes which specify the structures that develop on body segments [5,53]. In mammals there are about 40 genes containing homeoboxes. The genes are *HOX* in man. They are located in 4 clusters. They are numbered from 1-13 with 1 being cephalic and 13 caudal. *Hox a -6, Hox b-6 and Hox c-6* are called the paralogous group [54-56]. The codes for Hox genes have been identified in four different locations in the embryo as follows – an axial code specifying somites, a branchial code specifying somites, a branchial code specifying neural crest derivatives in the branchial region, and organogenesis code and a limb code. Other important developmental genes in vertebrates include non-Hox homeobox genes, Pax genes which have been associated with certain illnesses – Waardenburg's syndrome (***PAX*** -3) and aniridia (***PAX*** – 6) [56, 57-60].

2.8. Gene injection

The technology for gene injection concerns embryologists because to produce a true transgenic organism, change in its gene must be effected in the early embryo. Since we presently have the technology for manipulating early embryos in tissue culture, it is possible to manipulate the egg before implanting. The genes are obtained by recombinant DNA techniques either by synthesis (synthetic DNA) or by the use of the enzyme reverse transcriptase on tissue mRNA which then catalyses the formation of the gene called complementary DNA (cDNA). The genes are amplified by PCR technology and are either carried on a virus such as SV40 which is then allowed to infect cells or they may be injected directly into the nucleus. It is equally possible to use calcium phosphate on the cell membranes, which renders the cell permeable to a gene [50]. If the injection is into a very early embryo, the foreign gene may then be fully expressed in the transgenic embryo. Sometimes however, the foreign gene may be expressed in inappropriate places, like the expression of hemoglobin gene in muscle. It may therefore be necessary to provide tissue specific enhancers. It is now possible to use animal transgenic organisms to provide medical therapy as in the production of biomedically useful agents such as α – 1 antitrypsin in transgenic sheep mammary glands secreted into milk [61,62]. Methods are being developed to produce transgenic pigs capable of providing (HL –A) histocompatible organ transplants for humans in the future [63-65]. Given the long way in which this technology has come, it is gratifying to note changes in attitude to it [66,67].

2.9. Ante-natal diagnosis

The technique of Southern which detects specific DNA sequences in tissues [47] has allowed for the diagnosis of genetic conditions without first obtaining the primary tissue of affectation. Thus in sickle disease, red cells are not needed for diagnosis. Any cell of the fetus (and *ipso facto*, amniotic fluid cells for example) can be used for diagnosis. There is an initial need to obtain a gene probe which is radiolabelled. Hybridization of gene probe and specific DNA sequence (i.e., gene) being sought for can be picked up by autoradiography if

present. Hybridization to RNA is called Northern blot [50].

2.10. Oncogenes

Oncogenes are cancer genes which expressed lead to some of the characteristics of cancer cells or transform an otherwise normal cell into neoplastic cell. They have homology with certain retroviral sequences which infect certain animals that are then called viral oncogenes (v – onc). It appears that these genes in man (cellular oncogenes – c-onc) are activated during embryological development [68].

2.11. Cloning

The terminology 'cloning' continues to attract usage by lay and scientific public alike leading sometimes to much confusion. Cloning simply refers to making copies, which may resemble photocopy or indeed carbon-copy of any item. In biology, items to be cloned could be chemical, embryo or a complete adult. There are, therefore, basically three types of cloning. In biochemistry, cloning refers to making several copies of genes [69-72]. In embryology, it refers to duplicating the embryo through the elegant method of embryo splicing first made simple by Willadsen in Cambridge, UK [20, 21]. In gross anatomy, it refers to the duplication of a complete organism by the transformation of the constituent cell into another individual with the same genome. The last method is the most popular in modern times and was first reported for mammals by Wilmut in Edinburgh in 1997. Yanagimaachi in Hawaii [73] has perfected the technique in the mouse so much that he boasts of at least 8 generations of clones [73]. He uses cumulus cells to achieve cloning with cumulus cells providing the nucleus (diploid) to become the embryonic zygote nucleus and the preovulatory oocyte cytoplasm becoming the stimulating cytoplasm. In the initial experiment of Wilmut, udder cell of sheep was used to provide nucleus while cytoplasm was derived from the same source as Yanagimachi, through nuclear transplantation.

2.12. Genomics

The human genome has been completely sequenced – at least 97% of it. The implication for the future is that it is now possible to access genetic codes for any gene discovered in the future and therefore to attempt to synthesise proteins expressed by the genes in laboratory for therapeutics [45,46].

3. ***Microscopic anatomy***

3.1. *Cellular endocrinology*

Increasing study of cellular elements around the body has allowed the charaterization of more endocrine portions of the human body. Localization of APUD cells have been made in Skene's paraurethral glands in the female [74] and the urinary bladder[1].

Certain posterior pituitary hormones have been localized in the FRT such as vasopressin and oxytocin [75]. In the ovary, they are luteotrophic although we cannot rule out the possibility that they may affect water retention in certain stages of the reproductive cycle in the woman and may be responsible for such severe water retention pathology as in premenstrual tension. Prolactin is found in the endometrium and decidua [76].

Certain peptide hormones have been localized in the gonads. They include testibumin (in addition to inhibin) – Shaha [77], LHRH, relaxin, proopiomelanocortin (POMC) – derived peptides such as β-endorphin, α-MSH, ACTH, γ-MSH and N-acetylated derivatives of βEP such as NacEP [78]. Clements *et al* [78] suggested that the peptides function as paracrine (or indeed autocrine) regulators of intragonadal functions. It has been suggested that peritubular cells of the seminiferous tubules of the testis may be under the direct stimulation by androgens produced by Leydig cells. Peritubular cells in turn stimulate Sertoli cell via a paracrine factor P-Mod-S to induce their functions in spermiogenesis [79]. Factors known to be produced by the testins now include testins [80] produced by Sertoli cells in

order to effect spermiogenesis, transferrin, IGF-1, androgen binding protein, and testicular albumin [81,82].

One of the best known renin-angiotensin systems of the body is now found in the gonadal system. In the ovary, this system is thought to function in steroidogenesis, ovulation and angiogenesis in follicular and luteal tissues [83,84]. Immunopositive staining of Leydig cells of the testis and the human prostate gland for renin was reported by Inagami *et al* [85]. Using *in situ* hybridization techniques, Deschepper [86] was able to demonstrate renin immunoreactivity in testis, anterior pituitary, intermediate lobe of pituitary arteries, pineal gland and the ovary in addition to reactivity in the kidney and adrenal glands.

Vasoactive intestinal peptide has been found to be ubiquitous in the male and female reproductive tracts both in the epithelial cells and in neurons [87]. They have been shown to mediate sexual arousal mechanism in the sexes.

Several new hormones have been added to the list of gastro-pancreatic endocrine (GEP) system of Fujita [88]. They include galanin, (inhibits glucose-induced insulin release), glucagon-like peptide-1 (GLP-1) and helodermin[89]. Previously recognized gut hormones include bombesin, secretin, cholecystokinin, gastric inhibitory peptide, neurotensin and enteroglucagon. Although the functions of these hormones are not all known, some are known to affect gut motility while others are thought to influence exocrine (digestive) secretions of the gut [1].

The heart is now considered an endocrine organ being able to produce atrionatriuretic factor (ANF). Generally ANF elicits responses opposite to that of angiotensin II [85]. The secretion is by myoendocrine cells in the heart.

The brain is also a large endocrine gland containing considerable amounts of hormonal neuropeptides. New hormones have been described in recent times. Thus there is the brain natriuretic peptide (BNP) [90] and brain renin-angiotensin system [91].

The following hormones have been localized in the pancreas, not only in the islets, but also scattered amongst exocrine cells. These are glucagon (α2 cells), insulin (β), somatostatin and gastrin (D), pancreatic polypeptide (PP) and VIP (D1) [92].

The thyroid glands, apart from producing thyroxine and triiodotyronine, also produces calcitonin and katacalcin from C cells [93].

The thymus is now recognized as an endocrine gland with its secretions which control maturation of T lymphocytes. It produces the following factors-thymosis α1, β1, β4, thymopoietin I, II, thymosin B, B4, thymic humoral factor, thymostimulin and *facteur thymique serique* [94]. Several interleukins are secreted by thymocytes [5] and colony stimulating factors (CSF) for granulocytes and macrophages [5].

The placenta during pregnancy seems to take over the functions of hypothalamus –pituitary axis reproductive functions. It has now been shown to produce such hormones as gonadotropin-releasing hormone (GnRH), corticotropin-releasing factor (CRF), growth hormone releasing factor (GRF), thyrotropin releasing hormones (TRH) and also inhibin related peptides [95]. Hormones may modify local placental hormone secretions and affect maternal and/or fetal pituitary activity.

3.2. Cellular immunology

The cells involved in immunity have now been classified into subsets following the original broad classification into T and B lymphocytes. There are now 4 subsets of T lymphocytes (helper, suppressor, cytotoxic and delayed hypersensitivity T cells). Further division using monoclonal antibodies (Mab) which distinguish the OKTs have identified clusters of differentiations (CD) [96]. CD3, CD4, CD8. Others are CD2, CD11, CD14, CD20, CD21. Other cells involved in immunity are natural killer cells (NK) and null cells which are not classified into either T or B [97]. T cells are now known to produce chemical agents called lymphokines, which assist them in their function of cell mediated immunity. Other cells of the blood system such as macrophages also produce agents similar to lymphokines, hence

the new name cytokines. The largest secretion of such cells are the interleukins which now number more then 10. Other cytokines include tumor necrosis factor and the large family of interferons (α,β,γ).

3.3. Cellular haematology

Cells which are found in blood have been studied in detail in recent times. Firstly the genealogy of blood cells have been re-described. Burst-forming units of the erythroid line (FU-E) forms the colony forming units of erythroid lime (CFU-E). This then forms the pronormoblast. With the myeloid and mono-cytic series colony forming units of granulo-cytes and macrophages (CFU-G/M) lead to the formation of the well known mycloblasts [98].

Macrophages are now known to be ubiquitous and to be derived from single cell unit monocyte of the mononuclear system. The concept of a reticuloendothelial system is now fading away therefore [94] to be replaced by a mononuclear phagocytic system. Macrophages are involved in wound healing by promoting factors which aid fibrogenesis by fibroblasts, and monocyte numbers rise in blood stream concomitant with the healing of wounds [99, 100]. Macrophages are now known to produce over 100 cytokines with effects ranging from regulation of haematopoiesis to haemostasis [94].

3.4. Neuropeptides

The Amine Precursor Uptake and Decarboxylation (APUD) concept still continues to receive wide acceptance in the understanding of new peptide hormones figure 1, [1.101] long after Pearse had left the scene. But the postulated diffuse neuro-endocrine system (DNES), which originally said to include all known APUD cells has been extended to accommodate nervous system cells containing neuropeptides [102]. With new information coming in on the effects of neuropeptides on behaviour, the concept of the diffuse neuroendocrine system seems to receive corroboration from numerous reports. Baldwin [103] showed that several neuropeptides injected into the brains of rat, sheep and chicken alter ingestive behaviour. Early, Pearse and Polak [104] had postulated the behavioural modulatory role of the DNES.

3.5. Molecular microanatomy

Somatic cell hybrids: In order to localize genes on chromosomes, several methods have been developed. In somatic cell hybrids, the cell of a human is fused with that of a rodent using a virus or polyethylene glycol. As division of the hybrid cell continues in culture, human chromosomes are lost in culture and the remaining biochemical characteristics of the culture medium determine which chromosomes carry the gene(s) for the characteristics [105]. This is extremely important in modern genomics. Also Southern blot technique can be utilized on DNA from hybrids cells to identify a gene using radiolabelled specific DNA probe [106].

In situ hybridization histochemistry: Another method for the study of genes in cytological materials is *in situ* hybridization. This method is akin to Southern blot (for identifying DNA sequences) or Northern blot (for identifying RNA transcripts). Immunohistochemistry which is the commonest and widely used to detect peptides and their locations is not sensitive enough to localize small concentrations. In such situation, *in situ* hybridization can detect RNA transcripts which suggests the site of production of such peptides [102, 107, 108].

The technique is performed on slide sections of tissues. The slides are subbed with siliconized coverslips and reactions are incubated in moist chambers [109]. The cytological materials on the slides are then exposed to either DNA or RNA probes labelled with tritium (^{3}H) or radioactive iodine (^{125}I). The nucleic acid probes may be either single or double stranded. The technique for tissue processing in *in situ* hybridization must preserve the morphology of cells, and not extract or modify RNA or DNA, or change their localization. The nucleic acid must also be accessible to hybridization probe. After *in situ*

hybridization, counterstains can be performed using Giemsa stain, G-banding for human metaphase chromosomes or Coomassie blue.

Molecular ultrastructure: It is presently possible to apply the electron microscope to the study of molecular biology by the direct visualization of double stranded DNA filaments. Thus it is possible to characterise DNA structure as to whether circular or linear or its degree of supercoiling and the length or distribution of DNA fragments using transmission electron microscope. The electron microscope can also be utilized to analyse or detect complementary sequences which can be between two DNA molecules called heteroduplexes or between RNA and DNA called hybrids in addition to the study of protein nucleic acid interactions [110].

CONCLUSION

With the modern trends in anatomical research it is clear that anatomy still holds true as a discipline that requires an understanding that goes far beyond the appreciation of structure only. It has wide applications in various fields of medicine. Modern medicine can be seen to grow out of painstaking research and anatomy has a great deal to contribute through its traditional divisions into embryology, histology and gross anatomy.

REFERENCES

1. Williams PL Warwick R Dyson M Bannister LH (1989). *Gray's Anatomy* 37th ed. Edinburgh: Churchill Livingstone.
2. Lytle WJ (1974) The inguinal and lacunar ligaments. *Journal of Anatomy* 118:241-251.
3. Rizk NN (1980) A new description of the anterior abdominal wall in man and mammals. *Journal of Anatomy* 131:373-385.
4. Bojsen-Moller F, Flagstad KE (1976) Plantar aponeurosis and internal architecture of the ball of the foot. *Journal of Anatomy* 121:599-611.
5. Willams PL, Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Fergusin MWJ (1995) eds. Gray's Anatomy. *The Anatomical Basis of Medicine and Surgery*. 38th ed. New York: Churchill Livingstone.
6. Hjortsjo C-H (1956) The intrahepatic ramification of the portal vein. *Lunds Universitat Arkss NF Avd* 2, 52:1-30.
7. Finalyson NDC Bouchier IAD, Richmond J (1991) Diseases of the liver and biliary systems. In Edwards CRW, Bouchier IAD (eds). *Davidson's Principles and Practice of Medicine*. 16th ed. Edinburgh: Churchill Livingstone.
8. Blumgart L (1988) *Surgery of the liver and biliary tract.* Edinburgh: Churchill Livingstone.
9. McGrath P (1978). Aspects of the human pharyngeal hypophysis in normal and ancencephalic fetuses and neonates and their possible significance in the mechanism of its control. *Journal of Anatomy* 127:65-81.
10. Lister G (1985) Indications and techniques for repair of the flexor tendon sheath. *Hand Clinics* 1:85-95.
11. Del Campo CH, Ginther OJ (1972) Vascular anatomy of uterus and ovaries and the unilateral luteolytic effect of the uterus: guinea pigs, rats, hamster and rabbits. *American Journal of Veterinary Research*. 33:2561-2578.
12. Del Campo CH, Ginther OJ (1972) Vascular anatomy of uterus and ovaries and the unilateral luteolytic effect of the uterus: horses, sheep and swine. *American Journal of Veterinary Research*. 39:305-316.
13. Ogunranti JO (1992) Δ5-3β-hydroxysteroid dehydrogenase in rat oviduct and implication of oviductal steroidogenesis. *European Journal of Gynecology Obstetrics and Reproductive Biology* 44:145-150.
14. Ginther OJ, Dierschke DJ, Walsh SW, Del Campo CH (1974) Anatomy of arteries and veins of uterus and ovaries in rhesus monkeys. *Biology of Reproduction* 11:204-210.
15. Edwards R, Steptoe P (1980) A matter of life. *The story of a medical break through*. London:Hutchinson.
16. Bongso A (1992) Improved pregnancy rate after transfer of embryo grown in human fallopian tubal cell coculture. *Fertility and Sterility* 58:569-574.
17. Yao Y, Ho P-C, Yeung WS-B (1999) Effects of human oviductal cell coculture on various functional parameters of human spermatozoa. *Fertility and Sterility* 71:232-239.
18. Hewitt DA, England GCW (1999) Synthetic oviductal fluid and oviductal cell coculture for

canine oocyte maturation *in vitro. Animal Reproduction Science* 55:63-75.
19. Perez GI, Trbovich AM, Gosden RG (2000). Mitochondria and the death of oocytes. *Nature (Lond) 403:500-501*.
20. Johnson MH (1988) How are two cell lineage established in early mouse development? *Satellite Symposium of the 8th International Congress of Endocrinology on Development of - Preimplantation Embryos and their Environment*. New York: Alan R Liss.
21. Willadsen SM (1992) Micromanipulation of embryos of the large domestic species. In Adams CE (ed): *Mammalian egg transfer*, pp,. 185-210. Boca Raton: CRC.
22. Fehilly CB, Willadsen SM (1986) Embryo manipulation in farm animals. In Clarke JR (ed): *Oxford Reviews of Reproduction Biology*. Vol. 8. Oxford: Clarendon Press.
23. Mori T (1988) Regulation of steroidogenic function during luteinization. *Stellite Symposium of the 8th International Congress of Endocrinology on Development of Preimplantation Embryos and their Environment.* New York: Alan R Liss.
24. Lightman A, Palumbo A, Rzasa PJ, Culler MD, Jones C, Caride VJ, Negrovilar AF, Deschepper CF, Ireland JJ, Decherney AAH, Naftolin F (1988) The ovarian renin-angiotensin system (OVRAS). In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology*. *Vol 2, pp. 1201-1206. International Congress Series* 799. Excerpta medica.
25. Kola I, Lacham O, Jansen RPS, Turner M, Trounson A (1990) Chromosomal analysis of human oocytes fertilized by microinjection of spermatozoa into the perivitelline space. *Human Reproduction (Oxf) 5:575-577.*
26. Iritani A, Hosoi Y (1988) Microfertilization by various methods in mammalian species. *Satellite Symposium of the 8th International Congress of Endocrinology on Development of Preimplantation Embryos and their Environment*. New York: Alan R Liss.
27. Asch RH, Ellsworth LR, Balmaceda JP, Wong PC (1984) Pregnancy after translaparoscopic gamete intrafallopian transfer. *Lancet ii*: 1034-1035.
28. Kingsland CR, Manasse PR, Aziz N, Haddad N, Taylor CT, Richmond DH (1992) Transport *in vitro* fertilization: a novel scheme for community based treatment. *Fertility and Sterillity* 58:153-158.
29. Mills MS, Eddowes HA, Cahill DJ, Fahy UM, Abuzied MIM, Mcdermott A, Hull MGR (1991) a prospective controlled study of *in vitro* fertilization, gamete intra Fallopian transfer and intrauterine insemination combined with superovulation. *Human Reproduction 7:490-494*.
30. Chang SY, Lin PW, Soong YK, Guu HF, Chang MY, Wang ML (1991) A clinical pregnancy after a simple method of zona cutting, cryopreservation and zygote intrafallopian transfer. *Fertility and Sterility 55:420-422.*
31. Bongso A, Ng SC, Ratnam S (1990) Co-cultures; their relevance to assisted reproduction. *Human Reproduction 5:893-900.*
32. Cataldo P (1996) The Newest Reproductive technologies: applying Catholic teaching. In Smith RE (ed): *The Gospel of Life and the Vision of Health Care*. Massachusetts: Pope John XXIII Centre.
33. Gavaghan H (1994) NIH gets the green light on embryo research. *Nature (Lond) 371:370.*
34. McHale J, Fox M, Murphy J (1997) *Health Care Law: Text, cases and material pp. 627-695.* London: Sweet & Maxwell.
35. Enders AC (1988) Maturation of the blastocyst. *Satellite Symposium of the 8th International Congress of Endocrinology on Development of Preimplantation Embryos and their Environment.* New York: Alan R Liss.
36. Heyner S (1981) Immunogenetic study of the mammalian egg and early embryo. In Finn CA (ed). *Oxford Reviews of Reproductive Biology vol. 3, pp. 95-126*. Oxford: Clarendon Press.
37. Jacob F (1977) Mouse teratocarcinoma and embryonic antigens. *Immunology Review 33:3-32.*
38. Solter D, Knowles BB (1978) Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proceedings of the National Academy of Sciences USA 75:5565-5569.*
39. Nagai Y, Ciccarese S, Ohno S (1979) The identification of human H-Y antigen and testicular transformation induced by its interaction with the receptor site of bovine foetal ovarian cells. *Differentiation* 13:155-164.
40. Angell RR, Hillier SG, West JD Glasier Af, Rodger MW, Baird DT (1988) Chromosome abnormalities in human preimplantation embryos. *Satellite Symposium of the 8th*

International Congress of Endocrinology on Development of Preimplantation Embryos and their Environment. New York: Alam R Liss.

41. Papadopoulos G, Randall J, Templeton AA (1989) The frequency of chromosome anomalies in human fertilized oocytes and uncleaved zygotes after insemination *in vitro. Human Reproduction 4:568-573.*
42. Gluckman PD (1986) The role of pituitary hormones, growth factors and insulin in the regulation of fetal growth. In Clarke JR (ed): *Oxford Reviews of Reproductive Biology, vol. 8, pp. 1-60*. Oxford: Clarendon Press.
43. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. *Proceedings of National Academy of Sciences USA 74:5463-5467.*
44. Maxam AM, Gilbert W (1977) A new method for sequencing DNA. *Proceeding of the National Academy of Sciences USA 74:560-564.*
45. Anon (1994) Ownership and the human genome. *Nature (Lond*) 371:363-364.
46. Dickson D (1994) 'Gene map' plan highlights dispute over public versus private interests. *Nature (Lond) 371:365-366.*
47. Southern EM (1975) Detection of specific sequences among DNA fragments separated by Gel electrophoresis. *Journal of Molecular Biology 98:503-517.*
48. Baxter JD (1988) Molecular biology: from cloning to clinic. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol. 2. International Congress Series 799, pp. 1427-1431*. Excerpta Medica.
49. Itakura K, Hirose T, Crea R, Riggs AD, Heyneker HL (1977) Expression in E. coli of a chemically synthesized gene for the hormone somatostatin. *Science 198:1056-1063.*
50. Emery AEH (1985) *An Introduction of Recombinant DNA, pp. 161-179.* Chichester: Wiley and Sons.
51. First NL, Barnes FL (1988). Development of preimplantation embryos. *Satellite Symposium of the 8th International Congress of Endocrinology on Development of Preimplantation Embryos and their Environment.* New York: Alan R Liss.
52. Artley JK, Braude PR (1991) Gene expression and cleavage arrest in the preembryo. *Journal of Obstetrics and Gynaecology 11:162.*
53. Scott MP (1992) Vertibrate homeobox gene nomenclature. *Cell 71:551-553.*
54. Morris-Kay G (1993) Retinoic acid and craniofacial development molecules and morphogenesis. *Bioassays 15:9-15.*
55. Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J Sewright A, Hastie N, van Heyningen V (1992) The human *Pax6* gene is mutated in two patients with aniridia. *Nature Genetics 1:328-332.*
56. Satokata I, Maas R (1994) Mox 1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nature Genetics 6:348-355.*
57. Doll U, Niessing J (1996) A caudate type homeobox gene activity associated with the development and regeneration of the liver. *European Journal of Cell Biology 70:260-268.*
58. Soderman E, Mattsom J, Engstrom P (1996) The Arabidopsis homeobox gene ATHB-T is induced by water deficit and by abscisic acid. *Plur Journal 10:375-381.*
59. Logtan C, Wizenmann A (1996) Rostral optic tectum acquires caudal characteristic following ectopic Engrailed expression. *Current Biology 6:1006-1014.*
60. Kim T-K, Ko C, Nhan, S-U, Chung H-M (1996) Partial cloning of *Xhox3.1 gene in Xenopus laevis. Korean Journal of Genetics 18:111-116.*
61. Clerk AJ, Bessos H, Bishop JO, Brown P, Harris S, Lathe R, McClenaghan M, Prowse C, Simon JP, Whitelaw CBA, Wilmut (1989) Expression o human anti-haemophilic factor IX in the milk of transgenic sheep. *Bio/technology 7:487-492.*
62. Wright GH, Carer A, Cottom D, Reeves D, Scott A, Simons JP, Wilmut I, Garner I, Colman A (1991) High level expression of active human α1 antitrypsin in the milk of transgenic sheep. *Bio/technology 9:830-834.*
63. Latinne D, Gianello P, Smith CV, Nickekeit V, Kawai T, Beadle M, Haug C, Sykes M, Lebowitz E, Bazin H, Colvin R, Cosimi AB, Sachs D (1993) Xenotransplantation from pig to cynomolgus monkey – approach toward tolerance induction. *Transplantation proceedings 25:336-338.*
64. Holzknecht ZE, Platt JL (1995) Identification of porcine endothelial-cell membrane antigens recognized by human xenoractive natural antibodies. *Journal of Immunology 154:4565-4575*.
65. Rosengard AM, Cary NRB, Langford GA, Tucker AW, Wallworth J, White DJG (1995) Tissue expression of human complement

inhibitor, decay-accelerating factor, in transgenic pigs. *Transplantation 59:1325-1333.*

66. Mouneimne Y, Tosi P, Barhoumi R, Nicolau C (1990) Electroinsertion of full length recombinant CD4 into red cell membrane. Biochimi Biophysica Acta 1027:53-58.
67. Tuffs A (1993) Germany: Attitudes to gene technology relaxing. Lancet 341:106-107.
68. Emery AEH (1991) Genetic factors in disease. In Edwards CRW, Bouchier IAD (eds): *Davidson's Principles and Practice of Medicine, 16th ed., pp. 1-24*. Edinburgh: Churchill Livingstone.
69. Monkawa T, Yoshida T, Wakino S, Shinki T, Anazawa H, Deluca HF, Suda T, Hayashi M, Saruta T (1997) Molecular cloning of cDNA and genomic DNA for human 25-hydroxyvitamin D3 1 alpha-hydroxylase. *Biochemical and Biophysical Research Communications 239:527-533.*
70. Masuhara M, Sakamoto H, Matsumoto A, Suzuki R, Yasukawa H, Mitsui K, Wakioka T, Tanimura S, Sasaki A, Miasawa H, Yokouchi M, Ohtsubo M, Yoshimura A (1997) Cloning and characterization of novel CIS family genes. *Biochemical and Biophysical Research Communications. 239:439-446.*
71. Holzinger A, Kammerer S, Berger J, Roscher AA (1997). cDNA cloning and mRNA expression of the human adrenoleukodystrophy related protein (ALDRP), a peroxisomal ABC transporter. *Biochemical and Biophysical Research Communications 239:261-264.*
72. Tanaka T Nakamura T, Takagi H, Sato M (1997) Molecular cloning and characterization of a novel TBP -1 interacting protein (TBPIP): enhancement of TBP-1 action on *T at TBPIP. Biochemical and Biophysical Research Communications 239:176-181.*
73. Boyce N (1998). Go forth and multiply. *New Scientist 25 July pp. 4-5.*
74. Zaviack M (1986) Argyrophil and argentaffom cells in the human female prostate homologue (Skene's paraurethral glands and ducts) and urethra. *Acta Histochemica 79:93-96.*
75. Schaeffer JM<Liu J, Hsueh AJW, Yen SSC (1984). Presence of oxytocin and arginine vasopressin in human ovary, oviduct, and follicular fluid. *Journal of Clinical endocrinology and Metabolism 59:970-973.*
76. McRae MA, Newman GR, Walker SM, Jasani B (1986) Immunohistochemical identification of prolactin and 24K secretory endometrium. *Fertility and Sterility 45:643-648.*
77. Shaha C (1988). Localisation of gonadal peptides. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 1 International Congress series 799, pp. 457-463*. Excerpta Medica.
78. Clements JA He L, Risbridger GP, Smith AI, Funder JW (1988). Pituitary peptides in the ovary and testis. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol. 1. International Congress Series 799, pp. 469-474*. Excerpta Medica.
79. Skinner MK (1988) Peritubular myoid cell-Sertoli cell interactions. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 1. International Congress Series 799, pp. 469-474*. Excerpta Medica.
80. Cheng CY, Bardin CW, Boitani C, Grima J, Morris (1988) Testins and Sertoli cell proteins that may be used to study cell-cell interactions within the seminiferous epithelium. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp. 1157-1162*. Excerpta Medica.
81. Ganong WF (1987) *Review of Medical Physiology* 13th ed, p. 359. New York: Prentice Hall International.
82. Forti G, Vannelli GB, Barni T, Orlando C, Balboni GC, Casano R, Serio M (1988) Sertoli cell proteins in the human testis. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp. 1163-1168.* Excerpta Medica.
83. Edwards RG (1986) Follicular fluid. *Research in Reproduction 18:4.*
84. Lightman A, Palumbo A, Rzasa PJ, Culler MD, Jones C, Caride VJ, Negrovilar AF, Deschepper CF, Ireland JJ, Decherney AH, Naftolin F (1988) The ovarian reninangiotensin system (OVRAS). In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 1201-1206*. Excerpta Medica.
85. Inagami T, Parmentier M, Pandey KN, Naruse M, Pochet R (1988) Testicular renin-angiotensin system. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 1207-1212*. Excerpta Medica.
86. Deschepper CF (1988) The renin-angiotensin systems in endocrine glands: in situ

hybridisation and immunocytochemistry. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 1197-1200*. Excerpta Medica.

87. Ottesen B, Gerstenberg T, Ulrichsen H, Manthorpe T, Fahrenkrug J, Wagner G (1983) Vasoactive intestinal polypeptide increases vaginal blood flow and inhibits smooth muscle activity in women. *European Journal of Clinical Investigation 13:321-324.*
88. Fujita T (1973) *Gastro-enteric endocrine system. A cell-biological approach*. Tokyo: Igaku Shoiu.
89. Yanaihara N, Yaniahara C, Mochizuki T, Hoshino M, Zhang T, Iguchi K (1988) New gastrointestinal hormones. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 1545-1550.* Excerpta Medica.
90. Matsuo H (1988) Two families of natriuretic peptides: ANP and BNP. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 1499-1503*. Excerpta Medica.
91. Campbell DJ (1988) Angiotensinogen in the brain and peripheral tissues. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 1213-1218*. Excerpta Medica.
92. Buffa R, Capella C, Solcia E, Frigerio B, Saio S (1977) Vasoactive intestinal peptide (VIP) cells in the pancreas and gastrointestinal mucosa. An immunohistochemical and ultrastructural study. *Histochemistry 50:217-227.*
93. Hillyard CJ, Myers C, Abeyasekera G, Stevensom JC, Craig RK, Maclntyre I (1983) Katacalcin: a new plasma calcium-lowering hormone. *Lancet 1:846-848.*
94. Playfair JHL (1984) *Immunology at a glance. 3rd ed., pp. 30-32*. Oxford: Blackwell Scientific Publications.
95. Petraglia F, Sutton S, Zaccardo AM, Calza L, Giardino L, Marrama P, Coukos G, Volpe A, Genazzani AR, Lim ATW, Sawchenko P, Rivier J, Vale W (1988) Production and role of releasing and inhibiting factors in human placenta. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 407-412*. Excerpta Medica.
96. Reinherz El (1987) T-cell receptors. Who needs more*? Nature 325:660-663.*
97. Ferguson A (1991) Immunological factors in disease. In Edwards CRW, Boucher IAD (eds): *Davidson's Principles and Practice of Medicine*. 16th ed, pp. 25-44. Edinburgh: Churchill Livingstone.
98. Zucker-Franklin D (1985) Eosinophils. In Golde DW, Takaku F (eds): *Haemopoietic stem cells, pp. 45-63.* New York: Dekker.
99. Leibovich SJ, Ross R (1975) The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *American Journal of Pathology 78:71-100.*
100. Oladimeji M, Grimshaw AD, Baum M, Patterson KG, Goldstone AH (1982) Effect of surgery on monocyte function. *British Journal of Surgery 69:145-146*.
101. Pearse AGE (1966) Common cytochemical properties of cells producing polypeptide hormones, with particular reference to calcitonin and the C cells. *Veterinary Records 79:587-590.*
102. Polak JM, Bloom SR (1988) Neuropeptides and gut hormones: techniques for the localisation of mature peptide, mRNA and specific binding sites. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 2. International Congress Series 799, pp 1221-1226*. Excerpta Medica.
103. Baldwin BA (1988). Neuropeptides and food intake. In Imura H, Shizume K, Yoshida S (eds): *Progress in Endocrinology vol 1. International Congress Series 799, pp 705-710.* Excerpta Medica.
104. Pearse AGE, Polak JM (1978) The diffuse neuroendocrine system and the APUD concept. In Bloom SR (ed): *Gut Hormones*. Edinburgh: Churchill Livingstone.
105. McKusick VA (1980) The anatomy of the human genome. *Journal of Heredity 71:370-391.*
106. Shows TB, Sakaguchi AY, Naylor Sl (1982) Mapping the human genome, cloned genes, DNA polymorphism, and inherited disease. *Advances in Human Genetics. 12:341-352.*
107. Fuxe K, Cintra A, Aronsson M, Agnati LF, Kitayama I, Wilkstrom A-C, Okret S, Gustafsson J-A (1988). Localisation and distribution of glucocorticoid receptor immucoreactivity and of glucocorticoid receptor mRNA in the rat brain using immnocytochemistry and in situ hybridisation. In Imura H, Shizume K, Yoshida S (eds):

Progress in Endocrinology vol 1. International Congress Series 799, pp 875-883. Excerpta Medica

108. Cortes R, Villar MJ, Verhofstad A, Hokfelt T (1990) Effects of central nervous system lesions on the expression of galanin: a comparative in situ hybridisation and immunohistochemical study. *Proceedings of the National academy of Sciences USA 87:7742-7746*.
109. Pardue ML (1985) in situ hybridisation. In Hames HD, Higgins SJ (eds): *Nucleic Acid Hybridisation, a practical approach, pp. 179-202*. Oxford: IRL.
110. Oudet P, Schatz C (1985) Electron microscopic visualisation of nucleic acid hybrids. In Hames BD, Higgins SJ (eds): *Nucleic Acid Hybridisation, a practical approach, pp. 161-178*. Oxford: IRL.
111. Orkin SH (2000) Stem cell alchemy. Nature Nov 2000, pp 1212-1213.