

RECENT ADVANCES IN ANATOMY

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In this brief survey only those topics which appear to be of special interest to members of this section have been selected for discussion.

The number of human embryos of the first two weeks of development recovered by American embryologists has been almost doubled during the last six years. This is a remarkable achievement when it is realized that it was accomplished largely by the joint efforts of two men—Hertig and Rock, of the Staff of the Free Hospital for Women at Brookline, Massachusetts. Prior to their discoveries our knowledge of early human development was based on hardly more than a dozen embryos scattered widely among the laboratories of Europe and America.

Obviously the phenomenal success of these searchers for human specimens seldom or never seen, was not a matter of mere chance. They undertook a systematic and organized search for human ova in uteri of patients of high fertility who were willing to cooperate by keeping, while awaiting hysterectomy, a careful record of menstruation and coitus dates. These records enabled them to determine the likelihood of the presence of a conceptus and to judge more accurately the age if one were found.

In 1942, they announced the recovery of two normal human ova of $7\frac{1}{2}$ and $9\frac{1}{2}$ days of age respectively, which represented at the time the youngest human embryos ever observed. The younger embryo was in the process of implantation, the older one had just completed this process. Last month at the annual meeting of the Association of Anatomists at Cleveland, they reported the finding of a young human blastula free in the cavity of a uterus in the twenty-first day of the cycle. The probable fertile coitus occurred $4\frac{1}{2}$ days previously. This conceptus is judged to be 4 days old. It consists of nine blastomeres of varying size and shape. Since some of the blastomeres are multinucleated, it is concluded that the specimen is abnormal, hence the $7\frac{1}{2}$ day old embryo still remains as the youngest known normal human embryo.

A study of twelve very young embryos of their collection has been reported in some detail. Of this number seven were normal and five abnormal, the latter being so pathological as to indicate early abortion. It is interesting to note that all the normal embryos were implanted on the posterior wall, and all the abnormal on the anterior wall.

They conclude, 1) that nidation takes place between the fifth and eighth day following fertilization, 2) that the post-ovulatory phase of the menstrual cycle is less variable in duration than the pre-ovulatory phase, 3) that ovulation regularly occurs 14 days prior to the onset of menstruation, thus a woman with a menstrual history varying from 25 to 30 days has an ovulatory period ranging from the 11th to 16th day of the cycle.

The recent literature clearly shows a trend in anatomical investigations away from strictly morphological studies toward histo-physiological and histo-chemical problems. The aims of histophysiology are to interpret structure in terms of function and to provide a structural basis for understanding functional processes. In histochemistry the objective is the localization and identification of chemical substances in respect to the structural organization of the tissue elements.

Obviously histophysiological studies require the use of both histological and physiological methods. The usual procedure is to study the morphological changes associated with varying states of functional activity. Modern endocrinology owes much to this type of investigation. Of the very recent histophysiological contributions one only has been selected for discussion.

Dougherty and White, 1946, demonstrated that the adrenal cortex exerts a controlling influence over the number of lymphocytes in lymphatic tissue and in the circulating blood. Adrenalectomy in animals produces lymphoid hyperplasia and lymphocytosis whereas the injections of adrenotropic hormone or adrenal extracts produce lymphopenia in lymphatic tissue, lymphocyte degeneration and an increase in plasma cells. It has been shown also that augmentation of the normal quantity of circulating adrenal cortical secretion produces a significant increase in serum globulin and in circulating antibodies, which suggests that disintegrating lymphocytes are sources of these substances.

Histologists have long been interested in the localization and identification of chemical substances in cells and tissues. They have been over-zealous many times in interpreting their staining reactions on a chemical basis. Of the many methods that have been proposed in the past but a very few, such as the Sudan staining for lipids, the Prussian blue reaction for iron, and the nucleal reaction for chromatin have been accepted with certain limitations as trustworthy.

In the last two decades, particularly the last, investigators with experience in both histology and chemistry have reported many new histochemical methods. The procedures are diverse but all depend on the physical and chemical properties of the tissue constituents.

Differences in solubility and in rate of sedimentation on centrifugation have been employed to separate and isolate various cellular components for chemical analysis. The amazing feat of separating intact mitochondria from liver cells was accomplished by Bensley and Hoerr in 1934, submicroscopic particulates by Claude in 1938, glycogen particles by Lazarow in 1942, intact nuclei by Dounce in 1943 and nuclear chromatin in pure state by Claude and Potter and by Mirskey and Pollister in 1943. Methods and results are discussed in Volume X of Biological Symposia.

Analytical chemical analyses of the isolated mitochondria and submicroscopic particulates show that both are complexes of proteins, nucleo-proteins and lipids, the mitochondria containing more protein and less lipid than the particulates of Claude.

Identification of substances *in situ* in microscopic sections, either fresh or fixed, has been accomplished by various means; behavior in polarized light, fluorescence,

microincineration, ultra violet absorption and by highly specific and sensitive chemical reactions, the last mentioned has the advantage of superimposing a chemical picture on a morphological picture. An evaluation of these methods was published by Lison in 1936.

More recently introduced techniques that are now being extensively studied and applied are discussed in a recent review of histochemistry by Dempsey and Wislocki, 1946. It has been found that treatment of sections with the enzyme ribonuclease destroys the characteristic basophilic staining reactions of the Nissl granules in nerve cells, of the cytoplasmic granules of the basophiles of the anterior pituitary, of the cytoplasm of serous secreting glands, and of the trophoblast of the placenta. Thus, it appears that all those substances in cells which lose their characteristic basophilic staining reaction after digestion with ribonuclease represent or at least contain the ribose type of nucleo-protein.

It has also been demonstrated that the adrenal cortex, ovarian follicle, corpus luteum and trophoblast cells of the fetal placenta contain a lipid soluble substance that will convert phenylhydrazene when applied to sections to yellow phenylhydrazones. This is a well known test to aldehydes and ketones, but since this substance is lipid soluble and since it is present in the organs which are known to produce the keto-steroid hormones, it is concluded that this test demonstrates the presence of such substances in tissue sections.

The localization of the alkaline and acid phosphatases can now be visualized in microscopic sections. Since the phosphatases are believed to participate in the metabolism of phospho-lipids and sugars and in the absorption of sugars and fats through cellular membranes, the visualization of the precise location of these enzymes affords a valuable histological method for the study of cellular functions. A number of papers dealing with this subject has just been published. It seems that the enzymes are accumulated between the blood stream and the site of deposition of glycogen. They are present in the distal part of the absorbing cells of the small intestine. The prostate gland contains only acid phosphatases. Acid phosphatases accumulate in the syncytium of placentas near term, whereas alkaline phosphatases are present throughout gestation.

The interest of histologists in histophysiological and histochemical studies has prompted Dempsey and Wislocki to propose the term physiological histochemistry for this new discipline which correlates morphology, chemistry and physiology.

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