The Effect of Altered Plasma on Tissue Proliferation

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TISSUE PROLIFERATION IN ALTERED PLASMA

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by

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The Effect of Altered Plasma on Tissue Proliferations.

Tissue proliferation in plasma, altered plasma, and other media has been investigated by Carrel; Burrows; Harrison and others. The purpose of their investigation was to determine the intra vitro growth of tissue in plasma taken from animals after the removal of the thyroid and suprarenal glands. Our observations were confined chiefly to the cat. It was first deemed necessary to make cultures of normal tissue in normal plasma in order to obtain a fundamental knowledge of the rate, nature and detail methods of tissue proliferation.

Some preliminary work was done in chicken plasma in order to perfect the technique, since chicken plasma coagulated more slowly than that of the cat and consequently was handled with greater ease.

The technique consisted of the collection and centrifuging of the blood; the separation and retention of the plasma; the preparation and subsequent mounting of the tissues in plasma; and the incubation and microscopic study of both the living and stained preparations.

The animals when bled were anaesthetized with ether, and since bleeding was always from the carotids, the neck was shaved and rendered aseptic; the neck was opened by a single median incision, and the sterno mastoid fibres separated in order to expose the artery; after ligation of the cephalad portion of the artery a sterile, cold, oiled canula was inserted into the artery and 3 to 4 c.c. off blood withdrawn, into

one of the following containers.

a. Paraffined, sterile glass tube @ 0° C.

b. Sterile glass tube @ 0° C.

c. Sterile glass tube containing 2 c.c. of pure olive oil @ 10° C.

d. Sterile glass tube containing sodium citrate solution @ 0° C.

The tubes were then packed in ice and salted in centrifuge shields, and carefully balanced. A rubber cap over the mouth of the glass tube prevented contamination and was not drawn into the tube by the centrifugal force.

The tubes were then centrifuged for three minutes at 3000 revolutions per minute. The red corpuscles, erythrocytes, being the heavier were thrown to the bottom of the tube forming about 3/10 of the total blood volume; the leucocytes formed the next layer, or so called "blood cream" constituting 1/20 of the entire blood volume; the blood plasma constituted the remaining 13/20 of the original blood volume.

The supernatant plasma was then removed by means of a sterile ice cold pipette into sterile ice cold hermetically sealed tubes and placed in the refrigerator until needed.

The following table giving the dates, methods and animals relative to blood collections and the length of time elapsing before coagulation was used in determining efficiancy of certain innovations.

Results of Plasma Retention.

Animal	Method	Date collected	Date termination	Time retained
chicken	over blood	11/11/'12	11/20/'12	9 days
"	"	12/2/'12	12/10/'12	8 days

chicken	Pipetted off	11/11/'12	11/24/'12	13 days.
"	" "	12/2/'12	12/28/'12	26 days.
"	" "	12/9/'12	1/6/'13	28 days.
Cat #6	Over Blood	12/12/'12	12/12/'12	2 hours.
" #7	"	12/13/'12	12/13/'12	3 hours.
" #8	"	12/14/'12	12/14/'12	2 hours.
" #6	Pipetted off	12/12/'12	12/12/'12	8 hours.
" #7	" "	12/13/'12	12/13/'12	24 hours.
" #8	" "	12/14/'12	12/14/'12	10 hours.

The figures given in this chart represent average results, since only an average is necessary for this point. The conclusions then are, that blood plasma pipetted off from blood elements, will keep better than if allowed to remain in contact with blood elements; on an average of 13 days longer. Cat's plasma is much more difficult to keep and when kept is only kept for hours; the average length of time is in ratio with that of chicken plasma.

The tissues used in this work were of embryonic origin and were removed by a surgical as well as bacteriological regard for asepsis and placed in sterile normal salt solution 0.7% at 38° C. The tissues were subsequently divided either by teasing or by careful dissection with delicate tissue scissors; into sizes ranging from 0.5 tol. m.m. each. It was found that if the tissues were washed in Ringer's Solution, the plasma coagulated less readily; and that an isotonic salt solution consisting of Na Cl; Na CO_3; Ca Cl_2 and K.Cl. acts as an accelerator to the growth of all connective tissues. The tissue was next transferred under aseptic conditions to a sterile mount and the plasma added by means of a previously cooled pipette. The plasma coagulated in from 1 to 3 minutes after coming in contact with the tissue,

and formed a firm transparant bed of media.

There were two classes of plasma alteration used, the direct and indirect methods. The direct methods consisted in the addition of certain chemicals or other substances foreign to the normal blood content; among those used were agar agar, beef bullion, gelatin, blood serum and sterile water; the effects of heat and heterogenic serum and plasma were also noted.

The indirect methods consisted in the alteration of blood plasma by means of the removal of certain of the ductless glands especially thyroid and suprarenal or adrenals. The technique used was as follows: Animal was anaesthetized and placed upon an animal operating table; region of incision located, shaved and rendered aseptic; and in the case of the thyroidectomies, a mediam incision was made giving proper retraction for a lobectomy; the sterno mastoid muscle was separated and the sterno hyoid muscle pushed toward median line. The thyroid gland consists of two lobes, one lobe on either side of trachea and in the deep fascia internal to the carotid artery; each receives two arterial branches, an inferior from the subclavian and a superior thyroid from the internal carotid, both branches were ligated and the capsule opened and the gland removed; capsule left behind in some cases, in others it was also removed, the former garded against any parathyroid disturbance which is common since one of the parathyroids lies ventral and anterior to the thyroid and is intimately attached. The wound was carefully irrigated with bichloride solution and warm salt solution, and closed with a running suture, and the entire wound painted with saturated iodine solution. Certain modifications were used in these ectomies as follows; in a complete thyroidectomy, a unilateral lobectomy was often followed by a second lobectomy

resulting in the complete thyroidectomy.

The technique of a suprarenalectomy was practically the same except a median abdominal incision was made, and the suprarenal vessels ligated and the gland removed. The second gland was located from within and the area marked externally, to permit a subsequent lateral suprarenalectomy.

The technique of tissue mountings was practically all the same the only variation was in regard to the carrier for the mount. The coverslip method gave best results, since it enabled high power work on actively growing tissues, not possible with the heavier glass mounts. (a) The coverslip method consisted in removing a brass ring, 1/2 inch in diameter from melted paraffine onto a cool sterile slide adhering as paraffine cools, then the ring was coated with sterile vaseline, and inverted over the coverslip mount; thus the vaseline seals the mount into the air space made by the ring. (b) The petri dish mount was convenient for quantitative work, since tissues of any size and number may be placed on the inner side of the lid of the dish and the plasma added. The border of the dish was filled with oil thus sealing the dish and mount. (Small Petri dishes were usually employed.)

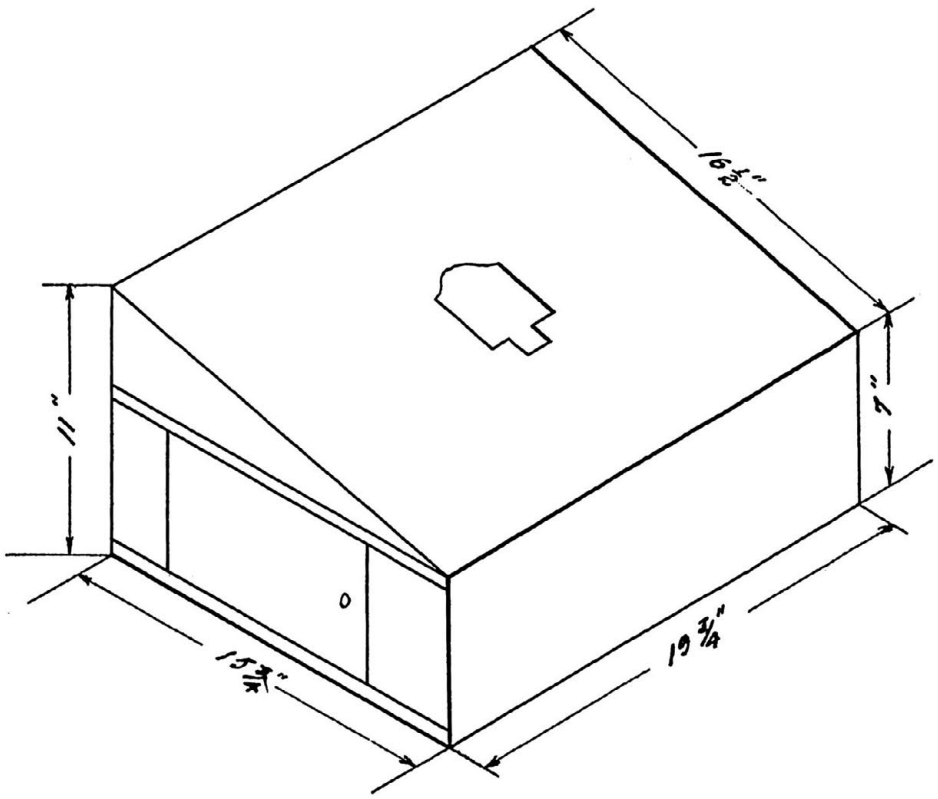
The Gabrilschenski Box described by Carrel; J. Exp. Med. Vol. 15 #4 is open to the same fault as is the Petri dish method, although it has in its favor better facilities for keeping tissues moist.

The mounted tissues were placed in labeled racks and put into the incubator, having first examined the mounts and noted an extravasation of tissue. The incubator was kept at

38° C. moist heat.

The tissue proliferation was found to be retarded by any chill so it was necessary to keep the mounts at 38° C. even when watching growths under the microscope. This was accomplished by use of box. (see cut below.) The box used consisted of a square container with a slanting lid, hinged at distal end , the lid had an opening to permit the ocular and focal adjustments to project and be used from the outside;an opening at either end, closed by a sliding door,permitted the adjustment of the mechanical stage and The side opposite the observer contained two sockets through which heat was furnished by an 8 and aI6 candlepower lamp. This side also contained a frosted glass to admit light for microscopic work

* Incubator Box. *



A Leitz Wetzlar scope was used in this work, both for the study of stained sections and of the living tissues, in the incubator box. The ocular #4 and three objectives,Low3, High6, and oil immersimn I/I2, aperatureI.3 were used, depending upon the tissue to be studied.

The tissues to be used for detailed microscopic study were killed and fixed before they were stained. Two methods of fixation were used, one by fumes, either of osmic acid or formaldehyde and the other by liquid formalin. The osmic acid turns the lipoid bodies dark and obscured the cell nucleus. The formaldehyde was much the better; the tissues were inverted over 40% formaldehyde solution and the fumes were allowed to act for two hours. The liquid fixation method consisted in washing the tissues in Ringer's Solution for one hour at 0° C. and then placing in an isotonic salt solution plus 2% of formalin. The osmic acid fixation requires no stain, merely a dehydration and clearance followed by chloroform balsam mount.

The best differential stain used was the Mann's acid haemotoxylin. The technique as follows was also varied by technician.

The tissues were now removed from the fixative to distilled water and then stained with acid haemotoxylin for five minutes or until the tissues, as well as the plasma assumed a deep red color. Next the tissues were decolorized in a 1% acid alcohol solution, either muriatic or sulphuric acid were used, watching under the scope until plasma and tissues were clear. The tissues were then washed in distilled water and a drop of a weak alkaline solution 4% NH_4OH. was added and the nuclei and when they were stained a deep blue, the tissues were removed to a 50% alcohol for a minute, then to 70% for five minutes; transferred to a saturated solution of Scharlack Rot in 70% alcohol one half to one hour or until all lipoid bodies were well stained; placed in 90% alcohol and mounted in glycerine on a hollow ground slide.

The following characteristics of normal growing tissue in normal plasma were soon self evident. In all new growing tissues, two types of cells appeared, a spindle shaped cell appeared early and very numerous, and a polygonal shaped cell appeared later. The spindle shaped cell was undoubtedly a connective tissue former; while the latter was the parenchymatous cell. (See Plate #1.) The least specialized the tissue the better the proliferation.

Confusion was avoided by an early examination of the mount, since during coagulation tubules as well as loose cells and blood elements were often drawn away from the tissue and simulated a new growth. Any glandular structures projecting were measured and tabulated. A well defined area of blood cells was often noted, varying with the tissue and the amount of washing it received prior to mounting; even then many of these faults could not be obviated as in the renal and thyroid tissues.

The normal suprarenal tissues developed many protoplasmic processes, (See Plate #1.) projecting into the normal plasma and forming new cells. These processes anastomosed through elongated processes with similar processes from other cells. The cytoplasm of these elongated cells was composed of many lipoid bodies and some granular material; the nuclei, usually spherical or oval, appeared homogeneous, with apparently neither chromatin bodies nor a reticulum. Later a second type of cell consisting of a few lipoid bodies and a rather large nucleus appeared. This was probably the parenchymatous cell and was of endothelial origin varying from 12 to 16 in diameter;

these cells grew less rapidly but more compactly; and were demonstrated on all growing tissue having an endothelial covering or lining.

Connective tissue growths in normal plasma were unusually rapid, the cells appeared as stellate, spindle shaped or in most any elongation. The freely branching anastomotic processes gave rise to new cells and formed a dense interlacement. (See Plate #4 for comparative growth and Plates #1,2 and 3 for individual cells under high power.) The cells vary from 20 to 60μ in length and 8 to 15μ in width with nuclei 2 to 4μ in diameter.

The normal thyroid glandular tissue carefully removed from its capsule and mounted in normal plasma and incubated @ 38° C showed the following growth in 24 to 36 hours. (See Plate #5) New epithelial buds appeared at the margin of the tissue and as they grew assumed a columnar appearance and from these radial columns cells branched at acute angles from either side; thus frequently forming queer enclosures which in turn often filled up with new cells. These cells varied from 10 to 16μ in diameter some with complete others apparently fragmentary nuclei, densely surrounded with lipoid bodies, the nuclei varied widely in size. No tendency to colloid formation was noted.

The normal suprarenal glandular tissue in normal plasma showed a growth in 12 to 24 hours, usually of connective tissue, corresponding to the above description and first appeared as lipoid bodies which radiated from the margin of the tissue and differentiated into cells. In 24 to 36 hours an epithelial budding, similar to that of the thyroid tissues only more extensive, was seen. The growth was exceedingly irregular, no tubular or radial arrangements were noted. The cells averaged from 15 to 25μ in diameter and

each contained an oval nucleous and a few lipoid bodies.

In the work on altered plasma media for this paper special attention was given to the thyroid and suprarenal tissues although other tissues were usually mounted at the same time.

The result of tissue proliferation in the plasma altered directly, gave the same characteristic growths as were noted above, the only differences noted were those of the intensity and rate as well as the manner of the proliferation. This work has been carefully written up and published by Ebelling and also by Carrel and Ingebringstein.

The following statements were derived from the results of a series of experiments carried on as described in the J. Exp. Medicine Vol. 15 No. 4 also Vol. 16 No. 4; and confirmed that work as follows:

1. Adult tissue grows, when at all, only in plasma media.
2. Embryonic tissue grows;
    (a) best in normal plasma media.
    (b) good in plasma 3/5 plus water 2/5.
    (c) fair in plasma 1/2 plus serum 1/2.
    (d) poorly in serum 1/2 plus agar 1/2.
    (e) poorly in plasma 1/2 plus bullion 1/2
    (f) poorly in Ringer's Solution.
3. Embryonic tissue grow better in homogenic than in heterogenic serum media, but better in heated heterogenic than heated homogenic serum media.

The results obtained in thyroid and suprarenal tissue proliferations in plasma media with plasma media altered indirectly were very marked. It will be noticed by the following charts, which should be examined in connection with this discussion, that a control of a normal tissue in normal plasma was run with each of the alterations. In studying the charts it should also be remembered that the tabulated results are not those of a single mount but rather those of the successful 80 or 90% of mounts; made following each bleeding. It was necessary to allow 10% of failure for tissue so injured in cutting as to give no new growth.

Early in November a number of thyroidectomies and suprarenalectomies, both partial and complete were preformed on cats in order that we might perfect our operative technique and then the following work was done.

The results then as seen in Chart #1 indicate that normal thyroid and suprarenal tissues which began to grow in normal plasma in from 24 to 36 hours following incubation did not grow nearly so well in blood collected 2 to 10 days following the thyroid lobectomy but did grow as well as normally in plasma collected both at the time of the lobectomy and 12 to 14 days following the lobectomy. The removal next of the other lobe of the thyroid gland so upset the metabolic function, that normal thyroid and suprarenal tissues would not grow in the plasma collected upon the day of the completed removal or as late as 8 days following the operation. In one instance when the animal was fed on the thyroid extract, three times daily an average dose of 1.5 gms. the thyroid and suprarenal tissues grew as well in plasma collected at the end of the feeding experiment as in normal plasma media.

The ultimate results of a complete thyroidectomy were quite different depending upon the manner of the thyroidectomy; as the following indicates.(See Chart #2) When the capsule was removed with the gland the plasma collected at any time absolutely prevented any new thyroid or suprarenal tissue proliferation, whereas if the removal was by an enucleation, we obtained no thyroid or suprarenal tissue proliferation from tissues embedded in plasma obtained following the operation; but tissue embedded in plasma taken 10 days following the operation, proliferated in 36 hours whereas the same tissue in normal plasma proliferated in 24 hours. There was still some retarding of tissue proliferation 30 days after the operation.

The effects noted upon the animal were also very marked. The result of a thyroid lobectomy was a slight upward and outward retraction of the eyeball on the side corresponding to the lobectomy, a temporary loss of appetite and a general lethargy lasting for one or two days, but the cat again appeared normal after five days had elapsed. A complete thyroid removal, however, proved interesting; the cat at once became listless and groggy with a loss of appetite and a desire to sleep, resulting in a general myxoedema with roughening of the pelt and finally death after 10 to 15 days.

The capsule if allowed to remain had the power of preventing these extreme results and the cat although it became somewhat myxoedematous did not succumb to these results. Symptoms of tetany appeared in many of our cases of complete thyroidectomies but if the capsule or a part of the gland remained the tetany was only temporary. Calcium lactate 2 gms. usually gave temporary relief from tetany as did also a transplant of a portion of the

gland.

The line of experimentation was next directed to the suprarenal gland. The results as seen in Chart #4 shows that thyroid and suprarenal tissue proliferate in normal plasma following 24 to 36 hours incubation. The same tissue embedded in plasma taken at the time and up to 16 days following a single suprarenalectomy absolutely retarded any proliferation but following an interval of 17 to 20 days the plasma again allowed the tissues to proliferate.

The results following a complete suprarenalectomy by a suprarenalectomy one month following the first were as follows; (See Chart #5) The thyroid and suprarenal tissues embedded in plasma collected at once sometimes proliferated but usually were retarded absolutely.

The complete removal of both glands (See Chart #6) so altered the plasma that no thyroid or suprarenal tissue proliferation occured even after five days incubation in plasma collected either at once or after any lapse of time.

The gross effects of the suprarenalectomies upon the animals were somewhat marked. The cat usually survived a removal of one suprarenal gland but became emaciated and the tissue at the site of the incision healed less readily. A complete suprarenalectomy usually proved fatal, the cat often never survived the operation and when it did appeared very weak and listless.

The conclusions derived from these results then were as follows:

1. All tissues grew better in normal plasma than in altered plasma media.

2. Embryonic tissues grew better than the adult tissues.

3. The ductless glands exercised certain metabolistic functions on growing tissues manifested through the blood plasma. The action can be compensated for in from 3 to 20 days in thyroidectomies by allowing a portion of the glandular structure to remain either in its normal position or transplanted to another region. Whereas in suprarenalectomies it was necessary for one of the glands to remain with an intact blood supply in order to allow tissue proliferation.

4. The nature of the compensation may be to neutralize a toxin secreted by other cells. This toxin when present and not neutralized by these tissues prevented tissue proliferation.

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Laboratory Record

Chart I. Experiment:- Thyroid Gland.
Lobectomy.

Animal	No.	Operations	Bleedings	Incubation Records.				
				12 hr.	24 hr	36 hr.	48 hr.	5 dys.
Cat	5	Normal	November 4/12	0	+	+		
			November 8	+	+	+		
			November 16	0	+	+		
Cat	6	Normal	November 6	+	+	+		
			November 14	0	+	+		
			November 18	0	+	+		
Cat	7	Left- Nov. 4/12	November 4	0	+	+	+	+
			November 6	0	0	+	+	+
			November 8	0	0	0	+	+
Cat	8	Left.- Nov. 4/12	November 8	0	+	+	+	+
			November 14	0	0	+	+	+
			November 16	0	0	0	+	+
			November 20	0	+	+	+	+
			November 22	0	+	+		

Laboratory Record.

Chart II.

Experiment:- Thyroid Gland.
Complete removal of Thyroid
by lobectomies.

Animal	No.	Operation	Bleedings	Incubation Records				
				12hr	24 hr	36hr	48hr	3dys.
Cat	9	Normal	Jan. 6/13	0	+	+	+	
			Jan. 14	0	0	+	+	
			Jan. 20	0	+	+	+	
			Jan. 28	0	0	+	+	
Cat	10	Normal	Jan. 9	0	+	+	+	
			Jan. 11	0	+	+	+	
			Jan. 22	0	0	+	+	
Cat	11	Left - Nov. 4/12	Jan. 6	0	0	0	0	0
		Right - Jan. 6/13	Jan. 9	0	0	0	0	0
			Jan. 11	0	0	0	0	0
			Jan. 14	0	0	0	0	0
Cat	12	Left - Jan. 15/13	Jan. 20	0	0	0	0	0
		Right - Jan. 20/13	Jan. 22	0	0	0	0	0
			Jan. 28	0	0	0	0	0
Cat	13	Left - Jan. 15/13	Jan. 20	0	0	0	0	0
		Right - Jan. 20/13	Jan. 24	0	+	+	+	+
		Fed Thyroid Extract. 1.5 gr. 3 times Jan. 21 3 times " 22 3 times " 23						

Laboratory Record.

Chart III

Experiment :- Thyroid Gland,
Capsule Removed,
Enucleation.

Animal	No.	Operations	Bleedings	Incubation Records					
				12hr	24hr	36hr	48hr	3dys	5dys
Cat	14	Normal	Jan. 30/13	+	+	+			
			Feb. 8	0	+	+			
			Feb. 15	0	+	+			
			Feb. 25	0	+	+			
Cat	15	Normal	Feb. 4	+	+	+			
			Feb. 14	0	+	+			
			Feb. 26	0	+	+			
Cat	16	Capsule Removed	Jan. 30	0	0	0	0	0	0
		Jan. 30/13	Feb. 4	0	0	0	0	0	0
Cat	17	Capsule Removed	Feb. 8	0	0	0	0	0	0
		Jan. 30/13	Feb. 14	0	0	0	0	0	0
Cat	18	Enucleation	Feb. 14	0	0	0	0	0	0
		Feb. 14/13	Feb. 25	0	0	+	+		
Cat	19	Enucleation	Feb. 15	0	0	0	0	0	0
		Feb. 15/13	Feb. 26	0	0	+	+	+	
			Feb. 28	0	0	+			
			Mar. 3	0	0	+			
			Mar. 10	0	0	+			
			Mar. 15	0	0	+			

Laboratory Record.

Chart II

Experiment:- Suprarenal Gland Removal of one Gland.

Animal	No.	Operations	Bleedings	Incubation Records				
				12hr	24hr	36hr	48hr	3 dys.
Cat	20	Normal	Mar. 1/13	0	0	+		
			Mar. 3	0	0	+		
			Mar. 10	0	0	+		
			Mar. 17	0	0	+		
			Mar. 25	0	+	+		
Cat	21	Right March. 1/13	Mar. 1	0	0	0	0	0
			Mar. 3	0	0	0	0	0
			Mar. 10	0	0	0	0	0
			Mar. 17	0	0	+	+	+
			Mar. 25	0	0	+	+	+
Cat	22	Right Mar. 10/13	Mar. 10	0	0	0	0	0
			Mar. 17	0	0	0	0	0
			Mar. 25	0	0	+	+	+

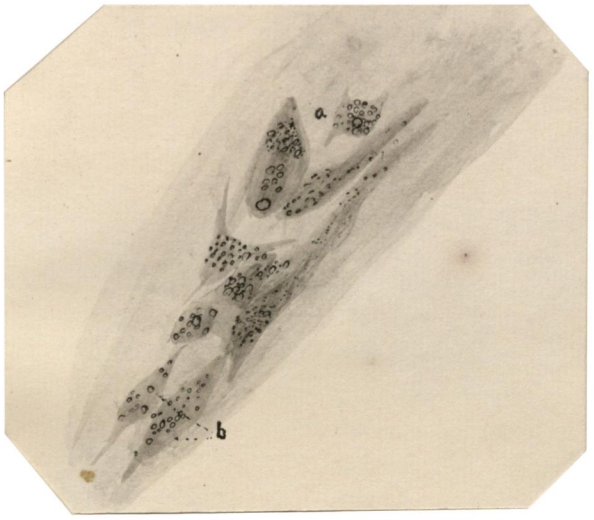
Laboratory Record

Chart V

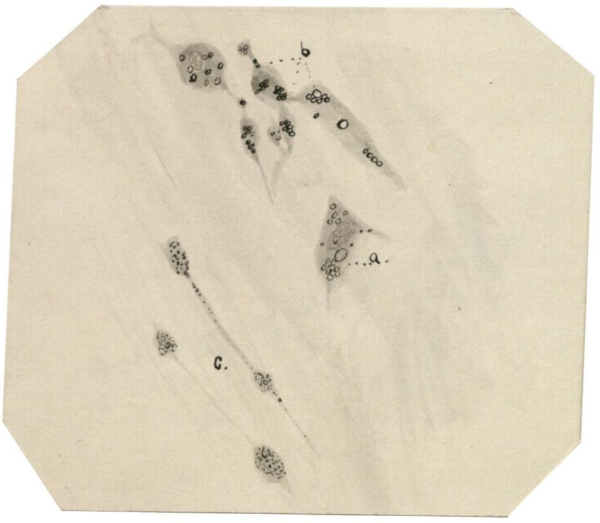
Experiment :- Suprarenal Gland.
Completed Suprarenalectomy

Animal	No.	Operations	Bleedings	Incubation Records					
				12hr	24hr	36hr	48hr	96hr	120hr
Cat	23	Normal	Mar. 1/13	0	+	+			
			Mar. 5	0	+	+			
			Mar. 9	0	+	+			
Cat	24	Normal	Mar. 3	0	+	+			
			Mar. 7	0	+	+			
Cat	25	Right- Feb. 1/13	Mar. 1	0	0	0	0	0	
		Left - Mar. 1/13	Mar. 3	0	0	0	0	0	
			Mar. 7	0	0	0	0	0	
Cat	26	Right- Feb. 1/13	Mar. 3	0	0	+	+	+	
		Left - Mar. 1/13	Mar. 5	0	0	0	0	0	
			Mar. 7	0	0	0	0	0	

Plate. I.



Supravenal Tissue Growth
a. Parenchymatous Tissue
b. Connective Tis.



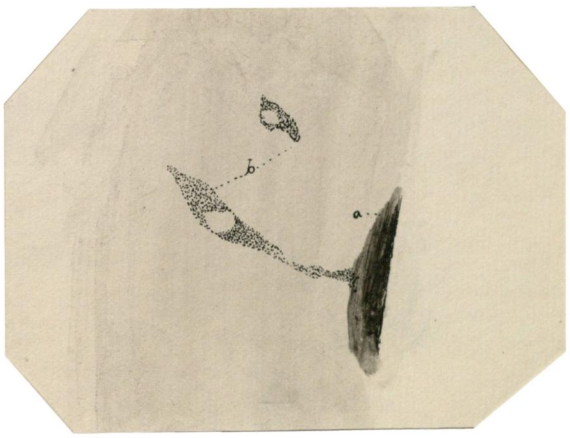
Supravenal Culture Cells.
a. Polygonal Cells & Nuclei
b. Cell anastomoses
c. Nerve cells.

Supravenal Culture
showing Common Types
a Fragmentary Nucleus
b Lipoid Arrangements.
c. Giant Cell (wandering)

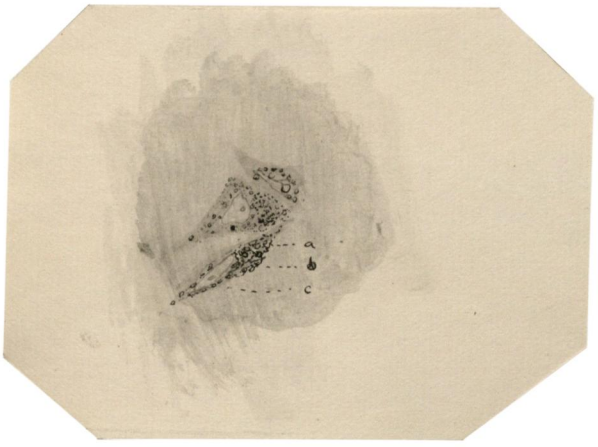
Drawings from Original Cultures
by
M. L. Hoffmann.

Plate 2.

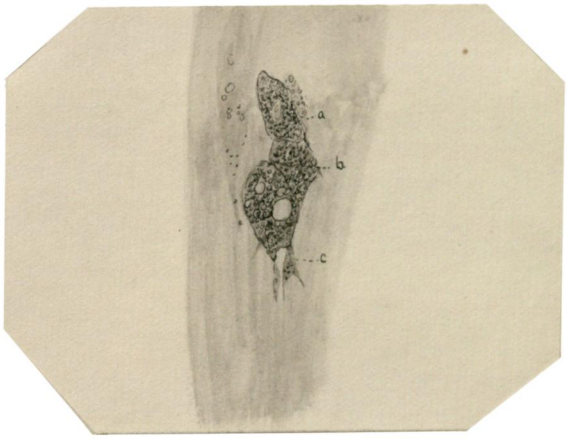
Growth Appearing on 5th day of Incubation (Thyroid)
12 hour growth.
a. Original Tissue with blood elements withdrawn from Tissue.
b Protoplasmic Processes
c Vacuole.
d Liboid bodies



Bacterial Contamination
Resembling New Growth. 12hour.
a. Original Tissue
b Bacterial Growth



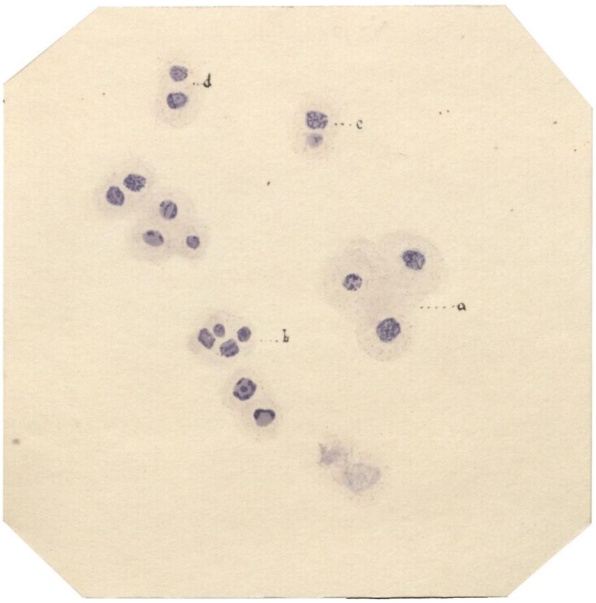
Connective Tissue Cells. (Aorta)
a Spindle Cell
b Liboid bodies
c Nucleus



Endothelial Tissue Cells (Peritoneum)
a Round Cell with Nucleus
b Excessive Amounts of liboid Matter
c Connective Tissue Pseudopodia.

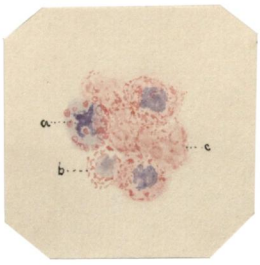
Drawings from living Tissues
by
R. L. Hoffmann.

Plate. 3.



Endothelial Tissue Cells

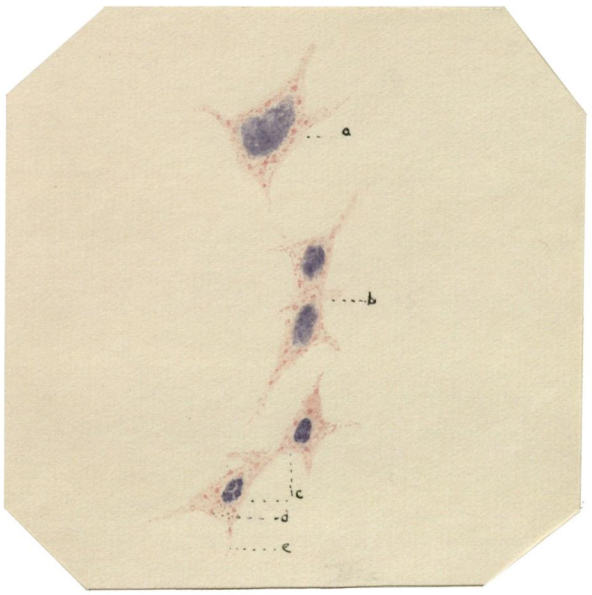
a. Three Mononuclear cells
b. Multi-nuclear Cells.
c Cells showing Chromatin Thread in Nucleus.
d Telaphase.



Endothelial Tissue Cells.

a. Cell with Irregular Nucleus.
b Cell with Compact Nucleus.
c Nucleus hidden by Lipoid bodies.

Drawings From Stained Preparations of New Tissue Growths by R.L. Hoffmann.



Connective Tissue Cells Mitosis

a Prophase
b Telaphase
c Daughter Cells
d Lipoid bodies
e Pseudopods

Plate. 4.

Successive Stages in Normal Tissue Proliferation.

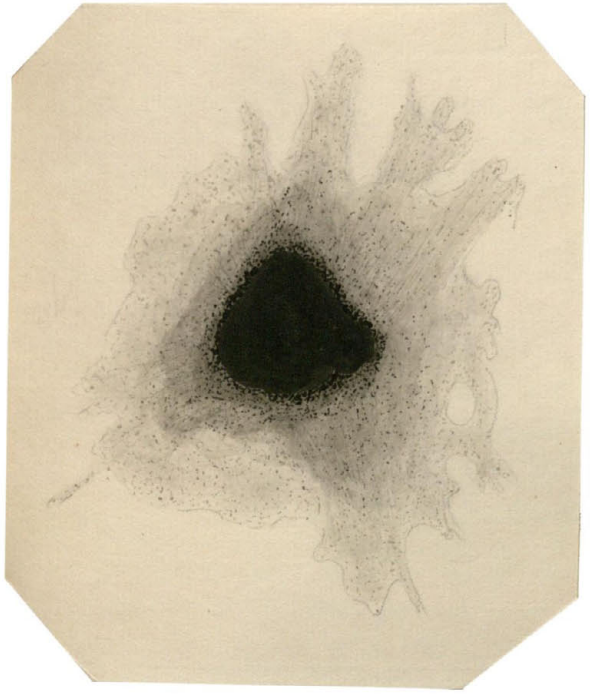
Tissue Mount after Coagulation, in Plasma media.

Tissue After 36 hour Incubation.

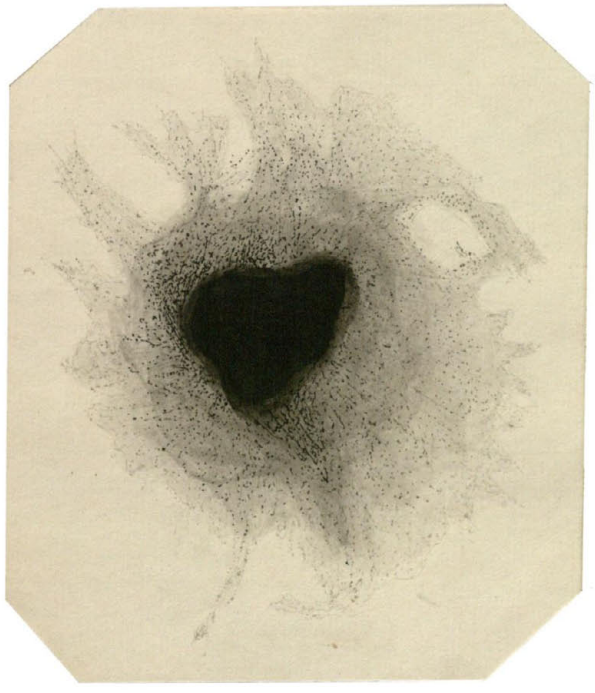
Tissue After 3 day Incubation.

a. Original Tissue.
b Zone of Blood cells.
c New growth
d Compact Cell Mass.

Drawings from New Growing Tissue
by
R. L. Hoffmann



Tissue after 5 day Incubation.

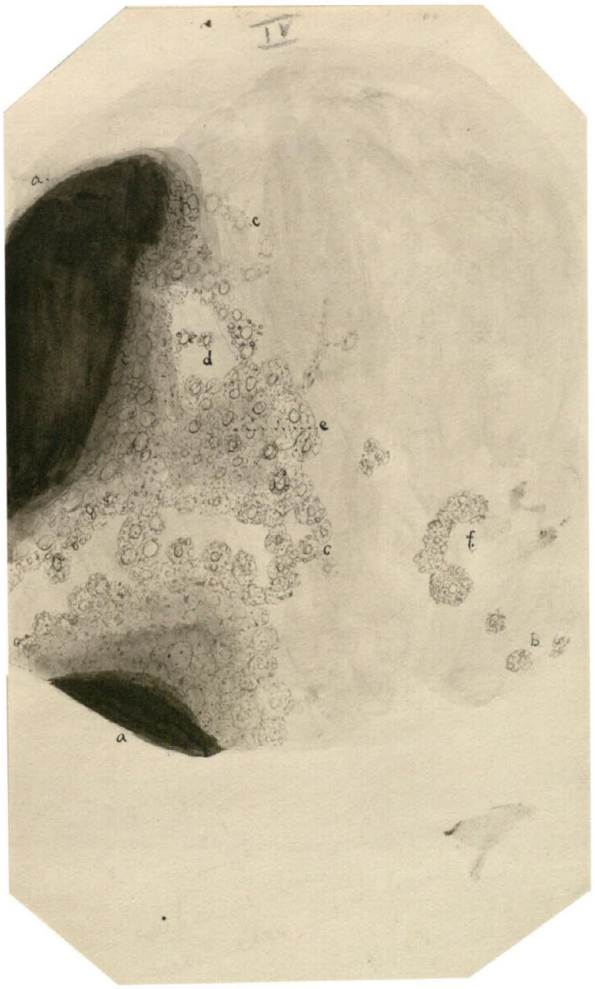


Tissue After 7 day Incubation.

Plate 5.

Thyroid Culture. 48 hr. Obj. 3
a Original Tissue
b. Lipoid bodies
c Endothelial Cell (Wandering.
d Protoplasmic processes

Nerve Culture. 24 hour Incubation Obj. 3
a Original Tissue
b lipoid bodies
c Protoplasmic processes.
d Division, or Pseudopodia



Culture of Thyroid Gland.
a. Original Tissue.
b. Wandering Cell. (Epithelial)
c. Columnar Arrangement.
d. Complete enclosure.
e. Enclosure Cell filled.
f. Proliferating Wandering Cell.

Drawings from Original Growing Cultures
by
R. G. Hoffmann.