**Histology.** — *The potencies and reactions of mesenchyma in fowls, in connection with the problem of avian leucosis.* By J. W. DUYFF. (From the Histolog. Laboratory of the University of Amsterdam, Dir. Prof. Dr. G. C. HERINGA.) (Communicated by Prof. J. BOEKE.)

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Since 1845, the year in which VIRCHOW gave his classical description of human leucaemia, "leucotic" processes in animals, i.e. diseases showing a more or less superficial resemblance to the leucaemia, have been subject to many researches by various investigators, and a great number of such 'leucoses" have been described. Now the human leucaemia is a circumscribed, well-defined disease, with typical anatomo-pathologic features, with typical alterations in the circulating blood, both quantitative *and qualitative*, and always fatal. Before concluding that there is an analogy, and a fortiori a certain identity of one of those leucoses with human leucaemia, the presence in this leucosis of the typical features of leucaemia should be ascertained.

One of those so-called parallels to our leucaemia is the leucosis in fowls, as studied and described by ELLERMANN and BANG, by HIRSCHFELDT and JACOBY, and others, and which includes "leucaemia", "pseudoleucaemia", "anaemia". ELLERMANN, who published a monograph on the subject in 1918, considers this disease as a specific infection with a filtrable virus, contained in the spleen, the liver, the blood and the bone-marrow of the affected animals; about 6—8 months after injection of emulsions of the above organs or the blood of leucotic fowls into healthy ones, some 40 p. 100 of the latter fall ill; after injection of filtered emulsions about 20—25 p. 100. In one and the same series, however, the distemper can show itself in very different ways, so that alternately intravascular myeloid resp. lymphatic "leucosis", the corresponding extravascular (i.e. aleucaemic) processes, disseminated solitary lymphomata and anaemic cases can be found. When we bear in mind that ELLERMANN himself states the difficulty in differentiating some of his cases from cases of tuberculosis, — that in his "Versuchsprotokolle" a case with 43.000 leucocytes, and without definite qualitative alterations [1]) of the white corpuscles (young and unripe stadia cannot be considered as being abnormal; they can be found in every non-

[1]) Pronounced qualitative alterations of the white corpuscules would be much more important for the diagnosis of a genuine leukaemia, than even rather important numeral changes. The "pathologic" forms as described by ELLERMANN (i.e. the forms observed in cases of his leucosis), can be considered as juvenile stadia (grand lymphocytes = lymphoid haemoblasts; polychromatophile erythrocytes etc.)

specific hyperleucocytosis), is announced as an intravascular leucosis (the normal number of leucocytes varying from 23.000—36.500) ; — that the anatomo-pathologic alterations in the organs consist principally of an increase of the accumulations of leucocytes (myelocytes and lymphocytes) in the periportal spaces of the liver, in the kidney, etc. and of the so-called leucostasis (i.e. an accumulation of white blood corpuscles in dilated capillaries) ; — that in a great number of cases only these lymphatic resp. myeloid hyperplasia can be found, the circulating blood being normal in a rather considerable number of the cases described, and in the others showing mostly but rather insignificant alterations, — then it will be clear, that investigators should be guarded in statements about a parallelism of this distemper and the human leucaemia and about a specific infectious morbific agent of the leucosis, all the more so, because the haemopoietic system in hens is rather labilely balanced, and might be influenced in some way by non-specific agents. Care should be taken not to identify a well-defined disease and a myeloid resp. lymphatic reaction, a syndrome.

In 1916, DANTCHAKOFF published some very interesting facts about the potencies and reactions of the mesenchyma of the chick-embryo and of the grown-up hen. She grafted splenic tissue of adult hens on the allantochorion [1]) of chick-embryos of about 8—12 days of incubation and studied the local reactions as well as the alterations in the tissues of the embryo. Very briefly resumed, her results were the following :

a. *Local reaction.* Hypertrophy of the mesenchyma of the allantochorion with change of mesenchymal elements into blood stem-cells (so-called lymphoid haemoblasts) and thus into "granuloblasts" resp. "histiotopic wandering cells". Necrosis of part of the grafted tissue, migration of most of the small lymphocytes and erythrocytes contained in it into the allantoic tissue, with subsequent necrosis of these elements, granuloblastic differentiation of the reticulum of the adult splenic tissue itself.

   Slight hypertrophy of the ecto- and entodermal epithelia, considered as due to a local and mechanical agent, viz. the hypertrophy of the allantoic mesenchyma.

b. *Reaction in the embryo.* Myeloid metaplasia of the mesenchyma, enlargement of the spleen, with granulo- or erythropoiesis.

The results of these very interesting experiments allow of the following conclusions :

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[1]) The allantochorion is the membrane, resulting from the growing together of the allantois and the amniogenic chorion (séreuse de von Baer of the French). It consists of a vasculated mesenchyma, covered at the outer side with an ectodermal epithelium, originating from the chorion, and at the inner surface with an entodermal, allantoic epithelium. Under the ectodermal epithelium lies a tight respiratory capillary-plexus. (DANTCHAKOFF).

1. In the products of the mesenchyma of the chick-embryo neither the functional adaptation and specialisation, nor the resulting morphologic alterations are necessarily attended by a limitation of their potencies.
2. The loose mesenchyma in the chick-embryo, as well as the reticulum of the adult splenic tissue in fowls are polyvalent (cf. in this connection LAGUESSES conception of the spleen as a "reliquat du mésenchyme embryonnaire").
3. In BRACHET's terminology, the haemopoiesis is a "différentiation provoquée".

When comparing the results of DANTCHAKOFF's experiments with the features of ELLERMANN's leucosis, we find a striking resemblance. Of course it should be remarked, that DANTCHAKOFF has been able to keep the embryos livng only up to the 18—19th day of their praenatal development. As a matter of fact, this made thorough examinations of the circulating blood impossible, (apparently basing her conclusions on the features of the blood in her coupes, DANTCHAKOFF describes it as having the properties of myeloid leucaemia, without giving further particularities), nor could the further course of the reaction be examined.

In repeating, and in several points extending DANTCHAKOFF's experiments we had a threefold purpose: first of all, to verify her results, then to isolate, if possible, the agent causing the myeloid reaction, and in the third place to form an estimate of the relation between her results and those of ELLERMANN. A preliminary statement of the results obtained may follow here.

A. On the whole we can corroborate DANTCHAKOFF's conclusions; in some points only, more especially concerning the adhesion of the grafted tissue to the allantochorion and the local reaction of the latter we obtained a somewhat different result, so that we need but give a short description of our results, only emphasizing the points in which we cannot completely agree with DANTCHAKOFF's views. Briefly, our results can be summed up as follows:

   **a. adhesion of the graft to the allantochorion.**
   In this respect, we can fully confirm DANTCHAKOFF's exact observations. The ectodermal epithelium is arroded and interrupted by the grafted tissue, some parts of this epithelium lying isolated in the stroma, and partly changing into something like cornified pearls. Attention should be drawn to the fact, that in some of the cases erythrocytes from the grafted tissue were found lying against the ectodermal epithelium, and dinting it in as it were; afterwards the epithelium cells take up the necrotic erythrocytes; the enlarged cells themselves show phenomena of vacuolisation and degeneration. In several cases,

we found an epitheloid extension of erythrocytes over the ectodermal surface of the allantochorion [1]).
(The erythrocytic character of these cells has been ascertained by means of the benzidine staining.)

**b. local response of the allantochorion.**
*General state of the membrane.* — The area surrounding the graft shows a remarkable thickening of the whole allantoic membrane (caused principally by the alterations in the allantoic stroma, partly, but in a less degree by the epithelial changes), and is characterized by a formation of crypts and papillae on the entodermal surface ; these alterations attain their maximum opposite the graft.

1. The *mesenchyma* shows in many cases, according to DANTCHAKOFF's results, an intense "myeloid metaplasia" : (in some cases this metaplasia presents itself as a diffuse process, mostly we find a diffuse alteration of the mesenchyma, attended by very intense haemopoietic foci in the perivascular areas ; in these perivascular haemopoietic foci we find lymphoid haemoblasts and an increasing number of granuloblasts, JOLLY's transitory cells, polynuclear "eosinophiles" [2]), and erythroblasts. — As a matter of fact, an interpretation of these phenomena as a myeloid metaplasia might be quite correct, though it is of course very difficult to exclude fully a myeloid infiltration.
2. The alterations of the *entodermal* epithelium, consisting principally of a stratification in about 5—6 layers of cells and the formation of papillous excrescentions, attain their maxium opposite the graft. Here we find mostly a real, solide, papillous epithelial tumor, consisting of fusiform elements, and in some cases showing central necrosis. Though the intensity of the entodermal reaction shows many variations, it is certainly not parallel to the extension of the mesenchymal hypertrophy, so that we cannot agree with DANTCHAKOFF's interpretation of the entodermal answer as being secondary and due to the hypertrophy of the allantoic stroma.
   Moreover, in the results of further experiments (cf. B, II: $\beta$) we found some additional arguments for the conception of the reaction as a specific and independent process.

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[1]) In one case, part of the graft was attached to the egg-shell-membrane. Here a similar extension of erythrocytes was found.

[2]) These "eosinophiles", (or, better, pseudoeosinophiles), cannot be compared with human eosinophile granulocytes, but have perhaps a certain analogy with our neutrophile granulocytes ; they form the majority of the myelogenic white elements in avian blood.

3. The *ectodermal* reaction cannot be separated from the process of adhesion of the graft to the allantochorion. Just as in the entoderm we find a hypertrophy and a stratification of the epithelium ; mitoses as well as amitoses are rather scarce [1]). In some cases the area near the periphery of the grafted tissue is characterized by the epithelium cells rankly growing into the stroma ; many of the cells leave the epithelial connection, but remain in relation with each other and with the epithelial layers by means of protoplasmatic expansions, and can only with difficulty be differentiated from mesenchymal elements.

**c. The alterations in the grafted splenic tissue** fully correspond to DANTCHAKOFF's description: small lymphocytes wander out of the reticulum and invade the mesenchyma of the allantochorion ; erythrocytes from the grafted tissue, too, can be found in the allantoic stroma; here erythrocytes as well as lymphocytes show necrosis, karyorrhexis and pyknosis. Part of the reticulum shows similar necrotic alterations ; the rest may be subject to myeloid metaplasia.

**d. Remote reaction in the embryo.** The intensity of the myeloid reaction of the embryonic mesenchyma shows many variations. About the factors influencing the extension of the myeloid metaplasia, cf. section B.

*A summary description* of a typical case may follow here :

> *Series I A N°. 2.* Graft of splenic tissue on the allantochorion (10th day of incubation) result after 2 days. Zenker—Formol (Maximov), Dominici. The grafted tissue contains some necrotic erythrocytes with karyorrhexis, some small lymphocytes, and swollen reticulm-elements. Slight reaction of the ectoderm (hypertrophy, formation of some giant cells, and fusiform elements) many interruptions of the epithelium. Epitheloid extension of erythrocytes from the graft over the ectoderm.
>
> *Entoderm :* proliferation, formation of crypts and rather plump papillae.
>
> *Mesenchyma :* hypertrophy, hyperaemia, diffuse haemopoiesis.
>
> *Embryo :* Diffuse myeloid metaplasia, slight enlargement of the spleen. The splenic tissue has lost its typical structure, and shows massive accumulations of myelocytes and eosinophile granulocytes. In the periportal spaces of the liver and in the interstitium of the kidneys, many myeloblasts, myelocytes and eosinophiles are to be found.

B. In consequence of DANTCHAKOFF's experiments, some questions present themselves.

[1]) Here and there a pluripolar mitosis may be found.

I. *Is the reaction necessarily bound to the grafting of living tissue ?* It is a well-known fact in human pathology, that necrosis is one of the most frequent causes of a myeloid reaction. In connection with this fact, the myeloid reaction in this case might be due to the necrosis of part of the grafted tissue, all the more so, because the remainder of the latter may be subject to a similar myeloid metaplasia itself. This view is borne out by the fact that the myeloid metaplasia is stronger in proportion as the necrosis of the grafted tissue is more important (the extension of the necrosis in the graft can be varied, e.g. by previously bruising part of it, etc.); (for the adhesion of the graft to the allantoic tissue, implantation of living tissue is required). In a case when instead of grafting tissue, a splenic extract was applied to the allantochorion, the general myeloid reaction was stronger than in any other case. This leads to the conclusion, that *the myeloid metaplasia is probably due to a chemical agent, proceeding from the decay of the cellular elements of the graft.*

II. *Is the reaction the specific result of the grafting of splenic tissue or of part of it ?*

*a.* Grafting of different organs (liver, gl. thyreoidea, gl. suprarenalis, kidney) led to the conclusion, that the answer to every implantation is different and specific. A notable myeloid metaplasia is obtained by grafting of spleen, bone-marrow, and (in a less degree) of liver. The answer to the grafting of different tissues will be described circumstantially before long.

*β.* After grafting of splenic reticulum, previously washed out with sterile Ringer (in this case the grafts were subject to an extensive necrosis) the myeloid metaplasia was very marked ; graftings of leucocytes and of erythrocytes, as well as application of extracts of these elements are in preparation. This experiment has some importance as to the interpretation of the epithelial hypertrophy ; the massive myeloid metaplasia was but accompanied by epithelial reactions hardly worth mentioning. The epithelial reaction might be especially due to the products of the small lymphocytes ; this would be in concurrence with Murphy's views about the relation between the autolysis of lymphocytes and epithelial proliferations.

*β. Series VI A N⁰. 2α.* Graft of washed-out reticulum (11th day of incubation).
Result after 7 days.
Zenker-Formol (Maximov), Dominici.

The *graft* consists of altered, partly necrotic reticulum-cells; no erythrocytes, an odd lymphocyte.
The *ectoderm* shows some very slender papillae, here and there a giant cell.
The *entodermal* reaction is hardly worthy of mention: flat epithelium without any trace of crypts or papillae. Some of the nuclei are small and dark, others enlarged and clear.
The *mesenchyma* is very dense and shows a pronounced angiopoiesis and extensive myeloid metaplasia; some of the myeloid foci are subject to central necrosis.
In the *embryo* the enlarged spleen contains but few erythrocytes; the normal structure of the organ cannot be recognized, the whole splenic tissue having been subject to an intense granulopoiesis. The periportal spaces in the liver show dense accumulations of granuloblasts; the renal interstitium is widened and contains many myelocytes.
The mesenchyma of the examined muscles shows a myeloid metaplasia. In the vessels, the number of granulocytes surpasses that of erythrocytes..

III. *Should the phenomena described be considered exclusively as an induced alteration of the elements of the embryonic mesenchyma?*

DANTCHAKOFF does not fully exclude the possibility, that in her experiments *invasion* of elements from the graft into the embryonic system and subsequent alteration of those elements might play a part. Now the results obtained by application of splenic extract, in which case there is no question about any invasion, show that the myeloid reaction need certainly not be due to any infiltration of the embryonic tissue by elements from the graft. Of course this does not exclude the possibility of invasion in the primitive experiments. At all events, if any invasion should exist, it is of very small importance. For the rest, there is another fact, which might tend to prove for an induced reaction; viz. the praedilective localisation of the myeloid foci round the blood-vessels.

We have tried to solve at least part of the question by application of previous vital staining of the grafted splenic tissue as well as of the allantochorion; only these experiments should be continued, as we have not yet obtained the results desired.

C. After many vain endeavours we obtained a series in which part of the setting matured [1]), so that repeated morphological blood examinations could be made, and the further progress of the reaction as well as its clinical features could be studied.

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[1]) This series consisted of 81 eggs, of which 16, (nearly 20 p. 100), matured.

I. *Clinical features.* A rather important part (7) of the chickens has expired already after having shown quickly aggravating symptoms ; limp paralysis of the legs (Nos. 1, 2, 5, 7), ocular (No. 2, 7 ?), and intestinal (diarrhoic) symptoms (Nos. 3, 5); in some cases ulcerating wounds (Nos. 4, 5), anaemia (Nos. 2, 4, 6, 7), progressive cachexia. As ELLERMANN does not describe any clinical observations as to his leucotic hens, a comparison with the features of his distemper could not be made.

II. *Anatomic alterations.*

a. Circulating blood : anaemia, in some cases a hyperleucocytosis up to 100.000 leucocytes with perhaps a slight relative increase (up to about 40 p. 100) of the myeloid elements ; in several cases a leucopenia with about 2000—3000 corpuscles.

We did not find obviously abnormal granulocytes, any more than ELLERMANN or DANTCHAKOFF ; of course young and unripe stadia were very frequent.

b. Alterations in the tissues. — The anatomo-pathologic features of our cases correspond with ELLERMANN's description ; in the hyperleucotic cases as well as in the leucopenic ones, myeloid hyperplasia and metaplasia, solitary myelomata and myeloid infiltration of different organs, loss of the normal splenic structure with alteration of the elements of the splenic reticulum into erythroblasts resp. myelocytes and "eosinophile" granulocytes, and leucostasis may be found.

Some typical cases may be cited here :

*Series D, No. 5.* Graft of adult splenic tissue on the 11th day of incubation . — Hatched Sept. 7th, 1928 ; died Nov. 5th.

Progressive limp paralysis of the legs, aggravating cachexia.

Blood-count : marked anaemia (2.300.000) ; many polychromatophile erythrocytes ; an odd erythrocyte shows a mitotic figure.

Spleen : enlarged, loss of normal structure, diffuse myeloid metaplasia. — Liver : slight increase of periportal myeloid accumulations. — Intestine : foci of myeloid metaplasia in the submucosa.

Lungs : hyperaemia, dense accumulation of lymphoid haemoblasts, changing into granuloblasts and eosinophile leucocytes.

*Series D, No. 3.* Graft of adult splenic tissue on the 11th day of incubation. — Hatched Sept. 7th, 1928 ; died Oct. 1th.

Progressive cachexia, intermittent paralysis, diarrhoea.

Blood-count : erythrocytes 4.000.000 ; white cells 97.500; lymphocytes about 35 % ; marked increase of the number of myeloid elements ; many unripe stadia.

Spleen : hyperaemia, myeloid metaplasia. — Kidney : widened interstitium with disseminated small accumulations of myeloid elements.

Liver and intestine : hyperaemia.

The material collected is too small still to allow of any definite conclusion as to the further course of the reaction ; the course of part of the cases with quickly aggravating symptoms, leading to a rather early exitus might point to a certain progression, as no defined intercurrent distemper could be made responsible for the symptoms described.

Lastly, the question arises, whether the reaction described could be carried over to other animals. — Using the technique as described by ELLERMANN, sterile emulsions of spleen and liver of the deceased animals were made, and intravenous injections of these emulsions in 6 adult hens were performed ; in 4 chickens intraperitoneal injection was used. The time elapsed since is too short to allow of a conclusion as to the appearance or non-appearance of alterations responding to ELLERMANN's description (in one of the cases a hyperleucocytosis of about 50.000 could be observed). Especially in the cases, where adult hens were used, a marked reaction might seen a priori rather improbable, as the products of the embryonic mesenchyma in the adult hens might have lost part of their potencies as a consequence of their further differentiation. On the other hand, DANTCHAKOFF's experiments show clearly, that in adult hens the mesenchyma of the spleen at least is still polyvalent ; moreover, as we observed already, the equilibrium of the haemopoietic system in hens is a rather labile one. In this connection it is important to observe, that the virus of ELLERMANN's leucosis is said to be contained exactly in the organs normally connected with the processes of blood formation and blood decay, and by decay of which, in accordance with DANTCHAKOFF's and our experiments, substances causing a myeloid metaplasia of the embryonic mesenchyma as well as of the adult splenic reticulum in hens, are produced.

For the rest, a positive result would mean nothing as to the etiology and the pathogenesis of the spontaneous avian leucosis ; it would be merely another warning to be guarded in admitting the existence of a specific infectious agent causing it ; another argument not to conclude that there is an anology with human leucaemia, if this conclusion is based merely on a more or less superficial resemblance ; — another argument lastly, not to transfer considerations about distempers in animals to "similar" diseases in man, without having serious and conclusive arguments for it.

## EXPLANATION OF ILLUSTRATIONS.

*Fig. 1.* General view of the allantochorion of a chick embryo, 3 days after the grafting of adult splenic tissue on its ectodermal surface. Hypertrophy of the allantoic membrane, complete adhesion of the grafted tissue to the allantochorion, slight ecto- and

entodermal reaction, beginning reaction in the allantoic mesenchyma (tightening of the stroma, hyperaemia).

*Fig. 2.* Allantochorion of a chick embryo, 2 days after the grafting of adult splenic tissue. Small perivascular haemopoietic focus.

*Fig. 3.* Allantochorion of a chick embryo, 3 days after the grafting of adult splenic tissue. Marked reaction of the entodermal epithelium ; formation of papillous excrescentions, tightening of the allantoic stroma in the corresponding area ; hyperaemia.

*Fig. 4.* Allantochorion 3 days after grafting. Typical papilliform excrescentions, vacuolisation of the elements, beginning of central necrosis.

*Fig. 5.* 2 days after grafting of adult splenic tissue. Formation of crypts and papillae on the entodermal surface of the allantoic membrane, hypertrophy and stratification of the entoderm.

*Fig. 6.* From the allantochorion of a chick embryo (entodermal side), 2 days after a particle of washed-out adult splenic reticulum has been grafted. Intense diffuse myeloid metaplasia of the allantoic mesenchyma, attended by a minimal answer of the entodermal epithelium.

*Fig. 7.* From the liver of a chicken, died 24 days after hatching. On the 11th day of its praenatal development adult splenic tissue was grafted on the allantochorion Periportal myeloid metaplasia : myeloblasts, myelocytes, pseudoeosinophilic granulocytes.

## LITERATURE CITED.

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5. ————————, The leucosis of fowls and leucaemia problems. London 1921.

*(This is a preliminary report.)*

Fig. 1. (A*. Zeiss; peripl. oc. 4×).

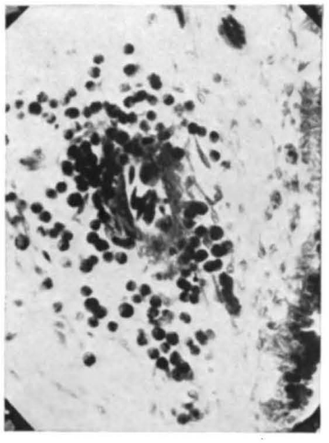


Fig. 2. (Achromate 7 mm. Leitz; peripl. oc. 4×).

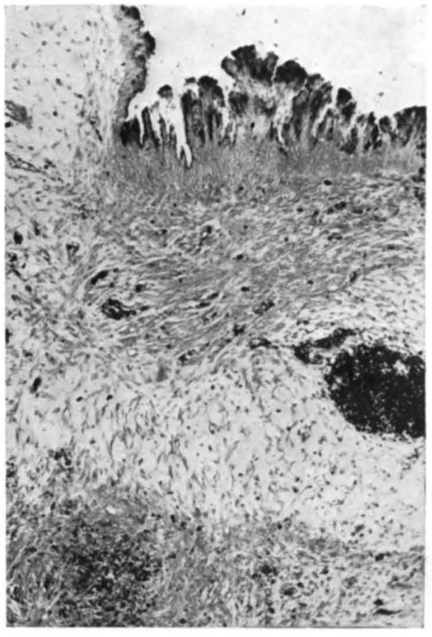
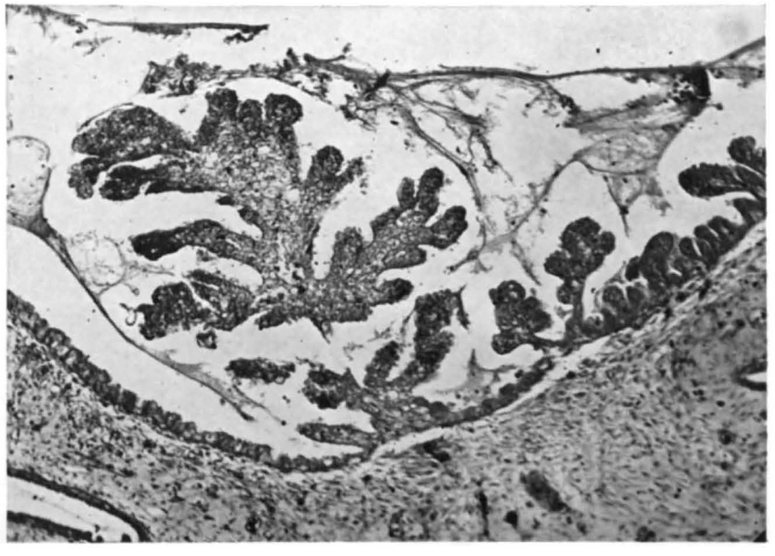


Fig. 3, (Apochrom. 16 mm. Leitz; peripl. oc. 4×).



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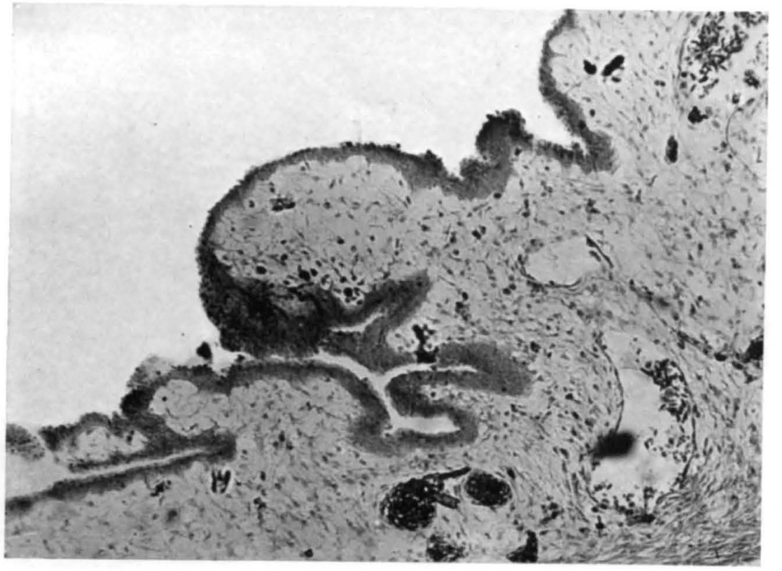

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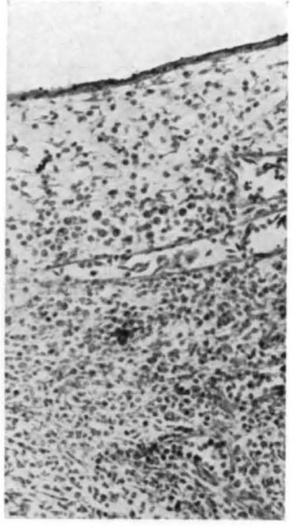

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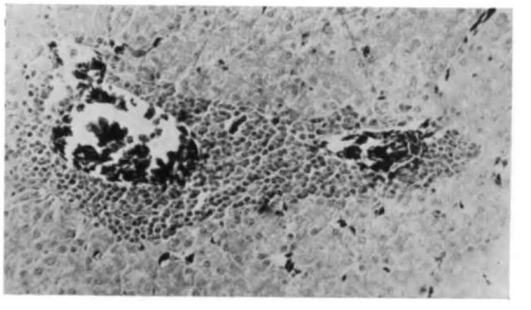

Fig. 7. (Apochr. 8 mm. Leitz; peripl. oc. 4×).