Is Human Leukemia a Somatic Mutation of Blood-Forming Cells due to a Virus?

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Two are the principal pathogenetic theories of leukemia. According to the first theory, the older one, leukemia is a systemic hyperplasia, caused by a disturbance of unknown origin, of the proliferation-maturation process of normal stem cells. The second theory, which is more recent, considers the leukemic blasts as pathological cells, functionally independent from the general control of the organism. Obviously, the pathogenetic process is markedly different, according to either theory. In the first case, we should think of an hypothetic cause of dysfunction, external to the blasts, and acting upon them. In the second case, the cause of the pathological behaviour of the blasts must be searched into the blasts themselves.

The experimental work based on the first theory has been unsuccessful for about half a century, and the theory of systemic hyperplasia is loosing ground. The effectiveness of cortisone on lymphatic leukemia seemed to bring support to this classical theory, but the disappointing observation that, inspite of repeated remissions, no patient was cured whatsoever, has convinced everybody that beyond every hormonal disturbance there is a deeper and more radical alteration which is the basis of the process. It will suffice to think of the profound morphological alterations of blasts, especially in acute leukemia, and of the monotonous reappearance of the same type of blasts at every relapse, regardless of the type of treatment used to obtain the remission. One of us failed, many years ago, to obtain any improvement in chronic myeloid leukemia with spleen extracts, which possessed, however, the ability to inhibit the normal myeloid cells. In the patients treated with these extracts only the mature cells were inhibited, while the proliferation of blasts remained unchanged (1).

If we accept the second theory as a working hypothesis, it remains to be established which kind of alteration is responsible for the change in the morphology and functional behaviour of the blasts. Very much discussed is today the hypothesis of a somatic mutation originating leukemia as well as tumors in general. Attempts to correlate the genesis of tumors with chromosome or gene changes are far from being recent. As far back as 1903, Farmer et al. (as quoted by Willis) supported the theory...
that the tumors were due to a «meiosis» and that neoplastic cells should be considered as «gametoids». This theory, as well as that by Boveri (1929), can be easily rejected today, thanks to the progress made in the chromosome study techniques. More attention should be given to the hypothesis of Lochart-Mummery, who, for the first time, invoked a gene mutation in the pathogenesis of tumors. This theory was unfavourably received. Willis observed that the mutation theory is not acceptable, because it implies the concept of a sudden change of a benign tumor into a malignant one, or the one of a sudden onset of a primitive malignant tumor. One may oppose to this objection that the sudden appearance of a mutant cell does not mean a sudden clinical manifestation of a tumor. On the other hand, it is well known that leukemia often has a sudden onset. Therefore, the hypothesis of somatic mutation, rather than to be rejected, is instead worth being more critically analyzed. The aim of this paper is just to review critically the experimental evidence in favour of the mutation hypothesis, and to discuss the possible causes of it. According to Auerbach, somatic mutations in mammals and man are possible, but hardly demonstrable. In other words, it is possible that gene changes in the chromosomes of somatic cells may lead to the formation of strains of mutated cells, but it is very difficult to establish whether and when this change has taken place. Once admitted the existence of a strain of aberrant cells in a given organism, it is necessary to establish whether this is due to a mutation process or to a phenotypic variation. When one is dealing with lower animals, especially mononuclear organisms, the main criterion for this distinction is that of clone culture. To do this, it is necessary to start from a single cell culture, which, from a technical point of view, is very difficult to obtain in higher animals and in man. Puck et al. (1956) and Marcus et al. (1956, as quoted by Penso and Balducci) proposed an interesting technique, based on the use of cells already cultured in vitro and irradiated with 2500-4000 r. A very diluted suspension of the cells to be cultured is implanted on the layer of dead cells, and the colonies derived from a single cell are checked with the microscope. By this method, one cannot utilize the cytologic analysis (after fixation and staining) of the starting cell; moreover, the observation of growing cells is not made in the ideal conditions. These limitations make the method useless for hematologic studies. We believe that the problem will be solved in the future when better techniques will be available. At present, we can only try to solve the problem indirectly, being aware of the provisional character of our interpretation. In other words, we should try to see whether it is possible to demonstrate in leukemia: (a) the presence of a cell strain which differs markedly, from the morphological and functional point of view, from the normal strain; (b) the regular transmission of cell changes from generation to generation; (c) the irreversibility of such changes. We will not avoid, however, the risk of interpreting as mutations, changes due to deficiency, nondisjunctions or somatic crossing-over (Auerbach), but it will be possible to suggest the hypothesis of a mutation as a likely one. We have been working for a long time on this hypothesis, trying to obtain some experimental support for the theory of viral origin of leukemia. We will summarize our observations, and discuss them in detail later on.
Our first experiments are dated 1939, and our first results have been published in May 1941 (2).

Choice material was the fertile chicken eggs before incubation, which were inoculated under the testacea with a drop of human leukemic blood, centrifuged in order to increase its leukocyte content. Such eggs were then incubated and, after 84 hours, the circulating blood, extracted directly from the embryo heart, was examined.

Results were satisfactory: in 4.6% of inoculated eggs, the blood showed an increase in hystioid cells (20%), while in the control group such an increase could be observed only in 14.4% of the eggs.

The “control” groups were eggs which had been inoculated with isotonic saline solution, normal blood, bone marrow material from nonleukemic individuals, a.s.o.

The most important morphological alterations to be found in the circulating histiocytes were increase in size of the cells, enlarged and polymorphic nucleoli, and, frequently, a lobular conformation of the nucleus.

The injection of such circulating blood into new fertile eggs, produced the same phenomenon. The presence in the circulating embryo blood of hystioid cells, which had increased in number and which were abnormal as to the morphology, was repeatedly obtained with serial inoculation up to the eighth and ninth transplantation, sometimes with an increase in intensity. On the contrary, the same experiment, repeated with blood from embryos inoculated with control materials having shown weak hystioid reactions, produced a negative response in one or two transplantations.

Encouraged by these positive data, we began a study on the morbidity and mortality of inoculated embryos in comparison with control groups, leaving aside the studies on the circulating blood, which obliged us to kill all embryos at an early stage. From 1941 to 1943 (3), we carried on this research examining 2,000 eggs. The control groups mortality was about 15%, while 60% — and in some strains even 70% — of the inoculated eggs died.

In these series of experiments, the transmission from egg to egg was obtained with the pulp of the dead embryo ground up in the same amount of isotonic saline solution. The control groups were inoculated with distilled water, isotonic saline solution, and material from normal embryos, killed by cold on the 8th day of incubation, and then incubated again for 24 hours after death. Mortality of experimental eggs reached its peak between the 6th and the 8th day of incubation, while the death rate of controls increased in the last days. It should be pointed out that the embryos inoculated with leukemic material, and that did not die within the first 14 days, reached maturity, but very seldom managed to break the shell, and often showed severe malformations (evisceratio, anencephalia) and hepatic steathosis with icterus.

Research work was taken up again in 1951/52 with the following results (11):

<table>
<thead>
<tr>
<th>1951</th>
<th>Embryos dead on the 7th day</th>
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<tr>
<td>Control eggs . . . . . 760</td>
<td>131 (16.9%)</td>
</tr>
<tr>
<td>Experimental eggs . . . 1,006</td>
<td>439 (43.6%)</td>
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351
Control eggs . . . . .494 82 (16.4%)
Experimental eggs . . . . .490 167 (34.8%)

The type of human leukemia used for inoculation had no influence on embryo transmissibility. The breed of the eggs did not show remarkable differences.

The months when the eggs appeared to be most receptive were the winter ones, especially January and February.

The anatomopathological picture of embryonal disease was studied in the researches of 1941/42 and in a more detailed way in 1951/52 (11).

We summarize it very briefly:

a) Chorial annexes were very richly provided with blood vessels (as could be easily observed also by means of transillumination through an intact shell). These vessels are abundant, clearly evident, thick, grouped together. Haemorrhages are frequent.

b) Perivascular "sheaths" formed by erythroblasts and histioid cells can be observed, while the endothelium is cushioned and shows desquamations. Presumably, this phenomenon causes the increase in histioid cells in the blood stream, which had first come to our attention.

c) At the same time, the endothelium loses the hemopoietic capacity, which is peculiar to normal embryos.

d) As soon as the embryos have reached a developmental stage, sufficient for the formation of the chorioallantois, the picture changes altogether. No "sheath" can be observed but, instead, perivascular nodules of histioid cells, intravascular clots and ectodermal "cushioning" are present.

Equally remarkable were alterations obtained by means of insemination of dead embryo pulp on ectoderm, following the classical Burnett technique. In this case histological examination shows much diffusion of the cushioning, groups of cells frequently atypical, and ulcerations. We must point out that, here, mesodermic reaction was absent, in striking contrast with what happens in the above mentioned embryos.

Inoculation in an adult chicken of dead embryo pulp, after a series of transplantations, had given us a positive result already in 1943 (3).

At that time we inoculated young chickens with the pulp of embryos which had also been inoculated with the material obtained by repeated transplantations on eggs. We were thus able to produce a disease in these chickens, which sometimes proved to be mortal, and that was characterized by mesenchymopathy, histioid reaction in blood stream, hepatic lesions, anemia, cachexia.

Our best results are those published in 1949 (6). From a case of subacute histioleukemia, showing a remarkable organ invasion, neoplastic-like, peripheral blood was taken (leukocytes content 104,000 per mmc) and inoculated following the usual technique. A typical transmission was observed with a very high death rate (79%) until the 9th transplantation.

Pulps of embryos dead within the first week at the 3rd and 9th transplantation
were employed as inoculation material in 5 young chickens. Both subcutaneous and intravenous inoculations were performed. All inoculated animals showed anemia and leukocytosis (between 30,000 and 40,000) in which histioid monocytes and undifferentiated histioid cells prevailed. Only one animal died spontaneously on the 20th day; the remainders were killed after three months. Observation showed that the reticuloendothelial system was in a state of irritation, with extravascular erythroblastic nodules and endothelial desquamation; i.e., this picture was entirely identical with the one observed in the embryo. The same results were obtained by transmitting intraveinously the blood of the five chickens to other adult chickens (3).

As to the biological peculiarities of the transmissible "unknown factor", we have the following data:

In 1943 (3), attempts to inactivate it by means of heat at 87° C for 1 h and at 60° for 90 minutes gave us very doubtful results. In 1947, Cardinali obtained a partial but sure inactivation with prolonged heating at 65° C of transmission material (5).

In 1953 (13), over a total of 1,496 eggs, 676 were inoculated with material previously filtered through Seitz, and 820 with non-filtered material. Both leukemic blood and embryo pulp were filtered at each transplantation. On the 7th day of incubation, the death rate was 38% in embryos inoculated with filtered material, 43% in those inoculated with non-filtered material, and 17% in the controls.

The χ² test shows no remarkable difference between the death rate with filtered and non-filtered material.

The histological picture was about the same in both cases; perhaps the perivascular infiltrations were less impressive, but on the other hand there were more marked mesenchymal and ectodermal reactions.

From the immunological standpoint we observed in 1943 (3) that, while intracutaneous infection of normal chicken embryo pulp in leukemia patients is not followed by any local skin changes, when the same test is performed employing sick embryo pulp, a zone of redness appears, followed by fever and often by the formation of an induration which lasts some weeks. Such experiments were repeated and confirmed in 19 cases (10).

All the above recorded experiments have been gradually reported to the Congresses of the Société Européenne d'Hématologie (Paris 1948, Roma 1951) and the International Society of Hematology (Cambridge 1950); we here summarize these results, since they are not very well known.

We now intend to carry on a critical review of these experimental data, in order to see whether they can be of any help in the solution of the problem.

The first and simplest hypothesis to be taken into account is that our findings might be due to a survival of injected leukemic cells, with a possible concomitant mesenchimal reaction.

To prove the inacceptability of this hypothesis, we underline the following data:

a) An amount of extraneous cell material, sufficient to provoke a reaction, as the one we described, is administered only with the first inoculation. It seems quite impossible to consider such a quantity of material really effective after seven, nine
transmissions from embryo to embryo, and in particular from embryo to adult animal, a multiplication of human cells during passages being biologically absurd.

b) There is not a single histological and cytological datum observed, which might justify the hypothesis of such a survival. All the histological pathological changes observed are composed by typical chicken embryo cells.

c) Filtration on Seitz of the first inoculum and of the material employed in all other transplantations rules out this hypothesis altogether. The transplantation of a whole and living cell through Seitz can be attributed only to a technical error or to an imperfection in the filter. Even if this possibility must be acknowledged, it is statistically impossible that it could happen constantly for many passages of the same strain and in all the experiments.

A second hypothesis, which has to be more seriously considered, is the one considering the reported phenomena as due to a toxication from heterogeneous proteins. Proteins pass through the Seitz filter; furthermore, Pentimalli in his recent and older researches was able to reproduce a disease entirely similar to leukemia in rabbits intoxicated with heterogeneous proteins.

If we consider in our researches the first inoculation, the possibility of a toxic condition from heterogeneous proteins must be granted. As a matter of fact, the amount of the first inoculum being about 0.05 cc and the average content of an egg around 50 cc, the dilution is of 1:1,000, a dosage sufficiently high.

But, as we proceed with the transplantation while the embryo receptivity to disease increases, having often observed the highest death rate on the ninth transplantation, the amount of heterogeneous proteins diminishes. On the ninth transplantation, the dilution of material is such that it seems quite impossible for the heterogeneous proteins to be held responsible for the picture. Our controls also exclude the hypothesis that embryonal proteins, denatured by death, might behave as heterogeneous proteins when employed for the transplantation. As previously stated, on the seventh day embryos were killed by cold, incubated again during 24 hours and then employed as inoculum. A death rate slightly higher than in other controls was thus obtained, but it never reached experiment death rates and, moreover, it must be emphasized that in this way the typical histological and macroscopic picture of experiments was never observed. Therefore, also this hypothesis must be ruled out. What has been said in regard to proteins can be repeated in relation to any other substance capable to produce in an aspecific way, the transmissibility we observed.

If we still wish to support the idea that heterogeneous substances are the cause of the lesions considered, then we should leave aside the possibility of an unknown aspecific substance introduced with the inoculum. We should rather take into consideration the continuous production of an unknown factor being at the same time the cause and the consequence of the observed lesions.

Although we do not believe the cause of leukemia to be a simple disturbance in the humoral regulation of normal blood cell production, however many experimental data have shown that substances are produced in the lymphoid or myeloid tissues which are able to induce lymphoid and myeloid proliferations.
Such a mechanism could explain the repetition of phenomena in every transmission, due to a new production of principle.

But the following facts must be pointed out:

a) A hyperproduction of any blood-forming tissues was never observed in our embryos and in our chickens. On the contrary, the picture is characterized by the loss of hemopoietic activity in the endothelial tissues with perivascular heterotypical foci. In more advanced stages of development, even these disappear, and only histoid nodules, devoid of hematic cells, are left.

b) Even if the egg to egg inoculum may be considered able to transport a sufficient amount of stimulating substance, this cannot be taken into consideration as far as transmission to adult chicken is concerned and in particular in the chicken to chicken transplantation (2 cc of peripheral blood).

c) Progress of disease both in embryo and in chicken, showing a long incubation time after a single injection, and its clinical course having both the possibility of death or complete recovery, does not show any resemblance with the well known pictures obtained by massive and repeated injections of myeloid and lymphoid substances.

Some enzymes are now being studied in the medical literature which, even if separated from the cells that originated them, are able to induce in other cells the production of substances having an action entirely similar to their own. Such enzymes could very well explain, in our case, transmissibility and also, to a certain extent, transmission to adult animal. This hypothesis would not contrast with inactivation by prolonged heating and with filtrability on Seitz. But, taking into account anatomical and functional peculiarities of the above mentioned embryonal disease, it must be pointed out that we believe it is highly improbable, if not altogether impossible, that a single enzyme can be the cause of a series of alterations involving an entire layer (mesoderm), ectodermal pathological structures, parenchymal alterations, etc. We should therefore admit the existence of a complex of enzymes, reproducing themselves by means of serial transmission, closely linked to the presence of living and indifferentiated cells. These enzymes would be able to produce a disease very often mortal, but sometimes ending by a complete recovery, and this happens with greater frequency, the farthest we proceed from the egg cell. As it is clearly seen, all these features correspond to the properties of a living virus.

There is the possibility of a virus coming from the environment, carried and made evident by transmission. Two points are against this hypothesis: first, that experiences, over a period of 15 years, were carried on in three entirely different places, far from one-another. As a matter of fact, experiences were performed from 1939 to 1945 in the Hematology Laboratory of the University Medical Clinic; from 1946 to 1949 in the Experimental Biology Laboratory of the Cancer Institute; from 1951 to 1952 first in the Biology Laboratory and afterwards in the Virology Laboratory of the “Istituto Superiore di Sanità”. These are four entirely different places where, over a period of many years, there would always have had to be the same type of viruses in the environment, reproducing themselves with absolute uniformity. The second point is that it is improbable that this infiltration of viruses would always take place in the
experiments and never in the controls, in every new environment and for every strain.

The above mentioned objections, in regard to a virus "picked-up" in the environment, can be repeated in connection with any other virus already existing in the egg and put into evidence by transplantations. In the four laboratories, where our researches were performed, four different chicken-breedings were employed, and some thousand eggs were also bought directly on the public market. Moreover it must again be pointed out that the virus would have shown its action only in experimental eggs and never in the controls. Under these circumstances, there is only one hypothesis to be considered: namely, the presence of a virus in the human leukemic blood employed for the first inoculum.

But also for this last assumption two possibilities are to be discussed. It is widely known that leukemia patients are extremely receptive to viral diseases as herpes zoster, viral hepatitis, gripe etc. Therefore, we must discuss the eventuality that the virus we observed existing with great frequency in leukemic blood, might be a virus in leukemia patients' blood, but not the virus of leukemia. Against this, we must point out the great uniformity of the anatomic and physiopathological picture in thousands of experimental embryos. Of course, some embryonal tissues give the same response for different virus types as in the case of ectodermal epithelium, when insemination is directly performed on the choioallantois. But when a deep derangement of angioblastic and hemoblastic structure and function occurs with great uniformity, it is highly improbable that such a uniform response might be due to different viruses. Furthermore this possibility cannot be supported, insofar as adult chicken disease, so similar to embryo disease, is concerned. Therefore, the existence of only one virus in the blood of many different patients occurring in the most different types of leukemias and in every stage of the disease must be acknowledged.

If we ignore the above observations, which point to the fact that a virus causes leukemia, we must admit the presence of an "unknown factor" having the biological features of a virus which always occurs in the blood of leukemia patients, and is apt to reproduce a mesenchymal disease by serial transmission. This would question the whole problem of the nature of viruses, rather than the viral origin of leukemia.

Therefore, we believe that the only logical conclusion to be drawn from our researches is that a living virus exists in the blood and in the bone marrow of leukemia patients, which is the cause of the disease.

Fertile nondeveloped egg cell is highly receptive to this virus, while embryo in advanced developmental stage is less receptive. Receptivity diminishes still further, insofar as adult animal is concerned. The virus shows a marked tropism toward mesenchyma, producing a deep derangement of hemopoiesis.

Next step will be the isolation and classification of the virus. This task has already been undertaken by expert virologists. The final step will be the study of the means to attack and destroy the leukemogenic viruses. It is too early to anticipate what the course of future work in this direction will be. We can say, however, that the
pathway of future research work will be very much influenced by our knowledge of the mechanism of action of the virus on the blood-forming tissues. If the theory we are discussing will be definitely ascertained, the research work on the cure of leukemia will be directed toward the study of preventive vaccine and possibly curative, probably still leaving a place for cytostatic agents, as means to destroy the mutated blasts.

Going back to the pathogenetic problem, we will try to see whether we can find elements of support for the hypothesis of mutation in our experiments. At the Moscow meeting (16) we mentioned that in the embryonal disease experimentally produced by us, numerous and profound morphological changes were observed in the blasts of embryonal annexes. Profound mitotic changes, aploidism and polyploidism, changes in the nuclear-cytoplasmic ratio, nuclear giantism, etc.: all these effects indicate undoubtedly a marked cellular alteration, but, as pointed out by Di Guglielmo, they are not demonstrative of a true mutation. Much more important for the mutational hypothesis is the severe functional alteration of the blasts, as it has already been pointed out by us at the Berna meeting (15). As we have already said, the most important aspect of the pathogenesis of the embryonal disease is the change in the functional behaviour of the sinusoidal endothelium of the annexes. They have lost the normal hemopoietic intravascular activity, while they have acquired the ability to produce perivascular infiltrates. Such alterations, which we could call inversion of functional polarity, are continuous and irreversible. The character of such an anomalous behaviour in a continuously proliferating tissue are in good accordance with the hypothesis of a mutated functional attitude, which is transmitted from cell generation to cell generation. In favour of the irreversibility of such alterations are the findings of severe anemia, profound malformations, fat degeneration in the liver, splenomegaly, etc., in the embryos which are still surviving after 14 days of observation and which will eventually reach the hatching stage. All these malformations are dependent on the functional deviation of sinusoidal endothelium. The loss of the property of the hemopoietic tissue of receiving and responding to the information originated from the needs of the organism is the most serious, most permanent and most irreversible loss which may take place in a tissue. It seems to us that all these facts satisfy the postulates we listed at the beginning of the paper. The mutation hypothesis, therefore, seems to be a very likely one, although not irrefutably demonstrated. Our observations, then, while allowing us to support the viral etiology of human leukemia also suggest the possibility that the blasts are mutated cells (17).

Is the viral etiology of leukemia compatible with the hypothesis of the blast mutation? We think that today it is possible to give an affirmative reply to this question, based on many observations in other fields of experimental biology and pathology. It is today accepted that the virus, or virus material, or even a single molecule of a pathological DNA may be taken in a genetic apparatus, which retains the anatomo-functional characteristics of a cell strain, but markedly changes its behaviour. After the successful attempt to split the DNA spiral, even the hypothesis of the uptaking of a splitted spiral is admissible, which, in given conditions, should form again the
other half, reconstructing the original DNA. But at this point we must stop, because the facts we have so far explored do not allow us to proceed further. We can say, however, that even if it is not known whether the mutagenic-viral hypothesis could be extended to other tumors, we think that this is possible for the tumors of the group of malignant lymphomas, which are the closest to leukemia. We will, therefore, confine ourselves to the following conclusive remarks: (a) Our experiments demonstrate, for the first time in the history of experimental pathology, that human leukemia is due to a virus; the isolation and characterization of the virus will be the task of virologists. (b) The same observations bring also support to the pathogenetic hypothesis of the mutagenic activity of the virus on the blasts. Such etiology and pathogenesis can be at present prospected for the diseases of the group of malignant lymphomas.

Summary

The authors briefly describe their old and recent research work on the etiology of leukemia, and discuss the genetic-viral hypothesis of human leukemia. The injection of human leukemic blood in fertilized chicken eggs, before incubation, causes a severe disease in the embryos. This disease is characterized by the fact that the hemoblasts are extremely atypical, and loose the ability to produce intravascular blood cells, while they form perivascular infiltrates. The disease is indefinitely transmitted from egg to egg, also using acellular filtered material. The transmissibility is inactivated by heat. In conclusion, we can say that the observations described strongly support the viral etiology of human leukemia and suggest the hypothesis that the virus might be responsible for a somatic mutation of the blasts.
References

2. — RIGGIO G.: Reazione istoiode indotta nell'embrione di pollo entro la 84a h. di incubazione dalla inoculazione precoce di materiale leucemico. Reale Accademia d'Italia. Rendiconti Scienze, 1941.
RIASSUNTO

Gli AA. passano brevemente in rassegna le loro ricerche sull'eziologia della leucemia, discutendo l'ipotesi genetico-virale della leucemia nell'uomo. L'iniezione di sangue umano leucemico in uova fertilizzate di pulcino, prima dell'incubazione, provoca agli embrioni una grave malattia. Essa è caratterizzata da emocitoblasti profondamente atipici, i quali perdono la capacità di produrre cellule del sangue intravascolari, formando infiltrati perivascolari. La malattia si trasmette indefinitamente da un uovo all'altro, anche se si usa materiale acellulare filtrato. La trasmissibilità viene inattivata dal calore. Si può dire, in conclusione, che le osservazioni descritte forniscono una prova notevole dell'eziologia virale della leucemia nell'uomo e suggeriscono l'ipotesi che il virus possa essere responsabile di una mutazione somatica dei blasti.

ZUSAMMENFASSUNG


Abschliessend lässt sich sagen, dass die beschriebenen Beobachtungen einen bemerkenswerten Beweis für die virale Ätiologie der Leukämie beim Menschen liefern und die Vermutung aufkommen lassen, dass der Virus für eine somatische Mutation der Blasten verantwortlich sein könnte.
Fig. 1. Membrane of a normal embryo (in toto, small magnification)

Fig. 2. Membrane of a diseased embryo (in toto, small magnification)
Fig. 3. The same as fig. 2, $\times 100$

Fig. 4. The same as fig. 2, $\times 400$
Fig. 5. Section of a normal chorionic villus, × 800

Fig. 6. Section of a diseased chorionic villus, × 800
Figs. 7-8. Magnified details of fig. 6
Fig. 9. Chorioallantois of a diseased embryo (small magnification)

Fig. 10. Another section of the above
Figs. 11, 12, 13, 14. The same as fig. 9: its epithelial stratus, highly magnified ($\times1,100$), on ultrasection.
Fig. 15. Slide of a perivascular infiltrate

Acknowledgement

We wish to thank “S. Karger, Basel - New York” for kindly granting us permission to reproduce figs. 2-4 from the following publication: