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ON THE ROLE OF THE REACTIVITY OF THE INFECTED ORGANISM IN THE DEVELOPMENT OF TUBERCULOSIS

(STUDIES ON EMBRYONIC TISSUES)

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The aim of this paper is to analyse the morphogenesis of tuberculosis, as an expression of the reactivity of the infected organism. Studies on the tuberculous changes in connection with the consecutive reaction forms of embryonic evolution shed light on the influence of reactivity on the pathological process. On the other hand, the fact that tissues of different reactivity are present within the same embryonic organism, makes it possible to investigate the connection between pathological changes and tissue reactivity.

To study the reactivity to infection, the embryonic complex was infected with Koch bacilli of the human, bovine and avian type, respectively. According to MENKES, the embryonic complex consists of the body of the embryo with all its appendages, with all its living environment. This concept includes the maternal organism as well; as we shall, however, deal with avian embryos, the term embryonic complex will mean only the embryo and the structures included in the egg.

A total of 297 white Leghorn and Plymouth chicken embryos was studied in 32 series. Bacterial infection of the hen's egg and of the avian embryo complex has extensively been studied, under both artificial and natural conditions [2—5, 14—23, 27, 31—33, 35, 36]. There seems to be general agreement in that the pathogens may penetrate the intact shell before or after the laying of the egg. This view is of particular biological significance, because it infers that the embryonic complex, which repeatedly contacts various pathogens during its phylo- and ontogenesis, would in some way adapt itself to the unusual circumstances created by the presence of the pathogen. Among others, the presence of one or more bactericidal factors in the egg white seems to corroborate this view. This adaptation develops probably in the most superficial living layers of the embryo complex, which are most exposed to contact with external pathogenic factors.

The few studies on the tuberculous infection of the embryo complex were focussed not so much on the reactivity of embryonic tissues, as on the circumstances of bacterial growth and the effects of certain drugs, and most of the morphological investigations on the chorio-allantoic membrane [1, 5, 13,

15, 19, 21—24, 28, 30]. These investigations yielded the following general conclusions:

(i) The chicken embryo complex (used by most workers) can be infected with all of the more important types of *Mycobacterium tuberculosis.*

(ii) The site of tuberculous changes depends to a certain degree on the mode of infection.

(iii) The nature and extent of changes are more or less influenced by the type, quantity and virulence of the pathogenic agent, though there is also some contradictory evidence as to the role of virulence.

(iv) The most marked changes occur in the chorio-allantoic membrane, as well as in the liver and spleen.

(v) The changes in the chorio-allantoic membrane are partly mesenchymal (infiltrations, foci, etc.) and partly ectodermal (epithelial proliferation, etc.) The presence of giant cells is characteristic. In the internal organs the changes are usually non-specific, consisting mainly of infiltration and necrosis.

6. The tuberculous changes develop much faster in the embryo than in the adult organism.

7. Infection is most successful when carried out on the 10th to 12th day of incubation.

8. According to certain authors, the nature of the tuberculous process is influenced also by the species of bird used.

Methods

1. *Direct infection of the chorio-allantoic membrane.* In one case minced tissues from a tuberculotic guinea pig were used. In other cases suspensions of *Mycobacterium tuberculosis* (about 1 mg/ml physiologic saline), or minced chorio-allantoic membrane soaked in the suspension were employed. In every case the chorio-allantoic membrane was infected after 7 or 8 days of incubation.

2. *Direct infection of the embryo* was carried out according to MENKES [27], placing a piece of chorio-allantoic membrane soaked in the *Mycobacterium tuberculosis* suspension on the neural tube opened on the third day of incubation.

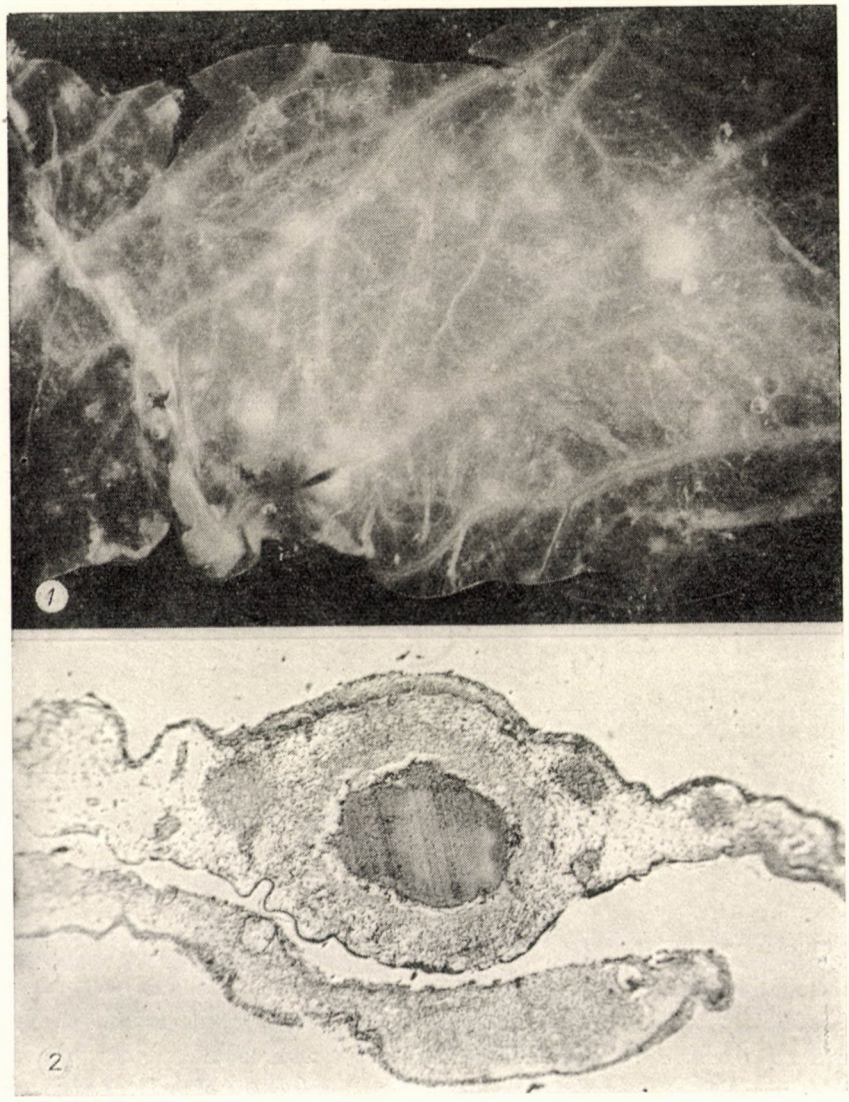
3. *Intraamniotic infection by injuring the body of the embryo.* On the fourth day of incubation a suspension of Koch bacilli was injected into the amniotic sac, afflicting with the tip of the needle on injury at the caudal end of the embryo.

4. *Intravenous infection.* The suspension of *Mycobacterium tuberculosis* was injected into the allantoic vessels of 12—13 day old chicken embryos, by the slightly modified technique of EICHHORN.

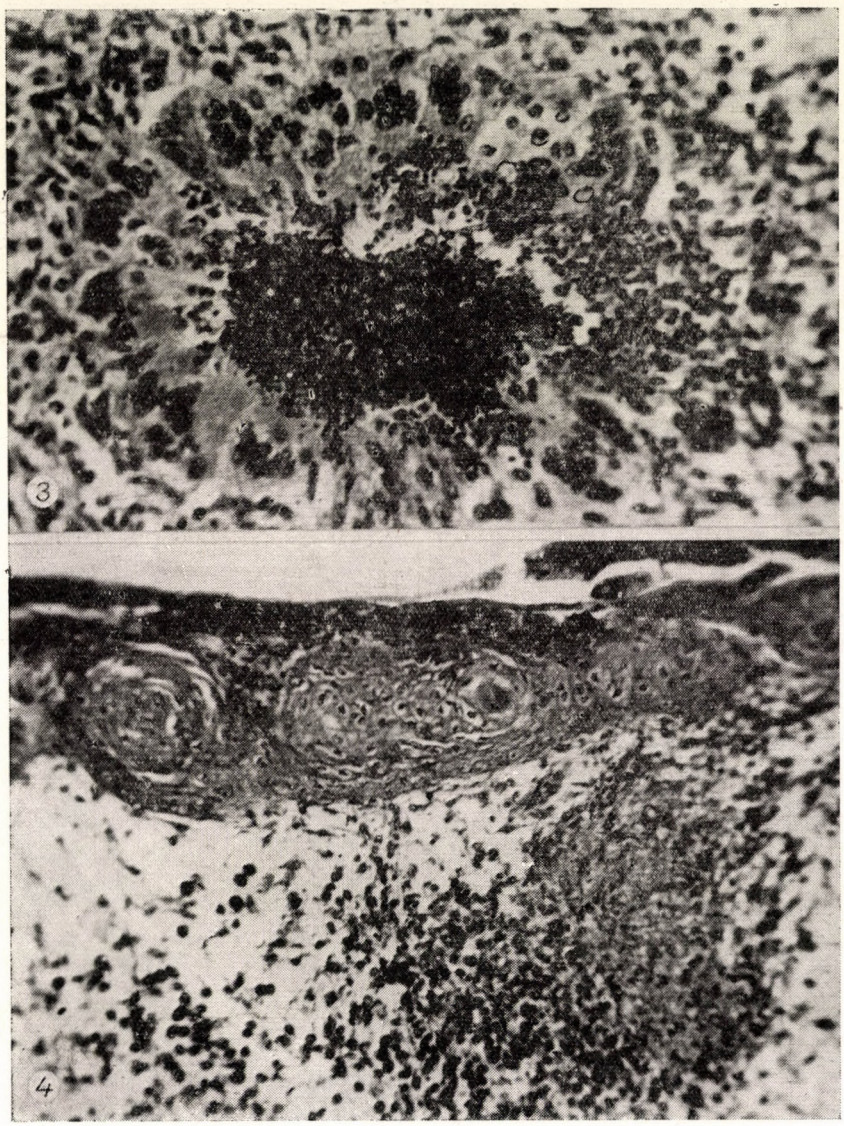
The embryos were worked up 6, 7, 8, 9, and 11 days after infection in case 1., 6 to 16 days after infection in case 2., 15 days after infection in case 3. and 6, 7, and 8 days after infection in case 4. The specimens were fixed in Susa's solution or in 4 to 5 per cent formol. The embryos were usually examined every day, by the naked eye or under a binocular lens. In some cases smears were made from the blood taken from the still beating heart, immediately after breaking up the shell, and were stained according to PAPPENHEIM, or with haematoxylin-eosin. The histologic sections were stained with haematoxylin-eosin and by a Ziehl—Neelsen technique adapted to staining tissues. Silver impregnation according to GÖMÖRI and PAPP was also carried out in some cases. Histologic sections and blood smears from normal embryos of the same age were used as the control specimens.

Results

1. Direct infection of the chorio-allantoic membrane with tuberculous guinea pig tissues. The typical changes developed in about 10 days. Unevenly distributed, white foci appeared on the chorio-allantoic membrane, which turned opaque and became oedematous. Under the microscope, infiltrations and foci, composed mainly of eosinophile cells, were seen. In the more advanced stage the foci contained polynuclear giant cells and necrosed areas *(Figs 1, 2, 3).*

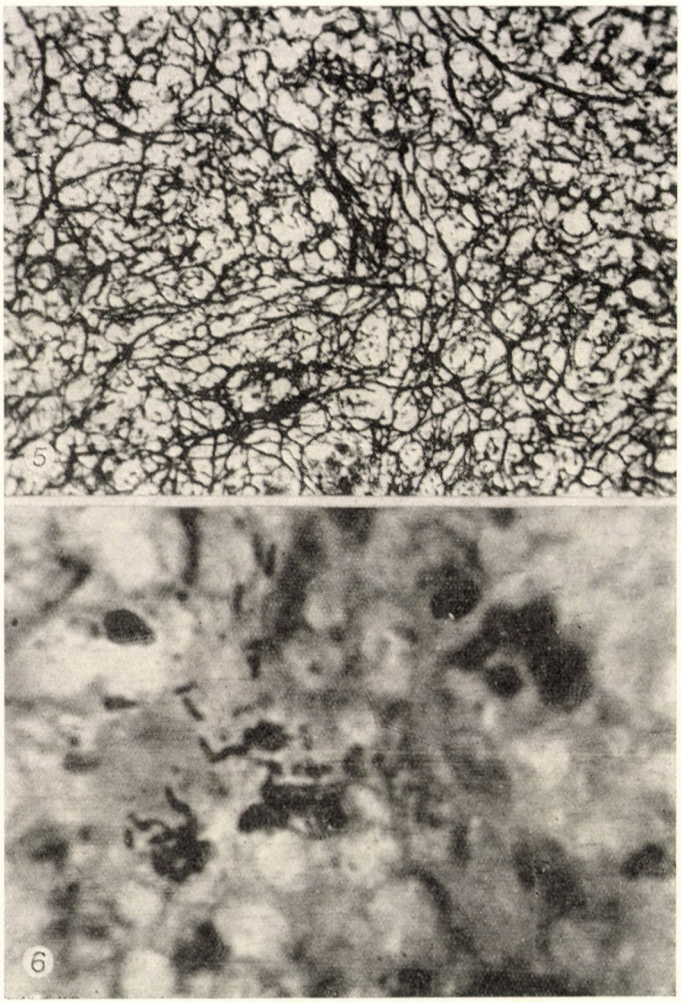


Figs. 1—2



Figs. 3—4

The inflammation was accompanied by an epithelial reaction. The ectodermal epithelium became multi-layered and sometimes cornified (metaplasia). At sites, epithelial bundles penetrated deep into the mesenchymal tissus of the membrane *(Fig. 4)*. In the inflammatory changes a considerable increase of the argyrophilic fibre ground substance was visible *(Fig. 5)*. Large numbers of Koch bacilli were present in the inflamed tissues *(Fig. 6)*.



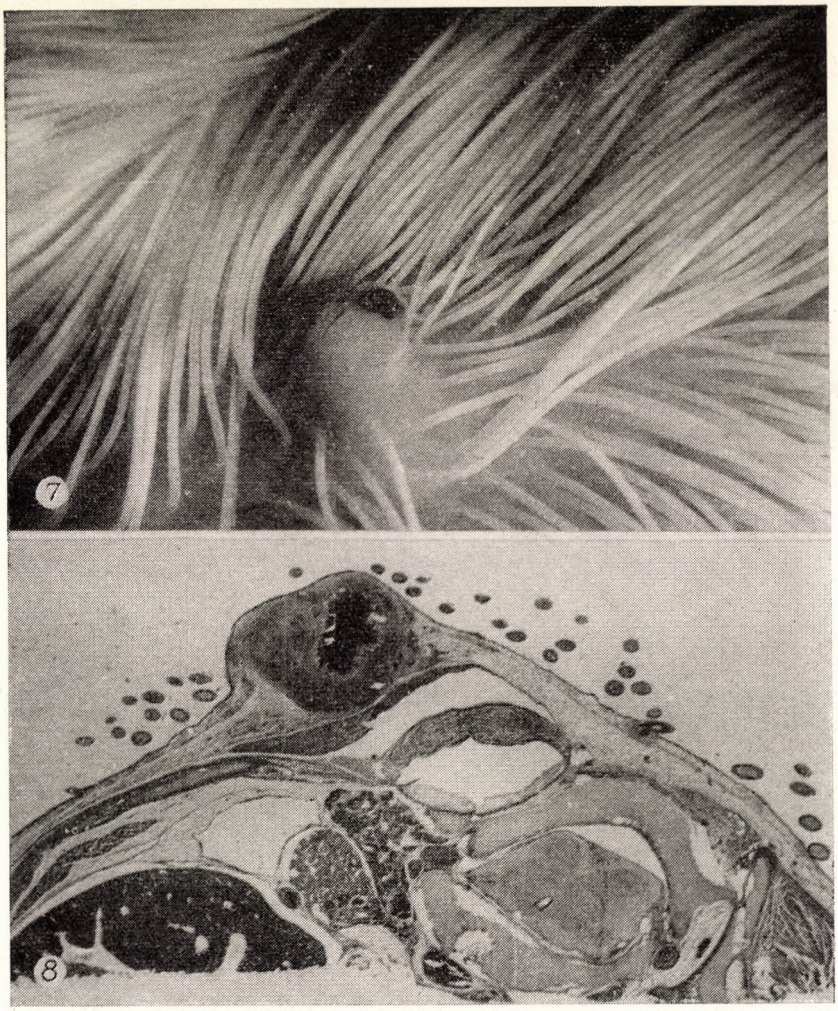
Figs. 5—6

Thus, the tissues of the chorio-allantoic membrane develop marked changes in response to the implantation of heterogenous infected tissues.

2. Direct infection of the chorio-allantoic membrane with a suspension of Mycobacterium tuberculosis or with infected membrane. The first changes appeared 4 to 5 days after infection, to become more and more marked subsequently. Essentially, the same focal changes developed as described above. Under the microscope, infiltrations, foci of similar nature, eventually necrotic changes and a few polynuclear giant cells were visible.

The epithelium showed diffuse metaplasia, which was less marked than in the former case. Sometimes small epithelial processes penetrated deep into

the mesenchyma. In the inflamed tissue the number of argyrophilic fibres was markedly increased and numerous Koch bacilli could be found. The internal organs exhibited no particular changes.

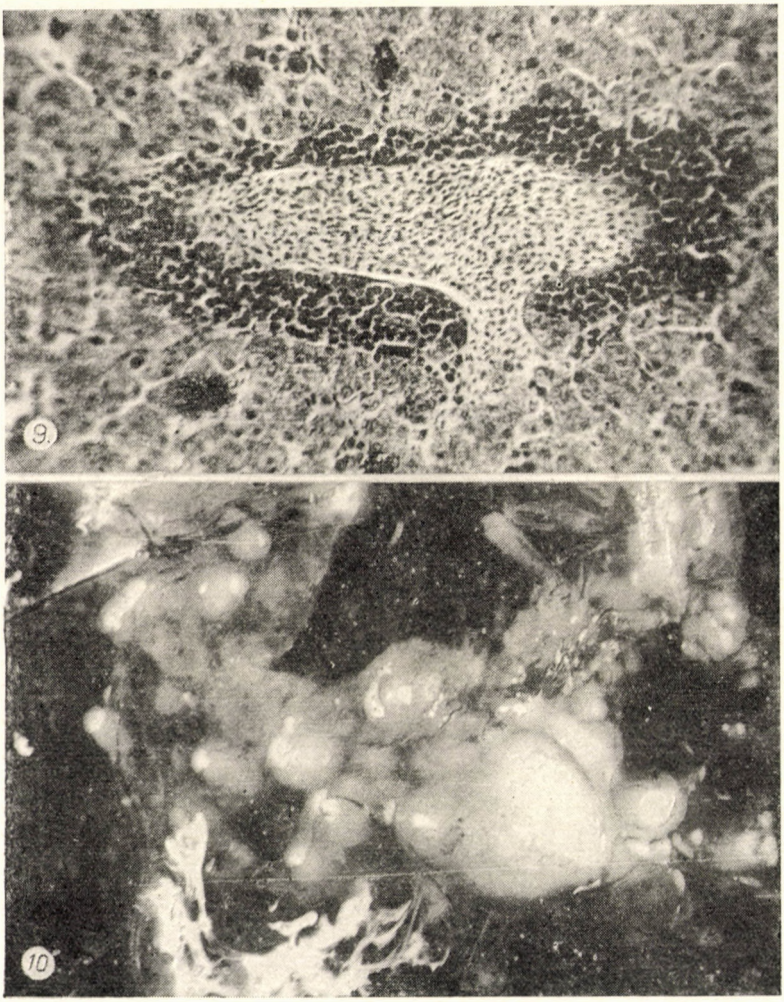


Figs. 7—8

Thus, the tissue of the chorio-allantoic membrane develop diffuse and marked changes in response to direct infection by various methods.

3. *Direct infection of the embryo.* In single cases circumscribed foci, similar in gross and microscopic appearance to those described above, were visible at the site of infection *(Figs. 7, 8)*. Except for a few perifocal infiltrations, there was no evidence of a spread of the process. These infiltrations were

often the sole changes, the skin and its epithelium showing none whatever. In some cases perivascular infiltrations composed of round cells or eosinophile cells were present in the liver, kidney, and lungs *(Fig. 9)*.



Figs. 9—10

The chorio-allantoic membrane showed changes similar to those found in the formed series. The changes were fully developed about 10 days after infection. The membrane was gradually thickened, turned opaque, and there was evidence of a circulatory disturbance. Some foci attained a considerable

size and even formed conglomerates *(Fig. 10)*. No epithelial response was visible even in the presence of a very intense inflammatory reaction, except when the epithelium had been infected directly. In the affected areas of the embryo, the number of argyrophilic fibres had increased and Koch bacilli were demonstrable.

The other annexes (yolk sac, amnionic sac) exhibited no marked changes.

Thus, under the same experimental conditions, within the same embryonic complex and at the same time, the various parts of the complex respond differently to the same pathogenic agent, in the present case to *Mycobacterium tuberculosis*. The response of the embryonic body is weak, local or totally absent, whereas that of the chorio-allantoic membrane is diffuse and marked.

4. Intra-amniotic infection, with injury to the embryo. Although a few embryos were only involved in this series, it was apparent that also on this kind of infection the changes develop in the first place in the chorio-allantoic membrane. They were essentially the same as those described above. In the body of the embryo there were only non-specific perivascular infiltrations, mainly in the liver.

5. Intravenous infection. No changes were usually demonstrable in any part of the embryonic complex 6 to 8 days after infection. In some cases a slight infiltration appeared in the chorioallantoic membrane at the site of the injection and mild perivascular infiltration in some viscera.

Biological tests were carried out to demonstrate the presence of virulent tubercle bacilli. Guinea pigs or rabbits were inoculated with the bacillus contained in homogenized embryonic tissue, whereafter typical, rapidly spreading tuberculous changes developed.

In a few cases living chicken were obtained from the infected eggs. As determined 4, 8, 22 and 23 days after hatching, productive, circumscribed foci were found in the liver, spleen and lungs. These were similar to the usual tuberculous changes. In some instances necrosis was also noted *(Fig. 11, 12)*.

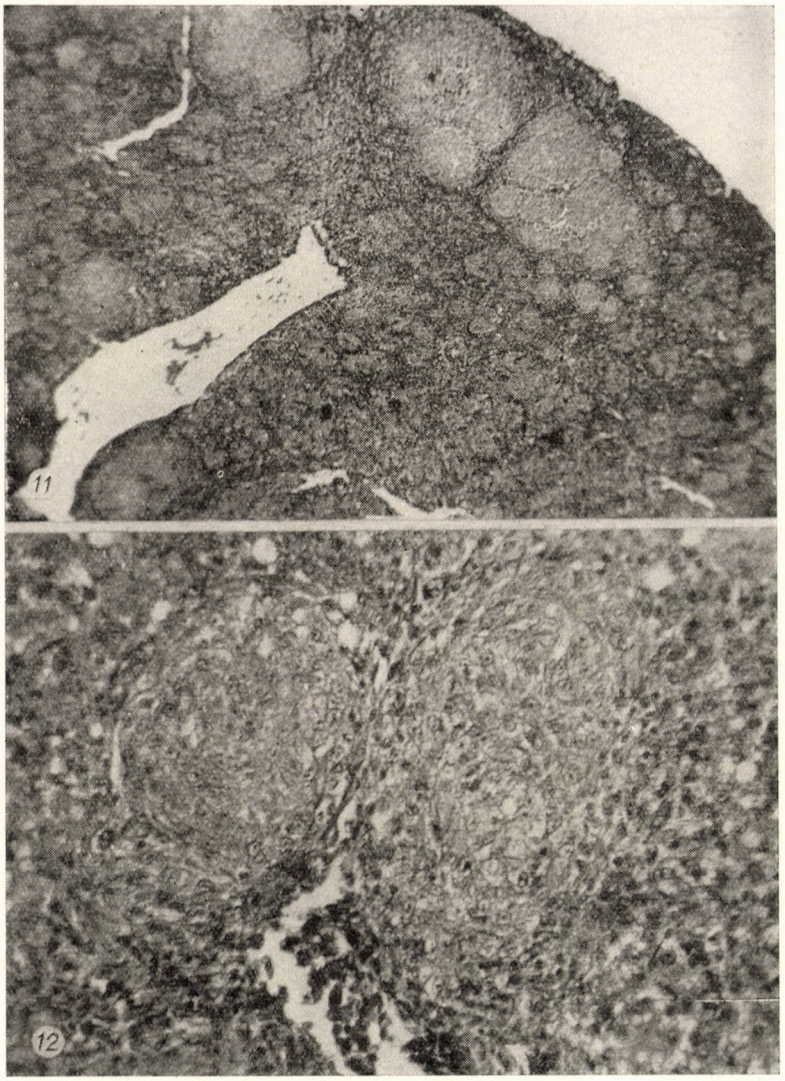
6. Morphology of the blood from normal and infected embryos. Comparative analysis of the differential blood counts of normal and infected embryos aged 18, 19 and 20 days showed the following changes for the infected embryos:

a) a relative decrease in the number of eosinophile meta- and promyelocytes;

b) a marked relative increase in the number of pseudoeosinophile (neutrophile) metamyelocytes;

c) a marked decrease in the relative lymphocyte (and sometimes promyelocyte) count.

These changes varied in degree. The erythrocyte changes were less marked only in some cases of intravenous infection was there a slight increase in the number of young forms.



Figs. 11—12

Discussion

I

Our investigations have elucidated several phenomena of the development of tuberculosis in the embryo complex. Some of the findings agree with the data in the literature. This applies to certain of the fundamental changes caused by tuberculous infection, to the specific reactions of the epithelium, as well as to the relative incidence of changes in the liver and spleen.

II

The other findings are somewhat at variance with the published evidence. From our few observations it seems that the type of the pathogenic agent is not of decisive significance from the point of view of morphological changes. This applies in particular to the "characteristic" difference claimed by MOORE to exist between the actions of human and bovine types. We are also unable to agree with the view that the optimum time for infection is the 12th day of incubation, as attained with reproducible and characteristic results with early infection.

The term "giant cell of the Langhans type" is used by many authors to denote the giant cells arising in the embryo as a result of tuberculous infection. We think that these giant cells differ in type, at least morphologically, from those seen in human tuberculosis. This applies to the shape of such giant cells (symplasts), to the localization of nuclei, etc. In our studies in guinea pigs the giant cells produced by tuberculous infection were morphologically different from the Langhans cells. As the shape or morphogenesis express the reactivity of the living substrate, these morphological differences indicate a difference in reactivity between the reacting organisms. It is interesting that the giant cells occur usually in the proximity of necrotic foci, sometimes in radial distribution. We have seldom found giant cells in the changes present in the body of the embryo, although such cells were frequently found by some authors.

In contrast with CANAT and OPIE, we have not observed a reaction of the embryonic epithelium in response to direct infection.

As regards the term "caseation" used by some authors to denote the necrotic process, we agree with the considerations put forward by LEE and STAVITZKY. In fact, the necrosed foci observed by us were markedly different from the usual pattern of caseous necrosis. The necrosed mass staining a dark red and containing cellular detritus and sometimes erythrocytes cannot be identified with tuberculous caseation. It has been suggested recently that caseation would be a morphological manifestation of tuberculous hypersensitivity. According to present knowledge, it is hardly justified to speak about an allergic reaction during embryonic life.

Finally, one is not fully justified to use the term "tubercle" to denote tuberculous changes in the embryo. The inflammatory foci observed considerably differ in both composition and structure from the human tubercles. For example, there occurs no lymphatic reaction. This fact alone makes it clear that the specific reactivity of the substrate is of a decisive importance in the development of morphological changes. It is obvious that the specific actions so often claimed for certain components of the tubercle bacillus (for instance, a mobilization of lymphocytes) can take effect only in the presence of adequate reactivity.

III

Our experiments have elucidated certain aspects of the problem investigated. From the point of view of methodology it is believed to be significant that we have used relatively small numbers of bacilli for infection ; this is thought to have certain advantages. Moreover, we have ensured a prolonged course of the tuberculous process by carrying out infection on the third days. Further, by means of experimental myelorachischisis the embryo was infected directly.

The following of our results should be emphasized.

(i) The tuberculous infection of the chick embryo produces an ectodermal epithelial reaction in the chorio-allantoic membrane and a reaction in the embryonic mesenchyma.

A) The epithelial reaction is composed of two phenomena.

a) A simple, non-specific metaplasia, which develops in response to various external effects and which corresponds to the adaptation that had developed on the chorio-allantoic membrane, as the most superficial living layer of the embryo complex.

b) A considerable non-specific epithelial reaction, which leads to a deep invasion by epithelial cells and to the development of epithelial islets and pearls. This seems to be the response to both the implanted tissue and the pathogenic agent. The most marked reaction of this type was characteristically produced by the implantation of infected tissue from another species (guinea pig). Similar observations have been made in connection with the transplantation of certain malignant tumours [25].

When infection had been carried out on the third day, *i.e.* before the development of the allantoic sac, the ectodermal epithelium of the chorio-allantoic membrane showed no substantial changes, despite the subsequent development of marked focal changes. The reactivity of the membrane growing in an infected medium seems to be altered in a short time. This is only natural in view of the extreme plasticity peculiar to embryonic tissues.

B) The epithelium or skin of the embryo, unlike the ectodermal epithelium of the chorio-allantoic membrane, exhibits no substantial reactions.

C) Notwithstanding the time of infection, the embryonic mesenchyma of the chorio-allantoic membrane responds with inflammatory infiltrations and with the development of foci. Necrosis and the appearance of giant cells are characteristic features. Likewise, there is a remarkable increase in the argyrophilic ground substance. The reaction is usually diffuse.

D) The mesenchymal response of the embryonie body is circumscribed. In our opinion, the focus which develops at the site of infection originates mostly from the implanted chorio-allantoic tissue and not from the organism's own tissues.

(ii) Like our previous experiments with malignant tumours [25], the present ones, too, showed that the reactivity of the body of the embryo differs morphologically from that of the chorio-allantoic membrane. This difference is related also to certain general biological and pathological laws.

A) It has been proved once again that the development of a pathological change (in the present case, of the specific inflammation) depends greatly on the reactivity of the substrate. Although the pathogenic agent is present throughout the embryo, the changes develop selectively, in accordance with the reactivity of the various parts of the embryo complex.

B) Our results suggest that (at least in our cases) reactivity is greatly influenced by certain local, cellular factors. Otherwise it would be rather difficult to explain the difference in reactivity between the body of the embryo and the chorio-allantoic membrane. Local factors, especially in the non-innervated chorio-allantoic membrane, may have an important role to play.

IV

The intravenous infection experiments showed, in agreement with the data published by LEE and STAVITZKY, that specific inflammatory changes are absent, even though virulent pathogens were demonstrated in the embryo by biological tests. One feels tempted to claim, especially in view of the results obtained by myelorachischisis, that the body of the embryos were in some measure immune to the tubercle bacillus. However, it would be premature to draw a final conclusion. According to our observations, the tuberculous changes develop fully in about 9 to 10 days in the embryo complex. However, the intravenous injections administered on the 12th or 13th day, only 8 to 9 days are left from the hatching time. Experiments are in progress to prolong the development of intravascular infection and it is hoped that their results will bring us nearer to the solution of this problem.

The tuberculous changes of productive nature found in the viscera of hatched chicks should be interpreted with caution. Hatching seems suddenly to alter the reactivity of the organism and, as a result, the form of systemic reaction. The occurrence of such an alteration is quite probable. According to data in the literature [5], by the end of embryonic growth the inflammatory reaction becomes similar to that seen in the post-embryonic stage. Nevertheless, the small number of our observations does not allow for final conclusions.

Comparative studies on normal and infected embryos have shown that in the period tested the haematological response of the embryo is similar to that occurring in human and animal tuberculosis. Neutrophilia and lymphopenia occur in man and experimental animal alike, according to data in the literature and our own observations.

Summary

1. The reaction of the chick embryo complex to tuberculous infection, as induced by different methods, has been investigated.

2. The morphological response of the chick embryo complex exhibited the following features.

a) Epithelial reaction of the chorio-allantoic membrane. This is either a simple, non-specific response elicited by the widest variety of external effects, or a more marked reaction, involving epithelial invasion and development of islets of epithelium. No epithelial reaction takes place when the chorio-allantoic membrane has developed in an infected medium.

b) The epithelium of the body of the embryo does not respond to direct mechanical intervention or to local inflammatory changes ; even the epithelial appendices develop normally.

c) Reaction of the mesenchymal tissue of the chorio-allantoic membrane. This involves the formation of infiltrations and foci of inflammation, necrosis and appearance of giant cells. The reaction is marked and diffuse.

d) The local inflammatory focus which develops at the site where the embryo has been directly infected is apparently due to the implanted tissue. A slight infiltration of the mesenchyma occurs exclusively in the proximity of the focus.

e) Intravenous infection produces no specific changes, only non-specific infiltrations. The process develops within a short time and therefore the problem requires further investigation.

f) Productive focal changes have been found in the viscera of chicken, on the 4th to 23rd days after hatching, following infection with tubercle bacilli during embryonic life. This phenomenon suggests that hatching may suddenly alter systemic reactivity.

g) The differential blood count from infected embryos showed marked changes on the 18th, 19th and 20th days of incubation. The number of neutrophile (pseudoeosinophile) leucocytes increased, that of the eosinophils and lymphocytes decreased.

3. The giant cells found in the tuberculous changes of the embryonic complex differ morphologically from the giant cells of the Langhans type.

4. The necrotic changes in the embryonic complex differ from the usual pattern of caseation.

5. The cellular composition of inflammatory foci is different from that of tubercles. Epitheloid cells and lymphocytes are usually absent and mainly eosinophilic cells (mono- or polynuclears) are found in their place.

6. A marked difference in reactivity is observable, especially between the body of the embryo and the chorio-allantoic membrane. This corroborates the earlier findings of MENKES et al. Under the same conditions of infection the chorio-allantoic membrane responds markedly and diffusely, whereas the response of the body is weak and circumscribed. The marked reactivity of the chorio-allantoic membrane is apparently due to a phylogenetic adaptation to repeated external injuries.

7. The different changes found in the tissues of different reactivity corroborates the significant role of the substrate's reactivity in the development of a pathological process. Moreover, in regard to the peculiar conditions prevailing in the embryo complex (presence of tissues without innervation, etc.) it seems that local factors, too, may play a role in the response to the pathogenic agent.

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О ПРОБЛЕМЕ РЕАКТИВНОСТИ ЗАРАЖЕННОГО ОРГАНИЗМА В РАЗВИТИИ ТУБЕРКУЛЕЗА

Й. ШАНДОР

Реакция эмбрионального комплекса цыплят в случае туберкулезного заражения проявляется в характерных изменениях. Эпителиальная реакция хорион-аллантоидной мембраны либо простая, неспецифическая, возникающая на многочисленные внешние воздействия (утолщение, метаплазия), либо она более выраженная, сопровожденная

разрастанием эпителия вглубь, образованием эпителиальных островов, и со сильной метаплазией. В противоположность этому эпителий зародышевого тела не реагирует даже на непосредственные воздействия, и развивающийся местный очаг не влияет на развитие эпителиальных придатков, или на характер эпителия. В мезенхиме хорион-аллантоидной мембраны развиваются инфильтрации и эозинофильные или же одноядерные круглоклеточные очаги с некрозами и образованием гигантских клеток. Реакция сильная и диффузная. В случае непосредственного заражения тела зародыша, кроме местного воспалительного очага, нельзя выявить значительных изменений. В случае внутривенного заражения (во второй половине инкубации) наблюдаются только небольшие инфильтрации в отдельных внутренних органах, однако, этот вопрос требует еще дальнейших исследований. После туберкулезного заражения во внутренних органах вылупленных цыплят обнаруживаются очаговые изменения продуктивного характера, предположительно в связи с изменением реактивной способности после вылупления. Туберкулезное заражение обусловливает выраженные изменения в картине крови зародышев цыплят (нейтрофильный лейкоцитоз, лимфопения и эозинопения). Образующиеся в зародышевом комплексе гигантские клетки и некрозы отличаются с морфологической точки зрения от гигантских клеток Лангханса и от створаживания. Состав клеток воспалительных очагов до вылупления отклоняется от обычного клеточного состава «бугорков». В пределах зародышевого комплекса наблюдается выраженная разница между реактивной способностью тела зародыша и хорион-аллантоидной мембраной. Выраженная реактивная способность последней развивается по всей вероятности вследствие повторных фило- и онтогенетических встреч с внешними воздействиями. Разница изменений тканей с различной реактивной способностью подчеркивает значительную роль, которую играет реакция субстрата в возникновении патологического процесса.

BEITRÄGE ZUM REAKTIVITÄTSPROBLEM DES INFIZIERTEN ORGANISMUS IN DER ENTWICKLUNG DER TUBERKULOSE

I. SÁNDOR

Die Reaktion des Hühnerembryo Komplexes nach tuberkulöser Infektion manifestiert sich in charakteristischen Veränderungen. Die Epithelreaktion der Chorioallantois besteht entweder in einer einfachen, nicht spezifischen, auf viele äußere Einwirkungen entstehenden Reaktion (Verdickung, Metaplasie), oder sie ist ausgeprägter, und mit Eindringen des Epithels in tiefen Schichten, Entstehen, von Epithelinseln und starker Metaplasie verbunden. Hingegen reagiert das Epithel des Embryokörpers selbst auf direkte Einwirkungen nicht, der sich bildende örtliche Herd beeinflußt nicht die Entwicklung der Epithelanhänge und den Charakter des Epithels. Im Mesenchym der Chorio-allantois entstehen Infiltrationen von eosinophilen bzw. einkernigen Rundzellen, mit Nekrosen und Riesenzellenbildung. Die Reaktion ist diffus und ausgeprägt. Bei direkter Infektion des Embryokörpers können außer dem örtlichen entzündlichen Herd keine wesentlichen Veränderungen nachgewiesen werden. Im Falle intravenöser Infektion (in der zweiten Hälfte der Inkubation) können bloß geringe Infiltrationen in einzelnen Innenorganen beobachtet werden, doch erheischt diese Frage noch weitere Untersuchungen. In den Innenorganen der nach tuberkulöser Infektion geschlüpften Kücken können Herdveränderungen produktiven Charakters nachgewiesen werden, vermutlich im Zusammenhang mit der Veränderung der Reaktionsfähigkeit nach der Schlüpfung. Die tuberkulöse Infektion bedingt eine ausgeprägte Veränderung des Blutbildes der Hühnerembryonen (Neutrophylie, Eosino- und Lymphopenie). Die im Embryokomplex sich bildenden Riesenzellen und Nekrosen unterscheiden sich in morphologischer Hinsicht von den Langhansschen Riesenzellen sowie von der Verkäsung. Die Zellenzusammensetzung der Entzündungsherde vor dem Ausschlüpfen ist von der üblichen Zellenzusammensetzung der »Tuberkeln« verschieden. Innerhalb des Embryokomplexes besteht ein ausgeprägter Unterschied zwischen der Reaktionsfähigkeit des embryonalen Körpers und jener der Chorio-allantois.

Die bedeutende Reaktionsfähigkeit der letzteren entsteht vermutlich infolge des wiederholten phylo- und ontogenetischen Zusammentreffens mit äußeren Einwirkungen. Die Verschiedenheit der Veränderungen von Geweben mit unterschiedlicher Reaktionsfähigkeit bestätigt die bedeutende Rolle, welche die Substratreaktion in der Entstehung eines Krankheitzprozesses spielt.

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