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STUDIES OF COCCIDIOIDES IMMITIS RIXFORD ET GILCHRIST

by

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VII. Immuno-Allergic Reactions in Experimental Infection of the Guinea Pig

Continuing our investigations of Coccidioides immitis (1,2,3,4,5,6,7,8), we present on this occasion a study of manifestations of allergy and immunity in experimental infection of the guinea pig. We chose that animal as being sensitive to inoculation with the fungus in question and easy to handle and to obtain.

This work was planned and carried out by Drs. Negroni and Bonfiglioli, with Dr. Vivoli in charge of the histopathological study.

Work plan. We proposed to investigate the following matters: 1) at what moment of the infection cutaneous sensitization to the coccidioidin appears; 2) what is the reaction of infected and allergic guinea pigs (sensitive to coccidioidin), when exposed to a second inoculation with a) material from a live culture and b) the same material after its vitality has been destroyed by heating; and 3) the appearance and course of circulating antibodies.

Methods and techniques. As shown in the accompanying tables, we used 5 groups of white guinea pigs weighing 350-400 grams, which we designated with the first 5 capital letters of the alphabet.

Groups A, B, and C, consisting of 15 guinea pigs each, were inoculated via the routes indicated with an infecting
dose of 10 mg (moist weight) of the product obtained by pro-
longed trituration in a sterile mortar of cultures of C. immitis,
Argentine strain No. 695, after incubation for 20 days at
28° C in agar broth.

Five guinea pigs in each group were subjected to sub-
cutaneous vaccination with a suspension of spores, in progress-
ively increasing concentration, in a physiological solution
with 0.3% carbolic acid added, heated in a water bath 30 minutes
at 65° C. Sterility controls made it possible for us to verify
the fact that this temperature destroys the vitality of the
"entospores" of the cultures. Four series of this vaccine were
prepared, of which the most concentrated corresponded in
opacity to tube No. 3 of McFarland's scale and each of the others
was prepared by dilution at 1/10. Each guinea pig was given 3
weekly injections of 0.5 ml during one month beginning nine days
after the infecting inoculation.

The guinea pigs of group D were subjected to vaccination
without previous infecting inoculation.

The study of cutaneous allergy was made by intradermic
injection of polyvalent coccidioidin prepared from Argentine
and US strains of C. immitis according to the technique recom-
manded by C.E. Smith and "standardized" with the coccidioidin
type (batch 29-31) kindly supplied by him.

It was decided to use the testicle in evaluating the
reactions to a second inoculation because that organ, as has
been demonstrated by E.R. Long (9), reacts intensely to tuber-
cular infection, to which coccidioidomicotic infection has
much similarity, and also with the object of getting uniformity
in the literature. The material used in reinoculations con-
sisted in a suspension of "entospores" in physiological solu-
tion, the opacity of which corresponded to that of No. 3 of
McFarland's scale. This suspension was divided into two frac-
tions: a) heated in a water bath at 65° C for 30 minutes to
destroy its vitality, and b) unheated. The guinea pigs were
inoculated with 0.2 ml and were killed after 18-20 hours and
after 5 days, with the object of observing tissue reactions
that might afford us an index of allergy and immunity.

Finally, group E received only inoculation via the
testicle with live and dead material. Groups D and E, then,
took as controls, the former for the variant introduced
into our experiments and the latter to appraise the reaction
to testicular inoculation with "entospores" in an organism
previously uninfected.

For the reactions of fixation of the complement we used
two distinct antigens. The first consisted in an emulsion of
the lipides extracted from the mycelium and the second in coc-
cidioidin, both conveniently diluted. In view of the negative
results obtained with the first, we continued our studies using coccidioidin exclusively for these reactions. This product was also used in the precipitin reactions, and for agglutination reactions we used a suspension of "entospores" whose vitality had been destroyed by heating.

**Results.** The intradermal reaction with pure coccidioidin gave positive results ++ at the end of one week of infection and +0+ with coccidioidin diluted to 1/10. These results were accentuated in intensity in tests made a week later.

Serological tests made on four guinea pigs that were infected and four others that were infected and vaccinated, all of group A, at the end of a month gave negative results. The same results were obtained with two guinea pigs of group B at the end of 20 days after infection and two of group C after 17 days.

Serological reactions repeated at the end of three months after infection with three guinea pigs from group A and one from group B showed intensely positive fixation of the complement, but negative precipitin and agglutination reactions.

In guinea pigs inoculated with culture material in other experiments nine months to a year before and still surviving, we got intensely positive results in the complement fixation reaction from three of ten animals. The agglutination and precipitin reactions were always negative.

The results of the tissue reactions are assembled in the accompanying tables, which also contain notes of the modifications that the parasite undergoes in microscopic appearance, number, and size.

Translator's note: The tables in the source cover a two-page spread and cannot be readily reproduced here. For convenience, all the material contained in the tables is given at this point in paragraph form.

**Group A. Guinea Pigs Infected Subcutaneously and Reinoculated Via the Testicle**


A789. Reinoculated with live material. Killed after


M4. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle of larger size and congested; granulations in the spleen. Histological lesions: nodular productive lesions and exudative lesions. Dropsical degeneration of the cells of the covering of the seminiferous tubules.


F25. Reinoculated with live material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 45 days. Macroscopic lesions: testicle enlarged in size and congested;

M5. Reinoculated with live material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size and congested; hypertrophy of ganglion. Histological lesions: lesions with productive predominance. Rare parasites, of medium size and with endospores.

Group B. Guinea Pigs Infected Intramuscularly and Reinoculated Via the Testicle


M11. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days.

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Group C. Guinea Pigs Infected Intraperitoneally and Reinoculated in the Testicle


A786. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated at the end of 20 days. Macroscopic lesions: testicle hemorrhagic, caseosis at the point of the infection; ganglion hypertrophied; nodules in lungs. Histological lesions: testicle: encysted granuloma with productive and exudative reaction, the former predominating. No parasites observed.


F97. Reinoculated with dead material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle enlarged in size, with adhesions and caseum; abscesses in liver, spleen, and peritoneum. Histological lesions: specific interstitial orchitis with predomiance of fibrous reaction. Epididymus: productive and exudative lesions containing parasites of medium size with radiate formations and others with polyhedral endospores.


F95. Reinoculated with live material. Killed after 5 days. Not vaccinated; reinoculated at the end of 43 days. Macroscopic lesions: testicle atrophied and caseated. Histological lesions: specific interstitial orchitis with predominance of fibrous reaction. Epididymus: productive and exudative lesions containing parasites of medium size with radiate formations and others with polyhedral endospores.


F90. Reinoculated with live material. Killed after 18-20 hours. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle unchanged. Histological lesions:
necrotic exudative lesions and interstitial orchitis without parasites. Ganglion: few parasites, of medium size and without endospores.

F91. Reinoculated with live material. Killed after 5 days. Vaccinated and reinoculated at the end of 43 days. Macroscopic lesions: testicle atrophied and casated. Histological lesions: exudative lesion with few parasites, one with polyhedral endospores.

Group D. Uninfected Guinea Pigs, Vaccinated in Various Ways, and Inoculated in the Testicle


R27. Inoculated with live material. Killed after 5 days. Vaccinated subcutaneously. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: nodular interstitial infiltration of monocytes and a great number of polynuclears; hemorrhagic foci with a great number of parasites in the infecting phase.


R29. Inoculated with live material. Killed after 5 days. Vaccinated intramuscularly. Macroscopic lesions: testicle enlarged in volume and congested. Histological lesions: nodular interstitial infiltration of monocytes and a great number of polynuclears; hemorrhagic foci with a great number of parasites in the infecting stage. Young and adult parasites almost all infectious sporangia, only one with cystic spores.


R31. Inoculated with live material. Killed after 5 days. Vaccinated intraperitoneally. Macroscopic lesions: testicle enlarged in volume and congested. Histological lesions: nodular interstitial infiltration of monocytes and a great number of polymorphonuclears; hemorrhagic foci with a great number of parasites in the infecting phase.

Group E. Uninfected Guinea Pigs Inoculated Via the Testicle


L41. Inoculated with live material. Killed after 5 days. Macroscopic lesions: testicle enlarged in size and congested. Histological lesions: nodular productive lesions alternating with exudative ones; a great number of eosinophiles and of parasites in the infecting phase.

Discussion. The experimental study we have just described enables us to confirm the facts scientifically discovered by Posadas in 1892 and interpreted in the light of our present knowledge of allergy and immunity by the Italian school — Cavallero (10,11), Redaelli (12), and Redaelli and Ciferri (13).

C. Cavallero (1941) in his studies of allergy and immunity in mycoses called attention to the fact that in Gilchrist's disease (the blastomycosis of US authors) the primary cutaneous lesion is of the granulomatous type, while the secondary localizations via hematogen lead to rapid suppuration and then a tendency to spontaneous cure, which the author likens to Koch's phenomenon. Farnia (cited by Cavallero) reproduced these facts experimentally and observed that the reinoculated germ disappeared after the sixth day. The reinoculated material sets up
a cellular reaction which passes through the following two
phases: 1) a reactive phase of granulocytic character and
2) a resolving phase of granulomatous aspect with giant cells.

C. Cavallero (1943) encounters identity of coccidioidomy-
cosis with tubercular infection, as had already been noted by
Posadas and later in the US by C.E. Smith, Jacobson, Chipman,
and Templeton. In coccidioidomycosis, besides the chronic form
in which a granulomatous reaction similar to tuberculosis is
observed, there exist the acute forms discovered by Gifford
and Dickson (1936-1937) in which exudative-vascular phenomena
predominate.

Redaelli and Ciferri (1942) described the reaction of
the host to the material inoculated in experimental coccidioido-
mycosis in the following terms: a reaction of non-specific
character is first produced, which tends to eliminate the
foreign matter by digestion, and in which polynuclears, mono-
cytic elements, and non-specific histocytic cells appear.
There are elements of the germ that resist, "anamalize," mul-
tiply, and diffuse the infection. Then there begins a reaction
of the conjunctive vascular tissue and particularly of the
local reticuloendothelial system, which is characteristic of
mycotic granuloma, the specificity of which is shown solely by
the existence of a particular parasite, in this case C. immitis.

Hyperreceptivity and allergy put a particular stamp on
the histogenic reactions of the animal infected. In hyperre-
ceptive individuals, in a state of negative allergy, an exuda-
tive vascular reaction is observed followed by a series of
regressive processes finally becoming necrotic and hemorrhagic,
while in hyperresistant, hyperergic animals we observe the
prevalence of hyperplastic reactive activity with the formation
of granulomata with epithelial and giant cells and with a great
quantity of plasmacellular and lymphoid elements arranged in
the periphery, accompanied by a succession, varying in extent,
of the fibroblastic series.

According to our studies intratesticular inoculation of
material from cultures of C. immitis into a guinea pig not
previously infected with coccidioidomycosis brings on in 18-20
hours a granulocytic and monocyctic non-specific reaction which
tends to eliminate the inoculated material as a foreign body;
this is completely accomplished by the end of five days when
material killed by heat has been inoculated. When on the other
hand living material has been inoculated, the spores of the
germ that have resisted this first non-specific defensive reac-
tion evolve into the infectious phase, forming sporangia with
a very fine peridial membrane which attain a diameter up to
83.5 μ and contain numerous very small polyhedral endospores
(Posadas's granulose forms). The histogenic reaction is
exudative-productive with abundant eosinophiles, as observed in
spontaneous infection in man (Wickoff and Usighi, cited by C.E. Smith, 1943).

Intratesticular reinoculation performed 26 to 43 hours after the first infecting inoculation gives rise after 18–20 hours to a reaction characterized by congestive-hemorrhagic phenomena and in some cases a necrotic exudative-productive reaction, which disappears by the end of five days when dead material has been inoculated but leads to a predominance of the productive reaction with formation of a granuloma when live material has been injected. This histogenic reaction, indicating a certain degree of immunity, is manifested by an almost complete disappearance of the parasites, and when they persist they appear in modified morphological and biological form. In fact, the parasites are medium to small, some 30 μ in diameter, without endospores and with the thick membrane frequently surrounded by an acidophilous areole or by radiate or claviform formations of the same staining properties.
Summary. Inoculation of material from cultures of Coccioidoides immitis in the guinea pig in various ways leads to a state of hypersensitivity and partial immunity. The cutaneous hypersensitivity is quite evident on the seventh day and is accentuated on subsequent days.

Reinoculation with culture material killed by heat provokes at 18 to 20 hours an intense congestive-hemorrhagic reaction and histologically discernible exudative-necrotic phenomena which disappear toward the fifth day. Reinoculation with living material, on the other hand, induces productive phenomena which lead to the total or partial disappearance of the material injected, depending on the degree of immunity existing. This reaction, similar to Koch's phenomenon, also follows the biological law established by Levendowsky and Jadassohn in the case of leprosy.

In a guinea pig not previously infected, 18-20 hours after inoculation we observe a non-specific granulocytic and monocytic reaction which leads to total elimination of dead material by the fifth day. After inoculation with live material, on the other hand, the endospores that have resisted this first tissue reaction evolve toward the formation of primary infection sporangia, which are characterized by their enormous size (up to 83.5 μ in diameter), their fine membrane, and their content of granulose endospores without any apparent membrane of their own.

Vaccination with material killed by heat does not create cutaneous or tissual hypersensitivity, nor does it seem to modify the course and the reactivity of the organism in the face of a new inoculation with either living or dead material. Neither have we observed any variations due to the manner of the infecting inoculation (i.e. subcutaneous, intramuscular, intraperitoneal).

The parasite which develops within an organism in a state of allergy and partial immunity exhibits the following characteristics: its volume is generally less, the production of endospores is notably restricted, and when such production does take place it leads to the production of cystic endospores (Posadas's vegetative or cystic phase). The peridial membrane is always thick and frequently surrounded with an acidophilous areole or with radiate or claviform formations of the same staining properties.
Figure 1. Sporangia with granulose endospores.

Figure 2. Sporangium with granulose endospores, in dehiscence.
NOT REPRODUCIBLE

Figure 3. Sporangium with cystic endospores.

Figure 4. Empty sporangium invaded by leucocytes.
Figure 5. Cystic parasite with radiate formations.

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Bibliography


