VI CONGRESSO INTERNAZIONALE DI MICROBIOLOGIA

ROMA 6-12 SETTEMBRE 1953

Segretario Gen.: E. BIOCCA *Presidente: V. PUNTONI*

ATTI
DEL VI CONGRESSO INTERNAZIONALE DI MICROBIOLOGIA

VOLUME VI

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SEZIONE XVII

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La pubblicazione degli Atti del VI Congresso internazionale di microbiologia ha presentato alcune serie difficoltà di ordine teorico e di ordine pratico, dovute alla complessità del Congresso stesso ed al numero molto elevato delle comunicazioni e delle discussioni.

A lato di contributi pregevolissimi sono stati inviati i testi di comunicazioni che non sarebbero degni di comparire accanto ai primi se non fosse prevalso il concetto della massima liberalità.

Tutti i lavori, seguiti dalle relative discussioni, sono stati inviati ai Presidenti di Sezione per la loro approvazione; il Comitato di redazione si è limitato a richiamare l'attenzione dei Presidenti su alcuni contributi poco raccomandabili, al fine di controllare se essi corrispondessero alle comunicazioni esposte in sede di Congresso.

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La maggior difficoltà incontrata dal Comitato nella redazione è stata rappresentata dalla raccolta e dalla pubblicazione delle discussioni; alcuni fogli manoscritti sono stati decifrati con molto sforzo ed il Comitato si scusa di eventuali inesattezze perchè, ad evitare notevoli ritardi della stampa, non è stato possibile inviare le bozze agli oratori intervenuti nelle discussioni.

Gli Atti del Congresso sono stati riuniti in 7 volumi, raggruppando in ciascuno di essi le Sezioni nella forma più razionale, a giudizio del Comitato di redazione, ed obbedendo ad esigenze editoriali.

V. Puntoni, *Presidente*

E. Biocca, *Segretario Gen.*

ATTI

DEL

VI CONGRESSO INTERNAZIONALE DI MICROBIOLOGIA

SEZIONE XVII A

MICROBIOLOGIA APPLICATA ALLA PATOLOGIA UMANA E SPERIMENTALE

MICROBIOLOGIE APPLIQUE A LA PATHOLOGIE HUMAINE ET EXPERIMENTALE

MICROBIOLOGY APPLIED TO HUMAN AND EXPERIMENTAL PATHOLOGY

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I.

MICROBIOLOGY OF CANCER

NEOPLASTIC INFECTION IN MAN AND ANIMALS

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(Cancer Research Laboratory, Newark, N. J., U. S. A.)

The degenerative, proliferative, collagen diseases of man and animals have yielded by bacteriologic methods, a group of pleomorphic, filtrable microorganisms probably belonging to the mycobacteria, a subdivision of the actinomycetales. These microorganisms are pathogenic for men and animals. The bacteriologic nature, the cultural properties and the pathology produced in animals as well as the results obtained by the study and use of specific antisera will be dealt with in a series of papers presented by other members of this group. The purpose of this paper is to relate the pleomorphic phases of this group of microorganisms to the pleomorphic group of collagen diseases present in man and animals.

The pathologic and clinical responses in the host to the pyogenic microorganisms are well konwn. The discovery of the spirocheta-pallida related the human etiology of syphilis to a wide spectrum of pathologic manifestations called by many names prior to the recognition of the specific agent. The same is true of Koch's bacillus. Before the identification of the tubercle bacillus, the clinical manifestations of tuberculosis were called by many names such as Pott's disease, scrofula, lupus, phthisis, depending upon the local tissue reactions. So it is with the widespread spectrum of diseases of the collagen group characterized by proliferation, fibrosis and degeneration of various degrees capable of involving any one or many of the host tissues whether brain, muscle, skin, spleen, mucous membrane, bone, gland, bone marrow, or connective tissue. The site of predilection, the degree of involvement or resistance depend primarily upon host properties such as genetic susceptibility, hormonal balance, and degree of immunity as well as upon biochemical and mechanical factors not entirely understood or demonstrated at this time.

The life span of man has greatly increased due to the victory of medicine over the common infections which are now preventable by immunization or amenable to treatment by antibiotics or antisera. But now the latter part of mans's life is made uncertain by many degenerative and proliferative diseases such as the various

kinds of cancer, scleoderma, sarcoidosis, Raynaud's and Berger's disease, arthritis and other members of the collagen group. The evidence for the specific microbial concept of the etiology of these widely described pleomorphic but fundamentally related diseases will now be presented.

The present speaker (VWC) first noted and published in 1947 the close similarity between the lesions of scleroderma (generalized systemic sclerosis) to leprosy — the ulcerations of the fingers and nasal septum, the anesthetic areas, the nodulations and hardness of the skin, the proliferative changes in joints as well as in soft tissue such as in lung, kidney, and liver, pointed to a possible microbial agent related to the mycobacteria. Smears and biopsies of the lesions showed a pleomorphic acid-fast microorganism, later confirmed by Alexander-Jackson and Wuerthele-Caspe by cultural methods.

Experimental animals inoculated with the cultures derived from scleroderma revealed proliferative lesions bordering on the neoplastic. This observation led VWC to study smears of neoplastic tissues using the acid-fast stains including the triple stain of Alexander-Jackson. Confirmation of these early results was obtained by later studies using many technics applied to isolated cultures, pathologic sections and tissue cultures. Many stains were used such as the Ziehl-Neelsen, the triple stain of Alexander-Jackson, the McManus, the Fite, the Supra-Vital (Toludin blue) and the tri-basic stain of Coidan as well as those stains demonstrating the phenomenon of bacterial fluorescence.

Although the most easily recognized forms are acid-fast, there are many non-acid-fast forms which are the same organisms but are not readily distinguished from common contaminants. Therefore, it was essential that a pure culture of the specific microorganisms be obtained. The first pure culture which recognized this microorganism as a mycobacterium was made by Wuerthele-Caspe in the summer of 1948. A series of Petragnani tubes were inoculated with material from living cancer tissue. Many were contaminated but one tube after six months after inoculation yielded a pure culture. Once the first culture was found the search for easier and more prolific cultivation went on with the cooperation and direction of Dr. Eleanor Alexander-Jackson. It was a time-consuming, back-breaking task requiring a number of years for fulfillment before sufficient amounts of culture could be obtained for animal inoculation. Prior to obtaining sufficient material by direct culture, the blood of terminal cancer patients was inoculated into the allantoic sac of ten day old chick embryos. A rapid destructive growth of the microorganisms in the chick embryo occurred in a matter of hours.

The search then began to find these bodies in the various wellknown neoplasms of animals known to be infectious in nature. Mr. Paul Little of the Lederle Laboratories collaborating with our group made a great number of cultures from the Rous Sarcoma on various media. Lesions could be produced with these cultures by serial passage through chick embryos. The chemically induced tumors of the

rabbit epithelium of Suguira of Sloan-Kettering Institute of New York as well as the sarcomas of the white rat of Margaret Lewis at the Wistar Institute, Philadelphia showed the same bodies on appropriately stained sections. Cultures made from material containing the Bittner factor as well as from Shope's rabbit papilloma yielded similar results. Since these various agents are known to be filtrable through the Seitz filter, the cultures were examined and found to be filtrable in all stages of growth. Then began a series of electron microscope studies with Dr. James Hillier of the RCA Victor Laboratories which showed the range in size of these bacteriological bodies. From these studies it became evident that a widely distributed group of microorganisms of a specific mycobacterial nature is etiologically involved in the production of a large group of proliferative and neoplastic diseases in animal and man.

In order to facilitate the recognition of these mycobacteria in tissues, it is necessary to review their pleomorphic forms. They vary from minute acid-fast intracellular and intranuclear bodies to larger globoid bodies which may enlarge and contain within them many minute granules. The freely growing form of the culture is a flagellated motile rod which on aging converts to a mycelial network. All these forms are demonstrable in tissue. Dr. Irene Corey Diller has done confirmatory work on this phase of culture.

Once an adequate amount of culture material was obtained, the studies in experimental animals began. The blood culture of a 26 year old young woman (BK) with widespread metastases to bone was inoculated into a series of 12 newly weaned Swiss heterozygous white mice. Of these, four died within six weeks of the lytic form of a neoplastic infection. The organs were soft, lyzed and showed widespread specific mycobacterial invasion. Four mice survived approximately three months. These all showed caseous lesions. The four remaining mice did not appear invaded but of these, two apparently in good condition dropped dead suddenly and two gradually became emaciated but survived a number of months These results correspond to Duran-Reynals figures in chicks infected with Rous Sarcoma — one third showing no resistance, with rapid invasion and death without tumor formation; one third showing partial resistance with tumor formation and one third showing almost complete immunity. We found, even in the latter group exhibiting high immunity, that there were many tissue changes of a chronic nature other than neoplasia. These results will shortly be discussed at greater length.

It was decided to dissect out a caseous lesion and trocar it into a series of mice in the same way as Sarcoma 180 tumor fragment is done. Twenty serial passages consisting of sixteen to eighteen mice in each passage were so treated. There were of course individual variations in the reactions of the mice — the first two or three passages showing fewer deaths at the end of one week but by the end of the 7th passage all mice so trocared died within seven days. When the tumor mass from these was passed, by the fifth to seventh day after implanation,

95% of the animals died. Passage from an animal surviving relatively longer led to a less invasive type of lesion. Here again immunity plays a vital role. These results coincided quite closely to those found in serial implantation of Sarcoma 180 in mice. However, the implants done by us gave only 10% incidence of gross neoplasm over all in spite of 95% death due to agranulomatous invasive lesion when rapid transplantation was done. However, focal atypical cells could be seen in almost every animal. The percentage of gross tumors is of relative unimportance in view of the wide manifestations of disease in these animals.

At this point, I wish to stress the fact that occurrence of gross neoplasia in the neoplastic disease is of no more importance relatively to the disease in general than the occurrence of a gumma in syphilis. It is an obvious manifestation but relatively only small evidence of the disease as a whole.

There were 320 mice injected serially with tumor masses originating from the initial inoculation of (BK) human strain of organism. Ten hundred sixty nine sections were examined by H & E and Ziehl Neelsen stains at 1500 magnification using an intense beam of light. For convenience and brevity a representative sampling of results obtained in the serial passages are given:

K1 50-134 Caseous lesions, neoplastic cells in small microscopic foci throughout many tissues. Spleen neocrotic with many areas of infiltration. A granulomatous mass was present in the testis.

K3 50-164 Site of inoculation shows massive growth of the microorganism with necrosis. Neoplastic cells in lung singly and in foci. Widespread growth of microorganisms in kidney.

50-193 Another animal in this group showed adenocarcinoma of the large intestine.

K4 50-196 Globoid bodies seen in connective tissues of almost every organ. Areas of degeneration of cardiac muscle with many of the specific bacteria present. Spleen shows widespread necrosis and massive growth with nuclear changes in the liver cells.

K5 50-188 This one was dead four days following implantation. Muscle fiber of abdominal wall invaded and largely destroyed at site of inoculation, marked invasion of heart muscle, large bacterial foci in liver and kidney. Areas in lung are neoplastic.

K6 50-192 Solid growth in lung. Growth of trocared mass extends directly into liver. Massive involvement of heart muscle with fibroblasts replacing large areas of destroyed heart muscle. Selective destruction of Isles of Langerhans. This animal would probably be diabetic.

K10 50-288 Peribronchial changes, pleurisy with effusion, carcinoma of the skin.

K10 50-287 Animal killed after fifth day. Site of inoculation shows high degree of resistance. Many macrophages engulfing globoid bodies. Little general tissue changes. Other organisms relatively normal. This type of growth coincides with the observations of Michael Levine as to the role of the reticulo-endothelial system in resistance. Where there is a high activity of the macrophages at the site of inoculation of tumor fragments from Rous Sarcoma, the tumor has been observed to regress.

K11 50-265 Infiltrated areas replacing one lung. Adhesive pericarditis, perihepatitis.

It can be seen by this sampling process what the wide range of tissue changes are. A human example of the wide panorama of disease possible when this microorganism is present in the blood stream and tissues is demonstrated by the case J. S. of Dr. Charles Crane of Newark, N. J. He had us see this white female because he thought the lesions would be of interest to us. Her blood stream readily yielded the specific culture. She had hard induration of the skin of the anterior chest wall and neck resembling scleroderma, widespread fibrosis of the lungs, enlarged liver, enlarged spleen, rheumatic heart disease.

The diagnoses were as follows:

cor-pulmonale
hypertrophy of right ventricle
mitral stenosis, rheumatic
possibly anemia
generalized malignant lymphoma
splenomegaly
hepatomegaly

sarcoidosis of the lungs, spleen, liver and lymph nodes
amyloidosis of the spleen, kidneys and lymph nodes
rheumatic mitral valvulitis
bronchiectasis
purulent bronchitis.

Recently Spoerci Coidan of Harlem Hospital has reported diagnosis and grading of human neoplasm by 24 hour tissue cultures. We have examined her tissue explants stained by her tribasic stain as well as by the triple stain of Alexander-Jackson and Ziehl-Neelsen. These segments are all positive by direct examination. It is of interest to observe that in the tissue explant of a human axillary metastatic node from carcinoma of the breast there are many microscopic foci consisting of one or two to several neoplastic cells arising around a small and invasive site where the specific mycobacterium can be clearly visualized. The grading of the

tumor as to degree of malignancy and the prognosis of the patient can be determined by the tissue culture growth of the explanted sample of the neoplasm surgically removed. A tissue culture in which there is rapid proliferation of the involved cells indicates neoplasia but good resistance and a good prognosis. An explant showing poor proliferation with liquefaction and lysis of the cells shows poor resistance and poor prognosis.

In conclusion the following statements are made:

1. A specific class of microorganism apparently belonging to the mycobacterium has been observed and is grown from the cancerous blood and tissues of man and animals.

2. They are filtrable and pleomorphic.

3. Inoculations of these microorganisms reproduce the specific disease in experimental animals thus fulfilling Koch's postulates. This group of microorganisms is associated immunologically with a wide range of physical and clinical manifestations in both animals and man, these manifestations including not only various diseases of neoplasia but also a wide range of other diseases such as scleroderma, interstitial myocarditis, perivascular infiltration, interstitial fibrosis, pleurisy, pericarditis, rheumatic fever, nephritis, hepatitis and arthritis depending upon the degree of host resistance, the invasiveness of the specific microorganisms, the strain specificity, and the site of tissue susceptibility.

Wuerthele-Caspe, V., Alexander-Jackson, E., Anderson, J. A., Hillier, J., Allen, R. M., and Smith, L. W., Cultural properties and pathogenicity of certain microorganisms obtained from various proliferative and neoplastic diseases, Am. J. M. Sc. 220:638-646, Dec. 1950.

Alexander-Jackson, E., Differential Triple stain for demonstrating and studying non-acid-fast forms of tubercle bacillus in sputum, tissue and body fluids, Science 99:307-308, April 14, 1944.

Wuerthele-Caspe, V., Mycobacterial forms observed in tumors, J. Am. M. Women's A. 4: 135-141, April 1949.

Alexander-Jackson, E., Hitherto undemonstrated zoogleal forms of mycobacterium tuberculosis, Ann. New York Acad. Sc. 46: 127-151, June 29, 1945.

Wuerthele-Caspe, V., and Allen, R. M., Microorganisms associated with neoplasms, New York Micr. Soc. Bull. 2: 2-31, Aug. 1948.

Von Brehmer, W., « Siphonospora polymorpha » n. sp. ein neuer Mikroorganismus des Blutes und seine Beziehung zur Tumorgenese, Med. Welt 8: 1179-1185, Aug. 25, 1934.

Villequez, E. J., Isolement de micro-organismes des tissus hyperplastiques et du sang des cancereux, Concours Méd. 74: 661-664, Feb. 23, 1952.

Glover, T. J., Bacteriology of cancer, Canada Lancet & Pract. 74: 92-111, March 1930.

Glover, T. J., Scott, M. J., Loudon, J., and McCormack, J. M., Study of Rous chicken sarcoma No. 1, Canada Lancet & Pract. 66: 49-62, No. 2, Feb. 1926.

Glover T. J., and Engle, J. L., Production of malignant growth in guinea pig, Pub. Health Rep. 48: 319-321, March 31, 1933.

Stearn, E. W., Sturdivant, B. F., and Stearn, A. E., Life history of microparasite isolated from carcinomatous growths, Proc. Nat. Acad. Sc. 11: 662-669, Sept. 1925.

Nuzum, J. W., Experimental production of metastasizing carcinoma in breast of dog and primary epithelioma in man by repeated inoculation of micrococcus isolated from human breast cancer, Surg. Gynec. Obst. 11: 343-352, Oct. 1924.

Diller, I. C., Personal communication.

L'Esperance, E. S., Studies Hodgkin's disease, Ann. Surg. 93: 162-168, Jan. 1931.

Smith, L. W., Wuerthele-Caspe, V., and Alexander Jackson, E., Malignant changes induced in experimental animals by a Mycobacterium derived from both human and animal tumors, Cancer Research 12: 298, April 1952.

Spoerci-Coidan R. 1948. A tribosic dye stain for nerve cells, Stain Tech. 23: 133-135.

Milford, J. J., and Duran-Reynals, F., Growth of chicken sarcoma virus in chick embryo in absence of neoplasia, Cancer Research 3: 578-584, Sept. 1943.

Diller, I. C., Cytological Studies of Relationships Between Microorganisms Isolated from Tumor Tissue and Normal and Malignant Cells of Host, Cancer Research, 12: 257, April 1952.

Wuerthele-Caspe, Brodkin, Mermod - Etiology of Scleroderma, A Preliminary Clinical Report, Journal of the Medical Society of New Jersey, Vol. 44, No. 7, July 1947.

DISCUSSIONE

(Emanuel Agius, 4, Victoria Avenue, Sliema, Malta).

The question was asked as to the number of cases from which the micro-organism described had been isolated.

(Risposta dell'Autrice).

Over 500 tumor cases (tumors and cancerous blood). Rous Sarcoma: 100.
Mixed tumors of animals: at least 100.

2.

A SPECIFIC MICROORGANISM ISOLATED FROM ANIMAL AND HUMAN CANCER: BACTERIOLOGY OF THE ORGANISM (*)

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A specific microorganism has been observed, isolated, and cultivated consistently from every one of hundreds of specimens obtained from both animal and human cancer of various types over a five year period. It has a polymorphic developmental pattern which a number of others (1-9) in various parts of the world have observed wholly or in part. Our chief contributions to the bacteriologic study of this organism are (a) the recognition of its potential acid-fast properties by Wuerthele-Caspé (10-13), and (b) the demonstration by Alexander-Jackson (14-16) of the similarity of its developmental morphology to that of the mycobacteria of tuberculosis and leprosy which she had studied closely for more than fifteen years. These contributions towards an understanding of this pleomorphic mycobacterium-like organism which we have tentatively called *Mycobacterium tumefaciens*, were made possible by utilizing easily repeated stain technics and methods of cultivation which have not heretofore been applied in cancer research.

The outstanding characteristics of the cancer organism apply to similar acid-fast microorganisms which we have isolated from various other proliferative diseases of obscure etiology, including Hodgkin's disease, scleroderma, sarcoid, arthritis, and Wilson's disease (hepatoventricular degeneration). An experiment was carried out with blood from scleroderma patients involving the intraperitoneal inoculation of 30 white mice and 30 controls. During the last three years 621 other animals, including mice, guinea pigs, and chickens, were inoculated with isolated pure cultures. A number of chick embryos were also infected by inoculation of cultures in the chorio-allantoic membrane. The invasive, slow-working pathogenicity and type of pathology produced by the cultures from scleroderma and the other afflictions mentioned above, suggest that this type of microorganism may play a major role in a variety of chronic proliferative diseases. The organisms in tissue and the pathology produced are discussed by Wuerthele-Caspé and L. W. Smith.

The following characteristics are important in the identification of these microorganisms:

(*) Published in part in « Growth », Vol. XVIII, 1954, pp. 37-51.

1) Acid-fast staining properties — especially in tissues.

2) Ability of the smaller forms to enter the cytoplasm and nucleus of host cells.

3) Filterability of the smallest forms through Seitz or fritted glass filters.

4) Large cyst forms within which develop acid-fast granules and rods.

5) Failure to grow on ordinary media, and scant growth of primary isolates, which in from five days to two weeks form a granular mat at the bottom of liquid media, which on staining and in hanging drops reveal many pleuropneumonia-like symplasms.

6) Development at times in necrotic tissue or in special media of highly motile rods which form a pellicle that sinks readily to the bottom of the tube or flask.

7) Ability of the motile rods, when once established, to grow on ordinary media, and even at room temperature.

8) Resistance of motile rods to (a) temperature — 90 degrees Centigrade for 14 minutes — and (b) penicillin — 0.1 ml of culture grew out in the presence of 1,000 units of penicillin per ml.

9) Ability of Seitz filtrates from motile rod cultures to redevelop the rods after some weeks of incubation.

10) Pathogenicity of both motile and non-motile cultures for animals. Death occurred in 24 hours when white mice received 0.2 ml and guinea pigs received 0.5 ml intraperitoneally of motile rods. Survivors develop a chronic systemic disease with granulomatous lesions, giving rise at times, in the case of the cancer organism, to true neoplosms.

11) Gram stain variability.

12) Presence in motile rods of colorless areas similar to those observed by some in rods and filaments of tubercle bacilli by Brieger *et al.*

13) Development in older cultures of mycelium-like filaments with rudimentary branching and budding.

14) Fluorescence as shown by Gerlach and Diller.

MEDIA

The principal media employed over a period of five years include Difco's brain-heart broth with and without glycerin, Dubos medium, Alexander-Jackson's modification of von Szabocky's glycerol lung broth, dextrose blood agar, Alexander-Jackson's adaptation of Bushnell's poi agar, Petragnani, Löwenstein-Jensen, and Dorset egg media, and Wuerthele-Caspé's chick embryo agar. The embryonated egg was utilized in early work, and in the first successful efforts to induce motile rods

to develop in non-motile cultures isolated from patients' blood. The living chick embryo proved an excellent medium. However, since approximately 10% of control embryonated eggs contained mycobacterial forms, this medium is not one of choice for work involving mycobacteria or other acid-fast organisms. Growth on Sabouraud's medium of non-motile isolates was scant or negative. Our best results have been obtained with Alexander-Jackson's broth, and recently, with Wuerthele-Caspé's autoclaved chick embryo agar. The method of preparation of these two media is given below.

Alexander-Jackson - von Szabocky Broth.

Ingredients:

water: 2,000 ml;
beef lung, cut up: 2 pounds;
peptones: 20 grams; 5 grams each of:
(a) myosate; (b) gelysate; (c) trypticase; (d) phytone [obtained from Baltimore Biological Laboratory, Inc., 1640 Gorsuch Ave., Baltimore 18, Md.];
glucose: 10 grams;
glycerol: 80 ml.

Boil the beef lung and water for 30 minutes. Filter through cotton or very coarse paper into a flask containing the other ingredients, and heat to dissolve. This crude lung broth can be autoclaved and stored in the icebox, and clarified subsequently. Autoclaving for a second time does not seem to produce any adverse effects.

Clarification:

A 1- to 2-mm layer of infusorial earth (Standard Filter Cel of Johns-Manville Co.) is deposited on a No. 42 Whatman paper disc by laying the disc on a Buchner funnel, applying suction, and then carefully pouring on about 500 ml of a 5 per cent suspension of Filter Cel. After the deposition of the layer, when the water goes through clear, the suction flask is well rinsed out. The hot medium can now be filtered through the prepared disc into the flask.

The medium should be filtered a second time through a Buchner-type funnel with a fine fritted glass disc, or else passed once more through the same Filter Cel.

pH Adjustment:

The pH of the medium should be adjusted to 7.3-7.4 with sodium hydroxide. The medium is then tubed into screw-top glass tubes 150 × 25 mm (Kimble Glass Co., Toledo, Ohio). The tops of the tubes are not screwed tightly until after autoclaving.