

# Between parasitic theory and experimental oncology: a proposal for systematizing oncological science in Portugal, 1889-1945

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## Abstract

This article deals with the bio-medical investigation of cancer studies in Portugal between 1889 and 1945. By examining the main works produced between the end of the nineteenth century and the middle of the twentieth century, it has been possible to illuminate and define a field of scientific endeavour which has been the scope of little study to date. Starting from the introduction and consolidation of the defining principles of experimental oncology, distinct phases can be discerned in the production of scientific material, alternating between support for the dominant theories and the application of methods for artificially creating the disease. In accordance with the principal phases of investigation, a brief systematic overview of the scope of these oncological studies is presented.

Keywords: cancer; oncology; science; Portugal.

Thanks to the experimental and clinical studies carried out, there has been a great advance in knowledge in this field. ... showing that cancer is not an evil of mysterious origin, but an illness like any other, capable of being reproduced in laboratory animals by means of various agents, which were without doubt the same as those that, in human beings, constitute the conditions capable of provoking and defining the appearance of malignant neoplasms

(Athias, 1941, p.58).<sup>2</sup>

### **Cancer in medical literature in Portugal**

From the point of view of compilations of the history of science, the approach to oncological illnesses in Portugal is a challenge because of the lack of substantial historical studies in the field. In contrast to the numerous and detailed works which already exist with regard to other areas, particularly Europe and the USA (Olson, 1989; Costa, 2011), the amount of historical material produced on the topic of cancer in Portugal is still sparse. There are some publications of institutional and biographical character edited by Silveira Botelho, dealing with the doctor and father figure of Francisco Gentil and the establishment that he founded, the Instituto Português de Oncologia (IPO, Portuguese Institute of Oncology) (Botelho, 2000; Botelho, 1978). The rest are restricted to the origins of the regional centers of the IPO in Oporto and Coimbra (Pacheco, 2002; Portugal, 2003), and it is only very recently that a start has been made on studies of a more wide-ranging nature with regard to the establishment and acceptance of oncology as an emerging scientific discipline in the context of contemporary Portugal (Costa, 2010a).

ol:As far as the different stages through which the development of bio-medical knowledge passed, expressed in the form of articles and reports, there are no interpretive lines that have been proposed up to the present time. Whilst it is true that there have been various approaches which have systematized and described in broad outline the different stages in the development of theories on the origins and etiology of the disease (Darmon, 1993; Wolff, 1990; Triolo, 1965; Triolo, 1964), or scientific treatises based on geographical considerations and specific policies, such as those by Wolfgang Eckhart (2000) in the case of Germany or Robert Proctor (1999) with regard to the Nazi period, in the case of Portugal the categorization of the stages by which oncological research developed between the end of the nineteenth century and the middle of the twentieth century has not received the attention it deserves.

### **The battle against cancer and the reform of Portuguese scientific medicine**

Thanks to laboratory research, the many achievements in the field of cancer studies led to a situation where experimental study was highly thought of and valued, and this was work which in the inter-war period was highly productive. However, this progress was very little known to the general public, even to the more educated sections of it, bearing in

mind that it remained, for the most part, confined within the laboratory and scientific circles. Although the first forty years of the twentieth century witnessed profound changes in the understanding of cancer, these changes did not have a radically significant impact on the way in which it was treated. In the case of Portugal, as with the rest of the world, the greater attention given to the disease was much more associated with the employment of curative treatments and much less with the field of research.

As an illness of lesser significance in the medical field when compared with conditions of greater social impact (tuberculosis and syphilis), the weight that it was gradually assuming in medical discussions meant that it had an important place among the health challenges of the first quarter of the twentieth century, without however displacing tuberculosis from its primary position. The fight against cancer in Portugal had its institutional beginnings with the foundation of the IPO in 1927, but scientific writing on the disease had already started in the nineteenth century, based on the reform of medical training at universities, as well as the need to fill the gaps in the scientific research programs of Portuguese doctors (Costa, 2010b).

Since the university reforms of 1911, plans for overhauling the teaching of medicine in university courses had been mapped out. These plans were not always successful, but they led to an improvement in the quality of the teaching staff, which became more attuned to the production of proper science rather than reproducing the discoveries made in other countries. Although the results had not been immediate, particularly because the legislation of 1911 had shown itself to be somewhat ineffectual in changing the ethos of the teaching bodies (Costa, 1917a, 1917b), the intellectual environment was favorable to the furtherance of reforms. The ground had been prepared beforehand by the campaign in favor of experimental medicine, which had been developed particularly by Miguel Bombarda and Sousa Martins in the nineteenth century, at both an ideological and political level. In practical terms there had been a few steps forward, such as the foundation of the Câmara Pestana Bacteriological Institute in 1892 and the setting up of the Portuguese Society for the Natural Sciences in 1907 by Marck Athias, Celestino da Costa and Abel Salazar.

Meanwhile study trips which were becoming common assisted in the dissemination of information which had already been passed on by means of medical reviews and periodicals, thereby facilitating the direct interchange of ideas rather than mere book learning. In 1909 there was a study trip which was important for the development of practical experimentation: Pinto de Magalhães and Marck Athias, through the intervention of Miguel Bombarda, were commissioned by the government to make a three month visit to certain laboratories and manufacturers of laboratory materials abroad (Amaral, 2005, p.267).

Moreover, the doctors who devoted themselves to studying the emerging problem of cancer did not fail to avail themselves of shorter or longer periods of training abroad, whether as students or on training or refresher courses. From Francisco Gentil, the founder and moving spirit in the battle against cancer, to Henrique Parreira, Francisco Bénard Guedes, Luís Simões Raposo, Álvaro Rodrigues, Lima Basto and many others who made their career at the IPO, they all spent shorter or longer periods of time undergoing additional training abroad. Whether it was in the field of oncology or in related disciplines, this training was influenced by the academic and scientific changes taking place. Every student

of medicine, or anyone who aspired to be such, absorbed the new developments abroad and reproduced in Portugal what he had learned; in some cases he set up his own school, which became a lasting institution and even today carries echoes of this original pioneering spirit.

This was the case with the bacteriologist Câmara Pestana and the histophysiologist Marck Athias, whose training mirrored the methods of German and French universities. The former was to some extent self taught, the latter had studied at the University of Paris where he came under the influence of the professor of histology Mathias Duval (Alves, 1999). Besides these names, there was a whole generation which looked at the problems of medical education, health and treatment in new ways, and among whom, in addition to the preponderance of a very intense ethos of scientific research, the acquisition of original knowledge was an essential element in the structuring of university teaching, far more important than the capacity to memorize and reproduce knowledge which had been tailor made by the work of foreign institutions.

### **Parasite theories in oncological research**

From being a specific problem associated simply with the sphere of medicine, from the first decades of the twentieth century cancer passed into the field of public health and came to be regarded as a global scourge.<sup>3</sup>

As Bruno Latour (1984) has shown, up to a point cancer demonstrates characteristics which are very similar to those noted with regard to microbes in the nineteenth century. After the work of Louis Pasteur showed the infectious nature of many illnesses, such as anthrax, tuberculosis, typhoid fever, leprosy and others, thoughts also turned towards a microbiological origin for cancer. There was a reappearance of the old ideas of Sydenham and Van Switen with regard to transmission, and a resurgence of the so called parasite theories, also known as microbial or infectious. In fact, as soon as it was thought to be proved that tumors of a tubercular or syphilitic nature were of microbial origin, the idea immediately grew up that cancer could similarly be due to the presence and growth of some unknown micro-organism (Triolo, 1965).

The impact of Pasteur and micro-biology in Portuguese medical and scientific circles was enormous, determining and defining the microbiological scope of the work then being carried out on the parasitical nature of the disease. In their lectures, some of those engaged in the renewal of medical science, such as Miguel Bombarda or Sousa Martins, had no hesitation in placing Pasteur and his theories at the apex of the medical revolution of their time (Bombarda, 1895; Martins, 1895).

If the contagious hypothesis for cancer was a subject which seemed to have been dismissed since the experiments of Jean Louis Alibert in 1808 (Shimkin, 1975), the controversy surrounding the matter was reignited with the new experiments, thereby re-opening a chapter which had never been totally concluded with regard to the possible contagiousness of oncological diseases and their transplantable nature. Von Langenbeck revived this possibility in 1840, but it was in the 1880s that the idea of a parasitical theory of cancer really became fashionable in scientific circles. While in 1872 Gustave Nepveu postulated

the existence of *Micrococcus* in cancer of the skin, in 1866 Gustave Rappin succeeded in cultivating various strains of *Micrococcus* taken from 13 cancers, having found them in tissues excised from malignant neoplasms, but he did not succeed in provoking the disease through its injection into animals (Shimkin, 1977; Triolo, 1964).

Experimental work proliferated, the most important being that of Hermann Lebert, E. Follin, Weber, Goujon, V. Wehr, Arthur Hanau and E. Hahn in 1888 and 1889, which was preceded by the work of Domingos Freire and Ernst Scheurlen in 1887. All of them aimed to observe the development of tumors through the inoculation of animals with cancerous tissue or 'cancerous fluid', evidencing the results by means of the histological analysis of the different tumors which resulted, and of the micro-organisms found within them. In Brazil, the professor of organic chemistry and biology Domingos Freire<sup>4</sup> took the lead when he announced the discovery of a microbial agent as the cause of cancer, obtaining in 1887 very similar results to those which Ernst Scheurlen was to present during the course of the same year. Publishing his results earlier, he arrived at the same kind of conclusions as the German doctor, and priority was disputed at the same time by a series of other researchers.

In addition to the impact of this work in almost all the internationally consulted medical reviews, dissension arose as the consequence of being the first to discover the elusive and feverishly sought pathogenic agent. Shortly afterwards various claimants arose with regard to the discovery of the supposed cancer microbe, including Gustave Rappin, Barbacci, Giuseppe Sanarelli, M. Koubassof and Rubino. The question would leave its mark on the medical publications of the time, and lead to real quarrels with regard to scientific priority (Freire, 1888; O micróbio..., 1888).

Carried out under the banner of positivist medicine, the work of Domingos Freire sought to show experimentally the microbial origin of carcinomas. When observing the blood of a cancer patient, he noted various bacilli with rounded extremities, similar to those of typhoid fever. The same micro-organisms were detected in the 'cancerous fluid'. These observations were followed by others in the same year, carried out by other European researchers, who despite adhering to the parasitic theory of cancer, observed the same micro-organisms and in addition others, the latter being very different from the former in shape, but they did not stop trying to associate them with carcinoma in some way.

Despite the fact that Ernst Scheurlen had announced a causal relationship between this bacillus and cancer – having come to this conclusion from the discovery of the carcinoma microbe – the results were not accepted with equal alacrity by the world scientific community, which looked at the conclusions of both Scheurlen and Freire with considerable reserve.

The fallout from this type of experimentation was not long in arriving in Portugal, where it awakened medical interest particularly that connected with bacteriology and histology. These and other experiments published in the scientific periodicals met with an attentive and receptive audience, which was sufficiently critical to try and reproduce the published experiments and to test concepts, methods and emerging technologies.

The various theories on the origin of the disease were followed closely, particularly the parasitogenic theories, which found a large number of adherents, and it is permissible to argue that the area which awakened most interest among Portuguese researchers in the nineteenth century was that surrounding the experiments which tried to prove and/or

test the infectious nature of the disease. The stage of observation and classification of tumors, which is a feature of clinical and anatomical methodology, was followed by experimentation linked to the hypothetical microbial agents, a collateral element in biomedical sciences in the second half of the nineteenth century, and which did not differ from the others with regard to the study of cancer. If in the first stage it was not possible to discern significant contributions, a few works of reference were produced in the second stage, such as those of António Lencastre (1881) *A natureza do carcinoma encefalóide* (The nature of encephaloid carcinoma) and the inaugural thesis of Câmara Pestana (1889), *O micróbio do carcinoma* (The carcinoma microbe).

### **Câmara Pestana and *The carcinoma microbe***

The influence of Domingos Freire and other adherents to the theory of the existence of a microbial agent was one of the themes in the thesis of the young doctor Câmara Pestana in 1889. Concentrating on the possibilities opened up by the parasitic theory, he not only shared the concerns of the late nineteenth century, but he also reproduced the methods and techniques employed internationally at this level.

The dissertation entitled *The carcinoma microbe* (Pestana, 1889) is an epitome of experimental knowledge, based on a systematic examination of the parasitical theories which dominated a significant section of French and German medical schools in the last quarter of the nineteenth century. The question persisted: whether the cause was internal and encouraged by diathesis (a predisposition of the organism) or if there was some external micro-biological agent, still unidentified, which was capable of causing the onset of the disease. The doubts of the scientist reflect the duality of a scientific problem imposed by different currents of thought:

Accepting therefore the infectious nature of carcinoma, and as from the moment in which there is infection, there is something which grows and reproduces itself; I ask whether this something is an autochthonous microbe, an actual cell of the organism, which through an evolutionary aberration has transformed itself into a foreign and harmful element, grafting itself and reproducing everywhere, resulting in primary and secondary neoplasms, as Dr. António de Lencastre would have it, or whether, on the contrary, the microbe is heterochronous, a stranger to the organism which, through the constant irritation that it produces, gives rise to the tumor (Pestana, 1889, p.10).

Despite its attractions, the hypothesis which placed the origins of cancer in the microbial sphere divided the principal researchers in the field, especially because of the difficulty in identifying the causal agent in the laboratory. This conflict was felt in the same way by the Portuguese doctor, who despite his best efforts did not succeed in detecting a microbial agent which he could connect directly and unequivocally with cancer. If the methodology employed was on the whole similar to that used by other contemporary researchers, technical and material deficiencies remained which Pestana did not omit to mention, and which in large measure prevented him from going on with more fundamental research.

In fact, at the time the thesis of Câmara Pestana was written, histology was still a branch of science of limited scope in Portugal, the systematic teaching of which in the



schools of medicine and surgery was still very backward in comparison with developments in France, Germany or Britain. The first initiative in creating a chair in histology was taken in Coimbra by Costa Simões, and in 1885 Ricardo Jorge continued the fight to establish a similar chair in the medical school of Oporto, recognizing that “the miserable science of the Bichats and the Virchows still does not have municipal authority for the course; and as the law did not recognize its existence in theory, it also had no existence in fact. Only a few years separate us from the time in which the poor old microscope lay unused and almost totally unknown” (Jorge, 2003, p.137).

Making use of anatomical parts of surgically removed tumors, Pestana submitted them to an array of tests running from microscopic analysis to bacteriological examination, producing various cultures from the ‘cancerous fluid’, without however succeeding in conclusively determining the definite existence of any carcinoma microbe. He studied four carcinomas and three sarcomas which had been removed by other doctors, observing the micro-organisms, which were sometimes disposed in chains and stained for examination, as well as their development in different types of culture. If on the one hand he succeeded in detecting the type of bacillus referred to by Domingos Freire, he showed that the multiple forms that it appeared to assume in the work of other medical writers were nothing more than the different phases in the evolution of the same agent. Following this, he inoculated various groups of rabbits with the ‘carcinoma bacillus’, obtaining tumors which in general resulted in the death of the laboratory animals. Although he reproduced cancer-forming experiments in animals, the results were not in the end conclusive.

Even so, Câmara Pestana remained hopeful of eventually identifying the causal agent, even though he recognized the need to undertake deeper research towards this end. The experimental evidence that he witnessed did not lead him to any other hypothesis except to advance cautious conclusions, without the boastful affirmations of Scheurlen (who two years previously had published a somewhat fantastic work) or the assertions of primacy on the part of Freire. It would be necessary to continue his work in a more intense and broad-based manner, not ignoring human experimentation in selected cases:

It seems to me that there is still a long and rugged road to travel, in order to arrive at a position where we can safely affirm the determinant cause of malignant neoplasms. After discovering the environment in which the microbe survives best, it will be necessary to transform the animal until it acquires the state of receptivity which is necessary for the development of the bacteria. It is necessary that the experiments on animals are more detailed, that instead of injecting the cancer microbe from a human being into an animal, it is injected from a human being to another human being, taking advantage of incurable cases of carcinosis, or making cultures of cancers from animals and injecting the microbe produced into animals of the same species. And only after obtaining a perfectly defined carcinoma, only after finding a sarcoma which cannot be confused with an inflamed neoplasm, only after having reproduced these tumors many times following a whole series of inoculations, only then will we have the right to assert with the certainty which must be a feature of all experimental science, that cancer is of a parasitical nature and that the carcinoma microbe has been found (Pestana, 1889, p.50-51).

In spite of everything, the impossibility of identifying a specific pathogenic agent did not result in the discouragement of efforts expended in this direction. At least up until

1906, a succession of works was published on alleged etiological agents, many of which were quickly discredited. One example, which had ample exposure in Portuguese scientific circles, was by Eugène Doyen, who in 1903 declared the discovery of *Micrococcus neoformans*, a bacterial agent that was discredited in 1906 as a cause of the illness, which suggested that the microbial theory of carcinoma should be abandoned (Karwacki, 1906).

Another doctor, Carlos Lemos, even carried out new laboratory tests in 1903, despite recognizing that the bulk of the work presented up to that time had been contested and refuted by various histologists (Lemos, 1903). In the view of the latter, the forms described as parasites were nothing more than degenerating cells produced by an agent, which was still unknown, or were simply due to the evolution of the cells themselves in uncontrolled proliferation. This did not stop the young doctor from summarily declaring his faith in parasitical theories, even though they remained manifestly unproved. In his inaugural dissertation, he adopted a point of view which even outlined suspicions of its highly probable contagiousness, by analogy with infectious diseases:

The failure of these experiments in no way detracts from the fundamental idea. The etiological agent of cancer remains unknown, but the parasitical theory, which is the only admissible one, has not been toppled from its pedestal. The observed facts, such as the actual epidemics of cancer, the cases of contagion – noted so many times – and even other facts of a purely clinical nature – such as the cancers which evolve rapidly, the generalization of cancer and the cases of acute carcinosis which make cancer come close to other diseases which are recognized as parasitic –, must force all concerned to the conviction that cancer has an infectious origin. An animate entity as the etiological agent of cancer is an unavoidable necessity. What remains is to identify it (Lemos, 1903, p.12).

The work which he carried out, based on a microscopic and histological examination of a mere ten operative samples, was not conclusive and served simply to assert the existence of cellular 'inclusions', which according to the author proved three points: that cancer showed inclusions whose parasitic nature was indisputable; that the parasites found belonged to the class of amoebas; and that, finally, these protozoa were the pathogenic agent of cancer. Published without the prudence shown years before by Câmara Pestana, its conclusions resulting from incomplete analyses are somewhat forced, and it contains many allusions to other experimental work and also statements without any kind of evidence, particularly with regard to the transmissibility of the disease among human beings, which served only to cast even more doubt on the validity of this theory.

### **Abandonment of parasitic theories or unproved hypothesis?**

In fact, the various studies which followed during the first decade of the new century ended by abandoning the parasitic theory in practice, already thrown into doubt during the course of the Fifteenth International Congress of Medicine at Lisbon in 1906. Through the absence of any conclusive proof, it was by the end of 1908 a theory on the way to obliteration in certain European scientific circles, notably in the United Kingdom (Bashford, 1908).



This did not stop certain passionate defenders of the 'cancer virus' from continuing to exist, many of them in Portugal, and in other places, particularly France and Germany, countries full of conjectures and explanatory hypotheses of this kind. On the basis of publications in French medical literature of the period, Oliveira Lima made such matters the subject of his doctoral thesis, setting out many of the theories and subscribing to some of them (Lima, 1907, p.7-12). He displayed great interest in the myxosporidine theory of Jaboulay, because he considered it to be the best supported by experimental observation, although there was no concrete and definite proof for it to be accepted without reservations. For this doctor from Oporto, as for many others, there were still strong indications that cancer should be brought within some form of parasitic theory.

This was the case with the eminent Carlos França, who in 1918 suggested that the cause of tumors might be found in protists. Attracted by the study of cellular inclusions in cancer, these latter seemed to him to be rare in the majority of cases, and were even absent in a large number of neoplasms. However, in a case of cancer of the bladder which he had studied, they showed themselves to be numerous and to have the appearance and structure of protozoa. In the article which he published, "Notes sur les inclusions cellulaires du cancer"<sup>5</sup> (Mira, 1927), he showed himself to be a faithful partisan of the parasitic theory, the only one that was in harmony with what was known about malignant tumors at the time. What seemed to him logical was to accept that different protists, tending to live by accident within the cells of metazoa, could be the cause of tumors, the type of which would vary according to the nature of the cells invaded and not with the kind of agent.

However, this was not a line of research in which the majority of Portuguese scientists had any faith. The evidence from laboratory tests was overwhelmingly against continuing even to consider this possibility, still less to spend scarce resources on it. From the end of the second decade of the twentieth century, Portuguese experimental science would be based largely on experiments with potentially carcinogenic agents in laboratory animals or on grafts of the disease, once more following the principal trends outlined in other countries.

### **Artificial generation of the disease: the paths of experimental oncology**

The absence of resources for the direct observation of cancer saw a redirecting of efforts towards transmission through grafts and the artificial production of the disease, practiced on a systematic basis from the beginning of the twentieth century. The work carried out was continuous, and its publication allowed a distribution by the international medical community of an extensive compendium of experiments, investigative trends and explanatory hypotheses, whether or not they were worthy of credit, in accordance with the reputation of those involved and the rigor with which the experimental work had been carried out.

The results were variable, and in many cases of short duration, but interest in medical matters was widespread, and the subject of cancer was very much to the fore, despite the fact that until 1910 the profile of the research area surrounding cancer had still not achieved the degree of coherence and stability which bacteriology and physiology showed by this time (Löwy, 1996, p.95).

The stage of experimental oncology was beginning, which was a new phase of research in an area which would become the basis for oncology itself as a distinct field of medical knowledge. Experimental oncology is an expression which defines those studies whose objective is the examination and understanding of the process of carcinogenesis artificially induced in experimental animals by the introduction of cancer-causing agents, whether physical (electro-magnetic radiation of natural or artificial origin), chemical, natural or artificially produced. It also studies the action of biological agents, especially viruses, in spontaneous or induced tumors in animals, concerning itself also with immuno-biological defense mechanisms in neoplasms which are spontaneous or induced by factors of a chemical or viral nature. Additionally it has an immediate support function in relation to clinical oncology, through its study of oncolytic or oncostatic agents, which are artificial (pharmaceuticals) or natural (hormones) chemical substances in tissue cultures or other experimental models. How did this phase in the study of cancer in Portugal develop? In what way did it contribute to form the scientific basis for oncology?

At the start of the twentieth century, there was no dominant theory to explain the disease. When in 1902 the Danish researcher Carl Jensen used the method of transmission by grafting on a large scale and re-published his results in 1903, it marked the beginning of a rise in international interest, which resulted in the creation of a large number of laboratories for the purpose of the experimental study of cancer. As far as Portugal is concerned, Marck Athias was the first to attempt the grafting of cancer in laboratory animals, in 1903, specifically in rats, with positive results which remained unpublished until at least 1925. This research, which Athias did not abandon and returned to with greater application in the 1930s, was also undertaken by Luís Simões Raposo, who had in the meantime been giving it particular attention since the first years of the century.

Both in methodology and in the inoculation techniques employed – fragment method and emulsion method – Simões Raposo was clearly influenced by the British school of Bashford, Murray and Cramer, all members of the Imperial Cancer Research Fund, which since 1902 had brought together the most experienced researchers in the world in the study of cancer grafting, and with whom Raposo maintained special contact. There was an exchange not only of experimental results, but also of samples of tumors, which he also engaged in with other scientific institutions in other parts of the world, such as the USA (Raposo, 1928a).

Although the study of grafts allowed various aspects of the pathogeny of malignant tumors to be clarified, it proved to be ineffective in investigating the etiological agents of the disease, in that it had been shown that the grafted fragment did not transmit the disease to the tissue of the inoculated animal, and grew only by virtue of the proliferation of the actual cells transplanted. Parasitic theories, which were the result of tests of animal parasites and bacteria, were always inconclusive and very difficult to verify, and failed to convince the principal researchers of the time; both Athias and Raposo showed themselves little inclined to accept them, influenced as they were by the results of their own experiments, but also by the close contact that they maintained with European research centers, and also by the numerous experimental studies then being published. It was necessary to produce cancer artificially, so as to learn, by means of a precise method, what transplantation had

failed to clarify. Recourse to substances of extreme carcinogenic power might resolve many questions, and this path was followed with particular interest by those few researchers in Portugal who devoted themselves to experimental investigation.

The numerous observations carried out, collected and compared, although not conclusive, proved to be useful material in considering the nature and action of various cancer-forming agents. Whether they were physical, chemical or biological, they combined with the role played by atmosphere, sun, profession and heredity in the appearance and growth of neoplasms; the evolution of a normal cell into a cancerous cell.

From 1920, the year in which Figiber and Bang introduced into Europe the method of cancer formation through the use of tar, many papers were published on this aspect. Five years earlier, the Japanese researchers Katsaburo Yamagiwa and K. Itchikawa published an

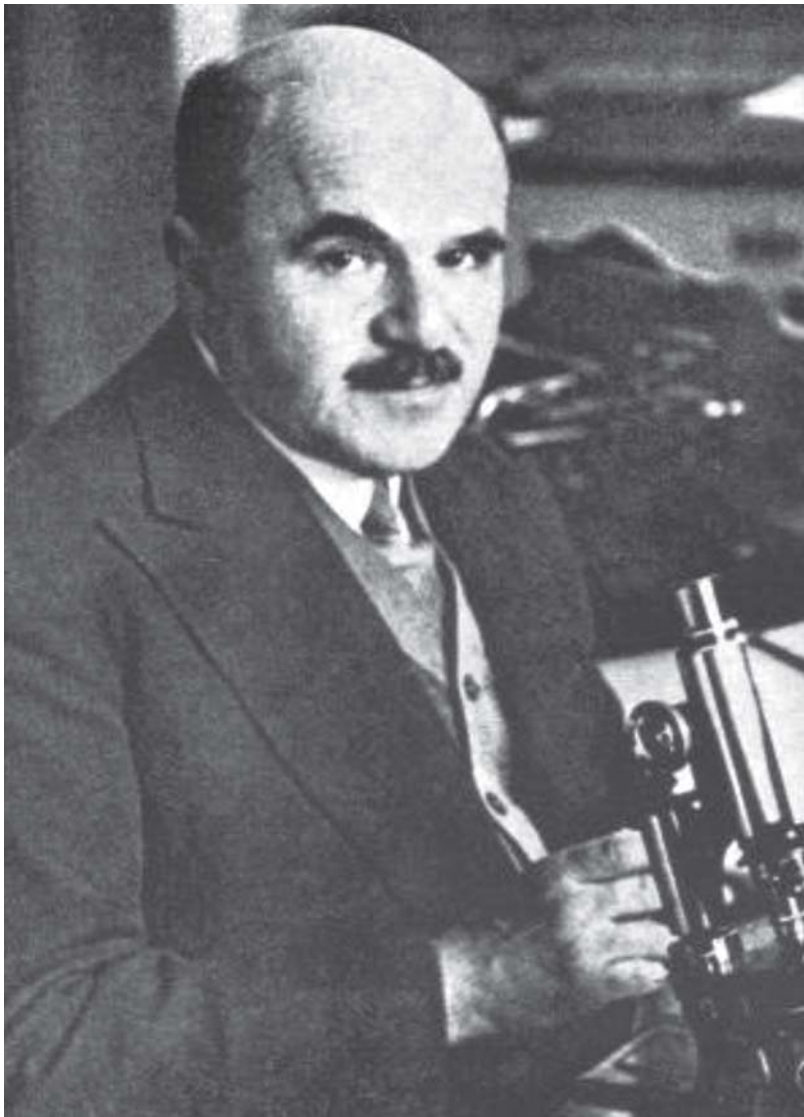


Figure 1: Marck Anahory Athias (1875-1946). Of Jewish descent, he was born in Funchal on December 11, 1875, and concluded the course in Medicine at the Faculty of Medicine in Paris in 1897. It was there that he associated with histologists and physiologists of international renown, among them Mathias Duval, a disciple of Santiago Ramón y Cajal, winner of the Nobel Prize in Medicine and Physiology in 1906. It was from the time he became director of research at the Portuguese Institute of Oncology in 1927 that he started a systematic program of investigation linked to experimental oncology, with the aid of a team which in 1929 became part of the staff of the institution. The group of doctors and scientists engaged at the time formed a small, carefully selected group of ten assistants, specializing in chemistry, physics, radiology, pathology and x-rays. Source: public domain photograph

article in which they claimed to have produced carcinomas in the laboratory, through the application of tar by brushing it onto the ears of rabbits. In 1919, Yamagiwa and Itchikawa succeeded in inducing the appearance of glandular carcinomas in the teats of female rabbits through injecting tar. In 1918, another Japanese, H. Tsutsui, chose the brown rat, because it was the cheapest animal, and painted its back, and since then it has become the classic animal in experiments involving tar-induced cancers.

In itself, 'cancer from tar' did not lead to any notable progress in the direct understanding of the disease. But it did open up a new phase of studies, representing a conceptual change in the way of looking at the illness. The possibility of developing tumors in laboratory animals through exposure to irritant chemical agents taught scientists many lessons, and contributed significantly to advances in scientific knowledge with regard to the etiology and pathogeny of cancer. It was possible to reproduce carcinomas experimentally, whether by painting the skin with products such as tar or hydrocarbons, by the ingestion of drugs, or even by injection. It was a safe and simple method, the use of which became general, allowing details to be clarified and results to be confirmed. It is in the aftermath of this time that we see in the 1920s an extraordinary optimism shown by doctors and scientists on the prospects of victory against cancer, but the results obtained continued to show the obduracy and complexity of the disease.

From 1915 onwards there followed around thirty years of the most varied experiments, with tests not only of tar but of a whole series of chemical and physical agents which showed themselves capable of inducing the illness in its different forms (carcinoma and sarcoma). These included hydrocarbons, vegetable oils, soot, arsenic, and *á* and *â* naphthylamine, and also x-ray, alpha-ray, beta-ray and gamma-ray radiation (Schinz, 1942).

In practice, the study of the conditions in which certain types of cancer could be induced led to a formal refutation of the theory that it was transmitted by contagion and that it was of an infectious nature, and there was a complete collapse of the analogies which had for a long time been made between cancer and infectious or contagious diseases.

### **Research and experimentation in Portugal**

From arsenic to tar, passing through the synergic action of both substances in cancer-forming processes, tests carried out in Portugal in the 1920s represented a clear return to the question of etiology.

Following what was already happening in most European countries, research in the 1920s and 1930s concentrated essentially on the external causes of cancer, and the bulk of it was carried out under the aegis of the IPO. The aim was to apply to the etiology of spontaneous tumors the knowledge obtained through these investigations into artificially induced tumors. The first to devote themselves to the task were Enrico Franco and Casimiro Afonso<sup>6</sup> (Franco, Afonso, 1925), but especially Simões Raposo (Raposo, Noronha, 1927; Raposo, 1928b, 1928c, 1928d, 1929, 1930, 1932). The latter, in particular, developed a regular scientific practice in the area of experimental oncology, within the IPO framework, publishing a significant part of his work in French scientific reviews which had a large international readership.

Meanwhile, Marck Athias also devoted himself to the subject, particularly to the use of methylcolantrene, a derivative of petroleum which, in very small doses, could be an even more effective cancer-forming agent than tar. He carried out this work in the IPO's laboratory of experimental pathology and histophysiology over several years until the end of the 1930s. Notable in particular were his studies of spontaneous tumors and their metaplasms, results obtained in continuing experiments on the inducement of cancer in animals through hydrocarbons (Athias, 1936, 1949, 1937; Athias, Dias, 1938, 1939).

The same path was also followed by his closest collaborator, Maria Teresa Furtado Dias, who not only took part in the experiments of Athias, but also concerned herself with *in vitro* experiments (Dias, 1937, 1941), continuing to examine the difficult problem of distinguishing between cultures of normal and cancerous cells.

One of the problems which needed the most time and experimentation was the transplantation of malignant tumors and the resistance showed by various organisms to the transfer and survival of the grafted fragments. Simões Raposo applied himself to the task with dedication after learning the technique in the laboratories of the Imperial Cancer Research Fund. His "Contribuição para o estudo da imunidade anticancerosa" (Contribution to the study of anti-cancerous immunity) summarized the conclusions of more than seven years of experiments (Raposo, 1933).

The greatest value of the results obtained lay in the fact that it was shown that there were no animals who were absolutely resistant to agents with strong tumor-causing powers, everything depended on the circumstances in which the experiment was carried out, the physical and/or chemical nature of the agent used, the dosage, the period of exposure and the form in which it was administered. Even so, the doubts of the researchers continued, given the varying reactions of animals of the same species to the same tumor-forming agent: everything led them to believe in other factors that must be considered in the cancer-forming process, which, in conjunction with other general, local and predisposition factors which were still unknown but whose existence was accepted, could be responsible for the emergence of the disease.

Among the studies dealing with the formation of cancers, one of the most interesting works was left to us by Amândio Tavares (1932), subsidized by the then National Education Board and carried out in the laboratory of pathological anatomy of the Faculty of Medicine of Porto, being recovered later (Tavares, 1935). Forming part of another work, published the previous year (Tavares, Morais, 1931), it is representative of one of the currents of thought on the formation of cancer. At the time, one of the questions which aroused the greatest interest and controversy was the behavior of the nerves in different tumors. His contribution to the influence of the nervous system in cancer experiments lay in the fact that the results he obtained were quite different from those of other researchers at the time; according to his findings, a sympathectomy (division of sympathetic nerve fibers) did not have such a marked effect in suppressing experimental tumors in laboratory animals as other researchers liked to believe. It was not possible, therefore, to attribute to the relationship between the nervous system and an artificial cancer induced by tar the great importance that other writers placed on it.

The preference for carrying out or reproducing cancer-forming experiments remained strong during the greater part of the 1920s, 1930s and 1940s, until the moment when the investigative pendulum swung back to the new factors associated with endocrinology and nuclear medicine.

On a smaller scale than the cancer-forming experiments, but influenced by them, there was also someone who tried therapeutic methods on an experimental basis. At the Society of Medical Sciences of Lisbon, the most important scientific platform in the country, Silvério Gomes da Costa announced in 1931 the preliminary results obtained through the local application of insulin to cancers of the skin, a work which was developed and became the basis for his thesis for an associate professorship at the Faculty of Medicine of Lisbon (Costa, 1934). On 8 December of the same year, he presented a similar paper to the Portuguese Biological Society, in association with Francisco Gentil, who had also taken part in an experimental study on the application of insulin to ulcerated breast cancers (Gentil, Costa, 8 dez. 1931).

If the relationship between hydrates of carbon and malignant tumors was already well known, injections of insulin having been tried in experimental malignant tumors in rats and rabbits, Gomes da Costa experimented with the action of insulin on malignant tumors in human beings, applying it locally to cancers of the skin. There was support for this technique in already published studies and also in others performed *in vitro* by various researchers. By carrying out the treatment on patients at the Portuguese Institute for the Study of Cancer and at the Institute of Pharmacology of the Faculty of Medicine of Lisbon, the author made a contribution towards clarifying the use of insulin in the reduction of tumors. Through the presentation of photographs of sufferers before and after treatment, operative excisions, histological preparations and microscope photographs, he showed how the neoplastic tissue had retreated, but could not produce a cure for the lesions caused by the tumors.

Of all the investigative methods employed, however, the one which showed itself most fertile in results was undoubtedly the experimental study of cancer, especially because everyone recognized that it was necessary to bear in mind the differences between the neoplasms induced in animals and those which developed in human beings. Perhaps for this reason the experimental research performed at IPO from the mid 1920s followed a course which was less inclined towards purely animal experimentation and was almost always related to clinical work:

Those who work in laboratories, such as the one at Gye, many miles distant from any hospital, are forced to restrict the field of their investigations and experiments, which I dare to call abstract, on animals. The observation of patients using the exact methods of scientific research and a diligent and watchful eye constitutes a real experimental study of the sick, which brings the problem closer to reality, suggests new ideas to the clinical specialist, and always presents the investigator with new doubts or, what amounts to the same thing, new horizons for his *in vitro* and animal studies. Without denying – for that would be to forget dozens of important discoveries – the possibility of carrying out beautiful and productive pathological studies far from the patients, I am among those who are convinced that the true field of experimental pathology lies within the hospital, and that the role played by the observation of animals, though of fundamental importance,



is merely to confirm and clarify any doubts raised by human symptomatology and pathological anatomy. (Raposo, 1925, p.60).

Even so, it was in the Experimental Pathology Laboratory of the IPO that much of this type of work was developed. An example of this was the study carried out in the 1930s and 1940s by Álvaro Rodrigues, on this occasion more concerned with the importance of the lymphatic system in the dynamics of cancer, but performed with recourse to tumor transplantation (Conde, 1982).

It was from 1930 onwards that he devoted himself to the study of the lymphatic system through lymphography, but it was from 1937 that this researcher made the IPO his place of work, where he would develop his most relevant researches in the area of oncology. On the basis of the most advanced biological conceptions of the time, and above all experimental surgery, he concentrated on the role of the lymph nodes in the mechanisms of organic defense against neoplasms, their local invasions and systemic dissemination. A debate was going on at the time over the question of whether the dissemination of malignant tumors happened via the lymphatic and blood systems or through the peripheral nerve sheaths.



Figure 2: Radium Building – Portuguese Institute of Oncology (IPO) in the 1930s. It was in the laboratories of this building, inaugurated in 1933, that most of the experimental oncology work was done until the opening of the hospital block in 1948. The IPO was conceived above all as a center for oncological studies, for which purpose it was endowed with various laboratories, a library, an oncological museum and a scientific review which was to have international influence. Successively, there were established sections for pathology, histophysiology, experimental pathology and the culture of tissues, chemistry and physical chemistry, and even radiation physics. The Institute united under one roof the potential for carrying out any work on neoplasms in human beings, but also in animals and even plants, such conditions being necessary for both clinical and experimental studies. Source: Biblioteca de Arte, Fundação Calouste Gulbenkian. Undated photograph, made by Estúdio Mário Novais, which existed between 1933 and 1983

At a time when he believed that the lymph nodes could constitute the means for the cultivation of cancerous cells, as had been mooted at the International Cancer Conference in 1938, Álvaro Rodrigues developed a series of experiments in the histophysiology laboratory of Marck Athias at the IPO, the results of which were published in 1940 in *Arquivo de Patologia*.

In what was clearly pioneering work in both subject matter and methodology, he demonstrated the regeneration of the lymphatic system and studied the histological reaction of the nodes, namely what came to be called sinusal histiocytosis, to express the organism's defense reaction when these nodes are found in the lymphatic vessels leading from an anatomical area which is the site of a primitive tumor. In various experimental models, after ablation of the lateral aortic nodes in a rat, he showed that the sarcoma in the animal, which had a tendency to remain localized, presented a larger volume in such an experimental situation, as well as greater and more rapid metastasis. He demonstrated that the absence of regional nodes allowed the transplanted tumor greater biological dynamism, as expressed by larger volume, accelerated rhythm of growth and greater capacity for systemic diffusion. He proved also that a sympathectomy always induced a more rapid growth of tumors transplanted into laboratory animals, with the formation of larger and more irrigated neoplasms. A factor still unknown at the time had been established: the influence of a sympathectomy on oncogenic neo-angiogenesis, a phenomenon which even today occupies a relevant place in the biology of carcinogenesis.

From his experiments he produced the hypothesis that actions developed at the level of the lymphatic nodes could interfere in the process of neoplasm dissemination, and he was a pioneer of sclerosant action around the nodes, at the laboratory level, a practice which would much later find its reflection in intra-lymphatic chemotherapy and intra-lymphatic injections of radioactive substances, a treatment which only much later came to be employed by other investigators all over the world. Between 1940 and 1942 it was at different conferences held at the IPO that he divulged his work on the lymphatic system and cancer, which he had been developing since 1935. This was the date when he first issued a statement on the possibilities opened up by visualization of the lymphatic system, in particular in studying the problem of cancer, expounded at the Cancer Week in Vigo (Rodrigues, 1936, 1940, 1941, 1942a, 1942b).

In showing that cancer was not an evil whose nature was a mystery, but a disease like any other, a large number of different experimental studies helped to transform concepts of contagiousness which had simply not been proved. There was indeed evidence of a set of external factors, under the influence of which could be observed the anarchic, tumultuous proliferation which was typical of the disease: physical and chemical agents, among which were tar and its derivatives, x-rays, radium, burns, chronic irritations and others. Internal dissemination also came to be better understood, which threw new light on possible therapeutic strategies.

The result of all this was a different view of cancer, which was now capable of being reproduced in laboratory animals through various chemical and physical agents, which were identical to those which in human beings constituted the factors capable of predisposing and occasioning the appearance of cancer. This notion, perhaps the most

important in the history of experimental oncology before 1945, encouraged a belief, in the words of Athias, “that one day, perhaps not so far off in the future, cancer will be finally conquered by medical science” (Athias, 1941, p.58), showing that the optimism of the 1920s had been replaced by more realistic expectations and perhaps by a greater degree of skepticism.

### **Final considerations**

Beyond the accumulated clinical and surgical experience, and even the repeated introduction of new therapeutic methodologies, the emerging Portuguese cancerology affirmed itself also in the field of investigation. Between Câmara Pestana and Marck Athias, there were various figures who dedicated themselves to acquiring knowledge of an experimental nature, oscillating between proof of parasitic theories and experiments inducing cancer through various agents.

In international terms, we may classify the different scientific approaches into stages, in accordance with the predominant type of investigation. Thus, three phases can be detected, starting from the introduction and consolidation of the principles defining experimental oncology. The first phase, based on histopathology, relates to the observation, differentiation and classification of tumors, a stage in which there do not seem to have been relevant contributions in Portugal. This was followed by a second phase, characterized by microbiology and based on the infectious diseases model, where the aim was to determine the existence of a parasitic agent, external to the organism and the cause of the illness. The third phase was based on studies of the formation of cancers in animals and the establishment of solid knowledge with regard to agents which are potentially carcinogenic for human beings, with a new direction being given to the understanding of the biology of malignant tumors. It is interesting to note that this road was taken before the information had been compiled and systematized by international health bodies, something which would only happen at the end of the 1960s.

Chronologically superimposed during the first twenty years of the twentieth century, in the last two phases that we can distinguish we do not see moments of total epistemological break, but periods in which the international trends or patterns of investigation most in vogue flourish with greater visibility. Without being the cause of immediate ruptures, scientific schisms do exist, but they are confirmed more by the impossibility of proving theories which cannot be experimentally reproduced than by their rejection pure and simple, achieved by means of irrefutable evidence. A parity therefore continues to exist between experiments involving transmission by grafting and those constituted by the artificial inducement of the disease, at the stage which saw the launch and the acceptance of experimental oncology, both at the international level and in Portugal.

In a transition as natural as it was foreseeable, the observation and classification of tumors proceeded to a phase of experimentation, whether involving processes of transmission by grafting or the artificial inducement of the disease. The medicine of the nineteenth century saw the birth of what would before long be called experimental oncology established on scientific principles, as they are known and practiced today.

During the course of a period which lasted a little less than thirty years, the first grafts were carried out and the first cancers were induced in laboratory animals; substances considered to be carcinogenic were tested and the way was opened to an area which, both in Portugal and in the leading anti-cancer centers, was at the limits of scientific knowledge. The thinking of the investigators followed contemporary trends, in particular those propounded in France, England and Germany, as there was no qualitative time lapse between the scientific methodology and the medical knowledge cultivated in the principal European centers and those pursued in the Portuguese investigatory equivalents. Among the latter, the IPO was the 'cathedral' of oncological science, the home of most of the small number of scientists who created out of experimental oncology the scientific foundations for a new medical discipline.

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#### NOTES

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<sup>2</sup> In this and other citations of texts from non-English languages, a free translation has been provided.

<sup>3</sup> For the transformation of cancer into a social 'scourge' in the first half of the twentieth century, see Pinell, 1992.

<sup>4</sup> For the impact of the Pasteurian revolution in the context of Brazil and Domingos Freire, see Benchimol, 1999.

<sup>5</sup> As well as the article on cancer, this work of Matias Ferreira de Mira presents a comprehensive list of 187 works by the author, divided into sections: histology, plague, cancer, trypanosomiasis, parasites, carnivorous plants, tropical medicine, etc.

<sup>6</sup> These investigators noted and stressed that the area where tar is applied is never totally transformed by cancer. Although the stimulation is identical with regard to all the cells, it is only at one or two points of a relatively extensive area that the process of malignant neoplasm commences.

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