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SCIENTIFIC KNOWLEDGE AND PROGRESS IN CONTROL OF PARASITIC DISEASES

" In any case I am the first to have no illusions on the immediate consequences of my studies. The application to industrial arts of results from Science is produced always very slowly. My present pretensions are very modest....."
(In the letter of Louis Pasteur, in August 1867 to the Ministry of Education asking funds for his studies on fermentation that gave origin, ten years latter, to the pasteurisation procedures).

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Human and animal parasites are known since the most remote antiquity. Chinese and Egyptian documents from thousands of years ago refers to ticks, mosquitoes, louses, round and flat worms etc.. However, it is curious to observe that the relation between parasites and disease is relatively recent, dating from the turning of the 19th to the 20th century. This came with the extraordinary development of scientific knowledge in basic Biological Sciences, together with the introduction of experimental procedures, associated to the development of optical, mechanical and chemical industries. The knowledge on the life cycle of parasites and the definitive proof on the relationship between their presence and disease developed progressively only after the brilliant discoveries in Microbiology by two genius of the end of the 19th Century, Louis Pasteur and Robert Koch, whose postulates were finally extended from bacterial diseases to parasite diseases.

Lymphatic Filariasis is a good example of the uncertain and slow rhythm of scientific discoveries in the area. Filaria worms and microfilaria were indeed known since the 17th and 18th century in domestic animals and birds but the first description of microfilaria in man was done by Delaquay in 1863, in quelous urine of a patient, quickly confirmed by Magalhães and Wucherer in Bahia. Only a few years after a microfilaria was found in the blood of a patient by Lewis in 1872 and in 1876 Bancroft in Australia described the adult filaria in lymphoid tissues. The description of the microfilaria development in the *Culex* mosquito by Sir Patrick Manson (1878-80) is celebrated as a mark in scientific knowledge in Parasitology but, in reality, Manson thought that the infected mosquito died in water during oviposition and infection of humans occurred by ingestion of contaminated water containing microfilarias. The first demonstration of the direct transmission of a parasite by an arthropod was done only in 1893, by Smith and Kilborne, in USA. They showed the role of ticks (*Boophilus*) in the transmission of babesia infection in the cattle. This was followed by the discovery of David Bruce, in 1895, on the transmission of *Trypanosoma brucei* by the tse tse fly. However, also here, only many years latter, in the 1900th, it was demonstrated that the tse tse fly is a real intermediate host and not only a mechanical carrier of the trypanosome. Ronald Ross, in 1897, discovered the transmission of bird malaria by the "grey mosquito". Grassi and co workers proved the transmission of human malaria by *Anopheles* mosquitoes in 1898. In summary, in relation to lymphatic filariasis, a delay of 30 years was observed between the “discovery” of the parasite and description of the life cycle that, finally, permitted to define control measures based on scientific knowledge.

Description of complete life cycles and the role of parasites as agents of human diseases extended along the first half of the 20th century: The American Trypanosomiasis (Chagas disease) was fully described by Carlos Chagas in 1909, (probably, the first parasitic disease where the Pasteur-Koch postulates were entirely satisfied); the demonstration of the role of *Phlebotomus* in the transmission of leishmaniasis (while previously suspected) was done by Shortt only in 1931. Hypnozoites of *Plasmodium vivax* were described only in 1948, by Shortt & Garnham and the life cycle of *Toxoplasma gondii* was clarified only in 1965 with the identification of the cat as the definitive host.

FIRST IMPORTANT VECTOR CONTROL CAMPAIGNS.

The description of complete life cycles and the role of arthropod vectors as intermediate hosts of parasitic diseases were quite soon followed by the proposal of control measures based on the attack to the vector. Mosquitoes as vector of important viral (yellow fever, encephalitis) and parasitic diseases (malaria, lymphatic filariosis) were particularly targeted in the proposed methods of control. In spite of the poverty of available weapons, the first half of the 20th Century has already registered some important victories against diseases based on vector control. Probably the first in the American Continent was the control of yellow fever in the first year of 20th century in Havana by Walter Reed, after the Finlay demonstration of the role of *Aedes* mosquitoes in the transmission of the disease. Followed the Gorgas campaign of malaria control in Havana and during the construction of the Panama´s Canal in 1905-1910. In Rio de Janeiro. Brazil, Oswaldo Cruz promoted an important sanitation campaign against mosquitoes and rats (1904-1909) that eliminated yellow fever and plague. It must be noted that weapons used against mosquitoes at this time were very primitive: fogs produced by burning sulphur or natural piretroides (extracted from the plant *Chrysanthemum*) against adults insects; petroleum oil, introduced in 1892 and Paris green (a mixture of arsenic salts), introduced in 1921 against larval stages in the breeding sites. As late as 1930-40, these limited and primitive gadgets were used successfully in the heroic campaign, directed by Soper and Wilson, from the Rockefeller Foundation, against the African *Anopheles gambiae*, that had invaded, by maritime route, the Brazilian North east area, provoking a terrible malaria epidemics with more than 14,000 deaths. However, these instruments, even if based on solid scientific knowledge, were of high cost, undesirable toxic side effects and limited efficiency. Therefore, they could never be used in large scale in endemic areas.

ORIGINS OF CHEMOTHERAPY OF PARASITIC DISEASES.

The progress of scientific knowledge on parasites’ life cycles and on parasites’ pathogenicity was possible thanks essentially to improvements of the Microscope. Another essential tool was the invention of staining procedures, in particular those derived from Romanowsky. Romanowsky was a pathologist of the Russian Army, a little bit lazy, never closing the flasks containing products. In 1891, leaving open a flask with methylene blue (used as disinfectant) he observed a contamination by fungi but used the product in spite of it. He mixed accidentally the product with eosin (used to stain malaria parasites) and verified that the nucleus of the parasite stained purple, while the cytoplasm stained blue. The methylene blues had been oxidized to azur A and B (tetra and tri methyl tionine). The Romanowsky derived staining methods, still used today, was not only very useful for studies of protozoan parasites and larval stages of helminthes but also, more than that, inspired another genius of the 19th Century, Paul Erlich. Erlich profited from the industry of colorants (stains), that had an extraordinary development with the textile industry at the end of the 19th century, after the discovery of the diazo reaction by Griess in 1850. In 1881, Erlich, still a Medical student, observed the staining of leukocytes granules by methylene blue and started a long career looking for chemicals with specificity for cellular organelles, and specific structures of bacteria and parasites. As an extraordinary experimentalist, Erlich started to analyze hundreds of new synthetic chemical colorants in laboratory models and clinical samples. One of his first discoveries was that methylene blue stained *Plasmodium* parasites in a lower concentration than required to stain leukocytes and proposed methylene blue as an anti malaria drug that was clinically used for various decades. This concept of selective staining (reflecting selective binding of a drug) was one of the basic concepts that made Erlich to be considered the father of Chemotherapy. Erlich tried also hundreds of colorants against *Trypanosoma equinum* in rats and described the trypanocidal activity of benzopurpurina. As this compound is insoluble in water, he convinced the chemists from Industry to look for soluble derivatives, which finally gave origin to a sulfonic derivative, trypan red, and a potent trypanocidal drug that was used for various decades. The interest for colorants remained a important source of inspiration in drug research and the first synthetic antimalaria drug, atebrine, synthesized in 1930 is a derivative of acridin orange.

In the 50´s, inspired by Erlich ideas, two young Brazilian medical students, Ruth and Victor Nussenzweig discovered that gentian violet, added to blood stored in Blood Banks, prevented the transmission of *Trypanosoma cruzi* by blood transfusions. This method has been used with success for various decades in some endemic areas of Chagas disease in Latin America.

Chemotherapy, in the first half of the 20th Century had two other empirical sources of inspiration. On one side, the analysis of “selective toxicity” of products known as toxic or poisons for humans and vertebrate hosts like arsenic derivatives. Arsenicals have been largely studied by Erlich himself with the discovery of the famous 606 and others derivatives, fantastic weapons for the treatment of syphilis, a world-wide plague from the 18th to the 20th centuries, equivalent to AIDS nowadays. Arsenicals and bismuth compounds were the only drugs available for the treatment of syphilis, before Penicillin, discovered by Fleming in 1934, but introduced in medical practice only at the end of the Second World War. Arsenicals, active against the syphilis *Treponema*, were extensively assayed against other parasites. Laveran, in the Pasteur Institute, after his studies on malaria, spent years of the final period of his life looking for arsenicals and antimonial drugs against parasites. Some showed moderate activity against trypanosomes. Antimonial drugs were used in 1914 by Gaspar Viana in Brazil with a remarkable activity against muco cutaneous leishmaniasis, which immediately inspired other scientists to use the drugs in Kalazar. Antimonial drugs were until recently the only available drugs against visceral and muco cutaneous leishmaniasis.

A second source of inspiration was followed by organic chemists and pharmacologists that worked to isolate and define active products of natural extracts that were used in traditional and popular Medicine to treat certain disease symptoms. Important drugs have been discovered following this approach in many areas of Medical and Veterinary Sciences but, in Parasitology , the most remarkable examples of success were anti malarial drugs. Cinchona bark was introduced in Europe by Spanish Jesuit priests in the 17th Century as anti-febrile products and later on shown to be active against the malaria attacks. The anti - parasite specific effect of quinine and quinidine, alkaloids components of the cinchona bark, extract and identified by the French chemists Pelletier and Caventou in 1820, was only shown in the beginning of the 20th century. At that time, the destruction and disappearance of malaria parasites in the blood of infected human and animal could be documented after administration of the drug. Quinine and quinidine chemical structures, identified in the beginning of the 20th century, inspired the organic chemists to search for analogues and chemicals with equivalent or similar structures, giving origin to the synthetic quinolein-family of anti malarial drugs. In particular chloroquine, synthesized in 1934 by the German chemists and universally used after the second world war in malaria control campaigns by WHO. A second case of an important natural product is that of quigaushu, a vegetal whose extract had been used in China for centuries against fever. When Quigaushu was analyzed, after 1950, by the Chinese chemists artesunate and arthemeter were isolated. These alkaloids with important anti malarial activity are used nowadays with success to treat *Plasmodium falciparum* infections resistant to amino-quinoleins and anti folic drugs that occur in almost all endemic areas of the world. Another anti-parasite drug that arose form the analysis of natural product is emetine, used against amebiasis and other intestinal protozoa parasites. Emetine is extracted from ipecacuanha, an Amazonian plant.

DISCOVERY OF SYNTHETIC INSECTICIDES AND DISEASE CONTROL

When considering the discoveries of drugs by means of semi-empirical approaches perhaps the most important is the discovery of and other organo-chlorides insecticides with residual activity. DDT was synthesized in 1939, by Swiss chemists, but its activity against mosquitoes was shown only latter. In 1943 DDT was used by the Allied forces during the Second World War to impregnate the clothes of soldiers in order to avoid lice proliferation and prevent exanthematic typhus transmission. After the war, when its strong activity against *Anopheles* was noted, DDT was used as the main weapon for the malaria eradication program, as approved by the 8th World General Assembly of WHO. The campaign started in 1957 and involved 60 countries. The results were spectacular with eradication of malaria from 37 countries, essentially from Europe and North America. Important reduction was observed in various regions of Asia and Latin America. In Brazil, for instance, the number of annual cases of malaria that was over 4 millions, dropped to less than 40 thousand in 1964. Malaria was eliminated from all the coastAL areaS and from the large hydrographic basins OF BRAZIL with exception of the Amazon area. The optimistic view that malaria couild be eradicated by DDT was challenged by the appearance of resistant anophelines (including to other organo chlorides), by the demonstration of harmful effects of DDT on the environmental and on humans´ health, particularly afer intense usage in Agriculture. Socio economic and demographic problems in poor areas of Africa, Asia and Latin America also contributed to the failure of the eradication campaign that was finally abandoned in the Inter Ministry World Conference on Malaria, in Amsterdan, in 1992, when the Eradication program was replaced by a Coordinated Control Project. However, the experience accumulated along this campaign, that demonstrated the feasibility of control and elimination of this terrible endemic disease, will be of great value for futures campaigns against malaria and other endemic diseases.

In Latin America a successful program of Control of Chagas Disease was developed in Brazil, based on the systematic use of BHC (benzene hexa chloride), for domiciliary spray in endemic areas were *Triatoma infestans* was the main vector. Permanent surveillance of houses for the presence of triatoma was accompanied by clinical and serological survey to detect new cases of Chagas disease in the infant population. As indicated in a recent report, the incidence of cases among infants, that was 5% in 1980, dropped to 0.28% in 1999. The very large endemic area of Chagas disease in Brazil was considered, in 2000, as free of transmission. The incidence of blood samples in the country with positive serological reactions for *T. cruzi* also decreased from 7% in 1980 to 0.73% in 1998. The campaign, started in 1974 was associated to a large mobilization of scientists working in laboratory or in the field. Annual meetings were organized in Caxambu (South East Brazil) that became a tradition International Meeting on Chagas disease, latter extended to leishmaniasis and finally to Research in Protozoology since 1996.

PROGRESS IN CONTROL MEASURES ORIGINATING FROM NEWLY ACQUIRED KNOWLEDGE IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

The development of Biochemistry after the 1930's, with the identification of metabolic and biosynthetic pathways of the eukaryotic cells provided a revolutionary impulse to Chemotherapy. This was followed in the 1950´s by the discovery of the structure of the DNA and in the 1970´s by its manipulation, giving origin to Molecular Biology. These discoveries and the hability to obtain monoclonal antibodies had a revolutionary effect on applied disciplines like Chemotherapy, Immunology and Vaccinology. It became feasible in Chemotherapy/Immunotherapy to identify targets of drugs or/and protective antibodies at the molecular level and to define mechanisms of action in terms of inhibitory activities against vital function of the invasive pathogen agent (virus, bacteria, parasite). It also became possible to develop experimental models for studying the targets of drugs or immune mechanisms using cell cultures or purified enzymes/protein in solutions, instead of experimental animals. In many cases the in vitro methodologies are less expensive and time consuming. Thus, when a specific molecular mechanism of action was known and the molecular target identified, chemical research was used to define the nature and direction of changes needed in an original drug to improve its activity and to create a new weapon. This scientific progress had enormous impact in the methodologies for development of new drugs in the second half of the 20th century that are nowadays in an explosive phase. Examples can be given in the control of filariasis

Onchocercha volvulus, responsible for the river blindness, affected 20 million people in Africa in 1970, according to WHO evaluation.. A Control Campaign was initiated in West Africa in 1974 focusing in the use of organo phosphate larvicide Temephos, against the larval stage of *Simulium* vector with favorable effects in the transmission rates and in the decrease of prevalence. In 1975 was discovered an macro lactone antibiotic, Avermectin, produced by *Streptomyces avermetilis* showing a powerful anti nematode effect. A dihidro derivative, less toxic, was synthesized latter and named ivermectin In 1987 ivermectin was introduced by WHO as the drug of choice in the *Onchocerca* Control Program (OCP) The program had an extraordinary success: The OCP practically eliminated *Onchocerca* transmission in that area and new programs were started in 1995 involving now other 19 countries in Central and East Africa. (Richards et al, 2001)

In 1893, Sir Patrick Manson though that it would be very difficult to treat lymphatic filariosis, since, according to him, it was impossible to develop a drug that would not kill the parasites without having serious side effects on the human host. Arsenicals and antimonial drugs developed against other parasites were tried against filariasis without practical effects. Suramin, a trypanocidal drug developed by Bayer in 1914 was used with moderate success in 1916. No other drug active against the parasite was known until 1940. Control of filariasis was, therefore, entirely dependent on anti-mosquito measures. Diethylcarbamazine, a piperazine derivative, was shown in 1947/8 to have a important anti microfilariaL activity and some activity against the adult worm. It has been used since to reduce transmission. Ivermectin was used for the first time against *Wuchereria bancrofti* in 1986, It showed an important microfilaricidal activiy. Remarkable results in decrease of microfilaremia and in vector infection rates were also obtained with the association of DEC with Ivermectin in control campaigns against lymphatic filariosis in India and Haiti and the excellent results obtained until now permit to expect that these two plagues of developing and under developed areas of Africa, South Asia, Pacific Island and Latin America will be soon eliminated After one century of intensive research efforts..

It was soon demonstrated that the macrocytic lactones act at the neuromuscular junction of the ventral cord of nematodes. Working with *Caenorhabditis elegans*, a neuromuscular junction receptor of the glutamate gated chloride type sensitive to ivermectin was isolated. The corresponding gene and mensager RNA were purified and the Pharmaceutics industries now have an molecular model to assay new macrolytic lactones and/or other drugs specifically inhibitory of the nematodes neuro- muscular junctions. A series of new drugs are in the process of development Ivermectine became the drug of choice against

Strongyloides stercoralis, that can cause a very serious infection in immunosuppressed patients. Ivermectine is also active against blood sucking arthropodes where it also inhibits neuromuscular junctions. Ivermectin is, therefore, extensively used against mites, lice and ticks affecting cattle and other domestic animals..

Another promising drug is now been studied against leishmaniasis. Miltefosine. It can be administered by oral route and was assayed in India against visceral leishmaniasis with success. It is a close analogue of lecithin (phosphatidylcholine) in which phosphorylcoline is attached by a ether bond to a carbohydrate backbone. It seems to modify cell-signalling pathways and membrane synthesis but The exact mechanism of citotoxicity to the parasite is unknown.

GENOMIC AND POST GENOMIC ERA AND PARASITE RESEARCH

The last decade of the 20th century was marked by the genomic revolution where the extraordinary technical development of automatic DNA sequencing permit rapid progress in the determination of complete genome sequence of *E coli* in 1990, then of yeast (1995) and a series of other prokaryote and eukaryote organisms, culminating with the sequencing of human genome published in the last year of the 20th Century. Parasites genome sequencing have also been pursued by many laboratories and, by March 2001 GenBank databases included 136,214 EST sequences from 41 different human and animal parasites. The major number of dbEST were from *Brugia malay, Onchocerca volvulus, Schistosoma mansoni, Toxoplasma gondii, Strongyloides stercoralis, Trypa.nosoma cruzi* and *Eimeria tenella*, all with more than 10,000 dbEST (Tarleton & J Kissinger, 2001). In addition malaria parasites and the main African mosquito vector were object of full genome sequence programs and it is expected that, by the end of 2002 the complete genome sequence of *P. falciparum* (25 Mb), of several rodent malaria parasites and of *Anopheles gambiae* (280 Mb) will be accessible to the scientific community (Hoffman et al, 2002).
In the same period, new important technologies have been developed for the manipulation of the genetic material of parasite by transformation. This permitted for the first time to define gene functions by knock out of genes or by introducing subtle mutations in defined domains. Transgenic parasites and vectors with modified biological and/or pathogenic behavior are produced and their study open new perspectives for molecular genetics analysis of parasites´ gene functions, with enormous opportunities for development of new vaccines and therapeutic agents. The beginning of the 21th Century is therefore characterized by an accelerated progress on new knowledge from genomics and post genomics methodologies including development of stage-specific transcriptomes, proteomic analysis, micro arrays technologies. The new approaches will complement methods in older disciplines like protein chemistry and Structural Biology. An important revolutionary development is that Bio -informatics is permitting now not only to collect structural information on macromolecules from Databanks, but to search for potential synthetic or immune inhibitors. Simulated " experiments" can be proposed in the

computer and, if they can not replace the bench, they will certainly avoid many experimental procedures.

II will try to give a few examples of practical results that can already be quoted as resulting for this new scientific developments. While I am talking they will already be considered old hat. Nonetheless, here are some examples: Analysis of genome sequences of filaria revealed a number of homologues of cytokines, including a MIF (macrophage inhibitor factor) that is secreted and has bioactivity on human macrophages. New therapeutic agents are being developed against these molecules. *Wolbachia*, a common infectious agent of insects, has been identified as an endosymbiont of filarial worms and now is considered as potential target for therapeutics and vaccines (Tarleton & Kissinger, 2001). Among numerous recent contributions in malaria, I selected a recent study on the transcriptome of sporozoite stage that identified 1,547 unique gene sequences specific of this stage. Blast search using database information show only 161 significant matches, whereas 1,386 unique sequences corresponded to novel sporozoite expressed genes. The gene MAEBL, though to be specific of asexual blood stage, and certainly involved in penetration in cells was identified. These results will certainly have a important impact in the construction of pre erythocytic malaria vaccines (Kappe et al, 2001; Hoffman et al, 2002).

Studies from a NIH group showed that saliva from *Phlebotomus* vectors we able to induce protection against experimental cutaneous leishmaniasis in mice. Imunochemical experiments could identify a series of immunogenic proteins in the saliva; N- terminal amino acid sequencing and access to data of *Phlebotomus* genebank permitted to identify the gene encoding the protein responsible for the protective effect and its complete sequence which will be used for elaboration of nnew vaccines againt leishmaniasis. (Valenzuela et al, 2001)

Genetic manipulation of vectors has been intensively worked by many scientific groups (Aultman et al, 2001). Transgenic mosquitoes have been produced in the laboratory that secret monovalent antibodies or active peptides able to inactivate parasite or virus agents when ingested with the blood meal. Para transgenic strategies have been developed in which symbionts or virus infected vectors present anti parasite activities. The search of manipulated vectors that transmit refractoriness traits to parasites is an active area of study with the hope of revealing new strategies to replace indigenous vector species Ito et al, 200, Lycett & Kafatos, 2002).

We have tried to show that the control measures available nowadays against parasitic diseases have been the result of a slow process of application to "industrial arts" of scientific knowledge developed in the turning of 19th to 20th centuries. Again, we witness in the turning of the 20th to 21th centuries an explosive development of scientific knowledge in Biological Sciences. This new knowledge will certainly dominate the strategies that will define the new control measures. In summary, we can say that tools and techniques necessary to control most of the parasitic diseases do exist and are efficient; new tools and techniques will certainly arise from application of the present revolutionnary development of Biological Sciences. More than that, in view of the nature of the accumulated knowledge it seems possible nd even likely that, in this century, terrible plagues that hav affected humans since their appearance in the Planet may be definitively controlled.

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Two final words. The first is to remind Louis Pasteur statement in 1867 and more recent François Jacob´s words on the non-predictable nature of Science itself. Jacob said that in a scientific enterprise a dose of incertitude is obligatory. Therefore, it is necessary to keep a certain degree of modesty when discussing the ambitious projects generated in the genomics and post genomics era. We have already seen, in the past, that projects supposed to give definite solutions to health problems have failed because they did not take in consideration the extreme plasticity and capacity for adaptation of parasites and vectors and of the complexe socioeconomic factors that block the access of people to benefits of Science. It is therefore important not to be excessively confidence in new developing methodologies.

A second comment comes from a special feeling of the modest scientist that has here the honor to talk to this eminent assembly. As a representative of professionals working in remote areas of the world, where parasitic disease still are the main source of human health distress, I would like to remind you of one thing: in many areas where I have worked, in Sub Saharan Africa, in South America, and in Brazil in particular, I spent time in the company of millions of humans that do not benefit from the development of scientific knowledge. This is true for AIDS, but also for malaria, leishmaniasis, trypanosomiasis, schistosomiasis filariasis and many other parasitic diseases.

In this beginning of a new milenium, when the promises of Scientific knowledge open enormous hope for the improvement of human health, let us expect that no more Humans of any continent, any country, any little county or village, will be left on the margin of benefits of Science. This is for me, an ethical principle to be adopted by the Scientific community in the World.

In the moment of celebration of its one hundred years the best wishes that we can propose is that PAHO will continue the fitting for the universality and equity of access to health care.

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