BUTANTAN INSTITUTE: FROM SNAKES TO VACCINES AND ITS DESTRUCTION

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Abstract

Butantan Institute, a public organization founded in 1914, was an example that developing countries could participate in the production of vaccines, the most efficient and inexpensive tool to avoid the spreading of infection diseases. Since 1983 I invested in developing technologies to produce effective anti-venoms sera and vaccines to distribute free for all the population in Brazil. This became the model to many development countries, to pursue independent research translating in safe and effective products. In 2001, with support of WHO, I became the first President of the Developing Countries Vaccine Manufacturing Network. By 2009 Butantan reached 710 million doses of six different vaccines and 700 million doses of anti-venoms supplied to our Ministry of Health, when began to produce the influenza vaccine. Creating a Center of Biotechnology I was able to attracted young PhDs that were free to work in new projects, like lung surfactants which could save 50.000 newborn each year of dying suffocated immediate after delivery, or the new technology to produce human plasma derivatives, which, according to Brazilian Constitutions, could not be commercialized for profit. Butantan, succeeding to be an important producer, became the “bad example” to the large vaccines and biologics producers from the developed countries. The official control agency was induced to demand that Butantan renovate its production plants and the new Direction hired private builders to take apart buildings and scatter equipments removed. Due to delay of the Congress release the budget of the Ministry of Health, funds have being saved in advance to guarantee the production of vaccines and sera each year.

Priority was given by the new Director to make Butantan beautiful, using the reserve funding, which was essential to start vaccine production each year, while the Ministry of Heath budget begin to import vaccines from the large pharmas, without taking notice of the increase of cost (Butantan low LPS DTP cost about US$ 0,16 per dose, compared with imported DTaP is US$ 15.00 per dose!). Meantime Butantan director used the Butantan Foundatiom rotating funds to make Butantan beauful and rebuinding a huge buinding for a central bureaucracyoffice, increasing the staff with ”administrators” without understanding what was the public health role of Butantan .The new generation of Directors of Butantan and the Large producers won the battle.

1914 Vital Brazil discover that anti-snake sera was species specific, and got from the São Paulo State a farm area in the limits of the city, where the first building of Butantan was constructed. Attend an international meeting in New York he brought a sample vial of anti-crotalus sera, which rescue the life of a veterinarian of the Zoo, bitten by a snake. President T. Roosevelt decided to visit Butantan Institute, which became known as the Snake farm.

In 1965 I became the chairman of the Department of Biochemistry from the School of Medicine of the University of Sao Paulo. In 1969 the mlitary government "retire" me and I left to US, at MIT and then a NY City College, were I stayed ten years. Idecided to return to Brazil to direct the Center of Biotechnology at Butantan (1983). I found out that, after immunizing horses to produce anti-sera, the blood was collected in rusted milk thanks and filtered to remove cells using dirty towels. The plasma was transfer to large bottles and kept at room temperature for several months until the sera became packed with mold and filtered to fill vials with the anti-venom sera. Obviously the preparation was not adequate and sera lost most it potency.

My first step was to build a new production plant, with stainless steel tanks, linked by tubes for the collection bottles, making the plant fully enclosed and hand free of the operators, avoiding contamination. This close system, which allows for cleaning on site, guarantee the product (sera or vaccines) was implemented for other plants, dedicate for each product, minimizing errors and contamination. The products require the transfer to vials containing a single dose. An automated central filling facility was acquired by Butantan, and a facility to assay each lot was created, using recommended quality control for safety and efficacy of each lot. All that seem obvious requirements, promoted me to be the director of Butantan Institute in 1991, by the State of São Paulo Office of Health, a position head until 1997, when I reach the age of 70 years, and was replaced by another professional selected by State.

The role of the Institute is complex, providing its products to the Federal Ministry of Health, that in Brazil will distribute vaccine and sera to each State to all population (about 130 million).

The Ministry of Health should receive each year the vaccines in time to transfer them to the States in January. The Federal Congress is suppose each year to release the budget early January, but it is done in June, too late to produce vaccines, which for lack of funds cannot purchase from Butantan or any international supplier. We solve the problems creating Butantan

Foundation, a not for profit organization, which save funds to begin vaccine and sera production early each year. The Foundation also hires the needed staff for production and purchase each year the chemicals and other supplies require to start each role in advance to guarantee the supply of vaccines in time. It sounds incredible to bypass the federal bureaucracy! Between 1984 and 2009 (when reaching 70 years I could not continue as the Presidency of Butantan Foundation), we were able to provide to the whole population the vaccines listed in table I.

Table I – Vaccines supplied by Butantan Institute from 1984 to 2009

Vaccines and sera	Year of introduction	Estimated number of doses
DTP (0-2years)	1984	300.000.000
Hepatitis B (0-2 years> 19 year)	1996	260.000.000
DT (19 year)	2008	120.000.000
Human rabies	2005	8.000.000
Influenza (from bulk)	2000	220.000.000
Total		908.000.000

A combined DTP vaccine, with diphtheria, pertussis and tetanus to be administer at 2, 4, 6 weeks was introduced in the USA in 1948. In Brasil 70 million children bellow one year of age received Butantan vaccine between 1990 and 2007, resulting in a drop of incidence of diphtheria and whooping cough and accidental tetanus:

Acellular pertussis vaccine

Bordetella pestussis strains used for vaccine production in the world were not uniform and some, like the strain used by Sato (from Japan NIH), were very toxic to less than one-year children. Bordetella contains pertussis toxin (that induces lymphocytosis), filamentous hemoagglutinin (FHA), adenyl cyclase (interferes with phagocytosis), (FIM) and pertactin (PRN) (which attaches to ciliate respiratory epithelium), tracheal cytoxin, dermonecrotic necrotic cytoxin (which causes dermal necrosis), BHK (which mediates adherence). It also includes in the Bordetella membrane the lypopolysacharide (LPS).

Sato approach was, using chromatography, to isolate pertussis components to create an acellular pertussis

vaccine. Several other European and American producers follow by, producing acellular pertussis vaccines, including FHA or FHA and FIM, or FIM and PRN. Acellular pertussis was combine with diphtheria toxin and tetanus toxoid to recompose a DTaP vaccine, avoiding adverse reactions.

DTaP rapidly conquer the market as compare with classical developing countries that were concern with affordable prices. It is easy to find the cost per vaccine using the annual vaccine price released by OPAS form Latin America: classical DPT cost US$ 0.16 per vial, while DTaP cost US$ 15.00, making impossible for Governments to distribute free to its populations.

Suddenly CDC became concern when the incidence of whooping cough increased dramatically even among vaccinated adults. Data appointed to Plow being a limited time affective vaccine.

Butantan's approach was to develop a technology to without breaking the bacteriamembrane decreasing LPS with an organic solvent. Electron microscopy shoved that the size and shape of the bacteria can be preserved, while decreasing its endotoxicity, creating the "Pertussis low".

DPT vaccination is done in three steps during the first year of life. A new idea that received attention was to protect the newborn by the administration of the DTP and other vaccines to the pregnant women, thus protecting the newborn by transferring antibodies from the mother's milk. Sounds effective and safe.

Inflluenza and pertussis

In 1918, during the war in Europe, the "Spanish flu" was carried by the American soldiers to the US and spread to Latin America, reaching Brazil and, specially, São Paulo city, with about 520.000 habitants, where there was 5.100 flue deaths. Those that could left the city used electric public transports.

Pandemic flu with different strains occurred in 1918 (H1N1, Spanish flu), in 1952 H2N2 (Asian flu), in 1957 H3N2 (Hong Kong flu). Only quite recently, WHO realized that the total world production vaccine e capacity could not supply all menaced countries.

Butantan decided to build its own plant to produce vaccine for Brazil, building a dedicated plant. I had become a friend with Charles Merieux, which helped us to build the plant and order the construction of the special equipment by Brazilian manufactures. Merieux helped us design and test the equipment, and agree to supply for a limited time vaccines produced by Merrier as bulk and to trainer the staff that will operate de plant and those in charge of the assay of the new vaccines. We acquired modern high-

speed centrifuges. Brazil have a large poultry production and we selected the producers that were capable to supply clean fertilized eggs as a media to produce influenza vaccine.

In the Center of Biotechnology, we were interested in finding effective adjuvants for the production of vaccines. The availability of our own monophosphoryl lipid A (MPLA), as a sub-product of LPS, removed from our low pertussis vaccine, was our obvious preference. Squalene from shark liver oil, replaced by synthetic product made by fermentation, became restrictive by the limits of availability to the large vaccine industry. Vitamins produced in large amounts for the food industry and vitamin market, were a potential choice of adjuvant. Those like squalene reveal that large vaccine producers would avoid marked competition. $Al(OH)_3$ is a traditional adjuvant since the 1920´s.

We conducted a wide trial for influenza using mice, using vitamins A, B2, B9, D and E, combined with MPLA, $Al(OH)_3$ or Squalene in 27 combinations, and evaluate immune response. Combinations $Al(0H)_3$ /MPLA and vitamin B2 (riboflavin) were the most promising adjuvant, tested in a double blinded human assay with 2009 influenza A (H1N1) vaccine (published in 2009, Arch. Virology in 2016).

With the adjuvants, it would be possible to use ¼ dose of the vaccine, reducing the cost of vaccination, increasing the capacity of Butantan's plant without any change in it. Renovation to increase the capacity of Butantan's flu vaccine production was ordered by present Director, imagining that it would sell to WHO, with a significant economical waste of reserves of Butantan Foundation,

The use of adjuvants would allow to offer a significant volume of influenza vaccine to other Latin America countries, if necessary, at a cost cut acceptable to Butantan if contributes to avoid the potential impact of visitors, tourists or migrants bringing flu virus to Brazil.

YIELD OF SPILT OR WHOLE INFLUENZA VACCINE PER EGG, WITH ADJUVANT

The most expensive item in our production of influenza vaccine is the fertilized chicken eggs. In a essay with different influenza vaccines, with splited or whole viruses, with $Al(OH)_3$ + MPLA as adjuvant we could obtain a 4 fold increase of vaccine yield per egg used, thus an increase of 4 fold vaccines yield per run with a significant reduction of cost per dose!

HEPATITIS B VACCINE

When Butantan decided to build its hepatitis B plant, it coincide with receiving a small group of russian expects, which decided to migrate to Brazil. While Butantan was building an proper plant, equipped with imported high speed continuous centrifuges, this group had expertize to construct producers strains and had one ready approved strains to began to produce hepatitis B vaccine.

Lots of Hepatite B vaccine were produced and properly assayed for efficacy. The migrant newcomers brought samples and seed lots. Vaccine was produced by Butantan that presented capacity to supply the demand of the Minister of Health, to be distributed to all population.

Hepatitis B vaccine, and the other vaccines in production continue until about 2016, when the past Director decided to renovated the plants, removing the installed equipment (to be discussed later).

THE ROLE OF BUTANTAN

When I came to Butantan I made clear what should, in my view, define its fundamental role:

- To innovate, translating innovation into products, thus becoming self-sufficient in the production of vaccines and other biologics so the Ministry could supply free to the Brazilian population, at affordable cost.
- To be able to identify the vaccines and hyperimune sera need to avoid frequent diseases in Brazil and in countries in the region, offering excess capacity through the rotating funds of OMS and UNICEF, making clear that are not moved by profits. Open our laboratories to investigators in Latin America as our partners.
- Establish cooperation with other public research centers like NHI and Pasteur Institute and with foundations which could help Butantan.
- Establish a cooperation with public medical schools in Brazil to join us in the clinical trials of the new vaccines.

This overview suggest guidelines to the Institute Director to approve and implement.

MORE VACCINES

The Center of Biotechnology under my direction, differs from other research laboratories when establishing its role in carry on research, not to publishing paper , but to develop new production process and products to be assayed in animals and, if approved, in human.

The group of young PhD recruited were assigned to develop different vaccines. Some other researchhers were ready to work in the dedicate plants or processes under development:

- Hepatitis B + DPT (at birth)

- *Haemophiluas influenza* type B capsular polyssaride
- *Neisseria menongites* capsular polyssacarideS
- *Strepteotococcus pneumonia PspA* and *PspCHaemophilus influena* type B capsular polyssacharide
- Rabies vaccine for human use (new bilding ready)
- Dengue vaccine NHI, production technology by Buftantan, bilding almost finished
- BCG expressing S1 pertussis or alfa TNF for intravesical cancer (with Prof. Srugi FM.USP)

VACCINES DEVELOPMENT ASSOCIATED TO US.NIH

In 2005 I began an agreement with Kapikian to develop the production of the pentavalent rotavirus vaccine, setting a small lab to produce vaccine for clinical trial. The Ministry of Health was eager to introduce the rotavirus vaccine purchasing for GSK the monovalent vaccine, which showed that it did not protect against G2 and G3 present in Brazil. I did get the support from the Ministry of Health. We got some support of Fogerty Center but no support from the Ministry and the production of the rotavirus pentavalent was not implemented.

In 2013 dengue became epidemic in Brazil and Latin America, reaching 995.000 cases diagnosed in Brazil. I mobilized the support of a team of experts that include Donald Francis, Steven Whitehead that directed dengue project at NIH and Annm Durbin from Bloomberg, the School of Public Health that maintained the support to Butantan for several years, beginning with a phase II-III trials. Butantan begin to build a dedicated plant, aiming to produce 100 million doses/year, for 3 doses of the vaccine per person. Butantan was ready for clinical assays with a pilote production.

Meantime Sanoffi began to produce its dengue vaccine, which was tested in Philippines and showed to be dangerous, killing a number of children, and end up with the punishment by jail for the person responsible for its use! This was not considered by a Minister of Health, a politician without any knowledge of public health that decided to purchase Sanofi vaccine just to be used in his State! Sanofi vaccine was removed from the market.

Butantan's dengue plant got about two years ago the support of the Minister of Health and of the State of São Paulo to finish the production plant. Francis, Ann and Whitehead continue they support and visit Butantan a few times to evaluate the result of the clinical trial.

BIOPHARMACEUTICALS

- **LUNG SURFACTANT**

A project documents how complex it is to transforms an idea through innovation into a product for public health. I became aware that 40% of premature neonates in Brazil, apparently normal at their delivery, died at birth in front of their mothers of asphyxia. It is about 50.000/year in São Paulo and 3 million /year in the world.

We developed at Butantan a simple process of surfactant obtaining, using porcine lungs (of no commercial value) to isolate by batch adsorption, followed by extraction with an organic solvent and further purification by SP-Sepharose resin, useful to treat pneumonia. The process allowed purifying also aprotinin that protects the lung from neutrophil elastase, useful to treat pulmonary edema and pneumonia.

The Ministry of Health committed to make surfactant available at maternities, to be administered by intra-tracheal tube. Meanwhile we worked at the development of an aerosol system to be used by nurses in the field without intubation.

A small plant was set to produce the first batches of surfactant to be tested. The surfactant produced was used for a large clinical testing in public maternities spread to Brazil, showing that it was secure, safe and efficient. Results were submitted to the regulatory agency and approved for use.

Immediate plans mobilized the help of a large meat slaughter company that accept to provide enough space in their plant to have a large tank where lungs were washed and start first steps of extraction to separate the crude lung surfactant, to be refined at Butantan and packed in single doses ready for use.

HUMAN BLOOD AND PLASMA

In Brazil the Constitution defines that blood, plasma and its products, cannot be purchased or sold for profit. Blood is usually collected by Hospitals from donors and used in patients. In Rio at the seventies, a known company created a plant to process blood, what they did without testing, and the result was spreading hepatitis B. The company let to it withdraw from Brazil without assuming its responsibility.

During World War, Brazil sent soldiers to Europe. The army had to have access to albumin to rescue wounded soldiers, while to be transferred to army hospital. An effort to separate albumin at the Medical School of the University of São Paulo did not succeed. By the eighties, the Clinical Hospital started to develop blood fractionation process. This was a signal to the Center of Biotechnology of Butantan to extend its activities to engage in human plasma fractionation.

The São Paulo State Government gave us funds to build a plant and begin to order equipments from Phamacia (later GE), based

in a chromatographic process. As Pharmacia was trying to sell small plants for blood fractionation to hospitals, they became interested in helping Butantan to develop its processes. At the time largest size chromatography columns became available.

Chromatography column and ultrafiltration system from hemoderivatives process

Butantan Institute is a branch of São Paulo State Office of Public Health. Its Director was selected by the State Secretary of Health from the staff of scientists with life time positions. Recently Director Prof Kalil, a scientist from the Medical School of the Univiversity of São Paulo, followed recently by by Prof. Dimas from the medical school of Ribeirão Preto University of São Paulo, which changed Butantan's fundamental role. The plasma derivative plant was never finalized.

THE END OF THE DREAM OF BUTANTAN ?

The new director stop completely the renovation of the plants, started by its predecessor, leaving the equipment outdoor in tends. He decided to invest all the available funds provided by the Ministry of Health and the rotating funds of Butantan Foundation to invest a large building to accommodate all the bureaucracy. Also invested at Butantan large park, gardens and internal streets, repainting and replacing the roof of the old builds, making Butantan "beautiful" for the visitors.

The sole plant that remain operational was the influenza vaccine plant. Even that plant undergone a reform "to increase the vaccine production", announcing in the newspapers that Butantan would supply WHO, which does not purchase vaccines!

There was an attempt by Sanofi to buy all the production plants, which I was able to stop by publishing in a brazilian newspaper. Sanoffi (which own the influenza plant in Mexico) purchased a long-standing South Korean plant that produced hepatitis B vaccine, and another in India, thus creating a monopoly in the developing world. Other large pharma offered to make a partnership with Butantan to market the production of our vaccines starting with dengue, in Latin America.

Vaccines produced by Butantan were replaced by purchasing bulk from large pharma. DTP that was produced by Butantan was replace by imported vaccine in bulk and nowadays is missing, delaying the vaccination of Brazilian children.

The economic impact with replacing Butant's DTP with Plow, by imported bulk of 3 million doses/year of acellular DTP to vaccinate pregnant women, expecting to protect their newborn. The estimated cost by OPAS was US$ 0.16 for classical DTP, for US$ 15.00 for acellular DTP! Either the Ministry of Health did not care for the new prices, or was part of a "deal" with the vaccine supplier. Also the Minister of Health did not follow CDC discovery that acellular DTP had a short effect, resulting in large number of adults with whooping cough.