SUMMARY OF THE PROGRESS ACCOMPLISHED AT

FOOT AND MOUTH DISEASE VACCINE LABORATORY, NONG SARAI

DIVISION OF VACCINE & SERUM

DEPARTMENT OF LIVESTOCK DEVELOPMENT

by

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Science, especially Biology, is making giant strides. We are now far from the viruses formerly defined by PASTEUR as: "rational but undefinable beings", whose multiplication or more correctly whose "replication" enables us today to penetrate to the very heart of the problems of heredity. And yet, I cannot resist quoting two texts of the illustrious Pasteur; one, because it seems very appropriate to today's ceremony; the other because it has long been our leitmotiv. Here is the first:

"I beg you to take an interest in those sacred abodes called by the expressive name of laboratories. They are the temples of the future, of prosperity and of welfare. It is there that Humanity grows, becomes stronger and better. It is there that it learns to read in the works of nature, of progress and of universal harmony." The second is his last piece of advice to his disciples:

"Never put forward any idea which cannot be proved simply and decisively. Practice the cult of a critical mind."

As I have already mentioned, these words have been our leitmotiv for a long time. It is therefore logical, at this point to ask oneself what has been happening at the NONG SARAI FOOT AND MOUTH DISEASE LABORATORY, from 1958, the year it was finished, until today's ceremony which celebrates our first industrial production of vaccine. I know that, according to the poets:

"Time measured by the year is not the measure of the days we live; therefore, consider man's heart as the true measure of time." But, if this time has not seemed long to us, may I be allowed briefly to retrace the stages of

our progress. It will also serve as a justification, in the eyes of everyone, of the **confidence** you, Dr. Chakr, Deputy Director of the Department of Livestock Development, and you, Khun Vichitr, Under–Secretary of the Ministry of Agriculture, have always shown in all of us, the workers at the NONG SARAI LABORATORY, in spite of the slowness of our progress.

The problem was apparently simple: to produce an anti foot and mouth disease vaccine. It was also a clearly posed problem: identify the virus, produce the virus, and transform it into an effective vaccine.

The identification of the virus raises the problem of deviating from certain techniques because: "one cannot for instance take a bit of aphtha from the mouth of an infected cattle, and put a smear of the material under the electron microscope to check at once whether the virus is or is not responsible." These indirect methods do what the electron microscope is unable to do, namely, to distinguish between the types of virus. Just to make this work easier, **seven** different types of foot and mouth disease virus are now recognised, all of them can cause a new disease identical to the preceeding one, from which an animal has just recovered. However, Thailand has been relatively priviledged up till now! Two classical types. O and A and its own Asia type: Asia I, thus numbered in case others should crop up!

I should mention that Asia I has given us some surprises, especially concerning its **slow evolution**. This slowness, when it attacks buffaloes is beyond belief: 13 days are needed before lesions are produced, whereas if the same animal is infected with other types of virus, lesions are produced in 4 days. This has certainly not helped to simplify the prophylaxis!

The production of diagnosing serum on guinea pigs for the identification of viruses caused no difficulty and took about 2 months for each type. In **March 1959**, the NONG SARAI LABORATORY was already able to identify foot and mouth disease virus, not only for the laboratory's needs, but also for the requirements of prophylaxis. We were thus able to establish that the 1959 epidemic was due to the Asia I type, whereas from the end of 1959 up till now, the present epidemic is attributed to type O. NONG SARAI LABORATORY was also able to help CAMBODIA by identifying the foot and mouth disease virus responsible for its local outbreak. And this, by the way, **poses the problem of the existence of a Far East Regional Laboratory** – who will deny its importance?

The present method of virus identification is routine work. I can even go further and say that serology no longer holds any secrets from our specialist

Dr. PINIT. Dr. PINIT is also grappling with little mice – because, unfortunately for them, suckling mice are receptive to foot and mouth disease! He applies statistic calutations to the readings of his results. By this means, we can find out the infectious powers of the virus. Thus it is not only a method for revealing the presence of viruses, but also for finding out their aptitudes.

The second problem was the production of virus. Just as in the case of pearls, we make the distinction between natural virus and cultured virus.

Natural virus is obtained after the injection of virus in the tongue of a live animal, and 24 to 36 hours after when the animal is slaughtered, all the epithelium is collected. Epithelium is the covering of the tongue. The average production varies with each type of virus from 5 to 30 gms. per animal. However, 1 gramme af this product, diluted in 100 **cubic meters** of water is still infectious for cattle. This method of production is economically possible if the meat can be consumed afterwards. But to slaughter the animal without more ado, in order to protect 50 or 80 others, is not justifiable in a non fatal disease. But this meat, which is not dangerous for human consumption can still spread the disease to other animals. So very soon, laboratories tend to adopt the production of virus by culture.

Cultured virus relies on the culture of tissue. In a way, it is an extension of CARREL's work, but great progress has since been made. It is hardly necessary to be reminded that in every organism general death preceeds the death of tissues. The fragment of a hen's heart, in CARREL's famous experiment, has proved that any tissue has eternal life. By making use of this property, it is possible to collect animal tissues just after slaughtering, and to give them the means of surviving. Up till now, viruses required the chemism of a living cell in order to multiply, and so virus culture is carried out on this tissue culture.

This living tissue can either be made to survive or else to start from a few cells and then produce a carpet of cells. With foot and mouth disease, the method of survival is that of a Dutchman, Dr, FRENKEL. The carpet of cells is made from kidneys: monkey kidneys for poliomyelitis, piglet's or cow's cells for foot and mouth disease. Here at Nong Sarai we have been successful in our **tissue cultures**, but we have only accomplished the first stages, and later we must take up this work again and concentrate all our efforts on Frenkel culture, since this method is the best adapted to our needs. **What exactly is Frenkel culture**? It takes the place of a live animal. The injection of virus in the

tongue epithelium of an animal transforms it into natural virus. If we maintain this epithelium alive in a flask, and inject virus, the latter will then multiply and become cultured virus. Theoretically, the idea and its execution are simple, but in practice, there are many hitches, especially in our case, as we started straight off with Asia I virus.

At the beginning, Asia I was very difficult lo cultivate and when it finally multiplied, its maximum growth took 28 hours (20 hrs. are usually sufficient for other types) and only provoked paralytic lesions in mice after 5 to 7 days, whereas 2 to 3 days are sufficient for its congenerics. But little by little we observed that it began to accept..... domestication! It now cultivates in 20 hours and passes in mice in 3 days. At the same time, it is true that our virus which was of buffalo origin was being slowly transformed into cattle virus. To adapt the virus to tissue culture, we had to make alternate passages from culture to cattle and from cattle to culture. This distinction is now quite clear in our minds, that is to say, a buffalo virus is no more like a cattle virus, even though it be of the same type, than say a Clos Vougeot and a Pommard, even though both belong to the famous Burgundy wine.

We also had to get to know the particularities of our filters. We now know a lot about their powers of adsorption, even to the extent of being able to tell others all about it.

Our virus is also extremely delicate, it requires pure air! Carbonic oxygen is too acid, it needs chemically pure oxygen.

Thus weeks and months passed with alternating failures and successes. Dr. PRACAL, the specialist, began losing his last.... hair, so that he can now without discussion, join the honourable category of eggheads! I should also add that what with all the grammes, milligrammes and microgrammes of amino acids, vitamins, antibiotics and hormones, Dr. Pradit has been working away with the conscientiousness of an alchemist. It is so funny! he says.

But today Nong Sarai can tackle with masterly skill the difficulties of the different types O,A, and Asia I.

The third problem was the realization of an effective vaccine against foot and mouth disease. The whole world produces vaccine against foot and mouth disease. When we came to testing our first experimental classical production, only 50% of buffaloes were protected!

We again thought of the difference between..... Burgundy wines! We decided to vaccinate buffaloes with either a stronger dose or with repeated doses in

order to obtain a booster effect. But in biology, reasoning may be correct, but in practice, it may be just the contrary. Fortunately, experimentation is there to help us in our decisions. This particular experiment did not help us to do any better since only 50% of the buffaloes were protected!

But man's imagination rarely lets him down, and a new idea occurred to us. The adaptation of virus to cattle produces a poor antigen for buffaloes; this is also true for pigs. It therefore seemed necessary to try a vaccine prepared with a **homologous virus.** But our vaccine made from natural buffalo virus only protected 55% of the buffaloes vaccinated!

After this failure we began to have doubts about the value of the **excipient which is used as the foundation of the vaccine.** Without entering into technical details, we must however name this substance–aluminium hydroxide is a medium in a state of colloidal suspension, and by nature an unstable substance after a time. Our stock was already more than one year old. Verification of its qualities in somewhat difficult and beyond our means at present. In spite of this, our theory was re–inforced by the successive failures when we tested several experimental productions on cattle. It has now been completely confirmed by the analysis carried out in Europe.

From there on, all the experiments **would have to be repeated with a new batch of aluminium hydroxide** However this product is made in Denmark and administrative slowness is international!

In the meantime we tried to do something new and different. THE WORLD REFERENCE LABORATORY IN FOOT AND MOUTH DISEASE whose centre is in Pirbright, England, is experimenting with a live vaccine; a **virus vaccine** obtained by the adaptation of foot and mouth disease virus to adult mice. This results in the virus conferring foot and mouth disease on mice, but experimentally, it protects cattle in England. It has also had practical success in East Africa.

In THAILAND our experiment was conducted and assisted by technicians from PIRBRIGHT and unfortunately was not so successful. The first experiment undertaken with an anti Asia I vaccine, protected only 50% of cattle and 44% of buffaloes. In the second experiment, carried out with Anti A vaccine, 100% of cattle and 35% of buffaloes contracted foot and mouth disease. Moreover the vaccine was not totally innocuous for buffaloes, since 30% showed foot and mouth lesions following the vaccination.

The problem was still not solved!

So, on the one hand the virus vaccine has not yet been perfected, and on the other, aluminium hydroxide is still in Denmark.

For a long time, the difficulty in the preparation and especially in the keeping of aluminium hydroxide had incited us to use another adjuvant with a less well-known action : **saponin**. This product is very stable and cheap, and is obtained by extraction from the crude drug with hot alcohol. At least 500 genera of plants can produce it. "Generally, saponins are neutral in reaction but some are acid and a few basic." Unfortunately our first tries were done with a "purified" saponin but of acid reaction, which had some curious and paradoxical result. The vaccine of cattle origin protected only 20% of cattle and slightly more buffaloes-37%. The vaccine of buffalo origin protected 64% of buffaloes and more cattle-90%. But these results were also incorporated into a learned theory, which later was contradicted by experimental data.

In the meantime, **by chance**, we received some non purified but neutral saponin. Since then, we have been repeatedly successful. In the first experiment, 100% cattle and 100% buffaloes were protected. In the second also, 100% cattle and 100% buffaloes were protected. A third experiment was carried out when foot and mouth disease broke out around the breeding station of TA PRAH. The corresponding vaccine was urgently prepared and used. This happened 3 months ago and TA PRAH has been fully protected,

Thus, thanks to the action of saponin, the problem of vaccinating Thai cattle and buffaloes, using the same type of virus, can be considered solved.

We now believe we are in a position to approach the industrial production of vaccine. We shall begin today by converting our stock of O virus, about 80,000 doses into a formolized, saponized vaccine.

You are here to celebrate this happy event!

On the occasion of a scientific meeting held in London, Sir Cyril HINSHELWOOD declared: "The full fruition of scientific work depends upon three things: the desire to know, the initiative to find out and the awareness to apply." I think that the team at Nong Sarai, under the brilliant leadership of its director Dr. UDOM CHARUTAMRA has never ceased to apply the above principle. Even though sometimes disappointment followed our failures, it has never destroyed our morale, but on the contrary has bound our team even closer together. in the end, the set-backs we have experienced have taught us a great deal. Today's success holds its own compensation. But our job is not yet finished. Today the final but not the least important step is to be started: **the industrial production of vaccine and the eradication of foot and mouth disease.** A vast and ambitious programme which is inscribed for "the basic freedom" in the WORLD-

WIDE FREEDOM FROM HUNGER CAMPAIGN started by Mr. SEN the Director General of the Food and Agriculture Organisation.

According to our estimations, foot and mouth disease costs THAILAND a loss of 300 to 400 million baht per year. The problem is therefore important. It comes third in what Mr. SEN calls the "action front" and is defined as follows: "The action front would, so to speak, be the culminating one of the campaign. It would seek to develop concrete projects to be carried forward in individual countries, projects which would range through the whole field of food production, distribution and consumption and would include measures to enhance the buying power of those sections of communities most in need of more and better food".

I will end by quoting Mr. SEN's most appropriate wish:

"I wish you good fortune in all that you undertake on behalf of the campaign and mankind itself.

Nong Sarai, 3rd December 1960
H.C. Girard

ตัวอย่างที่ดี

.....ข้าพเจ้าถือภาษิตของทหารที่ว่า การเป็นผู้ใหญ่นั้นหูต้องหนักหลายสะโตน เพราะหูนั้นมีมากกว่าปาก คือหูมี ๒ หู แต่ปากมีปากเดียว และข้าพเจ้าชักจะพูดมากกว่าหูเสียแล้ว การฟังอะไรจะต้องไตร่ตรองโดยรอบคอบ

คำปราศรัย พณฯ จอมพล สฤษดิ์ ธนะรัชต์ แก่นิสิตจุฬา

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