Deficiency of available silica may be caused by the nature of the parent material and also by leaching in the course of soil formation.

f) Iron.

For Japanese paddy soils one percent of active Fe_2O_3 as determined with Mg-ribbon reduction method is critical. Below this value unfavorable effects on the growth of rice may be expected. This threshold value has been adopted for the results of iron determination with a photo-chemical reduction method which is somewhat more drastic than the Mg-ribbon reduction, then soils having less than one percent of iron oxide may be grouped as "susceptible to deficiency", while these having less than 0. 5 percent as "severely deficient". The number of the iron deficient soils as grouped here is given in Table 4.

Among the three iron oxides, goethite, hamatite and lepidocrosite, which are common in Japanese paddy soils, no lepidocrosite has so far been detected in Thai and Malaysian soils.

g) Easily reducible manganese.

If criteria established for the Japanese soil are tentatively adopted, manganese deficient soils in Thailand and Malaysia are distributed as in Table 4. The term "manganese deficiency" as used here signifies physio-nutritional deficiency of the rice plant, while the iron deficiency as refered to above means probable insufficiency in keeping the environmental conditions for rice growth optimum. But both the deficiencies may be caused mainly by the nature of the parent material.

Due to the economical situation of rice in Southeast Asia it may not be practical at the present time to recommend the use of materials containing silica, iron and manganese. However the knowledge that not a few paddy field soils are deficient in these elements would have to be kept in mind in taking various actions, such as selecting varieties, improving cultural techniques, conducting fertilizer experiments, acting against diseases and insects, analyzing soils and plants, and so forth. It would be much more unreasonable to underestimate the importance of these elements besides N, P, K than to take the deficiency of these elements very serious.

Two Rinderpest Live Virus Vaccines, "Lapinized" and "Lapinied-Avianized"

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During the period from the second to the fourth decade of this century, several types of inactivated rinderpest vaccine were developed and contributed a great deal to the control of the disease in different countries in the world. Of them, the well known are the glycerin-inactivated (Kakizaki, 1917), the formalin-inactivated (Cursson and Delpy, 1926), the toluol-inactivated (Kakizaki et al., 1927), the chloroform-inactivated (Kelser et al.. 1928) and the formalin-inactivated and alminium-hydroxyde gel added (Jacotot, 1940). However, two facts, including (1) the vaccine is very expensive and (2) the immunity produced by its use lasts only for a few months, were considered as the great disadvantages common to these vaccines. And these became the main obstacles in massive application in the field, because control and eradication were usually urgent with this disease.

Edwards reported, in 1927 and 1930, of his success in the development of an attenuated

rinderpest virus as a result of serial passages of the original virus in goats. This attenuated virus was the first live virus vaccine of modern type applied to field immunizations against rinderpest. Since then, studies on live virus vaccines in rinderpest have become widespread. In addition to Edwards' caprinized vaccine, four other types of vaccine have been introduced by differet authors and have come into practical use in various parts of the world. These are the by rabbit-passage attenuated or lapinized virus by Nakamura et al. (1938), the by chickembryo-passage attenuated or avianized virus by Shope et al. (1949), the by rabbit- and chick-embryo-passage attenuated or lapinized-avianized virus by Nakamura and Miyamoto (1953) and the by calf kidney cell-passage attenuated virus by Plowright and Ferris (1959).

As a basic property of live virus vaccines in general, rinderpest live virus vaccines provoke from inapparent to slightly apparent infection of vaccinated animals before immunity is produced. This is actually a subject to which great attention should be paid in their use. However, these vaccines have commonly the great advantage, in contrast to the inactivated vaccines, for they are produced at much lower cost and in much greater quantity, and immunity produced by their use lasts much longer. The use of these vaccines has gradually outstripped the use of the inactivated vaccines in many countries, and their field applications have become, in recent years, common in parts of the world where the disease still exists. The complete erradication of the disease has been reported in a number of countries as a result of the advance of the disease control programmes which were based mainly on the massive application of live virus vaccines and strict execution of other control measures.

This paper deals with the lapinized and lapinized avianized live virus vaccines developed in Japan and describes mainly how these vaccine strains were established, to what breeds of animals the vaccines can be applied and how these vaccines are prepared.

Development of the Two Attenuated Strains

Lapinized Strain III

During years from 1934 to 1938, Nakamura and coworkers established seven rabbit-passage strains of rinderpest virus of which the first six originated from their old laboratory bovine strain "O" and the last one from a naturally infected case (cattle) which occurred at the time in Manchuria. By subcutaneous inoculation of blood virus at an early time of study as well as by intravenous inoculation later applied, there was no difficulty in passaging the virus successively in rabbits, and all the seven strains were maintained as long as desired. The strain widely distributed as a live virus vaccine in different countries in Asia and Africa and often called by the name of Nakamura Ⅲ strain is the third from 0 originating strain which was designated originally as strain Ⅲ, and is now over 1,200 rabbit passages in our laboratory.

The rabbit passaged virus has undergone two steps of distinct increase in pathogenicity to the passage host. The first alteration occurred in such early passage level as within 10 passages uniformly in all the seven strains, and was recognized by the development in infected rabbits by a distinct rise of body temperature and characteristic lesions in lymphatic tissues which were not in animals infected with the bovine virus. The second increase of virulence, which caused the death of animals, was very slow in occurring. Actually the death of rabbits due to virus became definite only after several hundreds of passages in strain Ⅲ. The longest passage history among the other strains was 180 of the fourth from 0 originating strain which had never acquired a definite lethal activity before the strain was discontinued.

Another important variation of virus property during rabbit passages was the decrease in pathogenicity to the original host. This alteration occurred gradually in the first 100 rabbit passages of strain Ⅲ, and was recognized by the gradual prolongation of the course of disease and finally by the escape from death

of inoculated Korean calves, which are known as one of the bovine breeds having the least resistance to rinderpest and succumbing practically 100 percent in acute courses when inoculated with the bovine virus.

The attenuation to cattle of the virus in strain Ⅲ, however, appeared to reach the maximum in 100 rabbit passages, and results of inoculation experiments to Korean calves carried out with virus specimens in a passage range between 100 and 361 were that all the animals revealed more or less strong reaction and about one-third succumbed on the average. No further attenuation has been noticed since then, and Lee reported even higher mortality rate in Korean calves inoculated with virus specimens after 700 rabbit passages.

Lapinized-Avianized strain LA

The lapinized-avianized strain LA is applied as a vaccine in Japan, Korea, India, Thailand, Cambodia and Egypt. This strain originated from the lapinized virus of strain Ⅲ, and has been established and maintained through the following 5 stages of chickembryo passages.

1st stage: Initiating from the 736th rabbit passage of strain Ⅲ, the virus was passaged alternatingly in 11 chickembryos and 11 rabbits (E_{11} R_{11}). Two successive chick-embryo passages of the virus in this stage were repeatedly tried and resulted always in complete loss of the virus.

2nd stage: The virus was transferred in 40 chickembryos by the help of 23 conjunctive rabbit passages (E_{51} R_{34}). The longest successive chickembryo passages available were 4 in two series which started from E_{24} R_{19} and E_{47} R_{33}, respectively.

3rd stage: The virus was maintained by 20 successive passages in chickembryos without any help of conjunctive rabbit passages (E_{71} R_{34}). Development of characteristic spleen lesions and death due to virus became evident in infected chickembryos at about E_{60} R_{34}.

4th stage: Again alternating passages in 7 rabbits and 6 chickembryos were made (E_{77} R_{41}=A). This was designed not from the necessity for maintaining the virus.

5th stage: The virus has been maintained up to the present by 617 successive chick embryo passages (A-E_{617}). Meanwhile, the virus has undergone remarkable attenuation to rabbits and cattle. Rabbits inoculated have not died of infection since about AE_{150}. Japanese calves inoculated have no longer developed any serious reactions since about AE_{169}. According to Fukusho, who has pursued the pathogenicity of this strain to Japanese calves during a long passage series from AE_{191} to AE_{572}, no alteration has occurred during those passages and the animals inoculated developed clinical reactions from nil to a few days' temperature rise plus some loss of appetite, and all survived and acquired immunity. (Japanese cattle are equally sensitive to rinderpest virus as Korean cattle, and calves inoculated with the lapinized virus die usuallly with a rate of one-third or higher.)

To the contrary, the virus has shown some further increase in its growing ability in chickembryos. This was recognized by some rise of virus titers in chickembryo tissues, which appeared to reach the limit by the passage level of AE_{200}, and by constant demonstration of specific complement-fixing antigen in infected tissues which served for a definite judgement of infection.

Choice of Vaccine

It is fundamentally required for a live virus vaccine that the infection with the attenuated virus should result without any adverse reactions in a solid and lasting immunity in vaccinated animals. With regard to rinderpest live virus vaccines, how animals respond clinically to a vaccine depends mainly on the degree of attenuation of the virus strain used and the susceptibility of the animals inoculated.

Among three vaccine strains which were developed by passages through different animal hosts, i. e., goats, rabbits and chick-embryos, the goat passaged or caprinized strain is the least attenuated, the chickembryo passaged or avianized strain the most attenuated and the rabbit passaged or lapinized strain in-between.

The influencing factors on the side of

animals are the species, the breed, the age, physical conditions etc., among which the most important are the first two. As suggested by data obtained either in experiments or in fields, some breeds of cattle, buffaloes and pigs may be graded in the following order as to the susceptibility to different strains of rinderpest virus.

1. Japanese cattle, Korean cattle.
2. East African Ankole cattle, Egyptian Balady cattle, Thai pig.
3. Holstein-Friesian cattle.
4. Asian water buffalo.
5. Asian cattle of Zebu type.
6. Egyptian water buffalo, Yorkshire pig.

The caprinized virus is safe and strongly immunogenic in animals of grades 5 and 6, but it often produces fatal reaction in animals of grades 3 or higher. The lapinized virus is safe and effective in animals of grades 4, 5 and 6, but has some risk of provoking severe reaction in Holstein-Friesian cattle, particularly of younger age. The lapinized-avianized virus is applicable with safety to all animals from grade 1 to grade 6.

The immunity following active infection with any of these virus strains is generally solid and last for years, regardless of the degree of clinical reaction the inoculated animal reveals. As an example, the lapinized-avianized strain gives strong immunity with no apparent clinical reactions to most breeds of cattle and buffaloes in Asia. However, it appears also true that the duration of immunity produced correlates roughly with the intensity of the preceding infection. Whether to raise the duration of immunity by the use of relatively strong vaccine or to minimize the clinical reaction by the use of relatively weak vaccine should be discussed from various aspects, taking epizootic state, geographical situation, capacity of technical activities, economical condition, intellectuality of owners etc. into consideration.

Those which are mentioned above are the basic factors to be considered at the choice of a vaccine strain for a given animal breed. However, in areas where the disease exists in enzootic form or invades frequently from outside, it is often noted that some old animals are immune in different degrees as a consequence of natural infection or vaccination in the past, and some young animals keep maternally derived serum antibody in different concentrations. The effect of a live virus vaccine among animals in such areas varies with individuals, since the immune response of each animal is modified and varies with the degree of preexistent immunity or antibody. Under such condition, vaccination should be repeated in relatively short period if it is desired to keep the majority of animals immune all the the time.

Random tests of complement-fixing and virus neutralizing antibodies in pre- and post-vaccination serums may serve for the evaluation of the result of vaccination. The test of virus neutralizing antibody is also applicable to assessing the incidence of animals with lasting immunity in a herd or in an area where natural infection or vaccination has taken place in the past. Data accumulated from such serological surveys may provide valuable informations at the programing of the primary vaccination in young animals or the revaccinating in older ones in the same locality.

Preparation of Vaccine

Lapinized-Avianized Vaccine

Source of vaccine: To prepare the seed virus, fresh chickembryo bodies infected with the LA strain is emulsified and diluted to 10^{-3} with phosphate-buffered saline (PBS). Leghorn hens' eggs incubated for 10 to 11 days* are inoculated intravenously with 0.05 ml of the seed virus and reincubatad for further 4 to 5 days. The infected embroys are then harvested, and, after removing the head, wings and limbs, the remaining part is

* In earlier days, 13-day-old embryos were inoculated. The use of younger embryos has brought some increase in the average virus titer.

used for vaccine. Its chickembryo infective titer** is 10^{-5} to 10^{-6}/0.05 or 0.05 g of the tissue contains 10^5 to 10^6 chickembryo infective units (EU).

Infected tissue emulsion and its freeze-drying: The infected chickembryo tissues are emulsified by a blender with equal amount of 1% glucose solution. The emulsion, after light centrifugation, is distributed into ampoules of 2ml each, shell-frozen and rapidly freeze-dried. The virus titer remains unchanged or drops only 1 log or less during the process of drying, so that the product usually has a final titer of 10^{-4} to 10^{-5}/0.05.

Keeping quality of the product: The keeping quality of a freeze-dried vaccine is greatly influenced by the sort of virus emulsion before drying. A heavy emulsion of infected chickembryo tissues in 1% glucose solution was found as best for the purpose among different emulsions comparatively examined. A product prepared from this sort of virus emulsion actually has a surprisingly good keeping quality, and, in experiments, vaccine lots prepared following the method described above exactly still maintained a titer of 10^{-3}/0.05 or higher after preservation for 480 days at −20°C, 360 days at 2 to 6°C***, 60 days at 20 to 26°C or 10 days at 37°C. However, to assure the effect of application in fields where different unfavourable conditions may be encountered, it is advisable to keep the vaccine at ice temperature possibly all the time during storage and transportation.

In contrast to relatively strong resistance of the virus in a dried state, the virus reconstituted in liquid is extremely fragile. Most vaccine specimens reconstituted in PBS were found non-infective to chickembryos after exposure to room temperature for 30 hours. It is recommended to keep the reconstituted vaccine on ice and to use it within several hours.

Dosage: In Japanese calves inoculated with different amounts of the LA virus (from 1 to 2 EU to 200,000 EU), the incubation period varied in a range from 4 to 12 days, roughly correlating to the virus amount used, but no difference was recognized in the type and degree of clinical reaction which followed the incubation period. This fact indicates that the use of excess amounts of the vaccine does no harm and has rather the advantage of making the occurrence of infection and, consequently, the production of immunity surer and sooner. It is, therefore, advisable to make the practical dose involve as much margin of virus as economically permitted.

The content of an ampoule of the vaccine prepared by the mentioned method is ordinarily reconstituted in PBS and inoculated to 20 head of animals. As the titer of the vaccine after storage is 10^{-3}/0.05 or higher, an ampoule to wich 1 g of infected tissue is added contains 20,000 EU or more, and, therefore, 1 dose 1,000 EU or more at the time of use. The minimum EU value required for causing active infection in an animal can be decided from results of comparative titrations carried out in animals of the same breed and in chickembryos. Minimum infective EU values so determined for certain animals are given below for reference.

Japanese calf	1–10 EU
Thai calf	100 EU
Thai water buffalo	10–100 EU
Thai local pig	1–100 EU
Cambodian calf	10–200 EU

A more direct assay of potency of the vaccine is to test each lot of product by animals of the same breed. Ordinarily, the animals are inoculated with a few dilutions of the vaccine and subsequently challenged by a virulent virus. According to Zahran et al. in

** To measure the virus titer of a specimens, 10-fold serial dilutions are inoculated intravenously with an amount of 0.05 ml to groups of 13-day-old eggs. Results are determined after 4 to 5 days' reincubation by examining the characteristic lesions of the spleen, i. e., enlargement and reddening, in individual embryos and more accurately by testing the specific complement-fixing antigen in embryo spleens pooled by groups.

*** Results of recent tests showed 3 lots of the vaccine stilll maintained a titer of 10^{-3} or 10^{-4}/0.05 after storage for 76 or 94 months at 3 to 6°C.

Egypt (1963), their local products of LA vaccine which were prepared exactly following the techniques described above were assayed by the direct method and found immunogenic to their local Balady calves with 1 ml of 1 : 500 dilution.

Lapinized vaccine

Preparation: Healthy rabbits are inoculated intravenously with 0.5 ml of the seed virus which is either fresh undiluted blood or adequately diluted emulsion of lymphnode harvested from passage rabbits of strain Ⅲ. Infected animals usually have high fever after an incubation period of 1 to 2 days, and are sacrificed on the 4th or 5th day of inoculation to harvest mesentheric lymphnodes* on which necrotic folicles are easily recognized from outside as the characteristic lesion. The lymphnodes collected are then emulsified by a blender in 1 : 10 with 50 % normal chickembryo extract which is previously prepared by emulsifying the tissue in equal amount of 1 % glucose solution and centrifugation. The lymphnode emulsion, after being centrifuged again, is distributed into ampoules with amounts of 2 ml, shell-frozen and freeze-dried.

The fresh, infected lymphnode is usually infective to normal rabbits by intravenous inoculation with 1 ml of 10^{-7} dilution or occasionally 10^{-8}. Since the loss of virus titer during the process of freeze-drying is within 1 log, the product usully has a final virus titer of 10^{-6}/1 or higher, and, therefore, it contains 10^{6} rabbit infective units (RU) or more per 1 g of original tissue.

Keeping quality of the product: The keeping quality of the lapinized vaccine prepared by the above method is comparable with or seemingly even a little better than that of the lapinized-avianized vaccine. So far as experiments conducted are concerned, all the products maintained a virus titer of 10^{-5}/1 or higher after the preservation for 480 days at −20℃, 480 days at 2 to 6℃, 90 days at 20 to 26℃ or 10 days at 37℃. For practical purposes, however, it is advisable to keep the vaccine at ice temperature all the time during storage and transportation.

Dosage: It appears also true with the lapinized virus that inoculation of excess amounts does not make the clinical reaction of the animal stronger. A practical dose should, therefore, contain enough margin of virus which may cover the loss of titer during storage and transportation and nullify the susceptibility differences among individual animals vaccinated.

The content of an ampoule of the vaccine prepared by the above method is ordinarily reconstituted in PBS and inoculated to 20 head of animals. As the vaccine has a virus titer of 10^{-5} RU/1 or higher after storage, an ampoule to which 2 ml of 10% virus suspension is added contains 20,000 RU or more, and therefore, 1 dose 1,000 RU or more at the time of use.

As already mentioned, the lapinized virus is not applicable on practical basis to Japanese and Korean cattle breeds because it is still too strongly pathogenic to them. However, so far as comparative titrations made for experimental purposes between calves and rabbits have shown, 0.1 to 1 RU is required for infecting a calf of either breed. So the dosage of the lapinized vaccine given above is balanced with that of the lapinized-avianized vaccine in the meaning that 1 dose of either vaccine contains at least 1,000 infective units for these very highly susceptible breeds. From information available in countries where this vaccine has been applied, it is reasonably presumed that the dose of the vaccine given above is sufficient in confering immunity on Zebu cattle and other susceptible animals. It is, however, advisable to carry out some preliminary experiments to check the safety and efficacy of the vaccine in the local animals to which the vaccine is desired to apply for the first time.

(This paper was presented as a part of the text

* Some use the spleen and blood of infected rabbits in addition to the lymphnode for the source of vaccine. These two tissues, however, are not utilized by us because their virus titers are lower than that of the lymphnode by logs.

for Dr. S. Kishi's lecture to the training course on the differential diagnosis of rinderpest held under the auspices of Food and Agriculture Organization of the United Nations at Mukteswar, India, in November 6-28, 1966.)

Rice Insect Control by Granular Insecticide

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When granular insecticide is applied over the rice plant, little is deposited on the plant surface and most reaches the surface of the paddy soil after falling into the irrigation water. Therefore, granular application to rice field means the application of insecticide into irrigation water or paddy soil.

In recent years, the possibility of applying insecticide into soil or irrigation water of rice fields instead of spraying or dusting it over the rice plant, has been studied. Some of these studies have revealed information now being used in the field.

It was firstly shown by Koshihara and Okamoto (1957), that the treatment of paddy soil with γ-BHC at the time of puddling before the trans-plantation of rice seedling was effective for control of the rice stem borer, *Chilo suppressalis* Walker, one of the most important pests of rice, in the first generation. They observed that BHC 3 percent dust or lindane 3 percent dust applied in paddy soil at the rate of 90 to 180 kilograms per hectare was strong enough to control the insect damage to the rice crop in the first generation.

On the other hand, Okazaki, Kikuchi and Funabasama (1957) applied BHC emulsions carefully to the surface of the irrigation water in paddy field avoiding direct splashing off rice plants with insecticides, and concluded that number of the stems damaged by the rice stem borer in the first generation decreased significantly in the treated plots.

Concerning these effects in such treatments, special attention has been paid so far in Japan to the behavior of this insecticide in and on the rice plant. Okamoto and Koshihara (1959) inferred that the effect of γ-BHC treated in paddy soil had resulted from the absorption and translocation of the toxicant through the root. Horiguchi (1960, 1964) observed similar effects of γ-BHC, but he attributed the effect mainly to the insecticide in the paddy water, which had crept up along the leaf sheath by capillary action and was concentrated due to the evaporation of water. Ishii, Enjoji and Sekiguchi (1959) studied systemic action of γ-BHC in plants using radioactive γ-BHC as the emulsion, and concluded that γ-BHC was not easily translocated into other parts of the rice plant. But they also pointed out that the systemic action of this compound in the rice plant might be proved if a radioactive compound with a stronger specific activity were used.

Besides these studies, a number of workers attentively observed the effects of such treatments. Nevertheless, it was not clear whether the γ-BHC was absorbed from the root and translocated into the stem and the leaf sheath or reached the borer zone of the stem by capillary action.

In order to make clear this point, Ishii and Hirano (1962) carried out a study on the translocation of γ-BHC in the rice plant. In this experiment an aqueous solution of radioactive γ-BHC was used. As a result, they pointed out that γ-BHC dissolved in water was absorbed by the root and then translocated to the stem and leaf of the rice plant, but it also crept up along the leaf sheath by