multiplication. For example, the virulence of some virus strains, e.g. ectromelia virus[16] and encephalomyocarditis virus[17], is associated with the capacity of growing in mouse macrophages. It has also been demonstrated that, in the mouse[11], ectromelia virus is rapidly removed from circulation by Kupffer cells, multiplies in them and then spreads to the parenchymal cells of the liver: growth in liver macrophages seems to be an essential preliminary to the hepatic cell infection. On the other hand, this same author demonstrated that different viruses, such as vaccinia, influenza and mixoma viruses in the mouse, and ectromelia virus in the rat, are taken up by the liver macrophages and then they fail to reappear. This was interpreted as an intracellular destruction of virus particles within the digestive vacuoles of the macrophage cell.

In conclusion, it seems that, during viral infections, macrophages can act either as vehicles of viruses or as viricidal cells. Therefore, it may be supposed that, for a certain species, most nonpathogenic viruses are taken up and destroyed by macrophages, while pathogenic viruses are able to multiply in these cells. Since in our experiments HBsAg particles were no more detectable within phagocytizing cells after long time incubations (24 and 48 h), it is conceivable that macrophages destroy the antigen respectively in the mouse and in the man. However we are unable to exclude the possibility that antigen particles could be present within the cells under a masked or defective form which is not detectable by the immunofluorescence method. Further studies are being carried out to elucidate this problem.

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Carcinogenicity examination of betel nuts and piper betel leaves[1]

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Summary. A dry powder of betel nuts, piper betel leaves and lime was administered to rats. Epidermal thickening was frequently observed in the upper digestive tracts of rats in groups fed the betel nut diet mixed with lime and the betel leaves diet, and a forestomach papilloma was seen in 1 rat given betel leaves diet. These epidermal changes were scarcely seen in rats given either betel nut or normal diet alone.

A high incidence of oral cancer is recorded in South-East Asian countries. Many investigators[2-7] suggested that the high incidence of oral cancer is due to the habit of chewing betel quid containing betel nut, betel leaf, lime and other organic materials. Attempts to confirm the carcinogenic activities of the betel quid using experimental animals have also been made. It was reported that mice in which extracts of betel quid with tobacco were painted in the ears[8] or instilled in the vaginae[9], developed a low incidence of papilloma and squamous cell carcinoma at the treated sites. Similar changes were observed in the hamster buccal pouch[10] treated with extracts of betel nut alone or in combination with tobacco. However, another investigator[11] could not induce tumours in the hamster buccal pouch treated with the pellets that contained betel quid ingredients. Recently, Ranadive et al.[12] and Kapadia et al.[13] reported that s.c. malignant tumours were induced in animals by s.c. injection of aqueous extraction from betel nut. Thus, carcinogenicity examinations of betel quid have been carried out entirely by local administration of the extracted substances in animals and the results obtained were affirmative in some cases and not in others. In the present study, we tried to administer a dry powder of betel nuts, piper betel leaves and lime, which are the main ingredients of betel quid, separately or in combination, to rats by feeding and examined the histopathological changes of the organs including the upper digestive tracts to know what constituents might play an important part in the carcinogenic activity of the betel quid.

Materials and methods. The inbred strain ACI rats of both sexes, 1.5 months old, were divided into 4 groups and treated as follows. Group I. 8 male and 8 female rats received betel nut diet until termination of the experiment (480 days). To prepare the betel nut diets, betel nuts imported from Indonesia was dried, milled and mixed with a rat basal diet CE-2 (CLEA Japan Inc., Tokyo) in 20% of the total. Group II. For 480 days, 11 male and 8 female rats received the diet containing 20% of dry powder of betel nut and 1% of calcium hydroxide. Group III. 9 male and 8 female rats were fed a diet containing 20% of dry powder of piper betel leaves imported from Formosa, for 300–327 days. After the termination of feeding of the experimental diet, rats were returned to the normal diet. Another group of 9 males and 10 females served as controls; they were fed the normal diet. All the animals were autopsied at death or

Incidence of squamous cell hyperplasia and other tumours

Groups	No. of animals	Effective No. of animals*	Squamous cell hyperplasia Tongue	Oral mucosa	Esophagus	Forestomach	Squamous cell papilloma	Miscellaneous tumours
Group I (betel nuts alone)	16	15	1					2 (1: urinary bladder transitional cell carcinoma, 1: myeloid leukemia)
Group II (betel nuts + $Ca(OH)_2$)	19	19**	5	1		4		1 (Myeloid leukemia)
Group III (piper betel leaves alone)	17	14**			3	5	1	
Control	19	18				2		

* Rats surviving beyond 249 days. ** 2 rats showed squamous cell hyperplasia in tongue and forestomach (group II) and in esophagus and forestomach (group III) simultaneously.

when killed due to moribund condition or at the termination of the experiment.

Results and discussion. The amount of food consumption per rat and rate of weight increase in each experimental group were almost similar to those of control group. In group I, except 1 female rat which died of pneumonia early after the start of experiment, 15 rats survived beyond 295 days. 1 rat of this group showed epidermal thickening with hyperkeratosis of the tongue. Transitional cell carcinoma of the urinary bladder and myeloid leukemia was observed in each rat. All animals in group II survived longer than 393 days. 8 rats of this group developed epidermal thickening with hyperkeratosis, parakeratosis or acanthosis in the tongue, buccal oral mucosa or forestomach (tongue: 5, oral mucosa: 1, forestomach: 4) and 2 of them had similar lesions simultaneously in the tongue. In group III, 14 rats survived beyond 249 days, 6 animals of this group had epidermal hyperplasia (esophagus: 3, forestomach: 5), 2 of them had the similar lesions in esophagus and forestomach simultaneously, and 1 of the 2 rats accompanied a forestomach papilloma. Neither tumours nor characteristic findings were observed in rats of the control group, except epidermal thickening of the forestomach in 2 animals (table). Statistical analysis indicated that the incidence of the epidermal hyperplasia in group II or III was significantly higher than that of group I or control group ($p < 0.05$).

As noted above, malignant tumours were not induced in any group of this experiment; however, the epidermal thickening was frequently observed in the tongue, esophagus or forestomach of rats in groups fed the betel nut diet mixed with calcium hydroxide and the betel leaves diet, and papilloma of the forestomach was seen in 1 rat given betel leaves diet. These epidermal changes in the upper digestive tracts were scarcely seen in rats given either betel nut or normal diet alone. Therefore, it was suggested that these findings were due to the administration of betel nuts mixed with calcium hydroxide and piper betel leaves. It may be noteworthy that the epidermal hyperplasia of the rats fed betel leaves was mainly found in the esophagus and forestomach; on the other hand, in the rats fed betel nut with calcium hydroxide, mainly in the tongue except a few cases in the forestomach. Suri et al.[10] reported that leukoplakia appeared before tumours in their experiment with dimethyl sulphoxide extract of betel quid. Atkinson et al.[4] stressed the shaked lime and calcium oxidase with some traces of calcium carbonate in the lime preparation as an important factor in the carcinogenic activity of the betel-quid mixture. Dunham et al.[14] demonstrated that atypical epithelium in buccal pouch of the hamster was induced by repeated treatment with calcium hydroxide. As far as we know, experimental studies on carcinogenicity of betel leaf have not yet been reported. It seems likely that the appearance of epidermal thickening in the esophagus and forestomach and a papilloma in the forestomach in rats does not directly mean the carcinogenicity of the betel leaf itself. However, Jussawara and Deshpande[15] described that there was sufficient evidence available to indict chewing tobacco as a factor of great importance in the etiology of esophageal cancer. To examine the carcinogenicity of betel leaf itself, further experimental studies, such as combinated administration with lime or separate administration of betel leaf for longer period, are necessary. In the present study, significant changes induced by the experimental diet were seen only in the upper digestive tracts. Ranadive et al.[12] reported that on s.c. administration of betel nut extract, mice developed fibrosarcomas at the site of injection. Recently, Kapadia et al.[13] also described induction of local malignant mesenchymal tumours in rats which received s.c. injection of extraction from betel nut. However, carcinogenicity examination of betel quid by feeding has not yet been undertaken. The carcinogenicity of betel quid was not demonstrated in the present study, but it seems that the results of this study represent a carcinogenic hazard for the chewing habit of betel quid.

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