This qualitative difference is more marked in the case of lysine, threonine and tryptophan.[11,12] This observation suggests that milling results in not only quantitative but qualitative losses as well. Hence, deep-seated and diffused protein bodies belonging to the type 4·3 pattern would be desirable for retaining the nutritive value of milled rice. The rapid technique described in this paper would be of use in screening world collections and mutagen-treated populations for this trait.

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ENHANCED VIRAL HAEMAGGLUTINATION WITH TRYPSINISED ERYTHROCYTES

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MORTON AND PICKLES[1] observed that treatment of erythrocytes with trypsin resulted in marked increase in agglutinability of M, N, S and A, B, O and P receptors by their corresponding antisera. Trypsin treatment of erythrocytes was also found of great help in the demonstration of Rh incomplete antibodies. On the other hand, marked reduction or abolition of viral hæmagglutination with trypsin-treated erythrocytes has been reported for influenza,[2] ECHO[3] and reo type 1 and 2 viruses.[4] We are not aware of any report in literature describing enhancement of agglutinability of trypsinized red cell by any virus. The present report describes the increased hæmagglutination titres of arboviruses against trypsinized cells.

The experiments were carried out on the erythrocytes of ducks, roosters, sheep, guinea-pigs, human blood group 'O' subjects, rabbits and frogs (*Rana tigrina*). The blood was collected in Alsever's solution and stored at 4° C. for at least 24 hours to avoid non-specific agglutination and were used within 5 days of collection. The red cells were washed thrice with isotonic saline. The packed cells were then divided into two aliquots. To one aliquot 0·1% trypsin (Difco 1 : 250) was added in proportion of 1 : 5. The trypsin solution was prepared in phosphate buffered saline pH 7·2 and was Seitz-filtered for sterilization. To another portion of packed erythrocytes, phosphate buffered saline was similarly added for control. Both the test and control erythrocytes were then incubated at 37° C. for 1 hour. After incubation the cells were immediately washed thrice with isotonic saline. The erythrocytes were then suspended in 0·4% concentration in virus-adjusting diluent of pH 6·6 for KFD virus and pH 6·4 for JBE virus antigens. The two arboviruses used were Kyasanur Forest Disease (KFD) and

Japanese B. Encephalitis (JBE) viruses belonging to group B. The antigens were acetone-ether-extracted mouse brains prepared from P9605 strain of KFD and P20778 strain of JBE viruses and were obtained from the Virus Research Centre, Poona, India. The hæmagglutination tests were set up with *trypsinized and untreated control erythrocytes* against the above-mentioned viruses using micro-technique of Clarke and Casals.[5] With each type of cells, control tests were set using virus-adjusting diluent in place of antigen. Two drops of viral dilutions mixed with equal amounts of erythrocytes were taken in microtitre HI plates and incubated at 4° C. for 90 minutes. The highest dilution of the viral antigen giving complete agglutination was considered as the titre of the virus for that erythrocyte. The experiments were performed on 3 to 5 animals of one particular species and were repeated at least three times on erythrocytes from one animal.

Table I shows the mean values of hæmagglutination titres obtained in repeated experiments with trypsinized and untreated control erythrocytes of different animals. The untreated cells of the duck were highly agglutinable giving a titre of 2,560 with KFD and 1,280 with JBE viruses. After trypsinization the titres were 20,480 with both the antigens showing an 8- and 16-fold increse respectively with KFD and JBE viruses. Rooster cells showed 160 and 1,280 titres respectively with KFD and JBE viruses which after trypsinization became 5,120 and 40,960, showing as increase of 32 folds with both the viruses. Sheep cells were weakly agglutinable and gave a titre of 80 with both the viruses but after trypsinization the titres were 320 and 640 respectively for KFD and JBE antigens, the increase being 4 and 8 folds. Guinea-pig cells were poorly agglutinable but after trypsinization the titres increased markedly being 32-fold with KFD and 16-fold with JBE viruses. Rabbit and human 'O' cells showed little alteration by trypsinization. The results with frog erythrocytes were striking. The untreated cells were found unagglutinable in the lowest dilution of 1 : 10 with KFD virus antigen. After trypsinization they were agglutinable upto 320 dilution of KFD virus antigen.

TABLE I

Shows hæmagglutination titres of KFD and JBE virus antigens against untreated and trypsinized erythrocytes

Erythrocytes	Hæmagglutination titres					
	KFD virus antigen			JBE virus antigen		
	Untreated	Treated	Degree of increase	Un reated	Treated	Degree of increase
Duck ..	2,560	2,0480	8-folds	1,280	20,480	16-folds
Rooster ..	160	5,120	32 folds	1280	40.960	32-folds
Sheep ..	80	320	4-folds	80	640	8-folds
Guinea-pig ..	20	640	32-folds	40	640	16-folds
Human 'O' ..	20	40	2-folds	20	80	4-folds
Rabbit ..	20	40	,,	20	40	2-folds
Frog ..	< 10	320	>32-folds	ND	ND	..

N.D. : Not done.

The findings of the present study thus demonstrate enhanced agglutinability of the trypsinized erythrocytes of different species of animals to KFD and JBE viruses. This is similar to (Hubener)-Thomsen-Friedenreich phenomenon, in which a pre-existing concealed receptor in the erythrocyte membrane is unmasked. The view is in accordance with Thomsen's[6] hypothesis that a latent receptor is uncovered by the influence of a catalytic agent. The conversion of unagglutinable frog erythrocytes to agglutinable by trypsin treatment is similar to the observation of Coombs *et al.*[7] who rendered unagglutinable ox erythrocytes equally agglutinable by antibodies after trypsinization. Our findings thus show that

differential agglutinability of the red cells to arboviruses depends upon the presence of unmasked receptors on the cell membrane. These receptors are present on all the erythrocytes tested but the presence of unmasked receptors varies from species to species and among individuals of the same species. The findings suggest that the receptors for arboviruses are not protein in nature as they are not destroyed by trypsin treatment.

The increased agglutinability after trypsinization raises the possibility of the routine use of enzyme-treated erythrocytes as a more sensitive indicator in hæmagglutination inhibition test in diagnostic virology and would affect a 4 to 32 times economy in viral antigen depending upon the erythrocytes used.

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EFFECT OF AUXIN SYNERGISTS IN ROOTING OF FRENCH BEAN (*PHASEOLUS VULGARIS* L.) CUTTINGS

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GORTER[1] noted that indole greatly synergised the effect of IAA (3-indole acetic acid) in root production of French bean (*Phaseolus vulgaris* L.) cuttings. She quoted the results of van Raalte's[2] work on synergism in suggesting that the enhancing effect of indole on IAA-induced root formation was caused by its inhibiting effect on IAA-oxidase activity. In a later investigation Gorter[3] used indole, α- and β-naphthols and phenol in combination with IAA, NAA (α-naphthyl acetic acid) and 2, 4-D (2, 4-dichlorophenoxy acetic acid) and concluded that synergism between those chemicals and auxins did not depend on a resemblance in molecular structure of the two compounds. She assumed that the exogenously applied auxins are attacked by an unspecific oxidizing enzyme which was antagonised by chemicals like indole, α-naphthol and β-naphthol. Hess[4] used a number of mono and polyphenolic compounds in rooting of mung bean (*Phaseolus aureus* Roxb.) cuttings and stated that structural requirements for a phenolic compound to stimulate rooting were presence of at least two hydroxyl groups in an *ortho* relationship and a free *para* position in the ring.

In the present investigation the influence of a number of chemicals including indole, α- and β-naphthols and several mono and polyphenolic substances, some of which have been shown to affect the IAA-oxidizing system,[5–8] has been studied. The objectives were to study (*a*) the structural requirements for a compound to act as auxin synergist in rooting and (*b*) the effects of such chemicals on the levels of exogenously applied auxins, activity of IAA-oxidizing system and penetration and transport of auxins in cuttings, in relation to adventitious root formation.

Experiments were carried out on cuttings of 12 days old seedlings of *Phaseolus vulgaris* L. cultivar "Tender green" which had been raised under controlled conditions. The method of Gorter was followed for rooting of cuttings.

Indole, α- and β-naphthols, pyrogallol and coumarin promoted rooting in absence of auxins. Figure 1 shows that indole greatly synergised rooting induced by IAA, IBA (γ-indole butyric acid), and NAA. Both the naphthols synergised rooting induced by all the auxins; α-naphthol being more effective than β-naphthol. Cinnamic acid also enhanced the root-promoting effects of the auxins. *Para*-hydroxy benzoic acid and salicylic acid synergised rooting by IBA and NAA, ferulic acid proved to be an effective synergist of

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