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.

We are thankful to Dr. T. R. Rao, Director, Virus Research Centre, Poona, for supplying the antigens.

1. Morton, J. A. and Pickles, M. M , *J. Clin. Pathol.*, 1951, **4**, 189.
2. Hirst, G. K. *J. Exptl. Med.*, 1948, **87**, 301.
3. Philipson, L. and Bengtsson, S., *Virology*, 1962, **18**, 457.
4. Gomatos, P. J. and Tamm, I., *Ibid.*, 1962, **17**, 455.
5. Clarke, D. H. and Casals, J., *Amer. J. Trop. Med. Hyg.*, 1958, **7**, 561.
6. Thomsen, O., *Z. Immunitætsforsch*, 1927, **52**, 85.
7. Coombs, R. R. A., Gleeson-White, M. H. and Hall, J. G., *Brit. J. Exptl. Pathol.*, 1951, **32**, 195.

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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IBA. Pyrogallol, though highly effective alone, antagonised the auxin effects. Most of the polyphenolic compounds antagonised rooting of 2,4-D-treated cuttings. Although indole was synergistic to IAA and IBA, the

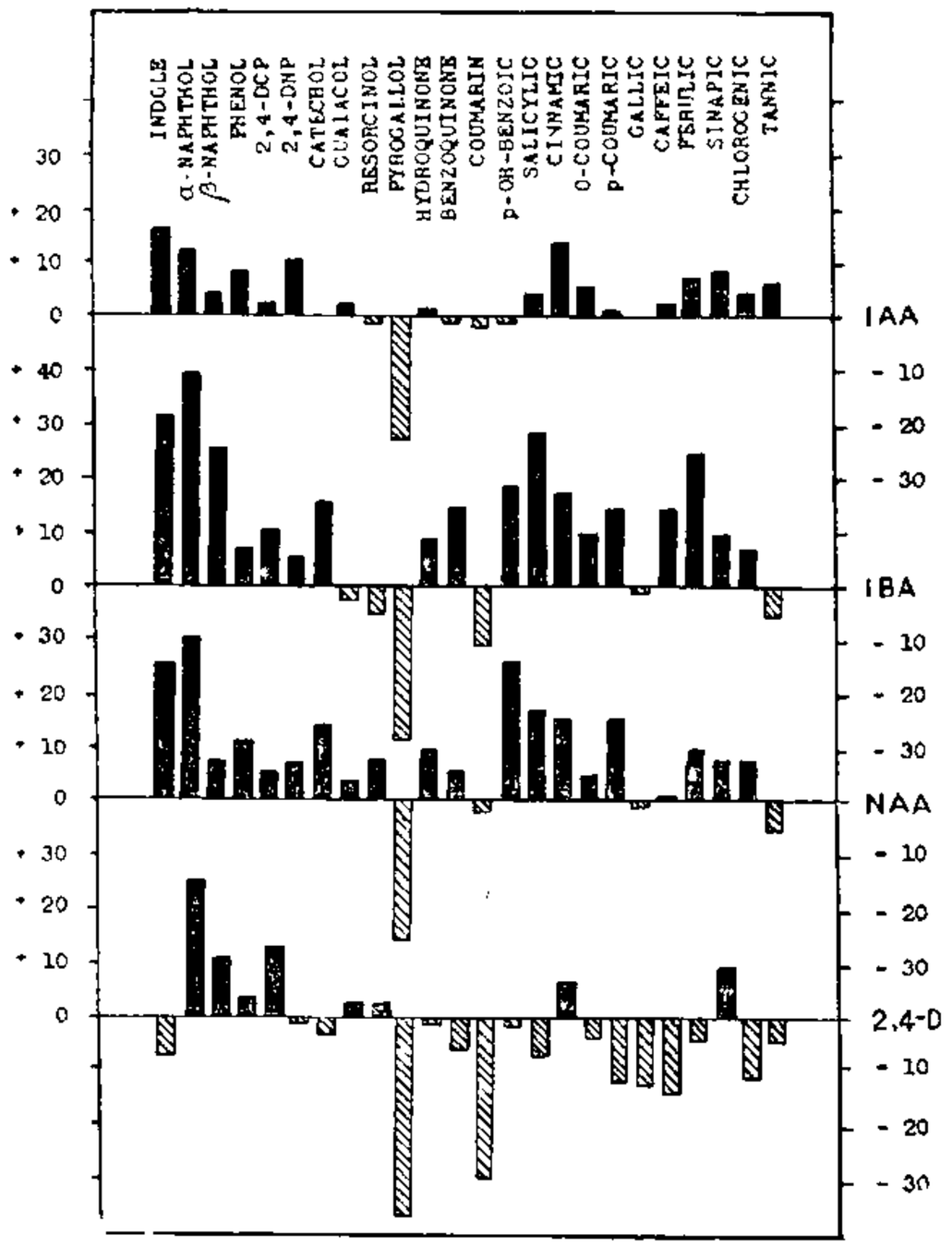

FIG. 1. Histograms showing increase (solid bars) or decrease (hatched bars) in number of roots per cutting due to synergism or antagonism [values obtained by subtracting individual effects of auxins and synergists or antagonists from mean auxin-*cum* synergist or antagonist effects, + (*plus*) denotes synergism, − (*minus*) denotes antagonism]. Concentration of IAA 10^{-4}, IBA and NAA 5×10^{-5} and 2, 4-D $2{\cdot}5 \times 10^{-6}$ M. Synergists or antagonists at 10^{-3}, except DCP 2×10^{-4}, DNP 5×10^{-5}, tannic acid 10^{-4}, catechol, salicylic and cinnamic acids at 5×10^{-4} M.

naphthols to NAA, and 2,4-dichlorophenol (DCP) to 2,4-D, the synergists also synergised rooting by auxins structurally unrelated to them. This confirms the views of Gorter that synergism does not merely depend on a close resemblance in the molecular structure of the synergists and the compounds synergised.

The structural requirements for a phenolic compound to stimulate rooting as stated by Hess are not satisfied by *para*-hydroxy benzoic and salicylic acids since both of these monophenolic acids synergised rooting by IBA and NAA in French bean cuttings. Moreover, in p-hydroxy benzoic acid the *para* position is not free. *Para*-hydroxy benzoic and salicylic acids also synergised rooting by IAA and NAA in cuttings of *Eranthemum tricolor* Nichols.[9]

Samples of cuttings taken 24, 48, and 120 hours after treatment with carboxy labelled IAA (3-indole acetic acid-1-C^{14}) indicated that more radiocarbon was present in tissues in the presence of catechol, pyrogallol, guaiacol, α- and β-naphthols, *p*-benzoquinone, hydroquinone and gallic, chlorogenic, sinapic, caffeic, ferulic and tannic acids than in their absence. Radiocarbon content after treatment with indole and coumarin was similar to that of control. These results point out that extent of rooting was not related to total radiocarbon content in tissues. Radioactivity in the tissue was, however, not confined to IAA only. Autoradiograms of extracts of IAA-1-C^{14}-treated cuttings showed the presence of four radioactive compounds (unidentified). The same compounds were found after treatment with different synergists but minor differences were observed in relative intensities of the spots under the different treatments. Radiocarbon content of NAA-1-C^{14} and 2,4-D-1-C^{14}-treated cuttings was not much affected by the phenolics or other chemicals tested.

Preparations of IAA-oxidase from bean tissues were incubated with IAA-1-C^{14} and a range of different substances. Enzyme activity was greatly inhibited by all the polyphenolics tested, and also by α- and β-naphthols, *p*-benzoquinone and hydroquinone. Indole did not effectively inhibit IAA-oxidase. IAA-oxidase preparations from *Phaseolus*, however, failed to oxidise NAA-1-C^{14} or 2,4-D-1-C^{14}, and addition of an IAA-oxidase cofactor like DCP or inhibitor like α-naphthol did not have any effect on the levels of these auxins in the incubation mixture (Table I).

The contentions of van Raalte and Gorter that indole acts through inhibition of IAA-oxidase are not supported by the present findings. Although autoradiograms of labelled auxin-treated cuttings showed incorporation of C^{14} into a number of compounds, may be in part, due to refixation of $C^{14}O_2$, IAA-oxidase of *Phaseolus* has shown good specificity. Unlike IAA-1-C^{14}-treated cuttings, total radiocarbon in NAA-1-C^{14} and 2,4-D-1-C^{14} treatments was unaffected by the IAA-oxidase inhibitors. The question of an inductive synthesis[10] of an oxidizing enzyme system during rooting is, however, not clear. Even after five days when root initiation was well advanced, no reduction in total radiocarbon of NAA-1-C^{14} and only slight reduction in 2,4-D-1-C^{14}-treated cuttings could be observed but by that time a large fraction of radioacti-

TABLE I

Specificity of Phaseolus *IAA-oxidizing system*

	Residual radioactivity (CPM) in incubation mixture of labelled auxins under different treatments after 6 hrs. at 28° C. (α-Naphthol, Indole and DCP. all at 10^{-4} M)			Radioactivity (CPM) in samples of labelled auxins treated cuttings (α-Naphthol-5×10^{-4} DCP-10^{-5} and Indole 10^{-3} M)		
	IAA-1-C^{14}	NAA-1-C^{14}	2,4-D-1-C^{14}	*IAA-1-C^{14}	**NAA-1-C^{14}	*2,4-D-1-C^{14}
Control (no stimulator or inhibitor)	443	941	595	840	704	1,275
α-Naphthol	812	931	586	1,944	719	1,251
DCP	450	925	609	834	707	1,274
Indole	465	914	585	896	733	1,268
Control (killed enzyme)	801	923	579	..	..	..

* 24 hours after application.
** 48 hours after application.

vity of these auxins was distributed to a number of unidentified compounds.

The synergism of the different chemicals with the auxins cannot therefore be explained merely by assuring that the synergists prevent oxidation of the auxins by IAA-oxidase.

The question of differential uptake of auxins under the different synergistic chemicals was not a major factor in the present experiment as the cuttings were allowed to draw the whole of the solution supplied. Transport of 2, 4-D-1-C^{14} within the cutting was, however, greater as compared with IAA-1-C^{14} or NAA-1-C^{14}; the latter moved particularly slowly. Some minor differences in the movement of auxins were found in the presence of the synergistic chemicals. Differences in penetration and translocation of auxins in cuttings cannot, therefore, be major reasons of root-promoting effects of all synergistic chemicals.

It is suggested that metabolic reactions other than those mediated by the IAA-oxidizing system are involved in synergism. Whether the auxins become biologically more active by forming addition products with the chemicals (*vide* Leopold and Plummer[11]) remains to be seen. Identification of the compounds into which the radiocarbon from the labelled auxins was found to be incorporated and the study of their role in rooting seem to be fruitful lines of study.

The author thanks the British Council for a grant under the Commonwealth University Interchange Scheme, the Calcutta University for a Sir R. B. Ghosh Travelling Fellowship and Professor M. B. Wilkins for laboratory facilities and his keen interest in this study.

1. Gorter, C. J., *Physiol. Plant.*, 1958, **11**, 1.
2. van Raalte, M. H., *Annales Bogoriensis*, 1954, **1**, 167.
3. Gorter, C. J., *Physiol. Plant.*, 1962, **15**, 88.
4. Hess, C. E., *Proc. Sixteenth Intern. Hort. Cong.*, 1962, **1**, 382.
5. Goldacre, P. L., Galston, A. W. and Weintraub, R. L., *Arch. Biochem. Biophys.*, 1953, **43**, 358.
6. Stutz, R. E., *Plant Physiol.*, 1957, **32**, 31.
7. Gortner, W. A. and Kent, M. J., *Nature* (*Lond.*), 1958, **181**, 630.
8. Ray, P. M., *Arch. Biochem. Biophys.*, 1960, **87**, 19.
9. Basu, R. N., Bose, T. K., Roy, B. N. and Mukhopadhyay, A., *Physiol. Plant.*, 1969, **22**, 649 (In press).
10. Filet, P. E., *Les Phytohormones de croissance*, Mason & Cie Ed., Paris, 1961.
11. Leopold A. C. and Plummer, T. H., *Plant Physiol.*, 1961, **36**, 589.