

group of amphetamine treated rats and have been reported previously<sup>16</sup>. Briefly, there was a progressive augmentation in response characterized by a more rapid onset and an increased magnitude of stereotypy with a contrasting shortening of both the stereotypy and the poststereotypy hyperactivity periods. Similarly treated rats demonstrated a 31% decrease in striatal dopamine concentration<sup>16</sup>.

The major finding of the present study is the 'down regulation' of [<sup>3</sup>H]agonist binding as well as [<sup>3</sup>H]spiperone binding following chronic amphetamine administration. Similar findings using [<sup>3</sup>H]spiperone have recently been reported<sup>17,18</sup> and are confirmed by the present results. Because [<sup>3</sup>H]agonists label a population of dopaminergic binding sites in the striatum that differs, in part, from those labeled by [<sup>3</sup>H]antagonists<sup>19,20</sup> it was important to determine if amphetamine treatment modulated [<sup>3</sup>H]agonist binding sites and [<sup>3</sup>H]antagonist binding sites in a similar fashion.

It is clear from these data that the enhanced behavioral sensitivity following multiple daily injections with amphetamine is not the result of an enhanced number of striatal dopamine receptor binding sites for either [<sup>3</sup>H]agonists or [<sup>3</sup>H]antagonists, as is the case following chronic antagonist treatment. These findings are consistent with previous observations that the amphetamine response pattern following antagonist pretreatment is markedly different from that after multiple amphetamine injections<sup>5</sup>. Such chronic amphetamine treatment would be expected to result in the continuous stimulation of dopamine receptors by increased synaptic levels of dopamine<sup>21</sup>. This hypothesis is reinforced by the finding of a marked dopamine depletion following the cessation of such chronic amphetamine treatments<sup>16</sup>. In the present situation it would appear that the chronic stimulation of striatal dopamine receptors by released dopamine is probably responsible for the significant decrease in both [<sup>3</sup>H]agonist and [<sup>3</sup>H]antagonist receptor binding. Such findings have been observed in other neurotransmitter receptor systems<sup>22</sup>. How this neurochemical finding relates to the enhanced behavioral sensitivity to amphetamine under these circumstances is still unclear. However, such a decrease in dopamine receptors may underlie the apparent tolerance that develops to oral stereotypies induced by apomorphine following such chronic amphetamine administration<sup>6</sup>.

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#### Amphetamine-induced changes in dopamine receptor binding

Ligand	Saline (n = 5)		Amphetamine (n = 5)	
	K <sub>d</sub> (nM)	B <sub>max</sub> (fmol/mg protein)	K <sub>d</sub> (nM)	B <sub>max</sub> (fmol/mg protein)
<sup>3</sup> H-spiperone	0.15 ± 0.02	1449 ± 35	0.13 ± 0.01	1157 ± 108
<sup>3</sup> H-NPA	0.51 ± 0.02	1092 ± 55	0.40 ± 0.03	802 ± 95

Striata from individual rats were subjected to saturation analyses using <sup>3</sup>H-spiperone and <sup>3</sup>H-NPA as described in figures (a) and (b). Using Student's t-test for paired observations (2-tailed) there was a statistically significant (p < 0.05) difference in the B<sub>max</sub>-values between the amphetamine- and saline-treated groups for both <sup>3</sup>H-spiperone (20% decrease) and <sup>3</sup>H-NPA (27% decrease). There was also a significant (p < 0.05) difference in the K<sub>d</sub>-value for <sup>3</sup>H-NPA between the 2 treatment groups. The number of animals in each group is indicated in parentheses (n).

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#### Occurrence of an anti-Thomsen-Friedenreich-like lectin in jackfruit seeds reacting with a receptor in ant egg glycoprotein

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**Summary.** The occurrence of an anti-Thomsen-Friedenreich-like lectin in the seeds of jackfruit and of its receptor-dominant disaccharide in an ant egg glycoprotein is described by agar-gel precipitin reactions.

It has been reported in several earlier papers<sup>2-4</sup> that peanut (*Arachis hypogaea*) lectin recognizes the carbohydrate part of the Thomsen-Friedenreich (TF) receptor, which occurs

as a cryptantigen in various glycosubstances from different sources and represents an interesting marker in medicine and oncology<sup>5,6</sup>. The finding of another anti-TF-like lectin

in the seeds of the jackfruit (*Artocarpus integrifolia*) is reported here, as well as the occurrence of its receptor, the TF-disaccharide  $\beta$ -D-galactosyl-(1  $\rightarrow$  3)-acetyl-D-galactosamine, linked O-glycosidically either to threonine or to serine, in a glycoprotein from eggs of an ant (*Oecophylla smaragdina* Fabr.).

The ant egg glycoprotein was prepared by 90% phenol/saline extraction followed by purification by gel-filtration on Sephadex G-100 column. The homogeneity of the glycoprotein was proved by high voltage electrophoresis in phosphate buffer (pH 7.1) followed by spraying with 1% ninhydrin in acetone. GLC analysis shows that the glycoprotein contains glucose, galactose and N-acetyl-galactosamine in the ratio 5:1:1.1, which apparently indicates the presence of Gal  $\rightarrow$  GalNAc. The structure of the carbohydrate part of the glycoprotein and the linkages between the monosaccharides are established by methylation and periodate oxidation studies. These studies will definitely give insight into the structural details of the carbohydrate part of the glycoprotein as well as the occurrence of TF-disaccharide in the glycoprotein.

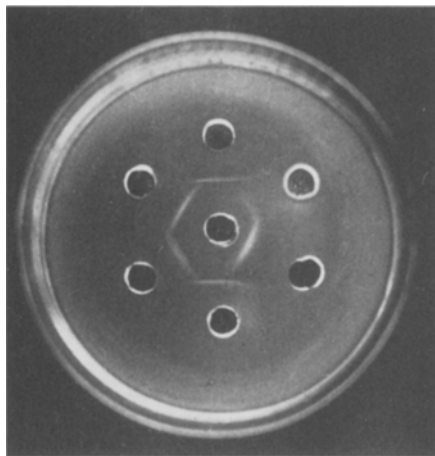


Figure 1. Precipitin reactions of an ant egg glycoprotein (middle well) with different lectins: 1, *Agaricus bisporus*; 2, *Areca catechu*; 3, concanavalin A; 4, *Artocarpus lakoocha*; 5, *Artocarpus integrifolia*; 6, *Arachis hypogaea*, read clockwise from 12.00 h (= 1) on.

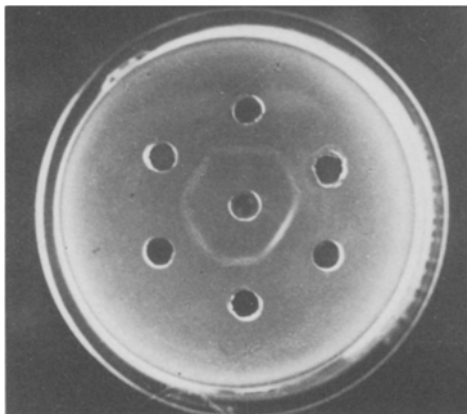


Figure 2. Precipitin reactions of the jackfruit (middle well) with glycoproteins from: 1, ant egg; 2, ant embryo; 3, desialylated human erythrocyte glycoprotein; 4, human blood group H substance; 5, blood group A substance; 6, edible bird's nest glycoprotein (read clockwise from 12.00 g (= 1) on. The concentrations of the lectins and glycoproteins used in the Ouchterlony diffusion plates are 10 mg/ml and each well in the plate was filled up by 20  $\mu$ l of test solutions.

Figure 1 represents an agar-gel diffusion pattern of ant egg glycoprotein obtained by 90% phenol/saline (v/v) extraction of the homogenized eggs, with different lectins. Peanut lectin forms a strong line of identity in precipitin reactions with jackfruit lectin, which has recently been purified on a Fetuin-conjugated Sepharose 4B column<sup>7</sup>, indicating that both lectins may react in a similar way. On the other hand *Agaricus bisporus* mushroom lectin produces a cross precipitin line with that of peanut clearly showing a different interaction in a non-identical manner. A similar result is observed with the plant lectin from *Artocarpus lakoocha*. This demonstrates that there are subtle differences in the reactivity between the lectins from *Artocarpus integrifolia* and *Artocarpus lakoocha*, although they belong to the same family and genus. Furthermore, in this picture concanavalin A and betel nut (*Areca catechu*) lectins show a close relationship in reacting with this glycoprotein, probably due to an interaction with nonreducing terminal mannosyl residues in alkali-stable carbohydrate chains (N-acetylglucosamine-asparagine) in the glycoprotein, which could be detected by a marker lectin from *Areca catechu* as reported earlier<sup>8</sup>. Desialylated ant egg glycoprotein also forms precipitations with anti-TF lectins even more intensely than the sialo-glycoprotein.

On the other hand, when comparing the reaction of the jackfruit lectin with certain other TF-containing glycoproteins from different sources a close relationship among these glycoconjugates could be observed (fig. 2). The fusion of the precipitin band of the ant egg glycoprotein with those developed by others gives some information on the occurrence of TF-disaccharide, the presence of which in these glycosubstances has already been established by GLC<sup>9-12</sup>. This additional chemical method is necessary, because the peanut lectin can give certain cross-reactions with N-acetyl-lactosamine structures<sup>4,10</sup>.

It is interesting to note that during development of the ant egg to its embryonic stage there are no changes in this chemical structure of the carbohydrate part (fig. 2) and that, in addition, the glycoprotein contains an 'A-like' receptor, which can be detected by the *Helix pomatia* lectin<sup>12</sup>. The practical significance of this precipitin reaction clearly indicates a possible method for purification of ant egg glycoproteins on jackfruit lectin immobilized affinity columns. It also enables us to use the jackfruit lectin as a substitute for the peanut lectin, which is much used for studies of membrane glycoproteins from different lymphocyte cells which carry peanut receptors<sup>11</sup> or for the occurrence of TF-receptors on tumor cells.

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