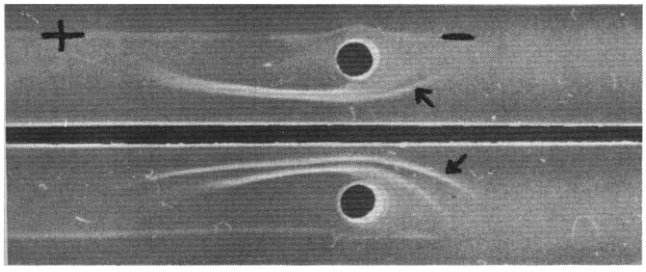

Fig. 2 Two variants of CRP. Arrow points to IgA. Upper and lower wells contain different CRP+ sera developed with goat anti-human CRP and goat anti-human IgA.

When more than 300 CRP+ sera were examined, only these two forms were seen. There was no correlation between CRP pattern and any specific disease.

Table 1 summarizes the response of human lymphocytes to CRP and certain other stimuli. Preliminary studies of lymphocyte cultures established that in this system unstimulated cells take up an amount of ^{3}H-TdR varying in individual experiments from 500 to 2,000 d.p.m. (disintegrations per minute). The standard deviation (s.d.) was 0.23. Experimental values twice that of the control were considered significant and levels three times that of the control had a value of $P<0.001$. Phytohaemagglutinin stimulation evoked a response 80–120 times that of the control. Cells from tuberculin or mumps skin test positive individuals responded to PPD or mumps vaccine by DNA incorporation two or more times control value (range 2.3–22 times control). The response to foreign histocompatibility antigens fell in the same range.

In all ten experiments in which lymphocytes were exposed to CRP, there was a dose-dependent response to this protein. High concentrations (20 μg/ml.) of CRP were toxic to the lymphocytes and resulted in loss of viability (80% dead) as evidenced by morphological studies (dye exclusion), DNA synthesis (20% of control value) and failure to respond to PHA stimulation. Lower concentrations of CRP were stimulatory in every case with maximal stimulation occurring at a CRP concentration of 10 μg/ml. The range of stimulation was from 3.5–21 times the control value. Complete removal of CRP gave values comparable with controls. The CRP was soluble, fully active biologically and free of normal serum proteins. All CRP had beta mobility but was composed of two subtypes both reacting with C polysaccharide and goat anti-CRP. They varied from person to person independently of disease. Genetic determination of this variance is a possibility.

We have shown that CRP can modify lymphocyte behaviour *in vitro*. Lymphocyte membranes are strongly negatively charged; this may reflect the presence of phosphorylated carbohydrate similar to that of the pneumococcal membrane.

Table 1 Response of Lymphocytes to C Reactive Protein and Other Stimulants

Stimulant	No. of trials	Ratio experimental/control Average	Range
Unstimulated cells	46	1.0	0.5–1.7* (s.d. 0.23)
CRP 20 μg/ml.	10	0.2	0.1–0.2
	$P<0.001$		
CRP 10 μg/ml.	10	7.2	3.5–21.4
Mumps virus 0.5 CFU	8†	6.8	2.7–17.2
PPD (25 μg)	6†	12.5	2.3–22.7
Foreign HL-A antigens	>100	7.8	2.0–15.3
PHA	>100	110.0	80.0–120.0

The response was measured by ^{3}H-TdR uptake in d.p.m. Values for unstimulated cultures were 500–2,000 d.p.m.
CFU, complement fixing units.
* Variation between duplicate tubes.
† Cells from skin sensitive subjects.

If CRP ability to stimulate lymphocytes *in vitro* reflects its function *in vivo*, it may be that locally released CRP activates lymphocytes which arrive in the area. If this were true, CRP would be an important factor in initiating lymphocyte reactions. Some further support for this hypothesis may be inferred from the observation[7] that peripheral blood lymphocytes are stimulated to DNA synthesis in acute viral and bacterial diseases, in active autoimmune diseases and following X-radiation and surgery. These unrelated conditions are all characterized by high levels of CRP.

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Indole(ethyl)amine N-Methyltransferase in Human Brain

TANIMUKAI *et al.*[1], using gas–liquid separation, correlated the appearance of a bufotenin-like substance in urine and the onset of psychosis in latent schizophrenics brought on by administration of a monoamine oxidase inhibitor with amino-acid precursors of indoleamines and methyl groups. Serious doubt about endogenous bufotenin as the cause of psychiatric disturbance was cast by research demonstrating that intravenously administered bufotenin produced nothing but bizarre cardiovascular symptoms in man[2,3]. One objection to such work is that bufotenin may not easily cross the blood–brain barrier. Recent preliminary evidence gathered in our laboratories from rats infused intraventricularly with bufotenin has suggested that this substance is at least as potent as its powerfully hallucinogenic 5-methoxy congener (unpublished results of D. Segal and A. J. M.).

A nonspecific N-methylating enzyme for aromatic amines has been reported in mammalian tissue. Julius Axelrod has demonstrated in rabbit lung a nonspecific N-methyltransferase which could methylate several aromatic amines[4]. But he failed to find it in the brain of several animals, although two other N-methyltransferases have been demonstrated in the brain of various species: histamine N-methyltransferase and phenylethanolamine N-methyltransferase[5]. We report here the presence of a relatively specific indole(ethyl)amine N-methyl transferase in sheep and three human brains after previous demonstrations of its presence in the brain of chick[6].

Tissue from the brains of sheep and humans was used. The three human brain specimens were one piece taken from the pre-frontal cortical area of a 54 yr old woman during a neurosurgical decompression following a cerebral haemorrhage; a piece of tissue taken from the parietal cortical area of a 5 day old infant during a shunting procedure for congenital hydrocephalus, and brain tissue from a 17 yr old boy who died of a testicular carcinoma was used for regional studies. The pieces

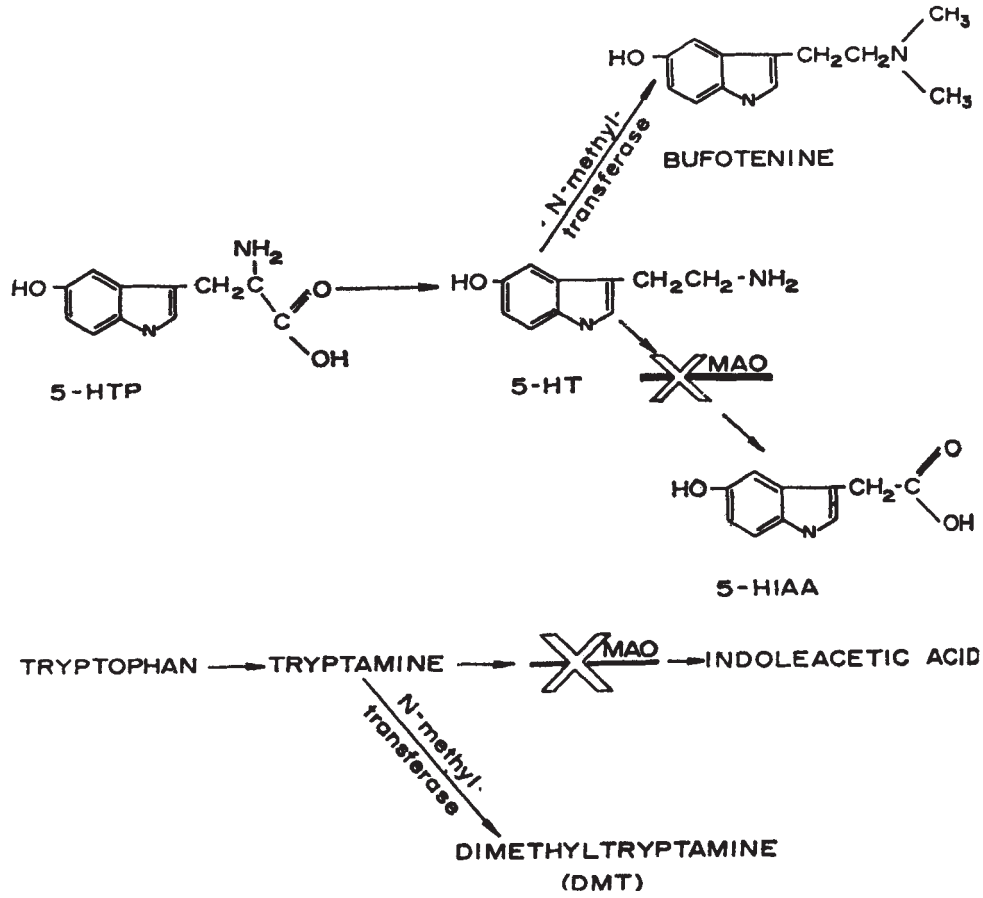

Fig. 1 Hypothetical indole(ethyl)-amine shunt acting when the normal degradation pathway of oxidative deamination is blocked by either a monoamine oxidase inhibitor or large doses of amphetamines.

were homogenized in five volumes of 0.32 M sucrose and frozen 2 h after death. Enzyme determinations were done on whole brain homogenate, 100,000*g* supernatant, 35–45% ammonium sulphate precipitate, and 'Sephadex G-200' column fractions. Incubations were in 0.25 M phosphate buffer (*p*H 7.9), with 5-hydroxytryptamine as a substrate and S-adenosylmethionine, methyl-^{14}C as a methyl donor. This mixture was incubated for 90 min at 37° C. The reaction was stopped by the addition of borate buffer, *p*H 10, and radioactive methylated products were extracted into water saturated isoamyl alcohol, placed in a toluene based fluor containing 15% 'Biosolv BBS-3' and counted in a scintillation counter with external standard quench correction. Reaction conditions in relation to substrate and protein concentrations were similar to those reported for chick brain[6]. Boiled enzyme was inactive. The K_m for S-adenosylmethionine was 2×10^{-5}. The K_m for 5-HT was 9×10^{-4}. Radioactive bufotenin, N-methyltryptamine and dimethyltryptamine chromatographed (thin-layer) with known compounds on cellulose in isopropanol, ammonia, water (200 : 10 : 20); in 20% KCl in water; and in methanol, butanol, benzene, water (40 : 30 : 20 : 10) (ref. 7).

Table 1 Specific Activity of Indole(ethyl)amine N-Methyltransferase from Various Regions in the Human Brain

Brain	Region	Specific activity*	Source
	Regional specific activity of human brain indole(ethyl)amine N-methyltransferase		
Infant male	Parietal cortex	95	Biopsy
Adult female	Frontal cortex	103	Biopsy
Adolescent male	Frontal cortex	<30	Two-hour post mortem
	Orbital surface of frontal lobe	69	
	Ventral midbrain (long tracts included)	74	
	Amygdala	136	
	Medulla	189	
	Uncus	218	

Each value is the mean of three determinations.
* nmol of 5-HT methylated/g/h.

Table 2 Substrate Affinity for Various Aromatic Amines after Progressive Enrichment of Indole(ethyl)amine N-Methyltransferase (Sheep) with Ammonium Sulphate Precipitation and 'Sephadex G-200' Fractionation

Substrates	Specific activities of fractions* 100,000*g* supernatants	34–45% NH_4SO_4	G-200
Tryptamine	0.22	0.86	2.13
Histamine	7.13	1.23	0.17
Normetanephrine	0.04		
Norepinephrine			

With purification, the affinity for the indole(ethyl)amine increases and that for the imidazole and phenylethanolamine decreases. Values below 0.02 nmol/g/h were regarded as zero.
* nmol of substrate methylated/mg of protein/h.

Table 1 is a summary of the regional specific activities of the enzyme from the three human brains studied. In the human brain studied regionally, the highest values are in the uncus, an area relatively dense in serotonergic nerve endings. Table 2 is a summary of the results of a study of the substrate specificity of the mammalian indole(ethyl)amine N-methyltransferase enzyme as progressively enriched by ammonium sulphate precipitation and 'Sephadex G-200' fractionation. Note that the progressive enrichment increases the activity of the enzyme for indole(ethyl)amine substrates and decreases the activity with respect to imidazoles and phenylethanolamines in the described reaction conditions. Table 3 shows the substrate specificity for the 100,000*g* supernatant and the 35–45% ammonium sulphate cut from human brain. As with sheep brain there is a relative absence of activity with phenylethanolamine substrates and a relative decrease in activity for histamine as a substrate compared with indole(ethyl)amines.

Table 3 Specific Activity of Indole(ethyl)amine N-Methyltransferase relative to Other N-methylating Enzymes from Human Brain enriched Only Partially

Substrate	Substrate affinities of methylating enzymes in human brain homogenate * Fraction 100,000*g* supernatants	NH_4SO_4; 35–45%
5-HT	100	100
Tryptamine	95	111
N-Methyl-5-HT	94	79
N-Methyltryptamine	62	55
Norepinephrine	0	7
Normetanephrine	41	40
Histamine	1,110	134

These data suggest (for these reaction conditions) the same specificity for indole(ethyl)amines in human brain as seen in sheep.
* With 5-HT as reference substrate (that is, equal to 100%).

The presence of a specific indole(ethyl)amine N-methyltransferase with a relatively reasonable K_m for its substrates (5-HT and S-adenosylmethionine) in human brain which has the capacity to convert serotonin to bufotenin, a centrally active substance, is interesting. Perhaps the activation of latent psychosis produced by methionine load after administration of a monoamine oxidase inhibitor may be a consequence of the shunting of either tryptamine or serotonin through this indole(ethyl)amine N-methyltransferase enzyme into methylated amine products instead of their usual pathway of oxidated deamination[8]. Fig. 1 is a diagrammatic representation of this speculated shunt. Whether this enzyme plays any role in normal cerebral physiology or in mental illness is a question awaiting further research.

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Water Stress and Apical Dominance in *Pisum sativum*

A RECENT study of apical dominance in isolated rhizomes of *Agropyron repens* L. Beauv. suggested that inhibition of the lateral buds by the rhizome apex largely depends on the supply of water, nitrogen and carbohydrate, any of which could act as a limiting factor and thus determine the degree of inhibition[1]. To test this hypothesis, further experiments were conducted with peas (*Pisum sativum*, variety 'Alaska'), which exhibit strong apical dominance and which are widely used in the study of this phenomenon[2]. The results agreed well with the concept of limiting nutritional factors, and suggest that for this species water stress may be particularly significant.

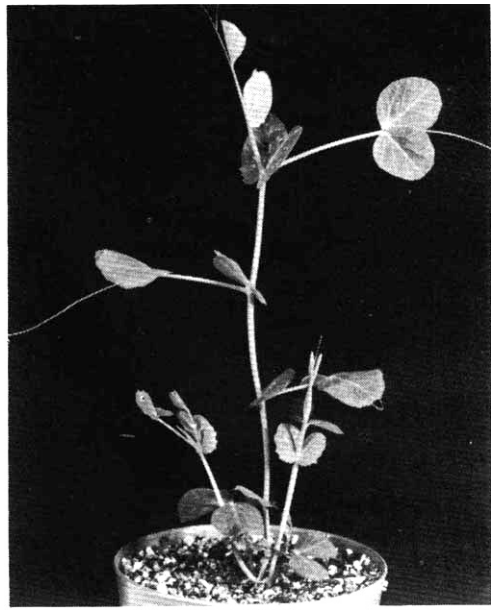

Fig. 1 Seedling of *Pisum sativum*, variety 'Alaska', illustrating the loss of apical dominance over the buds at the basal nodes and the stage of main shoot development at which lateral bud growth was recorded. Environmental conditions: temperature (day and night) 15° ± 1° C; relative humidity 85% ± 2%; photoperiod 16 h; light intensity 3,200 foot candles. Nutrient solution was half strength Hoagland + 0.6 g NH_4NO_3/l. (= 315 p.p.m. N).

Peas were soaked in distilled water for 6 h and germinated between wet paper towels at 20° C for 2 days. Seedlings of primary root length about 1 cm were planted in vermiculite in 12.5 cm diameter plastic pots. Four seedlings were planted in each pot and these were thinned to one seedling per pot when the leaf at node 3 was partly expanded. The seedlings were watered with Hoagland's solution[3], each pot receiving 200 ml. per day. Except where otherwise noted (footnote, Table 2), the Hoagland's solution was used at half strength with the nitrogen concentration increased to 210 p.p.m. by addition of 0.3 g NH_4NO_3 per l.

Transpiration rates were determined by the weighed pot method, using pea seedlings and 6.5 cm diameter plastic pots. Water stress was measured by determining the relative turgidity of the leaves using Weatherley's[4] method. Each determination recorded is the mean value for duplicate samples of twenty disks 7 mm in diameter. The disks were punched from each side of the midrib of the terminal leaflets of selected leaves at a uniform stage of development.

All experiments were terminated when the stipules of the leaf at node 7 had started to separate but were not yet fully expanded (Fig. 1). Effects of treatment on apical dominance were determined by recording the total length of all axillary buds and shoots at nodes 1 and 2 (nodes are numbered acropetally from the base of the shoot). Buds less than 10 mm long were measured to the nearest 0.1 mm under a low power microscope fitted with an ocular micrometer; those greater than 10 mm were measured to the nearest 0.5 mm using a ruler.

In preliminary greenhouse experiments, the plants invariably showed strong apical dominance. The growth of the lateral buds was arrested at a length of 1–3 mm and lateral shoots were produced only when the main shoot was decapitated. Because the plants were grown at a high nitrogen level (210 p.p.m.) and were strongly illuminated, it seemed unlikely that bud growth was limited by a deficiency of either nitrogen or