astronomical proportions.

In the alkaline range, the aromatic ring proton resonances of the tyrosine residues undergo shifts with the ionization of the phenolic groups. Thanks to the absence of other aromatic residues apart from phenylalanine, the spectrum in this region is relatively simple, and because the magnetic environments of the four tyrosine residues are all sensibly different, an ionization curve can be constructed for each, and the four peaks determined with good precision. Karplus *et al.* find, as did Masson and Wüthrich, that even in this alkaline range, some of the exchangeable peptide protons still remain unexchanged, and it seems that denaturation in these conditions is not wholly cooperative, and that even in so small a molecule independent cooperative units can remain tightly folded and resist the incursion of solvent.

Failure of cooperativity in denaturation, and the appearance of intermediates of long lifetime on the NMR time scale has now also been established for the archetypal case of ribonuclease. Thus Westmoreland and Matthews (*Proc. US Nat. Acad. Sci.*, **70**, 914; 1973), concentrating on the downfield histidine resonances—the separation of which because of the varied environments of these groups, is perhaps the most distinctive feature of the native spectrum—find that the acid denaturation profiles of the chemical shifts of these residues do not coincide. It is inferred that there are at least two separately unfolding regions in the structure, which is consistent with results of rapid kinetics of unfolding.

The reservation that secondary structure in general exists in acid-denatured proteins invests the guanidine hydrochloride system with particular interest, for here, as Tanford and his associates have shown, ribonuclease (and most other proteins) behave as random coils, possessed of no detectable intermolecular interactions. Nevertheless, here too the unfolding gives evidence of intermediate states when followed by NMR at high resolution. Benz and Roberts (*FEBS Lett.*, **29**, 263; 1973) have found that the downfield resonance from his-12 begins to decay at lower denaturant concentration than those of the other three histidines. When denaturation is complete only one C-2 histidine resonance, corresponding to all four groups in the coiled chain, is to be seen. His-12 occurs in the N-terminal "S-peptide" region, which is very possibly the most susceptible to disruption. This result is in a sense at odds with the kinetic and thermodynamic features of this system, which meet the criteria for a grossly two-state denaturation process, but as Benz and Roberts point out, the deviation from such behaviour in structural terms may be very slight.

PSYCHOLOGY

Depression and the Pill

from a Correspondent

As many as one in fifteen British women on the contraceptive pill may find that it induces a state of depression which can be severe enough for the pill to be an unsuitable form of contraception for them. The reasons for this have been examined by a group at St Mary's Hospital Medical School, London, who have related the problem to a deficiency of vitamin B_6 (pyridoxine) in some women on the pill (P. W. Adams *et al.*, *Lancet*, i, 897; 1973). It seems that treatment with vitamin B_6 may sometimes be effective.

The biochemical basis of this work is that the administration of oestrogens interferes with the metabolism of the essential amino acid tryptophan, increasing the amount metabolized by the nicotinic acid pathway. The metabolism of tryptophan involves the coenzyme pyridoxal phosphate derived from vitamin B_6, and therefore a pill-induced increase in the rate of metabolism of tryptophan uses up pyridoxal phosphate.

But one of the essential amines in brain function, 5-hydroxytryptamine, is also produced by a pathway which requires pyridoxal phosphate, and in patients with depressive illnesses an examination of the fluid surrounding the brain and spinal cord shows this amine to be present in abnormally low quantities.

Attempts have been made in the past to treat premenstrual depression in women on the pill with pyridoxine, but the importance of the work at St Mary's is that for the first time a double-blind crossover trial with pyridoxine has been made.

One of the problems in selecting patients for the trial was to ensure that the depression was a side-effect of the pill and did not have another cause. Most forms of depression give rise to symptoms of pessimism, tearfulness, anxiety and loss of sexual appetite, but also induce a disturbance of sleep pattern and appetite. These latter two symptoms are uncharacteristic of pill-induced depression, however, so women who had sleep and appetite disturbances or a history of mental disorder or obvious reasons such as marital disharmony that might lead to depression were excluded. The St Mary's team were then left with thirty-two women whose depression they felt to be caused by the contraceptive pill. Ten of these did not complete the trial, so the sample is small, but the results still seem significant.

The patients, divided into two groups, were treated with either 20 mg pyridoxine hydrochloride daily or a placebo and after two months the groups were interchanged. The women were on a variety of contraceptives though each contained 50 μg of oestrogen, and the length of time they had been on the pill made no difference to the results.

There was an alleviation of the depression in eleven women who at the start of the experiment were found to have an absolute lack of pyridoxine, but in the other eleven women whose biochemical assays were no different from other women on the pill the pyridoxine treatment showed no benefit over the placebo. So, as the St Mary's team point out, other causes must be sought for the depression in those women who did not respond to the pyridoxine.

Experiments along these lines may also conceivably throw some light on events in early pregnancy. Morning sickness has also been treated for some

Magnetic Effect of Cofactors

THE discovery that organic phosphates function as cofactors for haemoglobins, and are tightly bound to the deoxygenated state, has meant a re-evaluation of many physical properties, which were previously determined without regard to the presence and concentration of these ligands. The magnetic susceptibility of deoxyhaemoglobin, which is the subject of a re-examination by Alpert, Banerjee and Denis in next Wednesday's *Nature New Biology* (May 16), corresponds to ferrous haem iron atoms with an electronic spin of 2. This falls to zero in the liganded haemoglobin.

The classical value for the magnetic moment of deoxyhaemoglobin, obtained twenty-five years ago by Pauling and his colleagues, was about 5.4 Bohr magnetons. This turns out now to be the value for the complex with 2,3-diphosphoglycerate or inositol hexaphosphate. When the ligand is stripped off on a column, a drop to 4.9 Bohr magnetons is observed. This is exactly the theoretical value for independent spins. The higher magnetic susceptibility in the complex with cofactor must then be attributable to an added orbital contribution. The cofactor must therefore perturb the symmetry of the ligand field around the iron.

It is known from the X-ray work of Arnone that diphosphoglycerate binds in the central cavity of the protein at the junction of the two β chains, and in doing so induces displacements of up to 2 Å locally. Alpert *et al.* suggest that these can be sufficient to reduce the distortion of the ligand field around the iron from octahedral symmetry, which would have the effect of increasing the magnetic susceptibility. Both α and β chains probably contribute to this effect.