# d-Amphetamine Behaviour in Rats after a Wheat Protein Challenge, and its Reversal by Naloxone Hydrochloride

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***Abstract***

*When rats are reared on ordinary rat food and a special diet not containing wheat proteins and later subjected to d-amphetamine/wheat protein type behaviour experiments, there is a marked difference in their response output. Rats fed with rat food show d-amphetamine type behaviour, whereas rats not having been in contact with wheat protein show an enhanced behaviour pattern. After injection of naloxone hydrochloride, a marked difference was observed in the rats reared on special diet. It is suggested that this is evidence for the release of exorphins from the wheat proteins during digestion, their uptake and subsequent CNS action, which is naloxone reversible. A model hypothesis for absorption and distributed digestion of wheat protein is put forward.*

## Introduction

Evidence has been provided that the brain contains endogenous substances that act as agonists at morphine receptor sites (Goldstein, 1976; Terenius and Waldstrom, 1978). These substances are known as endorphins and are localised in some neurons; they also occur in the gastrointestinal tract (Schultz et al., 1977). The effect of endorphins can be blocked by the morphine antagonist, naloxone (Waterfield et al., 1977). These endorphins or endogenous opioid peptides are extremely potent substances exerting their effects at very low concentrations (Klee, 1977). Peptides with as little as 1% activity of the endorphin are potent substances which can have profound effects pharmacologically.

Some food proteins contain the peptide sequence of these endorphins and could well be a source of opioid activity. Peptic digests of protein containing the appropriate amino acid sequence contain peptides with an N-terminal tyrosine and exert opioid peptide activity (Klee et al., 1979).

As it is now accepted that proteins and peptides traverse the gut (Hemmings and Williams, 1978), it was thought that these products might have a direct or indirect effect on the neural system. Dohan (1978), Singh and Kay (1976) and Hemmings (1978) reported that ingestion of wheat had an adverse effect on a percentage of their

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subjects, especially those who suffered schizophrenia. Dohan et al. (1978) have shown that peptides derived from alpha gliadin by the action of pepsin, upon intraventricular injection may profoundly modify the behaviour of rats, even inducing a long lasting catalepsy, and Taylor (1978) found that amphetamine induced behaviour in rats was of a different nature after feeding wheat gluten fractions.

Williams et al. (1979) showed that when a rat was given different proteins by mouth, and later subjected to an amphetamine injection, the amphetamine induced or psychotic behaviour was quite different to that of the saline fed controls, suggesting that some proteins enhance the behaviour pattern with others inhibiting behaviour or response output.

It is now believed that some food proteins account for some antisocial behaviour and there is much research in progress throughout the world in relation to this problem and the problem of food allergies. The aim of the present study was to ascertain whether wheat proteins which are known to contain neuro-active peptides sequence had an effect on the behaviour pattern in the rat, and whether the morphine antagonist naloxone would suppress this action.

**Materials and Methods.**

*Animals.* Male and female rats of the Wistar strain were used in these experiments. One group was maintained on normal Lab Diet rat food (Christopher Hill, PRD diet). This is a cereal based diet containing 20% wheat and 20% wheat feed. The animals were given free access to water, (Group A). The second group (Group B), were maintained on commercial dog food not containing any wheat protein. This product was tested for the presence of wheat antigen by Ouchterlony tests against potent sheep anti wheat protein serum, and gave negative results. These animals were also given fruit, greens and water ad lib. Mothers from group B were started on this diet before the birth of the pups. When subsequently the pups were reared on this diet, they were never exposed to wheat protein. All animals were allowed to reach maturity, experimentation commencing at 90 days when weights were 200-260 grams.

*Proteins.* Wheat protein was prepared from milled Bouquet wheat (Rank Hovis McDougall) by stirring in a volume of 0.9% phosphate buffered saline (PBS) overnight at 4°C. This was left to settle and the supernatent was collected and concentrated to give a final concentration of 2% protein as determined by the Nessler method (Thompson and Morrison, 1951). This was a crude protein mixture which gave 5-6 bands on electrophoretic analysis. Four mls of a 2% solution was administered by stomach tube four hours prior to injection of d-amphetamine. Control animals were given four mls of a 2% rat serum in PBS prior to injection; this was done to maintain fed protein concentrations in the animals.

*Drugs.* Four mg/kg weight d-amphetamine in PBS was administered intraperitoneally 20 minutes before experimentation. In some cases this was in conjunction with naloxone hydrochloride (0.4mg/kg weight), this was injected intraperitoneally and also at the base of the neck. Drugs were administered so that all animals received an equivalent amount/kg weight.

*Behaviour.* Studies on the behavioural patterns were carried out in a 4 ft. x 4 ft. wooden box divided into two compartments. These were double skinned to minimise external noises. One rat/area was observed at one time, the period of observation being for one minute in every five, the whole period being for 75 minutes when behaviour patterns were considered normal. The d-amphetamine patterns obtained are head movements, back and side walking and circling which are typical of the "psychotic" animal and never seen in normal controls. If an overdose of amphetamine is given, the rat enters a convulsive state with death imminent. The rats behave normally prior to amphetamine and also when the effect wears off.

The behaviour patterns were broken down to the following categories: Active (A), Rearing (R), Walking (W), Sniffing (S), Immobile (I), Grooming (G), Head Movements (HM), Circling (C), Back Walking (BW) and Side Walking (SW); and for a

positive response, the pattern had to be shown for a full minute under observation (see Taylor, 1978; Taylor et al., 1974; Williams, 1978). As it can be seen, the first six categories are displayed by normal rats, but the latter ones portray d-amphetamine behaviour (HM, C, BW and SW).

Non-Parametric statistical analyses were carried out (Siegel, "Nonparametric Statistics" McGraw Hill) and these were found to be significant at the 1% or the 5% level.

**Results.**

The tables presented are of the behavioural patterns exhibited by control and experimental animals. The behaviour categories are listed as A, R, W, S, I, G, HM, C, BW and SW (see Materials and Methods); in all cases, behaviour was observed for a period of 75 minutes when the animals returned to normal behaviour. As a control experiment, untreated animals were taken at random from groups A and B. No abnormal behaviour pattern was observed after placing them in the behaviour box; they were content to sit and groom for most of the experimental period, the behaviour pattern being mostly walking and sniffing, showing curiosity at being in a different environment. The same pattern of behaviour was observed after the animals were given an injection of naloxone, so it was deduced that naloxone had no adverse effect on the behaviour pattern as neither had the diet change.

**Table 1**

| Time. | A. | R. | W. | S. | I. | G. | HM. | C. | BW. | SW. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| 5 | +o | | +o | + | | | | | | |
| 10 | +o | | +o | o | | | | | | |
| 15 | +o | o | + | o | | +o | | | | |
| 20 | +o | | +o | +o | | o | | | | |
| 25 | +o | | +o | +o | | o | | | | |
| 30 | + | | + | +o | o | o | | | | |
| 35 | +o | | +o | +o | | o | | | | |
| 40 | + | | + | + | o | +o | | | | |
| 45 | +o | | +o | +o | | + | | | | |
| 50 | +o | | o | o | | +o | | | | |
| 55 | o | | o | o | + | +o | | | | |
| 60 | + | | + | + | o | +o | | | | |
| 65 | + | | + | | o | o | | | | |
| 70 | +o | | +o | +o | | | | | | |
| 75 | +o | | +o | | | | | | | |

No. of rats tested - 6.
+ denotes special diet rats after wheat protein.
o denotes PRD diet rats after wheat protein.

Table 1 shows the results obtained from groups of animals after the administration of wheat protein. It can be seen that the behaviour pattern is very similar in both groups; Group B (special diet) could be said to be a little more active. This could be an interesting finding, as the enhanced activity could be due to the wheat protein affecting the activity of Group B rats. The effect is slight and could be monitored with the aid of a computer. Group A (PRD) behaved as group B with W,S,G, being the prominent factors; overall activity was observed to be less. The active (A) category was marked positive in all cases where any degree of activity was observed.

The first six categories of behaviour shown in the tables are of normal behaviour and can be seen throughout the experiments.

**Table 2**

| Time. | A. | R. | W. | S. | I. | G. | HM. | C. | BW. | SW. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| 5 | +o | +o | | | | + | | | | |
| 10 | +o | | +o | o | | o | | | | |
| 15 | + | | + | +o | o | + | | | | |
| 20 | +o | | +o | o | | o | | | | |
| 25 | + | | + | | o | | +o | | | |
| 30 | + | | + | + | o | | +o | | | |
| 35 | + | | + | | o | | +o | | + | |
| 40 | +o | | | + | | | + | o | +o | + |
| 45 | +o | | | | | | +o | | +o | + |
| 50 | +o | | | | | | +o | | +o | +o |
| 55 | o | | | | + | | + | o | o | |
| 60 | | | | | + | | +o | | o | o |
| 65 | o | | o | o | + | | + | | | |
| 70 | +o | | +o | + | | | | | | |
| 75 | + | | + | o | o | + | | | | |

No. of rats tested -6
+ denotes special diet rats after 4mg/kg d-amphetamine.
o denotes PRD diet rats after 4mg/kg d-amphetamine.

Table II is of the behaviour pattern observed after an i.p. injection of d-amphetamine. Group A (PRD) rats begin to show d-amphetamine behaviour patterns (HM, C, BW and SW) at 25 minutes after injection, the peak behaviour being at around 50 minutes, after which the d-amphetamine behaviour begins to decline. The other group, B, (special diet) shows a similar pattern, starting at 25 minutes, peaking at 45 minutes and then declining, returning to normal behaviour after 65 minutes.

**Table 3**

| Time. | A. | R. | W. | S. | I. | G. | HM. | C. | BW. | SW. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| 5 | +o | | +o | +o | | | | | | |
| 10 | +o | | +o | + | | | | | | |
| 15 | o | | o | | + | | | | | |
| 20 | o | | o | o | + | | | | | |
| 25 | + | | + | | o | o | | | | |
| 30 | + | | | | o | | +o | + | | |
| 35 | +o | | | | | | +o | +o | + | |
| 40 | +o | | | | | | o | + | | o |
| 45 | +o | | | | | | o | | +o | + |
| 50 | +o | | | | | | + | | o | +o |
| 55 | +o | | | | | | + | | +o | o |
| 60 | +o | | | | | | + | o | + | |
| 65 | +o | | | | | | | +o | | |
| 70 | +o | | | | | | | +o | | |
| 75 | + | | | | o | | + | + | | |

No. of rats tested - 5.
+ denotes special diet rats after 4mg/kg d-amphetamine and 0.4 mg/kg naloxone.
o denotes PRD diet rats after 4mg/kg d-amphetamine and 0.4 mg/kg naloxone.

To see if naloxone had an effect on d-amphetamine behaviour, an experiment was carried out where group A and B rats were given an i.p. injection of d-amphetamine in conjunction with naloxone (Table III). The pattern of behaviour is very similar to that of Table II in both groups, with d-amphetamine behaviour starting at 30 minutes and being most noticeable at 40-45 minutes, thereafter declining. This shows that naloxone has very little effect on d-amphetamine behaviour.

**Table 4**

| Time. | A. | R. | W. | S. | I. | G. | HM. | C. | BW. | SW. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| 5 | +o | | +o | +o | | | | | | |
| 10 | +o | (+ ) | +o | + | | | | | | |
| 15 | + | | + | +o | o | o | | | | |
| 20 | + | | | | o | | + | + | | |
| 25 | + | | | | o | | + | + | + | |
| 30 | +o | | o | | | | +o | + | | |
| 35 | + | | | | o | | +o | + | + | + |
| 40 | +o | | | | | | +o | +o | + | + |
| 45 | o | | | | | | + | +o | + | + |
| 50 | +o | | | | | | | +o | +o | +o |
| 55 | +o | | | | | | | +o | +o | +o |
| 60 | +o | | | | | | +o | +o | +o | |
| 65 | +o | | | | | | + | | +o | |
| 70 | | | | | +o | | +o | | | |
| 75 | | | | | +o | | | | | |

No. of rats tested — 5.
+ denotes special diet rats after 4mg/kg d-amphetamine and wheat protein.
o denotes PRD diet rats after 4mg/kg d-amphetamine and wheat protein.

Table IV presents the results obtained from A and B groups that had received a wheat protein challenge with an i.p. injection of d-amphetamine four hours later. Taking group A (PRD) it can be seen that abnormal behaviour starts at 30 minutes, peaking at 55 minutes, the most prominent behaviour pattern being circling with head movements and back walking; this is similar to patterns observed in Table A. Group B (special diet), however, show abnormal behaviour sooner, this starting at 20 minutes, HM, C, BW and also SW being prominent. Again, the peak is sooner showing at 45 minutes, and by the end of the session normal behaviour was approached. This shows that there is enhanced behaviour exhibited by the special diet (B) rats in comparison to group A (PRD) after the administration of wheat protein and d-amphetamine, suggesting that wheat protein affects abnormal behaviour by producing these effects at a previous time in group B, bringing forward abnormal or d-amphetamine behaviour patterns.

Table V presents results obtained after the administration of wheat protein and d-amphetamine and naloxone. Taking Group A (PRD) it is seen that the pattern of behaviour is similar to group A of Table IV which is of d-amphetamine wheat protein effect, abnormal behaviour starting at 40 minutes and lasting through to 70-75 minutes. The behaviour pattern here is slightly delayed when it is compared with the results of Table IV which started at 30 minutes. Group B, however, is totally different from Tables II and IV. Whereas normal behaviour started at 20 minutes with d-amphetamine and wheat protein, after administration of naloxone, the abnormal pattern of behaviour is seen to start at 40 minutes, peaking at 55 minutes with normal behaviour being observed at the end of the session. The interesting observation is that PRD fed rats given wheat protein, d-

**Table 5**

| Time. | A. | R. | W. | S. | I. | G. | HM. | C. | BW. | SW. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| 5 | +o | | +o | o | | | | | | |
| 10 | +o | | +o | o | | | | | | |
| 15 | +o | | +o | +o | | | | | | |
| 20 | + | | + | + | | o | | | | |
| 25 | +o | + | +o | o | | | | | | |
| 30 | +o | o | + | | | | | | | |
| 35 | o | | o | | + | | + | | | |
| 40 | + | | | | o | | o | + | + | |
| 45 | + | | | | o | | o | + | + | |
| 50 | o | | | | + | | +o | o | o | |
| 55 | +o | | | | | | o | +o | +o | + |
| 60 | +o | | | | | | o | +o | +o | |
| 65 | | | | | +o | | +o | | | |
| 70 | | | | | +o | | +o | | | |
| 75 | o | o | | | + | | o | | | |

No. of rats tested — 5.
+ denotes special diet rats after 4mg/kg d-amphetamine, wheat protein and 0.4mg/kg naloxone. o denotes PRD diet rats after 4mg/kg d-amphetamine, wheat protein and 0.4mg/kg naloxone.

amphetamine and naloxone are similarly though not as severely affected as PRD fed rats given wheat protein and d-amphetamine, whereas the special diet rats are very different in that the administration of naloxone has suppressed the abnormal behaviour (HM, C, BW, SW), and what is more interesting is the fact that there is a time shift from Table IV. Now the activity is at 35-70 minutes, depressing the wheat protein effect back to near normal d-amphetamine behaviour.

From these results, comparison should be made between Tables II, III, IV and V with emphasis on the d-amphetamine behaviour (last 4 categories). It is only then that the full effect of wheat protein behaviour is appreciated (Table IV). The effect of naloxone on rats fed wheat protein and injection of d-amphetamine is more pronounced in that there is a shift in time, particularly in the group B (special diet) animals.

Table VI is interesting in that the behaviour patterns were obtained from group B (special diet) rats after they had been on PRD diet for a two-month period. The behaviour pattern seen here is similar to that of Table II PRD diet rats, with d-amphetamine behaviour starting at 30 minutes and showing a peak at 50 minutes; d-amphetamine, wheat protein and naloxone show a similar pattern starting at around the same time and peaking at 55 minutes, both patterns sloping off at 60-65 minutes with normal behaviour exhibited thereafter. The interesting observation made here was that a naloxone injection had very little effect, suggesting that there is inhibition of uptake of wheat protein from the intestine. This is discussed later.

Comparing this with Table VI, the similarity ends with group A rats, peak behaviour is at 50-55 minutes after a period of time on PRD diet, the special diet rats portray behaviour patterns similar to those from Table V and the behaviour pattern is as if the animals had been on PRD diet all their life. Attempt to reverse this was not successful; when the rats were put back on a special diet, the pattern of behaviour was not brought forward to that previously observed.

**Statistical Analysis.**

There are two types of diet, A (o) and B (+) (+) being the special diet. There are four types of drug applications; 11 Amph, 111 Amph and naloxone, IV Amph and wheat protein and V Amph, naloxone and wheat protein. These can be combined to form eight treatments.
All, Bll, Alll, Bill, A1V, B1V, AV, BV. Four abnormal conditions were observed, HM, C, BW, SW.

The following table gives the onset in minutes of the conditions under the influence of each combination of diet/drug.

| | All | Bll | Alll | Bill | A1V | B1V | | | AV | BV |
|---|---|---|---|---|---|---|---|---|---|---|
| HM | | 25 | 25 | 30 | 30 | 30 | 20 | | 40 | 35 |
| C | | 40 | 30* | 35 | 30 | 40 | 20 | | 50 | 50 |
| BW | | 40 | 35 | 45 | 35 | 50 | 25 | | 50 | 40 |
| SW | | 50 | 40 | 40 | 45 | 50 | 35 | | 58* | 55 |

In order to have a ranking of the rows on which to apply Friedmans 2 way analysis of variance the missing values (asterisk) had to be estimated.

| | All | Bll | Alll | Bill | A1V | B1V | AV | BV |
|---|---|---|---|---|---|---|---|---|
| HM | 2.5 | 2.5 | 5.0 | 5.0 | 5.0 | 1.0 | 8.0 | 7.0 |
| C | 6.0 | 2.5 | 4.0 | 2.5 | 6.0 | 1.0 | 8.0 | 6.0 |
| BW | 5.5 | 2.5 | 2.5 | 4.0 | 5.5 | 1.0 | 7.5 | 7.0 |
| SW | 5.5 | 2.5 | 2.5 | 4.0 | 5.5 | 1.0 | 8.0 | 7.0 |
| T | 18.5 | 10.0 | 17.5 | 14.0 | 24.0 | 4.0 | 31.5 | 24.5 |

The test statistic for the 2 way ANOVA is $\frac{12}{\sum r^2} = Nk(k-1) \sum_{J=1}^{k} (Rj)^2 - 3N(k-1)$

where N= no. of rows, k= no. of columns, Rj = sum of ranks.

Null hypothesis $H_o$ rank total for the various drug/diet combinations differ only because of chance.

Alt. hypothesis $H_1$ Drug/diet treatments make a real difference.

The sampling distribution of $\sum r^2$ is approximately $\chi^2$ with k-1 degrees of freedom provided the number of rows / column is not too small. From the tables $\chi^2(0.99;7) = 18.48$. Thus if test statistic is greater than 18.48 we reject $H_o$ in favour of $H_1$.

$\sum Rj^2 + (18.5)^2 + (10.0)^2 + (24.5)^2. = 3129.$

Test statistic $= \frac{12}{4x8x9} \times 3129 - 3x4x9 = 22.37.$

As this is greater than 18.48 we can conclude that the drug/diet treatments do make a real difference.

Two drug/diet treatments stand out in the table. B1V is ranked first in each case and AV is ranked last or equal last in each case.

Removing these two columns from the analysis gives the following

| | All | Bll | Alll | Bill | A1V | BV |
|---|---|---|---|---|---|---|
| HM | 1.5 | 1.5 | 4.0 | 4.0 | 4.0 | 6.0 |
| C | 5.0 | 1.5 | 3.0 | 1.5 | 5.0 | 5.0 |
| BW | 3.5 | 1.5 | 5.0 | 1.5 | 6.0 | 3.5 |
| SW | 4.5 | 1.5 | 1.5 | 3.0 | 4.5 | 6.0 |
| T | 14.5' | 6.0 | 13.5 | 10.0 | 19.5 | 20.5 |

$\sum R_j^2 = 1329$

* $\frac{12}{4x6x9} \times 1329 - 3x4x7 = 10.93$

Test statistic $= 4x6x9$

$\chi^2(0.99;5) = 15.09$

Here we have estimated one missing value and so lost one degree of freedom, so the CR starts at $1_{0.99;4} = 13.28$ and we still accept $H_o$

Test statistic falls outside the critical region in this case so $H_o$ is not rejected, but the conclusion is that there is no significant difference between A11, B11, A111, B111, A1V and BV at the 1% level, but are significantly different at the 5% level.

**Table 6**

| Time. | A. | R. | W. | S. | I. | G. | HM. | C. | BW. | SW. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| 5 | +x | | +x | + | | | | | | |
| 10 | +x | | x | +x | | + | | | | |
| 15 | x | | x | x | + | | | | | |
| 20 | | | | | +x | +x | | | | |
| 25 | +x | | +x | | | | | | | |
| 30 | x | | x | | + | | + | | | |
| 35 | | | | | +x | | +x | | | |
| 40 | +x | | | | | | + | + | x | |
| 45 | x | | | | + | | + | | x | x |
| 50 | +x | | | | | | +x | +x | + | |
| 55 | +x | | | | | | | +x | +x | x |
| 60 | +x | | | | | | | +x | +x | |
| 65 | + | | + | + | x | | x | | | |
| 70 | | | | | +x | | | | | |
| 75 | | | | | +x | | | | | |

No. of rats tested — 4.

+ denotes special diet rats after 2 months on PRD diet, 4mg/kg d-amphetamine and wheat protein. x denotes special diet rats after 2 months on PRD diet, 4mg/kg d-amphetamine wheat protein and 0.4mg/kg naloxone.

**Discussion**

It is evident from the data presented that wheat protein has an adverse effect on the behaviour pattern of d-amphetamine induced behaviour in rats. When the rats have no previous experience of this protein the effect is greatly enhanced. When an abnormal state of behaviour is induced in rats a typical pattern is observed, and it is interesting to see how this is shifted in time with wheat protein challenge. There is very little difference when wheat protein is administered to rats that have had previous encounter with this type of protein.

When an animal that has not been in contact with wheat protein is given the protein, it was observed that a shock condition of the animal took place, this being very similar to anaphylaxis, but as no antibody to the protein is present in the animal, it must be put down to a condition stimulated by first encounter to that protein. (Maybe if rats were able to be sick they could have got rid of the protein).

The most useful information gleaned from these experiments arises from Tables IV, V and VI. Table IV shows the animals to have responded at a much earlier time than usual. Behaviour patterns were also more severe. Table V shows that this earlier effect was pushed back, suggesting that naloxone is blocking some neural pathway or brain receptor site. Statistical analysis showed this to be significant at the 1% level. As it has been suggested by many workers (Hemmings, 1977; Dohan, 1978) the effect of peptic products of digestion on the brain and/or nervous system can be great, and this study shows that this action was stopped by naloxone. Another interesting phenomenon was observed when these animals had not had previous experience of the protein and were placed on a diet containing it for a few weeks, the result reverted to the pattern of rats reared on cereal diet. It is suggested that this may be because of changes in the intestine of the animal building up a resistance, maybe by producing IgA antibodies to wheat proteins. It is hoped at a future date to examine these gut cells for production of antibody and to see if a morphological difference occurs.

It was observed by Woodley et al. (1980) that the wheat protein fraction, gliadin, altered brain developments when fed at an early age, and that intact protein may cross the various barriers, to affect the neural

development. Hemmings et al. (1977) proposed that large molecular weight fragments of protein reached the brain and demonstrated the presence of alpha gliadin in the brain after feeding suckling and adult rats. It was also observed by Taylor (1978) that fractions of wheat protein had an effect on behaviour after being administered to adult rats.

In previous work the wheat protein forming the starting material has usually been alpha gliadin (Dohan et al., 1978; Hemmings, 1978; Klee and Zioudrou, 1979). This is soluble in 70% ethanol. In the present work, the flour was extracted with saline, and the gluten containing the gliadins was discarded, so that another class of wheat proteins, the globulins, was in fact studied. It appears from the results that with the globulins as with the gliadins there are formed small molecules having a CNS effect on behaviour which is naloxone reversible, so clearly there is here evidence of formation of exorphins similar to the ones Klee et al. have isolated from alpha gliadin, casein and albumin. This is hardly surprising, but suggests the wheat globulin should be studied from this point of view as a matter of urgency. The same thing could be said of a great range of human foodstuffs which have been shown to cause immunological type disorders in clinical practice.

The particular importance of wheat proteins is that they cause behavioural disturbances. This may be associated, as Dohan suggests, with their richness in glutamine, but this is perhaps only part of the explanation, and the full picture must depend on the identification of sequences of pharmacologically active peptides hidden in the protein molecules. These may be released by the action, first of pepsin in the stomach, and secondarily by cellular proteases following the arrival in the tissue cells of larger molecular digestion products which have been shown to be absorbed from the intestine following a protein meal (Hemmings and Williams, 1978). This process has been termed "distributed digestion".

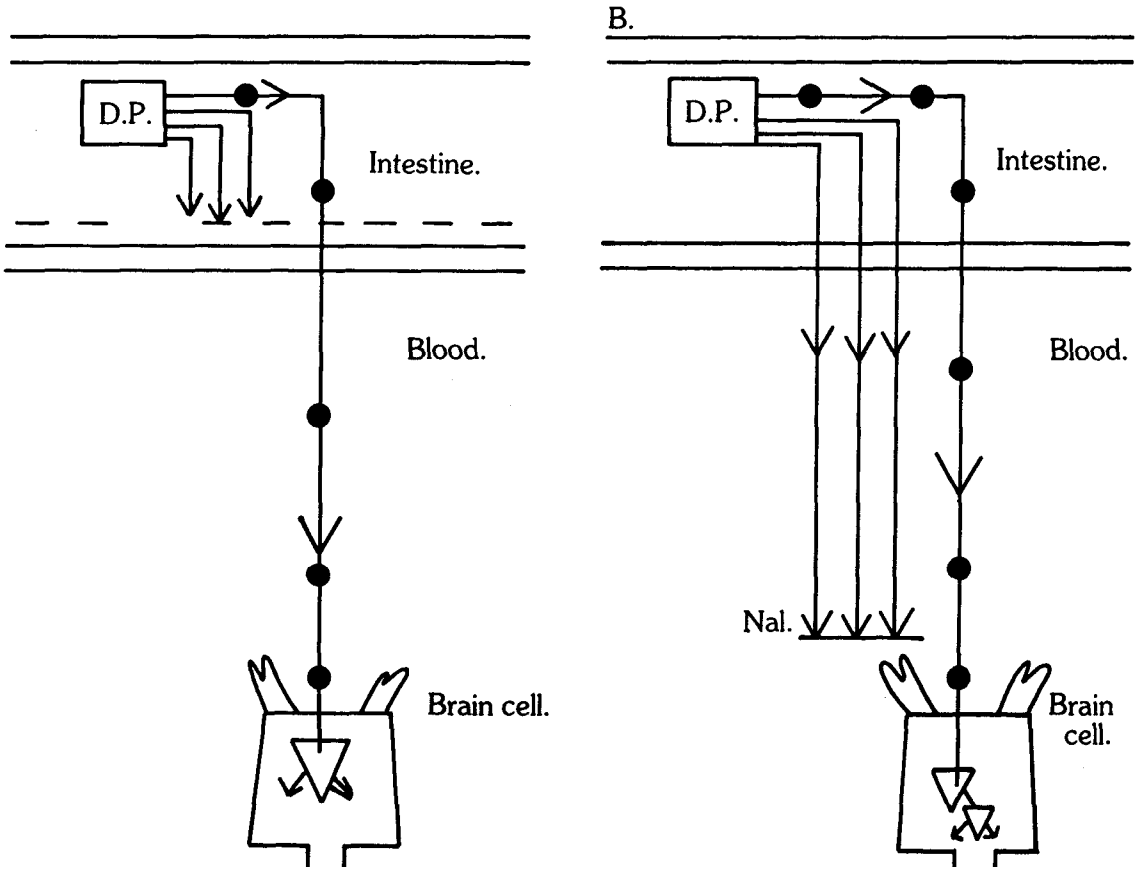
The present experiments are in some sense critical demonstrations that in the inexperienced animal, fed on dog food, small molecules are being absorbed from the gut and reaching the CNS cells and affecting behaviour, and this effect is naloxone reversible. There seems to be no other way to interpret the observations. The hypothesis put forward is that this effect is not seen in the experienced PRD animal because the latter is immune to wheat protein, and is secreting IgA antibody into its gut lumen which forms a barrier which the small peptides are unable to pass. There remains, however, in the PRD animal, the central effect of a wheat protein feed which was first described in a previous paper (Williams, 1979) and this, since it is not naloxone reversible, is likely to be due to the effect of the larger, protein component of the D.P. (degradation product) which has been shown previously to be present in adult brain tissue after a meal of wheat protein, even in animals fed PRD. Two hypothetical points may be made: first, the larger class of D.P. represented by those molecules giving antigen reactions in brain tissue (Hemmings, 1978) must be absorbed from the gut, despite the presence there of the immunological barrier; second, that the effect of the wheat meal on behaviour is mediated by this class of D.P. rather than by small peptides, since it is not naloxone reversible. It may however be that the pharmacological actions are in fact exerted by small fragments of the D.P. created or released by protease action inside the CNS cells after the entry of the larger D.P. fragment. It has been shown in muscle tissue that some time after feeding adult rats bovine IgG there are present two peaks of activity demonstrable by gel filtration: one is the larger D.P. fragment of about 50,000 daltons, the other is a peak of small peptides of 3,500 daltons or less (Hemmings, Jones and Williams, 1978). Thus it appears possible in muscle cells that small peptides may be released by the proteolytic action inside the cell upon the substrate of the larger D.P. and it may well be the products of that proteolytic action are peptides having pharmacological actions, possibly as yet unknown. In the context of the present work two possibilities are clear: The CNS effect of a wheat protein meal in wheat experienced animals may be due either to direct pharmacological

action of the larger D.P. molecules inside the cell, or to a peptide activity derived from the larger molecule by proteolysis by intracellular proteases. Presumably peptides released by such proteolysis inside the cell would not be subject to naloxone reversal, if it is true that naloxone acts at the cell surface by blocking opiate receptors concerned in the attachment of endorphins. It is questionable whether such attachment may be normally merely a first step in uptake, and if the normal biochemical target of the attached endorphin is not in fact inside the cell. The present work makes it seem likely that the latter may be the case.

This hypothesis is summarized for the sake of clarity in Fig 1. Fig 1A represents the uptake of large D.P.s from the lumen of the gut via the bloodstream to the brain cells, and their entry there. The peptides formed in the gut are represented as being stopped at an immunological barrier before penetrating the gut wall. There is evidence that such a barrier, of IgA antibody, would lead to enhanced proteolysis of the substrate in the gut lumen (Freed and Green, 1979; Walker et al., 1975). Fig. IB describes the situation in the inexperienced rat fed on dog food, where the peptides pass the gut wall into the blood stream, but are now stopped at a naloxone barrier of the brain cell. The larger D.P. is transported exactly as in Fig 1A.

This model has the advantage of suggesting much further work. As already noted,

Figure 1. A.




*A represents the "normal" PRD fed rat, or the rat fed dog food in infancy but later given PRD for two months.*

*B represents the rat gestated and reared in absence of cereal antigens, and therefore lacking the immunological barrier (IgA) of the gut shown in A. In these rats the naloxone barrier (Nal) may be present and effective, and is placed for the purpose of this hypothesis at the surface of the neural cell.*

*In both cases the larger D.P. indicated by the dotted line passes the gut wall and reaches the interior of the neural cell, where it suffers further digestion with the production of peptides (small arrows)* 290

the current work is concerned with globulins, and urgently requires repetition with gliadins. The peptides which are undoubtedly present and active in these experiments have yet to be identified in vitro by peptic analysis of wheat globulin followed by chromatography and the testing of fractions for biological activities. Conversely, the known exorphins derived from gliadins casein etc. need to be tested for activity when fed to both immune and inexperienced animals, to test further their transport from the gut lumen to the target centers in the CNS and the efficiency of the immunological barriers in preventing such transport.

**Legends.**

Table I. Results obtained after feeding wheat proteins to rats and the behaviour pattern obtained. (+ special diet, o PRD reared).

Table II. The behaviour pattern after an i.p. injection of d-amphetamine. As can be observed, the latter categories now show a positive response, (+ special diet, o PRD). Table III. Behaviour pattern obtained after an i.p. injection of d-amphetamine and an injection of naloxone hydrochloride, suggesting that naloxone has very little effect on d-amphetamine behaviour. (+ special diet, o PRD).

Table IV. Results obtained after feeding wheat protein and later injecting with d-amphetamine. There is now an enhanced behaviour pattern to be observed in the latter categories. (+ special diet, o PRD). Table V. Behaviour patterns obtained after wheat proteins, d-amphetamine and naloxone. The latter categories are diminished in the special diet animals. (+ special diet, o PRD).

Table VI. Results obtained after administration of wheat protein and d-amphetamine to special diet rats that had been exposed to a PRD diet (+) and x rats are similar but given an injection of naloxone.

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