Dietary Protein Reaches the Brain

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When a number of soluble proteins, labeled with radio-iodine, are fed to adult rats, considerable amounts of protein-bound radioactivity are found in the carcass, in all the organs including the brain. The specific activity of the brain is as high as that of skeletal muscle, indicating a considerable passage of the derivatives of the fed protein through the blood-brain barrier.

The derivatives retain the antigenic structure of the original molecule, in part, since antigenic activity can be demonstrated in the brain. The derivatives appear to combine with native cell components to form complexes of very high molecular weight.

Introduction

Chronic schizophrenics improve when placed on a gluten-free milk-free diet, and relapse when gluten is returned to their diet (Dohan, 1966; Singh and Kay, 1976). So far as I am aware this is the first evidence linking nutritional factors to mental disorder. Dohan (1977) uses coeliac disease as an analogue or model, but suggests that it may be more than this, and that coeliacs and schizophrenics may share a gene in common. The former often show psychiatric

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symptoms (von Kaser, 1961).

The gluten constituent protein, a-gliadin, has been implicated as a toxic factor in the etiology of coeliac disease (Hekkens and Pena, 1974), and therefore it is reasonable to assume it may be a-gliadin which is disturbing to schizophrenics. In experiments with rats in model psychosis it has been shown that gliadin has a marked effect on behavior when injected parenterally into the rats (Taylor, 1977).

The present work describes experiments on feeding labeled a-gliadin and other proteins to adult rats, and the subsequent location of these proteins or their derivatives in the tissues throughout the body, including the brain.

Results

A number of experiments have been carried out in which a variety of proteins labeled with radio-iodine have been fed to suckling and adult rats, or on occasion injected directly into the gut lumen. The proteins were iodinated at the level of approximately one iodine atom per four molecules of protein, by the gentle electrolytic procedure (Rosa et al., 1964). They were freed from iodide by prolonged dialysis in the cold. Rats were fed by gavage with polyethylene catheters. Rats were killed after the stated periods

and the complete alimentary canai removed. The skin was removed from adults, and the remainder of the body assayed by maceration and sampling as the "carcass" fraction.

Iodine has the great advantage for such experiments as this that proteins labeled with it, when degraded, give rise to products which are not reincorporated into further protein synthesis. Thus monoiodotyrosine and iodide, when administered orally, are excreted rapidly and quantitatively with the sole exception of the thyroid loop, which is unimportant in short-term experiments and easily blocked in longer ones. They do not give rise to protein-bound activity in the carcass (Jones, 1977). There is thus no problem of exchange of the label, as there is with proteins labled with tritium, carbon, or sulphur.

Samples were estimated as tungstic acid precipitable and soluble fractions, to distinguish between protein and amino acid or small peptides: samples submitted to sugar gradient ultracentrifu-gation were first clarified by spinning at 100000 G for 30 minutes to remove all organelles and solid matter.

A number of proteins have been employed in these experiments, which are listed in Table 1. They were deliberately chosen to include both proteins normal to the diet, such as rat IgG and the gliadins, and unfamiliar ones such as bovine IgG and hemoglobin. This was a simple attempt to determine if these two classes would be treated differently. The results, expressed as the percentage of the dose present in the carcass fraction as protein-bound radioactivity, are presented in Table 1. It can be seen that there is a massive carcass content, variable to some extent, but not obviously related to the dietary experience of the rats.

TABLE 1

Percentage of the absorbed dose present in the carcass as tungstic acid precipitable radioactivity after feeding various iodine-labeled proteins to suckling and adult rats.

Protein fed	Age	Number	Time (Hours)	Percentage	+ S.E.	References
Ferritin	S	**5**	**3**	25.4	5.1	Hemmings and Williams, 1974
Ferritin	30d	**5**	**6**	48.6	2.2	Hemmings, 1975a
Ferritin	60d	**5 .**	**6**	26.2	8.6	Hemmings, 1975a
Rat IgG	S	**5**	**3**	22.0	1.8	Hemmings and Wood, 1975
RatigG	A	**5**	**8**	34.1	5.8	Hemmings, 1975a
Bovine IgG	S	**5**	**3**	27.7	1.1	Hemmings and Wood, 1975
Bovine IgG	A	**5**	**8**	43.7	8.0	Hemmings, 1975a
a-gliadin	S	**10**	**3**	15.8	0.6	Hemmings et al., 1976a
a-gliadin	A	**6**	**24**	46.3	6.9	Hemmings et al., 1976a
B-gliadin	A	**5**	**8**	49.2	15.1	Hemmings et al., 1976b
V-gliadin	A	**6**	**8**	54.8	19.2	Hemmings et al., 1976b
Hemoglobin	A	**5**	**8**	24.8	9.9	Hemmings, 1976

S = suckling (15d.) A = adult (C. 300g)

Entry to the Brain

In certain of the above experiments the brain was removed, macerated, and sampled as above. The content of radioactivity found is presented in Table 2. Although the percentage of the dose shown here to be present in the brain in protein-bound form is a small figure, the mass of the brain is also small, and the specific activity of the brain is comparable to that of other tissues. Thus in sucklings the specific activity of protein-bound activity (counts/min/gm) three hours after feeding a-gliadin was found to be: skin 220, muscle 531, spleen 148, brain 133 (Hemmings et al., 1976a). In an attempt to ascertain whether the protein-bound activity genuinely represented intracellular location in the brain, and not merely retention at the

TABLE 2

Percentage of the absorbed dose present in the brain as protein—bound radioactivity after feeding a-gliadin and hemoglobin to suckling and adult rats.

Protein fed	Age	Number	Time	% in brain	i S.E.	References
a-gliadin	S	10	3	0^656	0.072	Hemmings et al., 1976a
a-gliadin	A	6	24	0.145	0.0199	Hemmings et al., 1976b
Hemoglobin	A	5	8	0.151	0.196	Hemmings, 1976

TABLE 3

Specific activities of brain fractions analyzed by differential centrifugation after feeding ^5 l-a-gliadin to rats. Means of six animals (Hemmings et al., 1976a)

Sample	Percentage of the total activity of brain			
	suckling rats		adult rats	
	mean	±S.E.	mean	i S.E.
p1) Nuclei, cell membranes	38.0	4.50	30.8	' 4.24
p2) Mitochondria and lysosomes	3.15	0.268	5.9	1.14
p3) Microsomes	3.12	0.310	4.3	1.15
p4) Tungstic acid precipitate of supernatant 3	12.88	3.23	29.8	6.19
s4) Tungstic acid precipitate of supernatant 3	44.28	1.96	29.2	2.85

surface of the blood-brain barrier, differential centrifugation was carried out on a group of both suckling and adult rats. The results are presented in Table 3. Roughly one-third of the activity is carried by the nuclear-cell membrane fraction and might be internal or external to the^ cell. But small but significant amounts are carried by the mitochondria and microsomes, and finally in the cytosol there is a considerable amount, another one-third in the adult, present as protein-bound activity in solution. Clearly the protein-bound activity is entering the cells of the brain.

Characterization of the Cytosol Protein

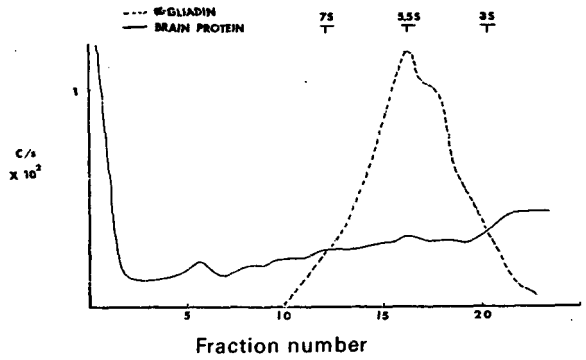
1. **Ultracentrifugation**

Since the specific activity of the protein in the cytosol of the brain extracts was significant, it became possible to carry out more elaborate studies on this material. Samples of adult rat brain after feeding labeled a-gliadin, bovine IgG, or hemoglobin were therefore submitted to an initial centrifugation at 100000 G for 30 minutes, to clarify them of all organelles. They were then layered on to sugar gradient tubes, and centrifuged as specified in the

legends of the figures.

Figure 1 presents the radioactivity profile through the gradient of the a-gliadin preparation, superimposed upon the profile of the brain macerate. It can be seen that there is little activity in the brain pattern in the region of the a-gliadin, or to the right of it, which would indicate breakdown products. There is a major peak right at the bottom of the tube in fractions 1 and 2, indicating the presence of a very heavy component. Figure 2 shows the profile of the serum of this rat, and again it can be seen that there is material to the left of the a-gliadin peak: this time as a minor component of approximately 7S. Figure 3 shows the profile of brain macerate after feeding bovine IgG, a 7S protein, and again, while there are marked peaks of size smaller than 7S, which could indicate breakdown products, the most considerable peak is in tubes 1 and 2, indicating a major component of a very large molecular mass.

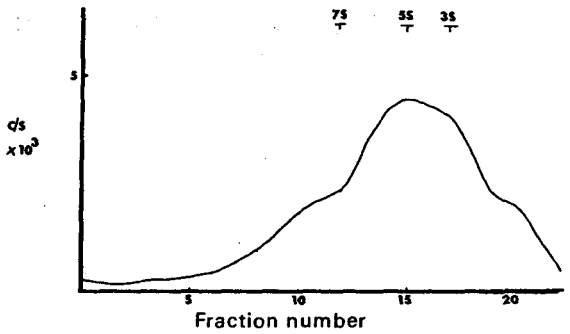
FIGURE 1



Sugar gradient ultracentrifugation profiles of radioactivity of a-gliadin (dotted line) and brain macerate from an adult rat after feeding the a-gliadin. Sugar gradients from 5 - 40% sucrose, run at 50000 r.p.m. for 24 hours in the Sw 50 rotor of a Spinco model L ultracentrifuge.

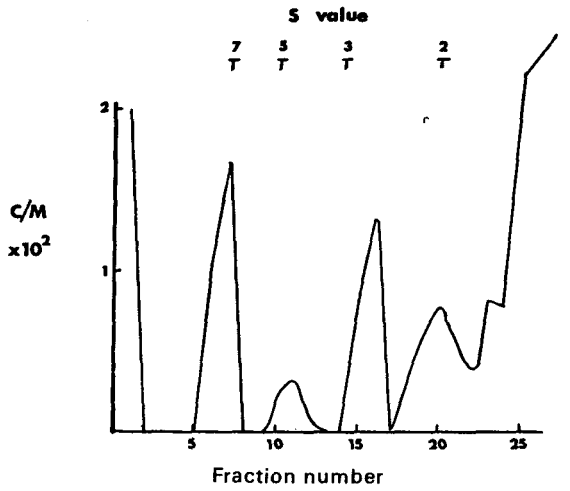
Figure 4 shows the profile of brain macerate after feeding porcine hemoglobin - ^{125}l, a protein of 4.5S. This run was designed to analyze the ultra-heavy components, and it is here seen that there are several peaks to the left of the 7S marker, and again a major peak in tubes 1 and 2. As a technical check, the brain

FIGURE 2



Sugar gradient ultracentrifugation profile of the serum of the rat of Figure 1. Same conditions as Figure 1.

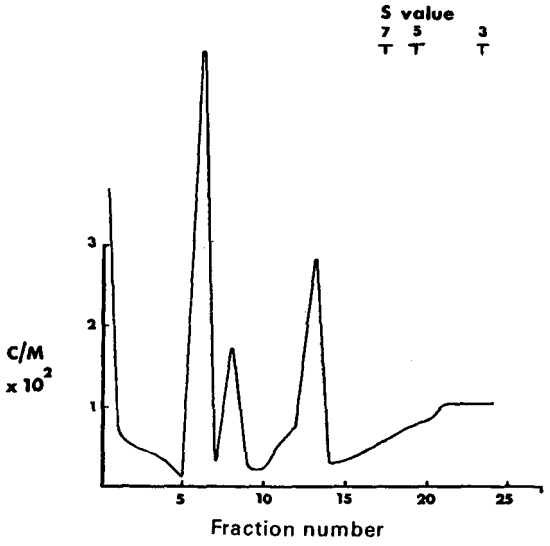
FIGURE 3



Sugar gradient ultracentrifugation profile of brain macerate from an adult rat after feeding bovine IgG. Spun for 30 hours at 50000 r.p.m.

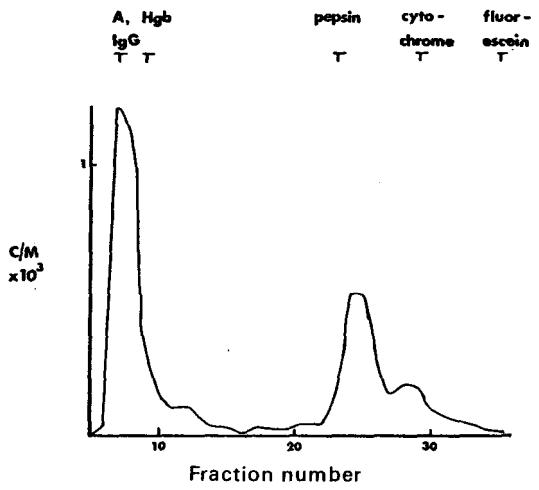
macerate following feeding of bovine IgG was subjected to chromatography on Sephadex G 75. The elution pattern of radioactivity is presented as Figure 5. Comparing the ultracentrifuge profile with this, it is clear that the three peaks to the left of the 5.5S marker have come off the Sephadex in the first fraction, leaving two peaks in the region of the pepsin and cytochrome markers which correspond with those found on the centrifuge. The chromatographic run was not carried far enough to elute the amino acid peak which is at the far right of the centrifuge profile.

FIGURE 4



Sugar gradient ultracentrifugation profile of brain macerate from a rat after feeding porcine hemoglobin. Spun for 18 hours at 50000 r.p.m.

FIGURE 5



Chromatographic elution pattern from running the brain macerate of Figure 3 on a column of Sephadex G75, 1 cm x 55 cms, eluted with phosphate buffered saline pH 7.0 at a flow rate of 26 ml/ hr. Markers of IgG, albumin, pepsin, and cytochrome, labeled with FITC, were incorporated with the sample.

2. Immunological Studies

The cytosol component of brain macerates and serum samples were put up in precipitation tests with specific antisera following the feeding of labeled a-gliadin and bovine IgG to adult rats, with appropriate controls. The results are presented in Table 4. In the case of a-gliadin there is approximately as much of the antigen present in the brain macerate as in the serum: in the case of bovine IgG there is greatly more antigen in brain than in serum, and indeed it would appear that most of the protein-bound activity of the brain retains its ability to precipitate with specific antiserum, in this case.

3. Visualization Studies

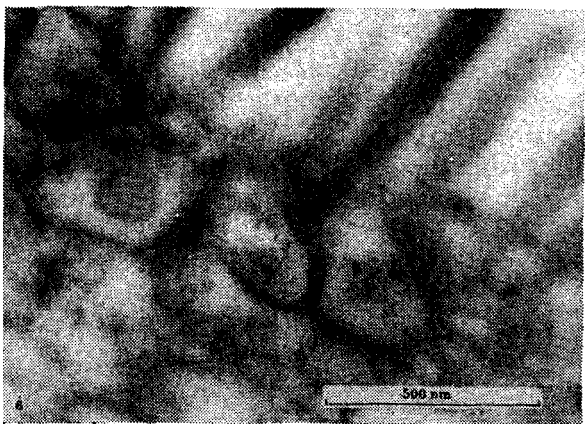
The process of absorption through the gut wall has been studied at the electro-nmicroscope level using ferritin as the trace molecule (Williams, 1975). Ferritin is found associated with the glycocalyx of the small intestinal epithelial cells, and in the ileum, as shown in Figure 6, it can be seen to be taken into vacuoles at the root of the microvilli. But there is on closer inspection a great deal of ferritin free in the cytosol of these cells: how it passes the unit membrane of the vacuoles is unclear, but Figure 6 shows some molecules of ferritin trapped apparently in the membrane as though they were "diffusing" through it. Figure 7 shows this

TABLE 4

The percentage of the radioactivity present which is specifically precipitable with immune serum, after feeding ^{125}I labeled a-gliadin or bovine IgG to suckling and adult rats. Means of five animals.

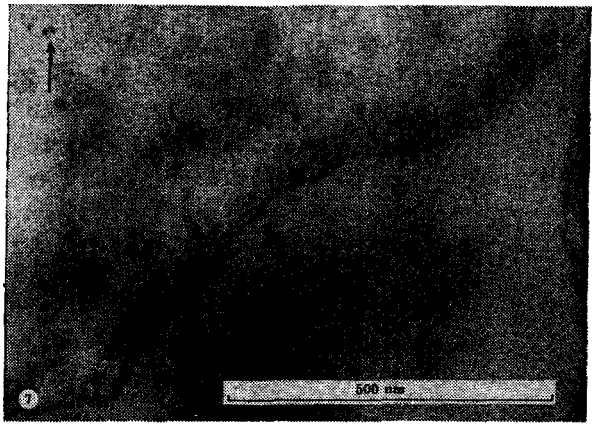
Protein	Sample	Suckling		Adult	
		% ppt	± *S.E.*	% ppt	± S.E.
a-gliadin	serum	13.1	2.1	6.02	2.57
	brain	13.7	2.7	7.4	6.9
Bovine IgG	serum	-		6.74	2.84
	brain	-		57.2	10.6

FIGURE6



Electron micrograph of a portion of the apical surface of an ileal epithelial cell after administration of horse spleen ferritin. Royal Society London, from Proceedings Royal Soc. B. In Press.

FIGURE7



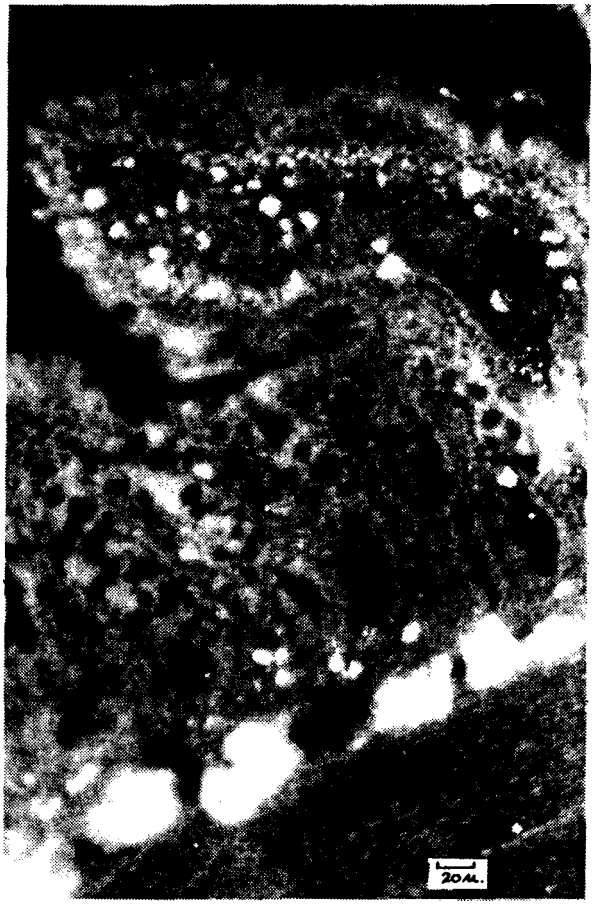
Portion of the lateral surface of a simila'r cell showing an intercellular space into which ferritin particles are discharging. Royal Society London, from Proceedings Royal Soc. B. In press.

cytosol ferritin present near the lateral membrane of the cell, and a group of ferritin particles apparently in process of passing through the lateral cell membrane.

To *date* it has not been possible to extend these studies to the tissues of the body because of the extreme dilution factors involved: using a reasonable fed dose of ferritin there is too little in such a tissue as the brain to pick up.

An alternative to the use of such a wildly heterologous and large molecule as ferritin is to employ immunohisto-logical techniques, relying on the identification of the antigen in the tissue by specific antiserum. At present, this has been done only at the light microscope level, feeding bovine IgG and identifying it by use of fluorescein isothiocyanate coupled (FITC) antibody from rabbit serum. Figure 8 shows a section of the ileum of an adult rat fed bovine IgG stained with this reagent, and clearly a very active traffic of antigen is going on across the epithelial cells, which show mild fluorescence, into the lymphatic channels of the villus, which show bright fluorescence.

FIGURE8



Light microscope fluorescence photograph of a portion of a section of the ileum of an adult rat after feeding bovine igG. Stained with FITC-rabbit IgG anti bovine IgG. CIBA Foundation Symposium 50: Peptide Transport and Hydrolysis Elsevier, Excerpta Medica, North Holland, Amsterdam. 1977.

Figure 9 shows a section of the forebrain of the same rat, stained in the same way, and definite very bright foci of fluorescence have appeared, not apparently blood vessels, but deep in the nervous tissue. It is proposed to continue

FIGURE9

Section of the forebrain of the same rat as Figure 8, stained in the same way. CIBA Foundation Symposium 50: Peptide Transport and Hydrolysis. Elsevier, Excerpta Medica, North Holland, Amsterdam. 1977.

this work at the E.M. level using ferritin conjugated antibody in sandwich technique.

Discussion

The first observation of which the present author, is aware on passage of antigens across the gut of adult animals was that of Uhlenhuth (1900). He reported that rabbits after being fed egg white developed circulating antibodies to that antigen. There was a good deal of activity in this field in the early years of the century. Notably Wells and Osborne (1911) reported experiments with guinea pigs fed on a variety of plant proteins, in which anaphylactic shock was induced in the animals by feeding the antigen, after parenteral immunization. They reported that guinea pigs habitually fed a protein could not be immunized against it by parenteral injection, an observation which surely predates the discovery of immune tolerance. They observed anaphylactic shock with a whole range of vegetable proteins, but some, such as the globulins of squash seed, vignin, excelsin, and castor bean globulin were effective in much lower doses than others. Edestin was the least harmful. A major first sign of the onset of anaphylactic shock in guinea pigs is "intoxication," and this is often the only symptom shown after the feeding of such a protein as edestin. "Intoxication" is a state of impairment of motor function, and is almost certainly cerebral, so that one might record that these authors were for the first time investigating the cerebral effects of diet.

If this effect of intoxication in sensitized guinea pigs is accepted as a central nervous system phenomenon, there is raised the question of first, how the sensitizing antibody has reached the brain; second, how the fed antigen reaches the brain, remembering that the time course of shock following a feeding is fairly rapid; third, what are the reactive elements. Is this effect in fact due to antibody-antigen reaction within the brain, or is it mediated as a response to histamine or bradykinin released by such reaction systemically?

There is some evidence that antibody from the plasma does reach the CNS (Frick and Scheid-Seydel, 1958). The present work shows that large cleaviage products of soluble dietary protein do pass freely across the gut wall into the circulation, and that they appear to enter the brain within short periods of being fed. Also, they retain their antigenicity to some degree both'in the serum and in the brain tissue. It cannot therefore be ruled out that we have to deal with antibody-antigen reactions occurring actually within the CNS. One must remember, however, the absence of the apparatus of hypersensitivity reaction, the lymphocytes and mast cells.

A further possibility of interference with normal function of the CNS is raised by the ultracentrifuge observations reported herein. With every protein so far

observed, the brain (and other tissue) cytosol protein has shown an ultra-heavy peak which accounts for a large part of the total protein-bound activity present. This may, of course, be a postmortem artifact: tissue extracts do tend to throw a precipitate on standing, however clarified initially. But the proportion of the diet-derived activity involved in this ultra-heavy component seems suspiciously large, and the hypothesis must be kept in mind that this foreign protein is becoming involved in reactions within the cell, possibly with native cellular constituents, which may well impede cell function.

To summarize, dietary protein in soluble form passes in large degree unchanged out of the digestive tract into the circulation and then into the tissues. It can provoke the production of antibody, or in some instances give rise to tolerance, and the mechanics of this choice are not yet understood. Some part of the influx reaches the CNS tissues. It would seem there is great need for intensive research into the effect of diet upon brain function.

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