Summary

The action of Adrenochrom on the brain was investigated electrophysiologically on the conscious rabbit. The moderate activation of the somatic behaviour and of the electrical brain-activity can be attributed to an increased activity of the ascending reticular system with simultaneous depression of the medio-thalamic intralaminary recruiting system, as well as to an increased activity of the hippocampus. Specific afferent (somesthetic) systems are only slightly moderated.

Clinical and Biochemical Analysis of Gluten Toxicity I

DICKE *et al.*[1,2] demonstrated that the gliadin component of wheat gluten contained the factor that is toxic in coeliac disease. Subsequently, several research workers[3,4] observed that the omission of gluten from the diet of adult patients with idiopathic steatorrhoea caused the fat absorption coefficient to rise.

Since 1956, we have been engaged in experiments to find out whether a certain amino acid structure of the gliadin is responsible for the toxicity. As a first step, gluten was treated consecutively with gastric juice and duodenal fluid, which were later supplanted by crystalline pepsin and trypsin. It was found that this treatment did not affect the toxicity. Thus, the observations of FRAZER *et al.*[5] on children with coeliac disease were confirmed.

The degradation of gliadin (conc. 100 g/2 l dist. water) is carried out at 37°C, first at pH 1–2 for two days with twice 0·5 g pepsin, after which the undissolved part is centrifuged off and the process is continued for two more days at pH 7–8, with the addition of twice 0·5 g trypsin.

The soluble fraction obtained after the enzyme treatment described above was split into an amino acid fraction, an acetone-precipitated fraction, and a residual fraction obtained by evaporation to dryness at 40°C under reduced pressure. Tests on two patients with thrush showed that only the acetone-precipitated fraction was obviously toxic. In view of the special amino acid composition of gliadin, we first isolated the acid peptide fraction from the toxic acetone precipitate by adsorption on alumina. The acid peptide mixture thus obtained was dialysed through a colloid membrane against distilled water and lyophilized.

Administration of the acid peptide fraction in doses corresponding to 24 g wheat gluten per day was found to have a strongly positive effect in two patients with thrush.

With one of the patients, the percentage of non-absorbed fat in the stool, after administration of 1·0 g of the acid peptide fraction, rose from 15 to 81·1% in one day. In the case of the second patient, the percentage of non-absorbed fat in the stool rose, after 0·5 g of the acid peptide fraction had been given for a period of 7 days, and subsequently 1·0 g for 3 days, from 12 to 86%.

Before proceeding further, we wanted to make sure that the mixture of acid peptides derived from the acetone-precipitated fraction of pepsin- and trypsin-treated gliadin consisted of straight chains. Since the amino acid cystine occurs in gliadin and – as demonstrated by two-dimensional paper-chromatography – also in the toxic fractions isolated by us, it is possible that by means of these cystine molecules, sulphur bridges are formed in one or more peptides, which cause ramifications in the molecule, making determination of the structure difficult. Similar structures have been encountered several times, for instance, as the investigations of SANGER have shown, in the hormone insulin.

Following SANGER[6], we treated the acid peptide mixture with performic acid, whereby one molecule cystine, even when it is part of a peptide chain, is converted into two cysteic acid molecules. Although a large portion of the cystine could be converted into cysteic acid in this way, we failed to transform all the cystine present, even though several modifications of the method were applied.

Treatment of the second of the patients mentioned above, with the acid peptide fraktion oxidized with performic acid, showed that this fraction was still highly toxic. After administration of 600 mg of oxidized acid peptides per day for 6 days, the percentage of non-absorbed fat in the stool rose from 15 to 51%.

Hence, it appears that, in spite of the performic acid treatment, the toxic factor is still present.

Since, according to these findings, not all the cystine present in the peptides was oxidized to cysteic acid by our treatment with performic acid, our results cannot yet be attributed to the occurrence of unbranched peptide chains.

J. H. VAN ROON, A. J. CH. HAEX, W. A. Seeder, and J. DE JONG

Department for Gastroenterology, State University Hospital, Leyden (Netherlands) October 21, 1959.

Zusammenfassung

Eine saure Peptid-Fraktion wurde aus dem Weizenprotein Gliadin durch Behandlung mit Pepsin und Trypsin erhalten. Das Verdauungsprodukt wurde durch Präzipitation mit Aceton und Adsorptionschromatographie an saurem Al_2O_3 weiter aufgearbeitet.

Die erhaltene saure Peptid-Fraktion wurde mit Perameisensäure nach dem von SANGER entdeckten Verfahren behandelt. Es wurde festgestellt, dass der in idiopathischer Steatorrhoe toxische Faktor nach dieser Behandlung noch immer vorlag.

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Urinary Excretion of 5-Hydroxyindoleacetic Acid and Histamine in the Pregnant Rat

It was recently observed that in the last third of pregnancy the rat excretes large amounts of histamine in the urine[1]. This increased output of histamine in the mother's urine is probably caused by an increased production of histamine by the fetuses[2]. Various observations indicate that there is a connexion between the content of 5-hydroxy-tryptamine (serotonin) and histamine in some tissues of the rat, possibly because the two amines are in

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