Journal of Nutritional & Environmental Medicine (1997) 7, 333–342

CARFAX

FOOD, BRAIN and BEHAVIOUR
Conference Proceedings October 1996
ALLERGY RESEARCH FOUNDATION

Assessment of Chemical Factors in Relation to Child Hyperactivity

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A questionnaire evaluation of 486 hyperactive children (HA) (82% boys, aged 7–13 years and 18% girls, aged 8–13 years) showed that more than 60% of cases reported a positive behavioural response (i.e. increased problems) in relation to consuming or being exposed to synthetic colourings and flavourings, food and beverage preservatives, cow's milk and associated products, chemical detergents and perfume. In contrast, 172 sex- and age-matched control children (C) reported only 12% of cases responding to synthetic colourings and flavourings and chemical solvents. The main health problems reported by the 96% of hyperactive children affected by synthetic colourings and flavourings were persistent thirst problems, the development of eczema, ear and/or chest infections, and the production of excessive amounts of catarrh. Trace element measurements undertaken by inductively coupled plasma mass spectrometry showed that a low zinc and iron status is associated with hyperactive children when compared with control children for blood serum, urine and washed scalp hair (HA < C). In many cases, hyperactive children also had very highly significant raised levels of aluminium, cadmium and/or lead (HA > C), particularly in urine and washed scalp hair samples. Hyperactive children with a known behavioural response following the consumption of a beverage containing tartrazine, E_{102} *(n = 23), sunset yellow,* E_{110} *(n = 12) and amaranth,* E_{123} *(n = 12) were given a dose of chemical food colour (50 mg) and their zinc levels (blood serum and urine) and behavioural activity were monitored for 120 min. A sex- and age-matched control group was also studied. Only hyperactive children showed a significant reduction in blood serum zinc levels and an increase in urinary zinc output following the consumption of* E_{102} *and* E_{110}*. Amaranth had no effect on their zinc status over the study time period. There were no significant changes in the zinc levels for control children for all three chemical food colours. The main behavioural changes were observed in the hyperactive children given* E_{102} *and* E_{110}*. For the 23 children who consumed a tartrazine beverage there were increased levels of overactivity (n = 18 children), aggressive (n = 16) and/or violent (n = 4) activity, poor speech (n = 2), poor coordination (n = 12), and the development of asthma and/or eczema (n = 8). Most of these were severe or moderate changes. Only one control child showed minor behavioural responses to tartrazine.*

Keywords: child hyperactivity, trace elements, zinc, synthetic food colours, tartrazine.

1359-0847/97/040333-10 $9.00

INTRODUCTION

Hyperactivity in children is a complex disorder characterized by a high level of motor activity, short attention span, low frustration, hyperexcitability and poor impulse control. Such children often show persistent symptoms of excessive thirst, poor appetite, multi-allergic reactions, and a tendency to develop asthma and/or eczema following exposure to a particular chemical, nutritional or environmental agent [1, 2]. Some studies have shown that chemical additives, widely used in certain beverages and foodstuffs, can trigger hyperactive behaviour problems in some children [3–10].

Azo colours comprise the largest group of certified colourants. More than 15 azo dyes are permitted in any food except raw or processed meat, fish, fruit, vegetables, white bread, tea, coffee and milk [11]. Tartrazine (E_{102}) is the colour most frequently studied in terms of food intolerance, behavioural changes and hyperactivity in children [7, 8, 10, 12–14]. One double-blind, placebo-controlled study showed that in hyperactive children tartrazine could induce a reduction in blood serum and saliva zinc levels, with an associated increase in urinary zinc output [15]. This change in trace element levels was also related to a deterioration in behavioural and emotional status. These findings suggest a possible chemical interaction between certain azo food colours and essential trace elements that may be linked to the aetiology of hyperactivity in children.

RESEARCH DESIGN AND METHODS

Evaluation of Chemical Factors in Hyperactive Children

Between October 1992 and July 1995, in association with the Hyperactive Children's Support Group (HACSG) and the Allergy Support Group of Oxford (ASGO), a series of studies was undertaken to assess the chemical factors in relation to child hyperactivity, in particular the influence of chemical food colours. A questionnaire evaluation of 486 hyperactive (HA) and 172 control (C) children was undertaken by parents. The comprehensive questionnaires (prepared by the HACSG) contained questions on early life, early symptoms, symptoms in the older child, other health problems and whether the child was affected by synthetic colourings, flavourings, preservatives and other food or chemical substances. The hyperactive group consisted of 82% male (all Caucasian, aged 7–13 years) and 18% female (all Caucasian, aged 8–13 years) children. The main features of the HA children were 54% fair hair (the others either brown or black), 48% blue eyes (others mainly brown) and 84% very fair skin complexion. A sex- and age-matched group of 172 control children was also evaluated. No control children were on special diets or nutrient supplements.

TRACE ELEMENT LEVELS OF HYPERACTIVE AND CONTROL CHILDREN

Blood serum samples were collected at a standard time of 8–9 a.m. from 93 hyperactive and 19 control children following overnight fasting. Similarly, mid-stream flow urine and scalp hair samples were taken from all 486 HA and 172 C children. All body fluids (2–5 ml) were wet-ashed in Teflon digestion vessels using 12 M-Aristar nitric acid and 30% hydrogen peroxide (heated at 100 °C for 12 h). The scalp hair samples (0.2–0.5 g) were washed using an acetone/distilled-deionized water ultrasonic procedure, dried at 60 °C for 6 h and dry-ashed at 450 °C for 12 h. The homogenized ash was digested in 6 M-Aristar nitric acid. All trace element measurements were undertaken using a Finnigan MAT SOLA inductively coupled plasma mass spectrometer (ICP-MS) located in the Department of Chemistry, University of Surrey. All reagent blanks, calibration standards and sample solutions were spiked with 100 ng ml^{-1} of In as an internal standard so as to evaluate and correct any changes in instrument performance throughout the measurement period.

Double-blind Evaluation of Synthetic Food Colours and Hyperactive Children

A group of 47 hyperactive children who were reported by their parents to be sensitive to various synthetic food colours were selected to evaluate the effect of synthetic food colours on both trace element and behavioural status. Various studies have reported that parents are reliable observers and raters of their children's behaviour in such circumstances [10]. This group of hyperactive children were given an oral dose following body fluid sample collection (i.e. after an overnight fast). The synthetic food colour (50 mg) was administered randomly as a 100-ml drink for each child. Twenty-three hyperactive children were given tartrazine (E_{102}), 12 sunset yellow (E_{110}), 12 amaranth (E_{123}). Fifteen control children were subdivided into three equal groups and also given the three synthetic food colours. This study does not include a placebo-hyperactive or control group as the subgroup number of children would have been very small. Blood serum and urine samples were taken (when possible) over a 120-min period following the consumption of the drink. All samples were coded to prevent group identification by the analyst. Behavioural changes were monitored by a paediatric neurologist and classified according to severe (virtually unmanageable), moderate (great difficulty in stabilizing behaviour), mild (manageable) or no reaction. Observations were recorded for changes in overactivity, aggressive and/or violent behaviour, poor speech and/or coordination and the development of asthma and/or eczema. All neurological assessments were undertaken double blind with respect to group and treatment. Informed written consent was obtained from all parents.

RESULTS

The questionnaire evaluation of 486 hyperactive and 172 control children (Fig. 1) showed that more than 60% of the HA children reported a positive response (i.e. increase in behavioural problems) in relation to synthetic colourings and flavourings, food and beverage preservatives, cow's milk and associated products, chemical detergents and perfume. In contrast, 12% of the control children also reported similar responses to synthetic colourings and flavourings and chemical solvents.

An evaluation of the associated health problems of the 96% of hyperactive children affected by synthetic colourings and flavourings showed that 60% or more had a persistent thirst problem, developed eczema, ear and/or chest infections and produced excessive amounts of catarrh. Many of these cases also reported a history of taking several courses of antibiotics in early childhood.

An analysis of blood serum (Table 1), urine (Table 2) and washed scalp hair samples (Table 3) from HA and C children showed significant differences for various essential and toxic trace elements. For all matrices the hyperactive children are low in zinc and iron; $HA < C$ as evaluated by a two-tailed Student's *t*-test, with a probability level of $p < 0.001$ (very highly significant difference). This is a similar finding to that previously reported for zinc in hyperactive and control children [15]. Interestingly, the hyperactive children selected in this study as a result of their sensitivity to synthetic food colours have much lower blood serum and scalp hair zinc levels than the hyperactive children used in the previous study. For example, the mean and range of zinc levels for the HA children in this study are ($\mu g\ ml^{-1}$) 0.56 (0.42–0.71) for blood serum and ($\mu g\ g^{-1}$), 82 (66–138) for scalp hair compared with previous levels for blood serum of 0.82 (0.52–1.00) and for scalp hair of 161.7 (113.4–207.6) [15]. In this study there were many very highly significant cases of raised aluminium, cadmium and/or lead levels in the urine and washed scalp hair samples from hyperactive children ($HA > C$).

A group of hyperactive children with known sensitivity to synthetic food colours were given a dose of tartrazine, E_{102} ($n = 23$ children) or sunset yellow, E_{110} ($n = 12$) or amaranth, E_{123} ($n = 12$). A sex- and age-matched control group ($n = 5$ children for each synthetic food colour) was also studied. Figures 2–4 show the zinc levels in the blood serum

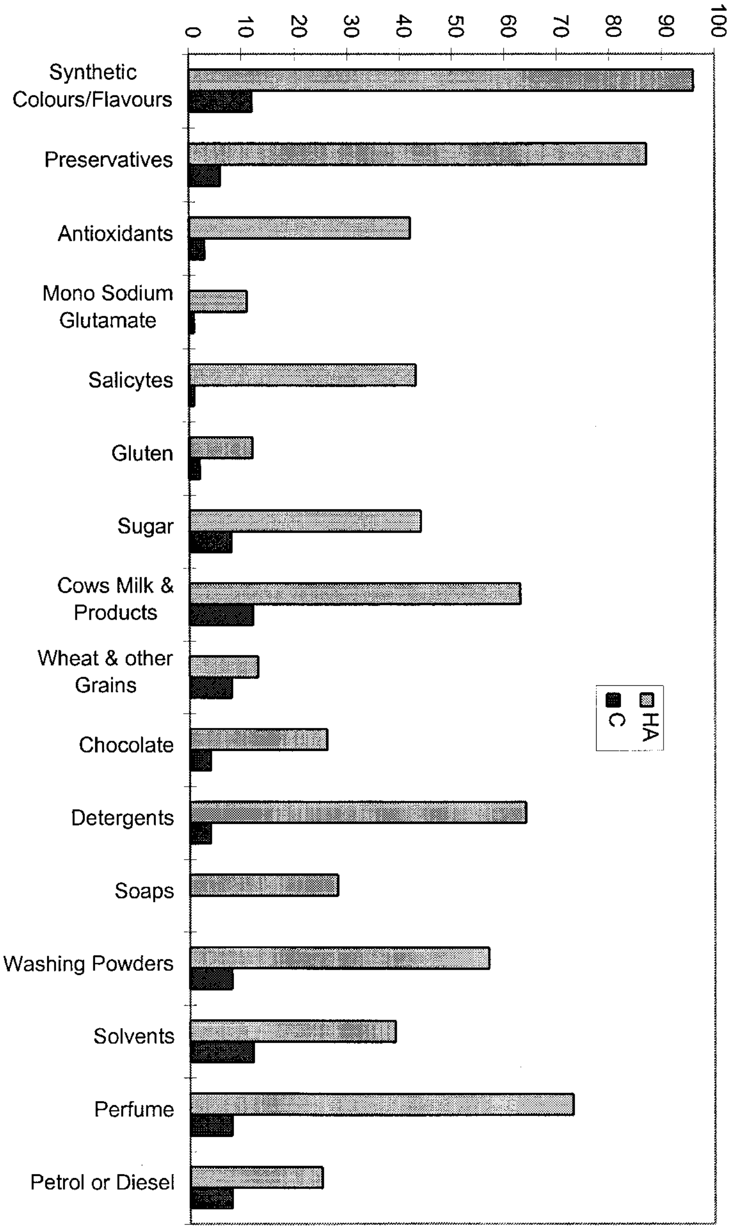

FIG. 1. Percentage of hyperactive (HA) and control (C) children reporting a positive response to foods or chemicals.

or urine samples collected at 0, 30, 60 and 120-min time periods (reported for only those cases who could provide a sample) after the consumption of the drink (with a 50-mg dose of synthetic food colour).

TABLE 1. Trace element content of blood serum for hyperactive and control children

	Trace element content		
Trace element	Hyperactive ($n = 93$)	Control ($n = 19$)	Statistical level[c]
Aluminium[a]	6.80 (1.5–16.7)	4.00 (0.8–8.7)	NS[c] HA > C
Cadmium[a]	1.40 (0.7–2.1)	1.10 (0.5–2.3)	NS HA > C
Iron[b]	0.74 (0.64–0.89)	0.96 (0.74–1.24)	** HA < C
Lead[a]	3.00 (0.8–6.7)	1.80 (0.8–3.6)	NS HA < C
Zinc[b]	0.56 (0.42–0.71)	0.93 (0.68–1.02)	** HA < C

[a] Concentrations reported as mean (range); values all ng ml^{-1}.

[b] Concentrations reported as mean (range); values all μg ml^{-1}.

[c] Statistical two-tailed Student's t-test: **very highly significant, $p < 0.001$; NS, not significant, $p > 0.10$.

Only hyperactive children showed a significant reduction in their blood serum zinc levels and an associated increase in urinary zinc output over the 120-min period following the consumption of tartrazine and sunset yellow. Hyperactive children given a dose of amaranth showed no significant changes in their zinc levels. The effect of tartrazine on the zinc status of this group of hyperactive children with a known sensitivity to synthetic food colours is a similar finding to that previously reported for hyperactive children [15]. Although this study does not contain a placebo-hyperactive or control group, the previous work showed that a non-tartrazine placebo beverage did not influence the zinc status of both groups of children [15].

Table 4 reports the behavioural changes following the consumption of these synthetic food colours for both the hyperactive and control children groups. The main behavioural responses were associated with the hyperactive children who consumed tartrazine resulting in increased overactivity ($n = 18$ children), aggressive ($n = 16$) and/or violent ($n = 4$) activity, poor speech ($n = 2$), poor coordination ($n = 12$) and the development of asthma and/or eczema ($n = 8$). The majority of these were severe or moderate responses. Sunset yellow and amaranth also had significant effects on the hyperactive children, in particular in relation to overactive and aggressive behaviour. Only tartrazine and sunset yellow induced the development of asthma and/or eczema after a 30-min period of consuming the beverage. One control child showed a mild response (aggressive, poor coordination and slight asthma) to tartrazine.

TABLE 2. Trace element content of urine for hyperactive and control children

	Trace element content		
Trace element	Hyperactive ($n = 486$)	Control ($n = 172$)	Statistical level[c]
Aluminium[a]	12.500 (2.7–38.4)	8.500 (1.3–18.6)	HA > C**
Cadmium[a]	1.800 (0.8–3.6)	0.900 (0.6–1.8)	HA > C**
Iron[b]	0.072 (0.046–0.140)	0.124 (0.065–0.160)	HA < C**
Lead[a]	23.000 (12–32)	16.000 (8–24)	HA > C**
Zinc[b]	0.210 (0.08–0.46)	0.64 (0.09–0.87)	HA < C**

[a] Concentrations reported as mean (range); values all ng ml^{-1}.

[b] Concentrations reported as mean (range); values all μg ml^{-1}.

[c] Statistical two-tailed Student's t-test: **very highly significant, $p < 0.001$; NS, not significant, $p > 0.10$.

TABLE 3. Trace element content of washed scalp hair for hyperactive and control children

	Trace element content		
Trace element	Hyperactive ($n = 486$)	Control ($n = 172$)	Statistical level[b]
Aluminium[a]	9.4 (3.8–27.4)	2.4 (0.9–9.0)	HA > C**
Cadmium[a]	1.3 (0.2–2.4)	0.4 (0.1–0.8)	HA > C**
Iron[a]	18.4 (4.6–42.8)	28.7 (15.8–48.3)	HA < C**
Lead[b]	8.2 (2.4–12.7)	3.2 (1.2–6.5)	HA > C**
Zinc[a]	82.0 (66–138)	138.0 (98–155)	HA < C**

[a] Concentrations reported as mean (range); values all $\mu g\ g^{-1}$.

[b] Statistical two-tailed Student's t-test: **very highly significant, $p < 0.001$; NS, not significant, $p > 0.10$.

DISCUSSION

A major finding of this study is that hyperactive children have statistically lower zinc and iron levels in relation to control children for blood serum, urine and washed scalp hair (all $p < 0.001$). Moreover, hyperactive children who are known to be sensitive to synthetic food colours showed a significant reduction in their blood serum zinc levels and an increase in urinary zinc output in response to consuming a beverage containing either tartrazine or sunset yellow. The poor zinc and iron status in these hyperactive children may be related to numerous factors, including poor dietary status, abnormal intestinal absorption or gut permeability and reduced utilization as a result of complexation or competitive interaction with other trace elements, such as copper, cadmium and lead.

Low zinc and iron status have been associated with increased susceptibility to infection and impaired cell-mediated immunity [16]. Hyperactive children are reported to suffer from more frequent coughs, ear and chest infections and skin problems, such as eczema. Many

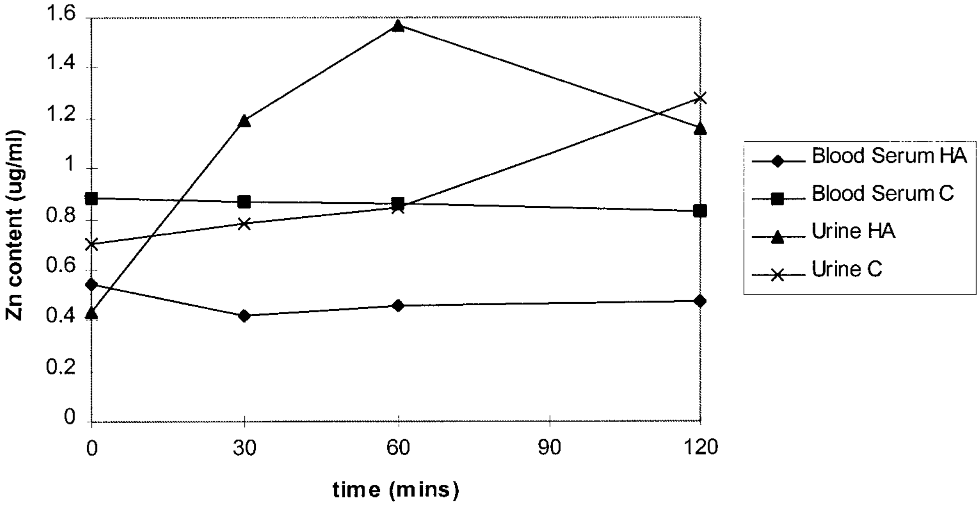

FIG. 2. Zinc content of hyperactive (HA) ($n = 23$) and control (C) ($n = 5$) children following a dose (50 mg) of tartrazine (E_{102}).

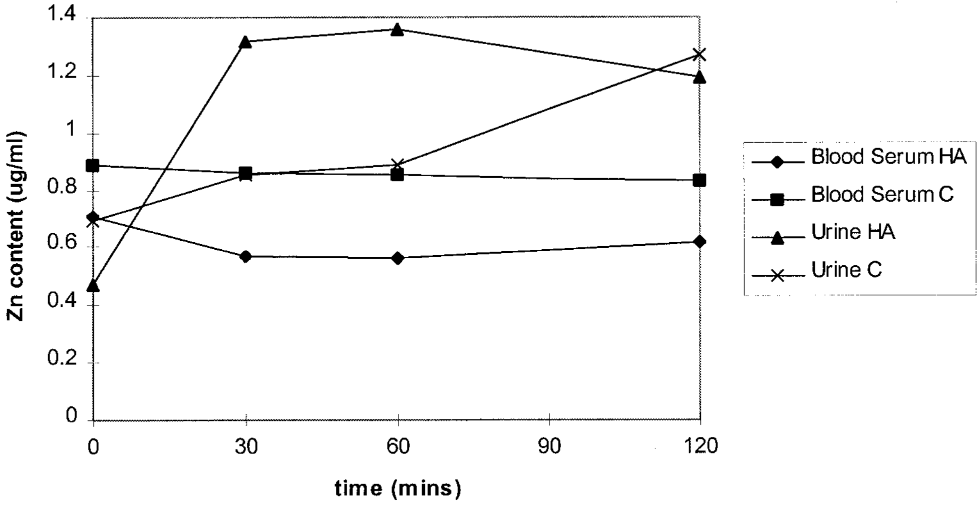

FIG. 3. Zinc content of hyperactive (HA) ($n = 72$) and control (C) ($n = 5$) children following a dose (50 mg) of sunset yellow (E_{110}).

cases seen by the HACSG have reported that oral zinc supplementation has a direct effect on reducing many of the health problems of hyperactive children [17]. Zinc deficiency has also been linked to gastrointestinal changes in the enterocytes and damage to the microvilli, which result in possible changes in gut permeability [18]. Several studies have shown that zinc-deficient animals are more prone to stress and are aggressive when compared with normal cases [18].

In a previous study it was suggested that the reduction in blood serum zinc levels and

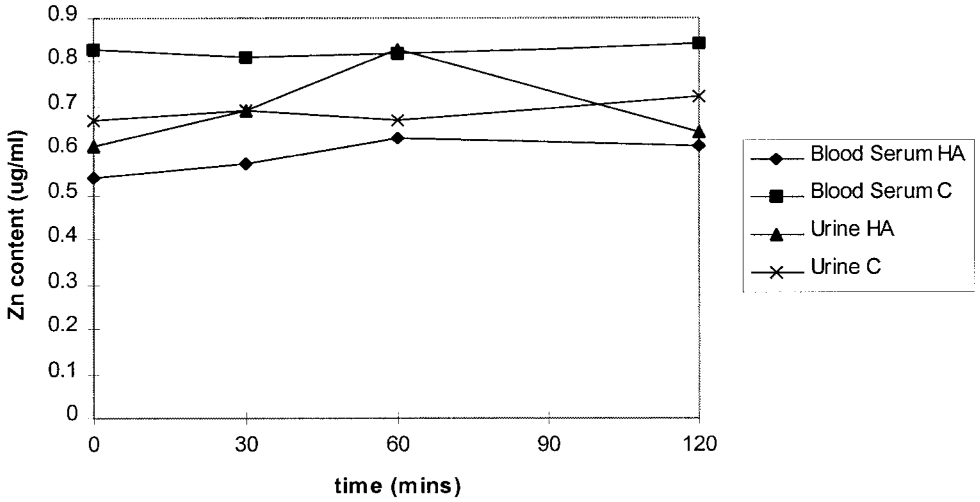

FIG. 4. Zinc content of hyperactive (HA) ($n = 72$) and control (C) ($n = 5$) children following a dose (50 mg) of amaranth (E_{123})

TABLE 4. Behavioural changes in hyperactive and control children following the consumption of a synthetic food colour beverage[a]

	Synthetic food colour					
	Tartrazine		Sunset yellow		Amaranth	
Behaviour	HA ($n = 23$)	C ($n = 5$)	HA ($n = 12$)	C ($n = 5$)	HA ($n = 12$)	C ($n = 5$)
Overactive	16 s, 2 mod	–	7 s, 2 mod	–	2 s, 3 mod, 1 m	–
Aggressive	14 s, 2 mod	1 m	2 s, 1 mod	–	2 s, 5 mod, 1 m	–
Violent	2 s, 2 mod	–	1 mod	–	–	–
Poor speech	1 mod, 1 m	–	1 mod	–	1–	–
Poor coordination	4 s, 6 mod, 2 m	1 m	1 mod	–	1 mod	–
Asthma/eczema	4 s, 3 mod, 1 m	1 m	3 s, 1 mod	–	1 mod	–

[a] Observed behaviour change after 30 min; s, severe; mod, moderate; m, mild.

associated increase in urinary zinc excretion in hyperactive children given a dose of tartrazine may be related to tartrazine acting as a chelating agent that binds any available blood zinc [15]. This should not be surprising in that various metal ions, such as chromium and cobalt, are used in the dye industry to coordinate to azo dyes and thereby improve washfastness and lightfastness properties. It is not clear whether ingested azo dyes are readily absorbed by the stomach or small intestine. In a review of this subject it was suggested that gastrointestinal absorption may be dependent on differences in gut flora [19]. It may be postulated that if hyperactive children have changes in gut permeability, especially a more leaky gut than normal children (M. P. Raymon, personal communication), free azo dye may be absorbed and thereby it could complex with zinc, with the subsequent excretion of a zinc–azo dye chelate in the urine. Spectrometric evidence has shown that zinc can complex with tartrazine and sunset yellow in ideal solvents, but this does not imply that the same complexes would be readily formed in biological fluids [20].

Many studies have investigated the relationship between the ingestion of synthetic food colourings and behavioural changes in hyperactive or hyperkinetic children. Any variation in the results can in part be explained by the use of different selection and diagnostic criteria for hyperactive and control children, differences in age and dietary status and the validity and reliability of behavioural measures. Many of these studies have used a wide range of differing synthetic chemical doses, ranging from 1.2 to 250 mg [5, 7–9, 14–15, 21, 22]. One study also reported a dose–response effect between tartrazine and hyperactive behaviour changes in some children [10]. Beyond a 10 mg dose of azo dye there was an increase in the duration of effect beyond 24 h.

Although tartrazine has been widely studied [10, 12–15] there are some cases involving other synthetic food colours. A double-blind, placebo-controlled study in which 50 mg doses of carmoisine were given to children with suspected reactors to food colourings showed two cases with a clear association between the ingestion of azo dye and behavioural symptoms of irritability, restlessness and sleep disturbance [9]. In the present study it is clearly shown that other azo dyes, such as sunset yellow, have similar effects on hyperactive children.

The fact that an ever-increasing number of studies have confirmed a link between the ingestion of synthetic food colours and hyperactivity raises the question about the possible mechanisms between azo dyes and biochemical systems. One study reported that azo dyes inhibit trypsin activity by 50% following the *in vitro* addition of tartrazine and sunset yellow [23]. Similarly, the enzyme activity of amylase was reduced by 66% with tartrazine, and 64% with erythrosine. If this effect could occur *in vivo*, low proteolytic enzyme activity would induce inadequate digestion. The direct consequences of this could be reduced nutrient availability, in particular of essential trace elements (zinc or iron) and essential fatty acids or increased antigenic loading of the gut. It has been hypothesized that hyperactive children may have a deficiency of essential fatty acids, either because they cannot metabolize linoleic acid normally or absorb essential fatty acids normally from the gut [24]. In addition, one of the early signs of essential fatty acid deficiency is abnormal thirst [25], a common health problem associated with hyperactive children.

Another important effect of azo dyes is their adverse effects on the integrity of the gastrointestinal tract. Tartrazine and sunset yellow have been linked to changes in the gut flora of rodents [19, 26], suggesting that if such effects can occur in humans, changes in the permeability of the gut may alter the absorption of both the azo dyes and metabolites.

Although at present the mode of action of these azo dyes is not proven, a diet free of synthetic food colourings has been shown to improve behaviour in hyperactive children [3].

ACKNOWLEDGEMENTS

The author would like to thank the Hyperactive Children's Support Group and the Allergy Support Group of Oxford for their support and encouragement of this work. Thanks are also due to the Allergy Research Foundation for providing a forum for this work to be presented.

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