Effect of Chlorinated Lipid and Protein Fractions of Cake Flour on Growth Rate and Organ Weight of Rats

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Chlorine gas is commonly used as a bleaching agent for cake flour but recent work has shown that chlorinated cake flour, flour lipids and wheat gluten have toxic effects when fed to rats over periods of 2 weeks (CUNNINGHAM et al. 1977a). Tracer studies showed that chlorinated cake flour lipids are absorbed and distributed throughout the body and remain in adipose tissue approximately the same length of time as normal lipids (CUNNINGHAM and LAWRENCE, 1976, 1977b, 1977c). Placental and mammary transfer of chlorinated lipids was also demonstrated (CUNNINGHAM and LAWRENCE, 1977d).

The present experiments were conducted to determine the effects of feeding chlorinated fractions of cake flour for longer periods of time and to determine if cake flour lipids were still toxic after baking.

MATERIALS AND METHODS

Male Wistar weanling rats in groups of 10 per treatment were used in all experiments. Except in Experiment 4 where the rats were pair-fed, all animals were housed 5 per cage and fed the experimental diets ad-libitum. Body weights were recorded weekly. At the completion of each experiment the rats were anaesthetized with ether, killed by exsanguination, and the heart, kidneys, liver and brain removed and weighed. Liver lipids were determined by Folch extraction (FOLCH et al. 1957).

Experiment 1 Cake flour containing 7.93% protein (N x 5.7) was chlorinated at 0.0%, 0.2% and 1.0% (w/w) using previously described techniques (CUNNINGHAM and LAWRENCE, 1976, 1977a). This was incorporated into a diet containing 87.4% flour, 4.0% corn oil, 5.0% soybean protein and 3.5% minerals and vitamins (CUNNINGHAM et al, 1977a). Each of the three diets was fed to 10 rats for 10 weeks.

Experiment 2 The lipids were extracted from cake flour by Soxhlet extraction with ether. The lipid and lipid-free residue fractions were each chlorinated with 2.0 g of chlorine per kg of original flour. The chlorinated

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fractions were incorporated into the same basal diet used in Experiment 1 with chlorinated lipids and unchlorinated residue replacing flour in one diet and chlorinated residue and unchlorinated lipids replacing flour in another diet. Each of these diets and a control diet containing unchlorinated flour lipids and flour residue were fed to rats for 2 weeks.

Experiment 3 Wheat gluten (Teklad Test Diets, 2826 Latham Drive, Madison, Wis.) was extracted with ether to remove traces of lipids and was chlorinated at 0, 2 and 5.0%. It was then mixed at a ratio of 1:9 (w/w) with ground rat chow to produce three diets which were fed to rats for 10 weeks.

Experiment 4 Two-kg batches of chlorinated (0.2%) and unchlorinated cake flour were each incorporated into 8 sponge cakes baked alternately using the following formulae; sugar, 250 g; extract of vanilla, 4.0 ml; flour, 250 g; baking powder, 15 g; NaCl, 2 g; and milk (2.0% fat), 212 ml. The ingredients were mixed with a small electric beater with each added in the order given above. The cakes were baked at 177°C for 40 min, cooled, crumbled, dried at room temperature and extracted for 8 hr with ether in a Soxhlet apparatus. The lipids (controls, 118.2 g; chlorinated, 121.5 g) were incorporated into ground rat chow replacing their own weight in 2 kg batches. Ten rats were individually fed *ad libitum* for 2 weeks the diet containing chlorinated cake flour lipids and control rats were pair-fed the same amount of the control diet.

RESULTS

Experiment 1 showed that flour chlorinated at 1.0% significantly ($P < 0.01$) reduced 10-wk body weight gain and increased the relative weight of the liver, kidney, heart and brain (Table 1). Flour chlorinated at 0.2% reduced weight gain ($P < 0.05$) and increased relative kidney weight ($P < 0.01$).

In Experiment 2, 0.2% dietary chlorine in the form of chlorinated flour lipids significantly ($P < 0.01$) reduced 2-week growth rate and increased the relative weight of the liver, kidney and heart (Table 2). When the same amount of chlorine was combined with the lipid-free flour residue the main effect was a significant increase ($P < 0.01$) in relative kidney weight.

Experiment 3 showed that 0.2% dietary chlorine in the form of chlorinated gluten significantly increased ($P < 0.01$) the relative weight of the kidney over a 10-week period (Table 3). The 0.5%

TABLE 1

Weight gain, relative organ weights and liver lipids of rats fed chlorinated flour for 10 weeks

	Controls	0.2 % Cl_2 flour	1.0 % Cl_2 flour
Number of rats	10	10	10
Initial weight, g	47.8 ±1.0	48.0 ±1.0	47.5 ±1.0
10-wk weight gain, g	254.9 ±6.7	224.7 ±9.7*	70.8 ±4.6**
Liver, % of body wt	3.38±0.04	3.20±0.11	4.72±0.14**
Liver lipids, %	3.92±0.11	3.89±0.19	3.94±0.16
Kidney, % of body wt	0.58±0.01	0.66±0.02**	1.05±0.03**
Heart, % of body wt	0.29±0.01	0.29±0.01	0.47±0.01**
Brain, % of body wt	0.62±0.15	0.69±0.03	1.39±0.05**

*P < 0.05 for SEM, **P < 0.01 for SEM

TABLE 2

Weight gain, relative organ weights and liver lipids of rats fed chlorinated flour lipids and chlorinated fat-free residue of flour for 2 weeks

	Controls	0.2 % Cl_2 flour lipids	0.2 % Cl_2 flour residue
Number of rats	10	10	10
Initial weight, g	46.5 ±1.0	46.1 ±1.2	46.4 ±0.9
2-wk weight gain, g	43.2 ±1.5	36.1 ±1.6**	39.3 ±1.5
Liver, % of body wt	4.11±0.19	5.83±0.22**	4.18±0.26
Liver lipids, %	3.33±0.10	3.53±0.12	3.65±0.08*
Kidney, % of body wt	1.11±0.02	1.38±0.04**	1.26±0.02**
Heart, % of body wt	0.51±0.01	0.55±0.01**	0.53±0.01
Brain, % of body wt	1.70±0.04	1.80±0.06	1.81±0.02*

*P < 0.05 for SEM, **P < 0.01 for SEM

TABLE 3

Weight gain, relative organ weights and liver lipids of rats fed chlorinated wheat gluten for 10 weeks

	Controls	0.2 % Cl_2 in Gluten[1]	0.5 % Cl_2 in Gluten[1]
Number of rats	10	10	10
Initial weight, g	50.8± 0.8	50.7 ±0.7	50.1 ± 0.6
10-wk weight gain, g	358.5±12.4	329.4 ±8.4	293.8 ±11.1**
Liver, % of body wt	4.31±0.09	4.21±0.05	4.08± 0.07
Liver lipids, %	3.50±0.10	3.77±0.08	3.61± 0.11
Kidney, % of body wt	0.70±0.02	0.80±0.02**	0.89± 0.02**
Heart, % of body wt	0.27±0.01	0.27±0.01	0.31± 0.03
Brain, % of body wt	0.48±0.01	0.51±0.01	0.54± 0.01**

*$P < 0.05$ for SEM, **$P < 0.01$ for SEM

[1]Gluten containing 2% and 5% Cl_2 incorporated at 10% of rat chow diet.

level decreased ($P < 0.01$) growth rate and increased ($P < 0.01$) the relative weight of the kidney. There was also a significant increase in the relative brain weight but this is normally expected when the growth of an animal is unduly restricted (WEIL, 1970).

Experiment 4 showed that lipids extracted from cakes baked from chlorinated flour reduced growth rate ($P < 0.05$) and increased actual liver weight ($P < 0.01$) of rats compared to pair-fed controls (Table 4). The brain weights, unadjusted for body weight were also significantly lower in the treated group and provided additional evidence for a significant reduction in growth. Liver lipids were not affected by the chlorinated compounds in any of the experiments.

TABLE 4

Weight gain, organ weights and liver lipids of rats fed lipids from cake baked with chlorinated and unchlorinated flour for 2 weeks

	Controls	Treated[1]	SEM
Number of rats	10	10	
Initial wt	45.1	45.2	0.2
2-wk weight gain, g	84.7	80.4	1.7*
Liver weight, g	6.54	7.34	0.22**
Liver lipids, %	3.34	2.93	.21
Kidney weight, g	1.40	1.38	0.04
Heart weight, g	0.49	0.50	0.01
Brain weight, g	1.57	1.50	0.01**

[1]Each kg of diet contained lipids extracted from cake that had been baked from 1 kg of flour which had been chlorinated with 2 g of Cl_2.

*$P < 0.05$ for SEM, **$P < 0.01$ for SEM

DISCUSSION

The reduction in growth rate in the present study resulting from feeding flour chlorinated at 0.2% and 1.0% for 10 weeks was quite similar to that found when the same levels of chlorinated flour were fed for 2 weeks in an earlier study (CUNNINGHAM and LAWRENCE, 1977a). However, an increase in relative liver weight was observed at both levels of chlorine in the earlier work but only at the 1.0% level in the present study. It is possible that over the longer period of time the liver was able to adapt to the lower level and not continue to show an enlargement.

Changes in organ weights must generally be viewed with caution since pregnancy, organ overload, severe feed restriction and many other conditions may affect organ:body weight ratios. However, statistical analysis of 45 experiments by WEIL (1970) indicates that in growing rats fed <u>ad libitumm</u> a decrease in body weight and an increase in relative organ weight must be considered as potential deleterious effects of the chemical on these organs.

Chlorine would appear to be more harmful to rats in the form of chlorinated flour lipids than as chlorinated lipid-free flour for a comparison of the same amount of chlorine combined in each fraction in Experiment 2 indicated greater weight loss and change in relative organ weights with the chlorinated flour lipids. Larger differences in organ weights have been obtained over a 2-week period by incorporating the same level of chlorine (0.2%) in flour lipids in a commercial rat chow diet and using pair-feeding to compare it with an unchlorinated control diet (CUNNINGHAM and LAWRENCE, 1977a). The chlorine was reported to increase the actual weights of the liver by 40%, kidney by 20% and heart by 10%. The increase in liver size in the present study would appear to be mostly in the form of proteinaceous tissue since the percent lipids in the liver were not affected by any of the chlorinated products tested.

When flour is chlorinated at the 0.2% level only about 45% of the chlorine is found in the lipid fraction (CUNNINGHAM and LAWRENCE, 1977a). Therefore, when the 0.2% chlorinated flour used in Experiment 1 was baked for Experiment 4 and the lipids incorporated into an equivalent weight of diet, 55% of the chlorine remained in the non-lipid fraction of the cakes and so any additive effect they may have had on the rats was not obtained. Similarly, the cake flour lipids contained only 45% as much chlorine as those which were extracted from flour in Experiment 2 and chlorinated with 2 g Cl_2 per kg of flour. It was therefore not surprising to find greater treatment effects from the chlorinated fractions tested in Experiments 1 and 2 than in Experiment 4. Never-the-less, even with pair-feeding which normally tends to reduce differences in weight gains, the baked chlorinated flour lipids caused a significant ($P < 0.01$) decrease in body weight gain and increase in liver weight. The experiment did not show if baking had any effect on the toxicity of flour lipids but it was apparent that the effects were not eliminated by baking.

The effects of chlorinated wheat gluten diet (0.2% Cl_2) in Experiment 3 were similar to those in Experiment 2 with a diet containing lipid-free flour residue chlorinated at the same level. Chlorine reacts with both the gluten and prime starch fractions of flour and although improvement in baking quality occurs in both fractions in about equal proportions it is difficult to determine the proportion of chlorine that reacts with either fraction (SOLLARS, 1958). No deleterious effects were observed in the rats given chlorinated lipid-free flour in Experiment 2 that were not observed in rats given chlorinated gluten in Experiment 4 and so the data cannot show if the products of chlorinated starch are also harmful to rats.

It may be concluded that both the lipid and non-lipid fractions of flour when chlorinated have deleterious effects when fed to rats. Lipid fractions have a greater effect that non-lipid fractions containing the same amount of chlorine and tend to increase the size of the liver. Chlorinated flour lipids are still harmful to rats after baking in cakes.

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