for muscle contraction. The intermittent nature of the paralysis and the variability in glucose tolerance demonstrated in our cases, in particular the decreased tolerance after paralysis, is not well explained. However, the latter observation is likely to be due either to exhaustion of a carrier mechanism in the cell membrane or to saturation of intracellular storage of glycogen.

The well-recognized association of periodic paralysis with thyrotoxicosis might be related to the ability of the thyroid hormone to uncouple oxidative phosphorylation.

Since Conn *et al.* (1956) demonstrated that sodium retention was important in the precipitation of attacks of periodic paralysis and that treatment with a low-salt diet after preliminary desalting was effective in preventing attacks, a number of balance studies in patients with periodic paralysis have been reported. Although all studies have confirmed sodium retention in relation to some attacks, sodium retention does not appear to be an essential accompaniment to paralysis (Jones *et al.*, 1959). The finding of only minimal sodium retention, during sodium restriction, in our patient in relation to a severe attack of paralysis supports this. The observation, together with the finding of marked sodium retention without the production of paralysis, suggests that sodium retention is not the primary defect in this condition. The discovery of sodium retention after glucose and insulin administration (Conn *et al.*, 1957) suggests a possible relationship between the abnormalities in sodium and carbohydrate metabolism. The mechanism of this association and the initial trigger to the chain of events leading to paralysis have yet to be determined. However, since the extrusion of sodium from the cell is an active process requiring energy (Hodgkin, 1958), it is possible that a transient reduction in the available high-energy bonds would lead to sodium retention by the cell. This would provide a common cause for sodium retention and the rapid peripheral uptake of glucose.

The failure, in our case, of a daily sodium intake of 22 mEq and diuretic agents to prevent attacks is similar to that described by Eales *et al.* (1958), although in their case such treatment initially appeared effective. More rigid sodium restriction to 8 mEq daily as used by Conn might be more effective, but from the practical therapeutic aspect such treatment seems to have little to offer.

Summary

Glucose and insulin tolerance were studied in four members of a family, including non-identical twins, affected with periodic paralysis. Evidence was obtained indicating rapid peripheral uptake of glucose as a regular finding in these patients. With the use of the intravenous glucose-tolerance test, the glucose disappearance rate was variable, being rapid before paralysis and reduced after.

In the one case specifically studied sodium retention and paralysis at times occurred independently of each other.

It is suggested that there is a lack of high-energy phosphate in the muscle cells, related to an inability to store creatine, leading to increased glucose uptake. A similar mechanism may be responsible for intracellular sodium retention.

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NUTRITIVE VALUE OF BREAD MADE FROM FLOUR TREATED WITH CHLORINE DIOXIDE

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The rejection of agene, NCl_3, as a flour improver, which followed the finding that flour so treated causes hysteria in dogs (Mellanby, 1946), raised in the milling and baking industries the problem of finding a suitable substitute. In the U.S.A. and Canada chlorine dioxide was used to replace agene from 1950 onwards (Horder, Dodds, and Moran, 1954), and later this substance came into general use in this country. For the formation of methionine sulphoximine, the poison responsible for canine hysteria, the nitrogen atom of agene must combine with methionine present in flour (Bentley, McDermott, Moran, Pace, and Whitehead, 1950). On theoretical grounds, therefore, it is clear that chlorine dioxide could not produce this particular poison. In agreement with this conclusion, experiments in the U.S.A. by Newell, Gershoff, Suckle, Gilson, Erickson, and Elvehjem (1949) demonstrated that dogs remain free from hysteria when given flour heavily treated with chlorine dioxide for 13 weeks. Rats, rabbits, and monkeys also remained well when given the treated flour for periods of 5, 6, and 22 weeks respectively. In human subjects no evidence of poisoning could be found after they had received diets containing flour treated with chlorine dioxide at 80 p.p.m., and wheat gluten treated with 400 p.p.m., for six weeks. For the commercial treatment of white flour in Britain the level of addition of chlorine dioxide is at present only 15–20 p.p.m.

Although chlorine dioxide does not cause the formation of methionine sulphoximine in flour, and although there is no evidence of the formation of any other highly toxic substance, the flour does not escape injury. Thus it has been clearly proved, by both chemical and biological methods, that even treatment with chlorine dioxide at about the ordinary commercial level causes almost complete destruction of the vitamin E which is naturally present in the flour (Moran, Pace, and McDermott, 1953, 1954; Frazer, Hickman, Sammons, and Sharratt, 1956a; Moore, Sharman, and Ward, 1957, 1958). Another indication of injury to the flour, apparently distinct from the destruction of vitamin E, may perhaps be seen in the results of feeding-trials with successive generations of rats by Frazer, Hickman, Sammons, and Sharratt (1956b). These workers observed that mean growth rates were always slower when the diet contained flour heavily treated with chlorine dioxide than when the flour was lightly treated. Between some groups, however, the differences were not statistically significant, and the authors' own conclusion was that even the heavily treated flour had no deleterious effect on rats.

The purpose of this paper is to report further evidence that the treatment of flour with chlorine dioxide causes reduced growth rates in multi-generation experiments with rats, even when doses of vitamin E are given to compensate for its destruction by the improver.

Diet

The experiments to be described were carried out during 1952–4, and the flour used was of the 80% extraction rate then current. Repeated batches of flour were necessary, but the quality was kept as uniform as possible. Some of the flour was left untreated, and was used for baking 2–10 weeks after milling. Treatment of the flour with chlorine dioxide was carried out weekly at the Cereals Research Station, St. Albans, and the flour was then sent, together with untreated flour, to the Baking Industries Research Station, Chorley Wood, for baking. Two levels of treatment with chlorine dioxide were chosen—30 p.p.m. (normal) and 300 p.p.m. (normal × 10). After baking, the bread was sliced, dried under gentle heat in a current of air, and ground to fine crumbs. The reason for this otherwise undesirable treatment was to allow for the mixture of the bread with other dietary components. The diet was composed as follows:

	Parts by weight
Dried breadcrumbs	80
Casein	8
Arachis oil	12
Salt mixture	5
Dried yeast	7

Each rat was dosed weekly with about 1,000 I.U. of vitamin A and 40 I.U. of vitamin D, supplied as one drop of halibut-liver oil. Vitamin E was supplied as 2 mg. of DL-α-tocopheryl acetate weekly, dissolved in two drops of arachis oil. Vitamin K was supplied as 0.05 mg. of 2-methyl-1:4-naphthoquinone weekly, dissolved in one drop of arachis oil.

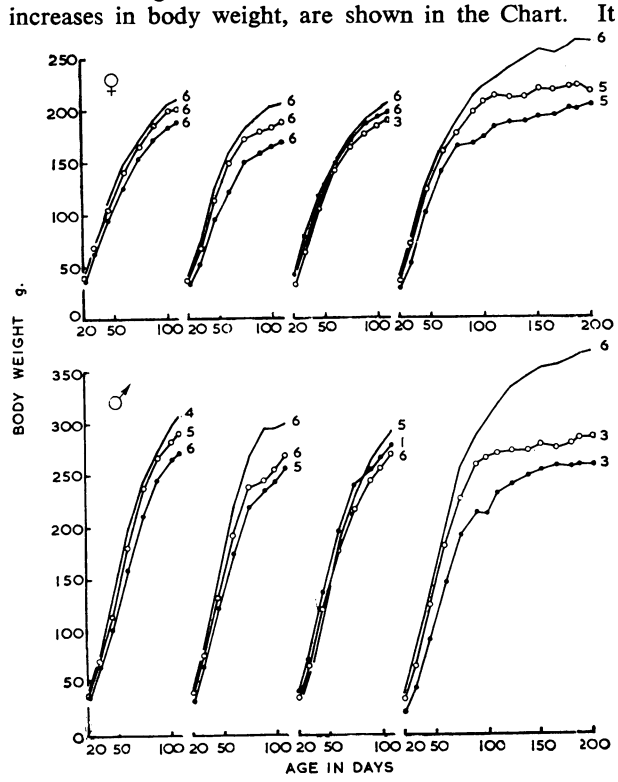
Preliminary Experiments with Weanling Rats

When groups of weanling rats, whose mothers had received a good mixed diet, were fed upon the diet containing breadcrumbs their mean rates of growth, over a period of 200 days, were virtually identical irrespective of whether the flour had been untreated or treated with chlorine dioxide at the N or N × 10 levels. In all the groups given diets containing breadcrumbs, however, growth was much slower than in rats given a good mixed diet. Hutchinson, Moran, and Pace (1956) found that supplements of lysine increase the growth rate in young rats fed upon bread. The inclusion in our diet of 8% of casein, however, makes it doubtful whether lysine was the limiting factor in our experiments.

Multi-generation Experiments

These trials were started by mating adult male and female rats which had previously been used successfully for routine breeding purposes. At mating, the experimental diets, containing breadcrumbs, were substituted for the previous mixed diets. For each experimental diet four pairs of rats were used. From their young six males and six females were taken at weaning, being selected to include the best available, subject to representatives being taken from each litter. The selected groups of males and females were kept on the same experimental diets for 110 days. Four males and four females were then taken from each group and mated. The procedure of taking six males and six female rats at weaning for growth trials, and later restricting them to four pairs for breeding another generation, was then repeated. In this way four generations of offspring were bred. In the final generation the growth trials were extended beyond the usual period, which was possible because no rats had to be taken for mating.

The mean growth curves of the rats, as indicated by increases in body weight, are shown in the Chart. It



Mean growth rates of young rats, from the age of 21 days onwards, in four generations reared upon diets containing breadcrumbs made from (1) untreated flour (plain lines), (2) chlorine dioxide at 30 p.p.m. (white circles), or (3) chlorine dioxide at 300 p.p.m. (black circles). The number of rats in each group is shown at the end of each curve.

will be seen that in both males and females growth was always somewhat more rapid when the breadcrumbs had been made from untreated flour than when the flour had been treated with chlorine dioxide. Comparisons between the groups given crumbs from N or N × 10 treated flours usually indicated more rapid growth with the lighter treatment. In generation 3, however, growth was more rapid with the N × 10 treatment than with N treatment for both males and females. This unexpected result coincided with failures in reproduction or lactation in generation 2, which prevented the full groups of six rats each being set up in generation 3. Thus only one male was available for the ClO_2N × 10 group, and only three females for the ClO_2 N group. No significance can therefore be attached to this change from the usual relationship.

In addition to the gaps in groups caused by failures in breeding, two rats died during the period of their growth trials. These were a male in the 2nd generation in the ClO_2 N × 10 group, and another male in the 3rd generation in the "untreated" group. It seems unlikely that these deaths were related to the nature of the diet. In plotting growth curves, and in statistical calculations, observations on both these rats have been excluded.

For statistical treatment the weight increases observed between the ages of 21 and 110 days for all four generations were combined according to sex and the treatment of the flour. From Table I it will be seen that in both sexes growth was most rapid in those rats which received breadcrumbs made from untreated flour. Growth was slower in the rats given crumbs which had received the N level of treatment with chlorine dioxide, and slower still with the N × 10 treatment. In all instances the differences between the untreated groups and the chlorine dioxide N or N × 10 groups were statistically significant.

In the experiments of Frazer *et al.* (1956a, 1956b) the young of rats from mothers fed upon flour treated with chlorine dioxide at the N × 10 level tended to be lighter, at an age of 14 days, than the young of mothers given flour treated at the N level. We have therefore compared the weights of our young rats at the age of 21 days, when they were weighed for the first time. From Table II it will be seen that our results were in agreement with those of Frazer *et al.* regarding the relative weights of the young in the chlorine dioxide N and N × 10 groups. For statistical purposes, however, it seemed more informative to make comparisons with the "untreated" group rather than between the two chlorine dioxide groups. Presumably this was not done in the investigation of Frazer *et al.* because the trials with untreated flour were discontinued after only two generations. It will be seen that in our experiments the differences between the weights in the young in the chlorine dioxide groups, with one exception, were significantly different from those in the untreated groups. The exception was for females in the chlorine dioxide N group.

TABLE I.—*Growth, as Indicated by Weight Increases, in Rats Fed Upon Diets Containing Breadcrumbs Made From Untreated Flour, or from Flour Treated with Chlorine Dioxide at a Normal* (30 *p.p.m.*), *or at* 10 *Times the Normal, Level* (300 *p.p.m.*). *Combined Results for Four Generations*

	Treatment of Flour	No. of Rats	Mean Weight Increase (g.) between Ages 21 and 110 Days	P ClO_2/Untreated
Males	Untreated	21	264	
	ClO_2 N	21	239	<0·001
	ClO_2 N × 10 ..	14	226	<0·001
Females	Untreated	24	174	
	ClO_2 N	23	161	0·05–0·01
	ClO_2 N × 10 ..	20	147	<0·001

TABLE II.—*Body Weights of Weanling Rats, Aged* 21 *Days, Reared by Mothers Fed Upon Diets Containing Breadcrumbs Made From Untreated Flour, or From Flour Treated with Chlorine Dioxide at N or N* × 10 *Levels. Combined Results for Four Generations*

	Treatment of Flour	No. of Rats	Mean Weight (g.) at Age of 21 Days	P ClO_2/Untreated
Males	Untreated ..	21	39·4	
	ClO_2 N ..	21	35·7	0·001<P<0·01
	ClO_2 N × 10 ..	14	32·1	<0·001
Females	Untreated ..	24	37·4	
	ClO_2 N ..	23	35·5	0·1<P<0·2
	ClO_2 N × 10 ..	20	32·8	0·001<P<0·01

Palatability

In experiments in which rats have been allowed a choice between various diets Moran, Pace, and Hutchinson (1955) have shown that diets containing fat extracted from untreated flour are preferred to diets containing fat from flour which has been heavily treated with agene or chlorine dioxide. These workers, and also Frazer *et al.* (1956b), have suggested that reduced palatability, leading to a decrease in food intake, may be an important factor in the interpretation of data on growth rates.

Undue emphasis of this factor seems hazardous, in the first place, because Moran *et al.* found that their rats usually had good appetites for a diet which was their second choice if their first choice was not available. Secondly, lowered food intakes may well be expected in rats which are growing slowly as the result of impairment in the nutritive value of their food. If we ascribe reduced growth rates to decreased palatability, without proof that this is the true and only explanation, we may defeat the purpose for which our feeding trials have been carried out.

In the present investigation the slower growth rates in the rats given breadcrumbs made from flour treated with chlorine dioxide, at least at the N × 10 level, was not due to reduced palatability. In experiments extending over eight weeks six young male rats were offered a choice between the chlorine dioxide N × 10 diet and the untreated diet. Food intakes of each diet were recorded daily. Table III shows the mean daily intakes of food for each rat in successive weeks. It will be seen that the diet treated with chlorine dioxide was consistently preferred to the untreated diet. Thus five of the six rats ate much more of the treated diet than of the untreated, and the sixth rat slightly more. An interesting point was the decided preference of all the rats for the treated diet in the first two weeks of the experiments. This suggests that they found the treated diet more "palatable." In later weeks larger amounts of the untreated diet were eaten, and in a few instances the mean weekly consumptions of untreated diet temporarily exceeded those of treated diet. This tendency seems in agreement with the findings of Harris, Clay, Hargreaves, and Ward (1933) that in some circumstances rats may learn by experience to discriminate between nutritionally adequate and inadequate diets.

Discussion

If we first restrict our attention to the difference between growth rates in successive generations of rats

TABLE III.—*Average Daily Food Intakes of Rats Given a Choice Between a Diet Containing Breadcrumbs Made from Untreated Flour or from Flour Treated with Chlorine Dioxide* (N×10)

Days on Experiment	Mean Daily Intakes (g.) of Each Diet													
	Rat 1		Rat 2		Rat 3		Rat 4		Rat 5		Rat 6		Means	
	Untr.	ClO_2	Untr.	ClO_2	Untr.	ClO_2	Untr.	ClO_2	Untr.	ClO_2	Untr.	ClO_2	Untr.	ClO_2
0–7	1·8	7·6	1·7	9·0	0	7·0	1·3	7·3	0·3	8·8	2·6	6·8	1·3	7·8
8–14	2·3	10·3	3·6	9·0	1·6	8·4	2·8	7·4	1·7	9·8	4·5	6·3	2·8	8·5
15–21	4·2	9·7	7·0	7·7	7·2	4·9	4·7	6·8	5·2	7·9	6·4	6·2	5·8	7·2
22–28	4·0	10·3	8·0	7·8	9·1	5·5	5·1	8·3	6·1	8·9	8·2	6·1	6·8	7·8
29–35	3·5	12·6	7·6	8·7	6·5	9·6	6·1	8·6	7·6	7·4	9·0	6·0	6·7	8·8
36–42	3·0	14·0	6·8	10·5	7·7	8·5	5·9	8·9	5·8	9·4	7·0	7·7	6·0	9·8
43–49	3·6	14·1	5·9	11·6	5·1	12·7	7·1	8·3	9·6	6·5	6·4	8·7	6·3	10·3
50–56	4·2	12·5	6·4	11·0	5·1	11·4	6·0	10·0	8·9	7·6	7·2	7·5	6·3	10·0
Means	3·3	11·1	5·9	9·4	5·3	8·6	4·9	8·2	5·7	8·3	6·4	6·9	5·3	8·8

Statistical comparison of the set of six means for the intakes of diet containing breadcrumbs made from untreated flour with the corresponding means for treated flour gave $P < 0{\cdot}001$—that is, a highly significant difference

given crumbs from flours treated with chlorine dioxide at the N and N×10 levels we find that our results are in good agreement with those of Frazer *et al.* (1956b). Thus growth was slower with the heavy than with the light treatment. Our results also agreed with the finding of Frazer *et al.* that suckling or weanling rats were smaller with the light treatment of the flour than with heavy treatment. Interest in these points of agreement may be increased by the different diets which were used by Frazer *et al.* and by ourselves. Thus in place of dried crumbs Frazer *et al.* included uncooked flour in their diets. Whereas our rats were specially dosed with vitamin E those of Frazer apparently received this vitamin in the dried milk and fish meal which were included in their diet. The retarding effect of heavy treatment with chlorine dioxide on growth, therefore, is not confined to generations of rats receiving one particular type of diet.

Our experiments differed from those of Frazer in the continuation of the breeding of control rats, given breadcrumbs made from untreated flour, throughout the whole course of the investigation. As we have seen, both the growth rates, averaged to include all generations, and the weights at weaning, for our three breeding groups, were in the order (1) untreated, (2) chlorine dioxide N, and (3) chlorine dioxide N×10. Similar results were obtained for both sexes.

In view of the known destruction of vitamin E in flour by chlorine dioxide, and of the possibility that the diets containing treated flour adversely affected reproduction or lactation, suspicion may be raised that our weekly dose of 2 mg. of DL-α-tocopheryl acetate was inadequate fully to supply the requirements of the rats. Against this possibility we may state that (1) the dosage of α-tocopherol was about five times greater than the minimum which we have found necessary to prevent all signs of vitamin E deficiency in the rat; (2) the weekly dose of α-tocopherol greatly exceeded in vitamin E potency the amounts of tocopherols which could have been derived from untreated flour in the same period; and (3) no signs of vitamin-E deficiency were ever observed in those of our rats which were eventually examined at necropsy, or in many rats similarly dosed and used for other experiments on flour improvers. It remains possible, however, that small amounts of vitamin E evenly distributed throughout the diet, as opposed to being given as a single massive dose, may have some nutritional virtue which is not yet appreciated. The destruction of some factor other than vitamin E, or the formation in the diet of a substance which adversely affects the nutrition of the rat, seems a more probable explanation.

Summary

Successive generations of rats were reared upon diets which consisted mainly of dried breadcrumbs. The bread was made from untreated flour, or from flour which had been treated lightly (30 p.p.m.) or heavily (300 p.p.m.) with chlorine dioxide.

Differences in the mean growth rates of the young rats were observed according to the treatment of the flour. For both sexes the growth was most rapid when the flour was untreated, somewhat slower when the flour had been lightly treated with chlorine dioxide, and slower still when the flour had been heavily treated. The weights of the young rats at weaning were in the same order in relation to the treatment of the flour.

In these experiments all the rats received adequate doses of DL-α-tocopheryl acetate. The slower growth rates when the flour had been treated with chlorine dioxide cannot therefore be explained by the known destruction of vitamin E by chlorine dioxide.

Experiments in which rats were allowed to choose between diets containing breadcrumbs made from untreated flour or from flour which had been heavily treated with chlorine dioxide indicated that the improver increased, rather than decreased, the "palatability" of the breadcrumbs to the rat.

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