OBSERVATIONS ON THE PROLONGED FEEDING TO RATS OF THE FLOUR MATURING AGENT, AMMONIUM PERSULFATE

AARON ARNOLD AND FRANS C. GOBLE
Sterling-Winthrop Research Institute, Rensselaer, N. Y.

TWO FIGURES

(Received for publication February 24, 1950)

Ammonium persulfate has been widely used abroad for treating freshly milled flour. The amounts used are small, being of the order of one part for 10,000 parts of flour, and it is known that the persulfate ion decomposes rapidly to innocuous sulfate in the dough stage of bread making (Kent-Jones and Amos, '47; Auerbach et al., '49). In view of the unforeseen and untoward effects observed in dogs fed flour matured with nitrogen trichloride (Mellanby, '46), there is great interest in the effects of all agents proposed for this purpose. As was brought out in a recent review (Arnold, '49a), many laboratories, including this one, have examined various agents as to their suitability for producing in flour the desired maturing without giving rise to toxic effects of any sort.

The purpose of the present communication is to report the results of feeding trials with rats fed diets which contained ammonium persulfate in amounts considerably above those which would be encountered in the feeding of flour containing the usual commercial levels of this maturing agent. Included also are feeding trials with diets which contained high levels of bread baked from flour treated with ammonium persulfate.

EXPERIMENTAL

Diets

The diets fed the rats had the following percentage composition: flour or dried bread, 78; lactalbumin, 10; hydrogenated vegetable oil, 8; U.S.P. salt mixture no. 2, 3.5; and liver concentrate powder (1:20), 0.5. The following water-soluble vitamins were added to the diets (given in milligrams per 100 gm of diet): thiamine hydrochloride 1, riboflavin 2, niacinamide 2, pyridoxine hydrochloride 1, calcium pantothenate 4, folic acid 0.1, inositol 10, and choline chloride 100. In addition, each rat received weekly 0.1 ml of oil which supplied approximately the following: vitamin A 2,000 U.S.P. units, vitamin D 400 U.S.P. units, α-tocopherol 10 mg, and menadione 0.1 mg. Food and water were before the animals at all times.

Flour samples

Untreated and unenriched standard patent grade flour was used in the formulation of the diets.

Bread samples

The bread was baked according to the following commercial formula (given in lb.): flour 100, water 64, corn sugar 4, milk solids 2, shortening 2, yeast 2, yeast food 6 ounces, and calcium propionate 1 ounce.

It may be instructive to indicate briefly the effect on the finished loaf of ammonium persulfate additions to the flour before baking. While a 0.02% addition of persulfate to flour was more than the usual commercial level, the corresponding bread was satisfactory from the standpoint of loaf volume and cell texture. The bread made from flour to which 0.1% ammonium persulfate had been added was slightly smaller in loaf volume than the bread made from flour which contained the 0.02% persulfate. This indicated that the flour had been

*Primex.
overtreated and that the gas-retaining power of the dough had been lowered. However, the product baked from flour which contained 1.0% persulfate resembled bread only distantly. The dough had obviously not expanded during fermentation and baking, so that the resultant baked product closely resembled in size the unbaked dough.

To preserve and to prepare them for use in the diets, all the breads were dried overnight at approximately 60°C. (140°F.) under vacuum in a steam-heated oven. This yielded a dry product showing no evidence of toasting or charring. The dried bread was ground in a feed mill to a powder or small granules before being mixed with the rest of the dietary ingredients.

Ammonium persulfate

Pure crystalline ammonium persulfate was milled with 10% tricalcium phosphate. The resultant powder (200 mesh) could be readily dispersed throughout the flour-containing diets. It was added to the flour at the time the diets were mixed, in the following amounts: none, 0.02%, 0.1%, and 1.0%. Similar amounts of ammonium persulfate were added to the flour from which the bread was baked. For this reason, ammonium persulfate was not added to the bread-containing diets, in contrast to the flour-containing diets described above.

Rats

The studies were begun with groups of 10 female and 4 male rats. Each group was fed one of the 8 diets described above. After three weeks of feeding in the case of the flour-containing diets and one week in the case of the bread-containing diets, the rats were mated, 5 females and two males to a cage. The females were with the males until they became pregnant. The females were not bred a second time after having cast a litter.

The aforementioned first generation rats were permitted to raise to weaning size 6 of each litter. At weaning, the
litters were reduced to three rats, one male and two female young, wherever possible.

The second generation litters were each kept separately and were allowed to breed so as to produce third generation young. The resultant litters were similarly reduced to 6 and kept until weaning, at which time they were weighed, observed for unusual symptoms, if any, and discarded.

The second generation female rats were bred a second time to test further the effect of the diets on the reproductive capacity of the rats.

Hematological examinations

The blood elements of the first generation rats were examined routinely, since they were exposed to ammonium persulfate for the longest period of time. In addition, the second generation rats were examined at autopsy. The following determinations were made: hemoglobin, calculated from oxyhemoglobin readings on the photoelectric colorimeter according to the procedure recommended for the Klett-Summerson instrument, using 4% ammonium hydroxide as the diluent; total red blood cell and total white blood cell counts, using special pipettes which allow for dilutions of 1:500 and 1:20 respectively; differential white cell count, following the procedure of Coffin ('45), the values being reported in absolute numbers rather than percentages.

Post mortem examinations

About half (54 in all, 6 to 7 rats for each of the 8 groups) of the first generation rats were sacrificed after 6 months of test. The remainder (47 first generation and 74 second generation rats relatively evenly divided among the groups) were sacrificed after one year of test, the conditions of the test being considered sufficiently rigorous for valid conclusions after that period of time. The following tissues and organs were examined: heart, lungs, liver, spleen, kidneys, adrenals, pancreas, and small intestine.
FLOUR MATURING AGENTS 463

RESULTS

Weight records of first generation rats

The average weight curves, together with the ranges within the groups of the first generation rats fed the flour- or bread-containing diets, are given graphically in figure 1. Comparison of the control group with the test groups fed

![Graph showing weight curves for first generation rats fed various diets.](https://example.com/graph.png)

Fig. 1 Average weight curves of first generation rats fed diets containing flour, or bread baked from flour, treated with various levels of ammonium persulfate. Each group consisted of 4 males and 10 females. The ranges of the individual weights within groups are indicated by the vertical bars.

the flour-containing diets indicates that the group fed the highest level of dietary persulfate gained less weight over the experimental period of 52 weeks than did the groups fed the intermediate levels of dietary persulfate. Outwardly, the flour diet 3 animals appeared normal, however. Alternatively, comparison of the control group with the test groups fed the bread-containing diets indicated no differences in weight gains among the groups.
Weight records of second generation rats

The average weight curves, together with an indication of the ranges within the groups of the second generation rats fed the flour- and bread-containing diets, are shown in figure 2. As in the case of the first generation animals, the group fed the flour-containing diet with the highest level of dietary persulfate gained appreciably less than the control group and the groups fed the diets with the intermediate persulfate levels. The poor condition of these animals was further evidenced by their unkempt appearance and loss of hair in irregular patches. On the contrary, the 4 groups fed the bread-containing diets were outwardly normal in appearance. The weight curves of the latter groups were quite similar.
and no evidence of marked differences was apparent whether they were compared among themselves or with the weight curves of the first generation animals.

Reproduction and rearing records of the first and second generation rats

The reproduction and rearing performances of the first and second generation rats are summarized in table 1. The

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Summary of reproduction and rearing records of first and second generation rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FIRST GENERATION RATS</td>
</tr>
<tr>
<td></td>
<td>No. of litters per no. of females bred</td>
</tr>
<tr>
<td>DIOETARY PERSULFATE LEVEL</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Flour diets</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>9/10</td>
</tr>
<tr>
<td>0.02</td>
<td>8/10</td>
</tr>
<tr>
<td>0.10</td>
<td>10/10</td>
</tr>
<tr>
<td>1.0</td>
<td>7/10</td>
</tr>
<tr>
<td>Bread diets</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10/10</td>
</tr>
<tr>
<td>0.02</td>
<td>9/10</td>
</tr>
<tr>
<td>0.10</td>
<td>10/10</td>
</tr>
<tr>
<td>1.0</td>
<td>10/10</td>
</tr>
</tbody>
</table>

1 Each group consisted of 10 females and 4 males.
2 At weaning the litters of second generation rats were reduced, where possible, to two females and one male. The litters cast by the second generation rats were kept until weaning and were then discarded.

results obtained from the breeding of the first generation rats are consistent with the growth records given in figure 1. Seven of the 8 groups on test had excellent reproduction and rearing records. The group fed the flour-containing diet with the highest level of dietary persulfate experienced difficulty in raising the young cast and only 4 poor litters of the 7 cast were weaned.
The second generation rats, however, did not reproduce or rear litters with success equal to that of the first generation. This was indicated through two breeding trials. Examination of the results, however, does not disclose trends which relate poorer performances to an increase in persulfate level. The rats fed the flour-containing diets with 0.1% persulfate reproduced and reared their young consistently better than the flour-fed control group. The rats fed the flour-containing diets with the highest level of persulfate did not reproduce satisfactorily, as might have been anticipated from the growth results above. The records of the second generation rats fed the bread-containing diets do not differ consistently among the groups. This is an indication that even at levels of persulfate (1.0%) which interfere seriously with fermentation and baking, no adverse effect of the bread can be detected, even in prolonged feeding trials.

Number and weaning weights of third generation rats

The data on the third generation young from the first breeding are summarized in table 2. The results confirm the trend
of those given above, in that no differences are apparent among the first three groups fed the flour-containing diets or among the 4 groups fed the bread-containing diets. The second generation rats fed the flour-containing diet with 1.0% persulfate had difficulty in rearing their young; the latter, in turn, did not reach normal weaning weights even after a prolonged rearing period.

The records of the young from the second breeding are incomplete, in that the weaning weights and times of most of the young weaned were inadvertently not entered into our records, and therefore cannot be given here.

**Hematological observations**

The hematological data obtained on the rats are too voluminous to present in detail. As an indication of the results obtained, the data obtained at the first reading (taken at 8 to 10 weeks on the test diet) and those obtained at the last reading (after 52 weeks of test) are given in table 3. It may be seen that there is a greater variation within groups than between groups for all categories. This demonstrates that no changes occurred which can be ascribed to persulfate ingestion in the instance of the flour-containing diets or to the ingestion of bread baked from flour which contained persulfate. The data obtained in the weeks (15 readings for most of the groups) between those given in table 3 fall in the same range as those given in detail.

The second generation rats were similarly examined before being sacrificed for histological studies. No dyscrasias or variations from the normal occurred in any of the rats receiving the bread diet with ammonium persulfate-treated flour or in any animals receiving the highest percentage of ammonium persulfate. In one of the rats receiving the 0.02% ammonium persulfate bread diet, the findings indicated a subacute myelogenous leukemia. This animal had a white blood cell count of over 488,000 (controls averaged about 16,000), of which 297,000 were myelocytes or metamyelocytes and
### Table 3

Results of initial and final hematological examination of the first generation rats (means ± s.e.)

<table>
<thead>
<tr>
<th>DIETARY PERSULFATE LEVEL</th>
<th>HEMOGLOBIN (g/l)</th>
<th>RED BLOOD CELLS $\times 10^6$</th>
<th>WHITE BLOOD CELLS $\times 10^9$</th>
<th>NEUTROPHILS $\times 10^9$</th>
<th>EOSINOPHILS $\times 10^6$</th>
<th>LYMPHOCYTES $\times 10^6$</th>
<th>MONOCYTES $\times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk. 10</td>
<td>Wk. 52</td>
<td>Wk. 10</td>
<td>Wk. 52</td>
<td>Wk. 10</td>
<td>Wk. 52</td>
<td>Wk. 10</td>
</tr>
<tr>
<td>% Flour diets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>14.2 ± 0.2</td>
<td>14.2 ± 0.1</td>
<td>8.8 ± 0.2</td>
<td>9.9 ± 0.5</td>
<td>18.7 ± 2.1</td>
<td>4.1 ± 0.6</td>
<td>7.6 ± 0.4</td>
</tr>
<tr>
<td>0.02</td>
<td>13.7 ± 0.2</td>
<td>8.5 ± 0.5</td>
<td>28.6 ± 0.2</td>
<td>3.7 ± 0.4</td>
<td>0.5 ± 0.2</td>
<td>24.2 ± 0.2</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>0.10</td>
<td>15.5 ± 0.3</td>
<td>14.2 ± 0.6</td>
<td>8.7 ± 0.3</td>
<td>17.9 ± 3.6</td>
<td>4.2 ± 0.5</td>
<td>0.5 ± 0.2</td>
<td>12.9 ± 1.1</td>
</tr>
<tr>
<td>1.0</td>
<td>15.3 ± 0.2</td>
<td>15.0 ± 0.5</td>
<td>9.5 ± 0.3</td>
<td>16.5 ± 4.2</td>
<td>5.1 ± 0.9</td>
<td>0.6 ± 0.2</td>
<td>10.7 ± 0.9</td>
</tr>
<tr>
<td>% Bread diets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>13.4 ± 0.5</td>
<td>13.4 ± 0.4</td>
<td>8.0 ± 0.4</td>
<td>15.3 ± 1.5</td>
<td>3.5 ± 0.4</td>
<td>0.4 ± 0.3</td>
<td>11.4 ± 0.3</td>
</tr>
<tr>
<td>0.02</td>
<td>13.6 ± 0.3</td>
<td>9.2 ± 0.6</td>
<td>18.3 ± 0.6</td>
<td>3.3 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>14.8 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>0.10</td>
<td>13.7 ± 0.5</td>
<td>13.5 ± 0.6</td>
<td>9.6 ± 0.6</td>
<td>28.3 ± 2.8</td>
<td>5.6 ± 1.4</td>
<td>0.6 ± 0.2</td>
<td>21.7 ± 2.4</td>
</tr>
<tr>
<td>1.0</td>
<td>13.5 ± 0.4</td>
<td>13.6 ± 0.8</td>
<td>7.8 ± 0.2</td>
<td>19.8 ± 2.8</td>
<td>5.4 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>15.4 ± 2.8</td>
</tr>
</tbody>
</table>

1. Standard error.
2. The bread 2 group was first read at week 29 rather than as indicated.
150,000 were neutrophilic band cells and incompletely segmented cells. This animal was not sacrificed at the time the others were killed but was kept under observation. Two weeks later the white cell count was approximately the same, with some tendency to more maturity of the neutrophilic series. The animal died 6 days later and at autopsy no gross abnormalities were observed. Myelogenous leukemia is a rare and interesting occurrence in the rat and its appearance in this one animal is considered to be incidental and not attributable to the conditions of the experiment.

The results based on hematological examination are thus negative, in that no significant deviations from the normal were observed.

Pathological examinations

On post mortem examination none of the rats showed any gross abnormalities. Microscopic examination of sections of the organs revealed no tissue changes attributable to the ingestion of flour-containing ammonium persulfate. No conditions were found which did not also occur in the controls except in the one rat with myelogenous leukemia, which showed the expected myelocytic hyperplasia of bone marrow and other changes secondary to this granulopoietic disorder. It is of interest to note that the gonads, in both sexes, of the rats receiving the highest percentage of ammonium persulfate in the flour diet were normal, even though this group had a poor breeding record.

DISCUSSION

From the results given above it may be noted that the rats did not exhibit any adverse effects when fed bread baked from flour treated with 0.02, 0.1, or 1% ammonium persulfate. These tests reflect the effects of the ingestion of food after the maturing agent had exerted its action, since the persulfate is known to disappear rapidly at the dough stage. The facts that the rats were fed the bread at levels considerably above those ordinarily consumed and also that rats consume more
food per unit of body weight than do larger species give added assurance of its innocuousness for larger animals. Studies with dogs fed flour treated with ammonium persulfate and steamed before feeding have led to similar conclusions (Bentley et al., '48; Arnold, '49b).

Data are presented on the chronic toxicity of ammonium persulfate obtained from diets which contained flour with 0.02, 0.1, or 1.0% of the agent. Since the usual amount of persulfate used in flour is 0.01% (Doty and Sherwood, '49), the experimental concentrations were twice, 10 times and 100 times, respectively, that likely to be encountered under practical conditions. Adverse reactions were not noted at the two lower persulfate levels, but growth and reproduction studies indicated that the diet with the hundredfold level of persulfate caused slower growth and impaired the breeding and rearing of young. Since this was in contrast to the results obtained in the bread feeding trials, and since flour consumed in appreciable amounts would come into contact with moisture at one stage or another in its preparation, thus leading to breakdown of the persulfate, it is evident that there is little likelihood of persulfate's being deleterious to the consumer when used in the treatment of flour.

Thus we are led to conclude that ammonium persulfate is an innocuous ingredient when used for maturing flour, even at levels substantially above those which could be used commercially.

SUMMARY

Flour treated with ammonium persulfate and bread baked from flour treated with ammonium persulfate were fed to rats. The treatment levels were 0.02, 0.1, and 1.0% by weight of the flour used, whereas more than 0.01% persulfate is seldom used in commercial practice. The growth of the rats and their reproduction records into three generations were followed. Additional observations included periodic hematological examinations and histological sectioning of representa-
tive tissues at the termination of the studies, after one year of medication.

The rats fed diets containing bread baked from ammonium persulfate-treated flour were not different in any observable respect from the animals fed the control bread-containing diet.

In the case of the rats fed the flour-containing diets, no differences among the rats fed the control diet and the rats fed the diets containing flour with 0.02 and 0.1% ammonium persulfate were detected. However, the rats fed the diet which contained flour with 1.0% ammonium persulfate (100 to 150 times commercial maturing levels) were definitely affected adversely. This was evident in the gross appearance of the second generation young, in the retarded growth rates of all the rats, and in the poor reproduction performances. However, hematological examination and examination of tissue sections at autopsy did not reveal the cause of the ill effects observed.

Since bread made with persulfate in amounts far in excess of those needed for maturing produced no untoward effects upon rats, since dry flour containing 10 to 15 times the normal amount of persulfate caused no ill effects upon rats, and since flour consumed in significant amounts is ordinarily moistened in some way before ingestion, thus changing ammonium persulfate to ammonium sulfate, it is concluded that ammonium persulfate may be used with safety at the levels ordinarily required for the commercial treatment of flour.

ACKNOWLEDGMENTS

The technical assistance in these studies of John C. Eggers, Christine Eddison, Giles D. Clark, Jr., and William F. Wuerdemman is hereby gratefully acknowledged. In addition, we wish to express our appreciation to Dr. Lloyd C. Miller for his assistance in the preparation of the manuscript.

The bread used in these studies was supplied through the courtesy of Earl F. Weisbrod.
LITERATURE CITED


