While this method was developed and used exclusively with milled sodium soaps, the method can be used with liquid potassium soaps if it is modified slightly. At room temperature, barium bromide precipitates diluted liquid potassium soaps of any fatty acid composition in a finely dispersed form. With liquid potassium soaps, as with milled sodium soaps, a wide variation in the anhydrous soap content of the sample does not introduce any inherent error in the method; consequently the moisture content of the sample does not introduce any error. As these soaps are likely to contain several per cent of glycerol, its content in the soap should be known and corrected, as shown in the expression for the calculations of hexachlorophene.

Potassium soaps will give slightly less light absorption than sodium soaps with this method so that the standard curve prepared from sodium soap will not be directly applicable to potassium soaps, and a separate standard curve should be prepared.

These soaps frequently contain an organic divalent sequestrant, ethylene diamine tetra acetic acid, but it will not introduce any error in the result even if up to 0.70% of the dry material is present. The trivalent sequestrant, nitritotriacetic acid, however, will give an error of +0.02% hexachlorophene for each 0.047% of dry material present in the soap. These are about the usual quantities of the two sequestrants used in liquid potassium soaps.

Summary

Hexachlorophene is determined in soap by measuring the color produced by reaction with ferric chloride in alcohol solution with controlled conditions of time and temperature. The precision is within ±5% of the hexachlorophene content at the ½% level or above. As a criterion, any soap that will give a finely dispersed barium soap and that does not form a precipitate with ferric chloride under the conditions of the method give good results. This includes all milled bar soaps. The effect of phenolic perfume ingredients on the result is negligible. Five per cent abietic acid in the fatty acid composition has no effect on the result. The method is slightly modified for use with liquid potassium soaps.

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The Chronic Toxicity of Lauryl Gallate[1]

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IN the quest for satisfactory antioxidants that might be used to retard development of rancidity in fats, oils, and similar materials, the higher fatty alcohol esters of gallic acid have shown solubility advantages over such powerful antioxidants as gallic acid and nordihydroguaiaretic acid. The antioxidant properties and advantages of octyl, dodecyl, tetradecyl, hexadecyl, and octadecyl gallates have been reported by Morris, Kraekel, Hammer, Myers, and Riemenschneider (1), and the direct esterification of gallic acid with higher alcohols has been described by Ault, Weil, Nutting, and Cowan (2).

Lauryl (dodecyl) gallate is typical of the abovementioned fatty alcohol esters of gallic acid in regard to antioxidant effectiveness in shortening and in retarding developments of rancidity in baking goods as judged by pie-crust tests. The use of lauryl gallate in edible substances requires data on the acute and chronic toxicity to permit evaluation of any undesirable effects that might result from the ingestion of small amounts of the compound at frequent intervals over a long period of time.

In discussing "Some Factors Affecting the Control of Oxidative Rancidity," Hilditch (3) stated that "the possible toxicity of ethyl gallate has been investigated by Prof. J. A. Gunn of Oxford, at the request of the Medical Research Council and by Prof. A. D. Macdonald of Manchester. Both reported that no symptoms of toxicity were observed in mice, which had received orally or subcutaneously massive doses of ethyl gallate, in concentrations far greater than could ever be approached by human beings when receiving foods stabilized against oxidation by the ester." This statement is not explicit as to whether the tests on mice were of an acute or chronic nature. In view of the use proposed for the gallic acid esters, data on chronic toxicity based on long term feeding experiments would be the more important.

If it is assumed that lauryl gallate is hydrolyzed in the digestive tract, there is little reason to believe that the resulting lauryl alcohol and gallic acid would exert any toxic actions. Lauryl alcohol might be oxidized to lauric acid, which occurs as a glyceride in many vegetable fats, for example coconut oil. Gallic acid is widely distributed in the form of gallo-tannin in vegetable foods. Gallo-tannin is not absorbed from the gastro-intestinal tract but is hydrolyzed to gallic acid, which is readily absorbed and oxidized in the body. Although such speculation suggests that the repeated daily ingestion of reasonable amounts of lauryl gallate would not produce chronic toxicity,

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TABLE I

Food Intake and Weight Increments of Albino Rats Fed Various Concentrations of Lauryl Gallate

Days on Diet	Per Cent Lauryl Gallate in Basic Diet															
	0		0.012		0.025		0.05		0.1		0.25		0.5		1.0	
	Food Intake*	Body Wt.†	Food Intake*	Body Wt.†	Food Intake*	Body Wt.†	Food Intake*	Body Wt.†	Food Intake*	Body Wt.†	Food Intake*	Body Wt.†	Food Intake*	Body Wt.†	Food Intake*	Body Wt.†
Females																
0	....	43	....	43	....	44	....	43	....	43	....	42	....	42	....	42
55	11.4	153	11.3	150	11.6	157	11.2	153	11.0	152	11.9	153	11.4	148	12.0	139
104	10.9	182	11.0	181	11.2	187	10.3	184	11.3	179	11.0	182	11.3	182	13.5	162
153	10.2	194	11.1	199	11.0	202	11.0	198	11.8	197	11.2	197	10.7	194	14.5	175
205	10.6	212	10.9	214	11.0	218	11.5	217	11.5	212	11.4	209	12.1	217	13.8	193
254	10.8	219	11.2	224	11.6	226	11.7	230	11.6	217	11.8	226	11.1	220	10.7	203
Males																
0	....	46					....	48							....	46
51	16.2	218					15.1	218							14.2	152
93	15.5	272					14.9	270							16.0	198
152	16.1	307					15.5	305							15.3	237
225	15.9	338					15.2	320							14.0	272

* Food intake values represent the average number of grams of food ingested per rat per day.
† The values for body weight in grams represent the average weight in each experimental group.

prolonged feeding experiments on albino rats were conducted for a maximum of 254 days.

A total of 55 female and 20 male weanling rats were used. Separate groups of five females and five males were maintained on a basic diet known to permit a satisfactory growth rate, healthy appearance, and normal activity. These groups served as the controls for feeding tests in which both sexes and various concentrations of lauryl gallate were used.

Thirty-five female rats were divided into seven equal groups and placed on the basic diet to which had been added 0.012, 0.025, 0.05, 0.1, 0.25, 0.5, and 1.0% lauryl gallate. Two groups of five male rats each were fed the basic diet containing 0.05 and 1.0% lauryl gallate. The experimental diets were prepared by grinding and thoroughly mixing the lauryl gallate with the basic diet on a percentage-by-weight basis. In all cases the rats were allowed free access to their respective diets and water. Food consumption and the weights of individual rats were determined at weekly intervals, at which time careful observations were made as to the general well-being of the animals. At the end of the feeding period, which lasted 254 days for female and 225 days for male rats, all animals were autopsied, examined for evidence of gross changes, and the various tissues saved for histopathological examination. The data on food intake and weight increments are summarized in Table I.

On all levels of lauryl gallate the average number of grams of food ingested per rat per day remained substantially the same as on the control diet. On all levels of lauryl gallate except 1.0%, the increments in body weights of female rats are in remarkable agreement with those of corresponding controls. In the case of the females on a diet containing 1.0% lauryl gallate, the decrease in weight increments as compared with the weight gain in all other groups does not exceed 10% and, in the absence of other evidence, cannot be considered significant. As compared with the control group of male rats, the males on diets containing 0.05 and 1.0% lauryl gallate had the same food intake, but the rats on the highest level showed a significant retardation of growth. This suggests that the much smaller inhibition of growth shown by the females on the same level of lauryl gallate is real and that males are more susceptible than females.

On the basis of data published by Morris, Kraekel, Hammer, Myers, and Riemenschneider (1) the high concentration of lauryl gallate given in Table I is at least 5 to 10 times the amount of this compound used in tests on antioxidant efficiency. Moreover the 1.0% of lauryl gallate used in the toxicity tests refers to 1.0% of the total diet consumed by the rats every day whereas the amount used in an edible oil or fat would represent a much smaller percentage of the total diet and would not necessarily be eaten every day. The difference between the amounts of lauryl gallate fed experimentally and the amounts likely to be consumed under practical conditions is sufficiently large to provide a large safety factor in estimations of potential health hazards of this antioxidant.

In addition, the following experiments were made to determine whether or not toxicity could be demonstrated with amounts of lauryl gallate in excess of 1.0% in the daily diet. Five female rats on a diet containing 0.006% lauryl gallate showed no evidence of toxicity after 76 days, at which time the lauryl-gallate content of the diet was raised to 2.5%. Three of the animals were dead by the 111th day. The remaining two survived for the entire feeding period of 254 days, at which time the rats weighed 189 and 192 grams but showed no decrease in food intake.

Previous experience in toxicity studies on a variety of compounds has shown that young rats frequently show toxic effects on dosage levels which have little or no effect on more mature animals. Therefore 5 female rats having an average weight of 42 grams were placed on a diet containing 2.5% lauryl gallate and a second group of five females having the same average weight were given a diet containing 5.0% of the antioxidant. All animals on 2.5 and 5.0% levels were dead on the 10th and 7th days, respectively. The daily food intake of both groups was so low that starvation was obviously a major factor in causing death. A group of five male rats having an average weight of 49 grams showed normal food intake and rate of growth for 38 days on a diet containing 0.012% lauryl gallate. The lauryl gallate content was then raised to 2.5%. A marked drop in food intake occurred at once, and three animals died by the 52nd day, one on the 59th, and the last one on the 73rd day. Again starvation was an obvious factor. A comparison of this group of five males with the five

females on a level of 0.006% lauryl gallate before being transferred to a level of 2.5% on the 76th day confirms the conclusion drawn from Table I that male rats were more susceptible than the females.

On dosage levels above 1.0% lauryl gallate in the basic diet, the shavings used as bedding in the animal cages became discolored brown, dark brown, or black. The albino rats also showed a blackening of the tails in varying degrees. The cause of this discoloration was not studied exhaustively, but the following simple experiment suggests the excretion in the urine of gallic acid in the free form or in a conjugated form involving one of the three hydroxyl groups. A small amount of lauryl gallate was dissolved in 1 ml. of ethyl alcohol and diluted with water. A solution of gallic acid was prepared in a similar manner. When each preparation was made alkaline with sodium hydroxide, the lauryl gallate solution turned yellow and the gallic acid solution became black. This observation suggests that the staining of the rats and the bedding was due to the excretion of gallic acid following hydrolysis of the ingested lauryl gallate.

At time of autopsy none of the rats, including those on the diet containing 1.0% lauryl gallate, showed any gross abnormalities that could be ascribed to the experimental regimen. The only gross abnormalities observed during autopsy of animals on higher dosage levels occurred in the females placed on 2.5% lauryl gallate after being on a diet containing 0.006% of the compound for the first 76 days. These animals were thin and had swollen abdomens. They had a minimal amount of intra-abdominal fat. The intestines were large, flaccid, and full of gas. These findings suggest starvation.

Tissues from all rats on all dosage levels of lauryl gallate were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Sections of the following tissues were studied histopathologically: heart, lung, liver, spleen, thyroid, kidney, pancreas, intestine, bladder, adrenal, and ovary and tubes, or testis. In some animals the parathyroids and uterus were examined. As compared with the same tissues of the control rats, no significant structural alterations attributable to the lauryl gallate were found.

During the preparation of the diets, especially those containing the higher concentrations of lauryl gallate, itching of the hands was noted repeatedly. This observation is believed to be correlated with the fact that rats on diets containing lauryl gallate showed greater activity and scratching than the controls. The degree of activity was roughly proportional to the concentration of lauryl gallate in the diet. On diets containing 0.025 to 1.0% lauryl gallate, bald spots, which we ascribe to the scratching, developed on the rats after 118 to 160 days.

The evidence of a mild degree of skin irritation on the hands of one of us was sufficiently clear-cut to rule out need for tests for skin irritation by lauryl gallate on experimental animals. However skin-sensitization experiments were made on albino guinea pigs by the method reported by Draize, Woodard, and Calvery (4). Four control animals were given intracutaneous injections of 0.05 cc. of 0.9% saline daily for 10 days. Four experimental animals were injected daily for 10 days intracutaneously with the same volume of a 0.1% lauryl gallate suspension in 0.9% saline. Two weeks after the last sensitizing injection each of the eight animals was given an intracutaneous injection of 0.05 cc. of a fresh suspension of 0.1% lauryl gallate on the flank just below the sensitizing area and the results compared with the initial responses. The diameter, height, and color of the reaction area was always noted 24 hours after each injection. No sensitization was observed. However the behavior of the animals showed clearly that lauryl gallate is more irritating than 0.9% saline. The control guinea pigs became accustomed to the injections and toward the end of the experimental period objected very little to the injections whereas the animals receiving lauryl gallate either showed no such adaptation or became more difficult to handle.

In view of the skin-irritant effect of lauryl gallate, tests were made to determine whether or not the motility of isolated surviving strips of guinea pig colon would be altered by the compound. The addition of lauryl gallate as a suspension to the excised organ bath produced no effect on the colon activity recorded on a kymograph. The addition of sufficient ethyl alcohol to dissolve the lauryl gallate produced an inhibitory effect which could be accounted for entirely by the alcohol as shown in control experiments.

Summary and Conclusions

Long-term feeding experiments with albino rats involving concentrations of lauryl gallate as high as 0.5% in the daily diet produced no deleterious effects as judged by food intake, rate of growth, gross observations at time of autopsy, and histopathological examination of tissues. Diets containing 1.0% lauryl gallate definitely inhibited growth of male rats and retarded growth of females slightly. Levels of 2.5 and 5% lauryl gallate caused no histopathological changes in various organs but decreased the food intake to a point where starvation was an obvious factor in death of the animals. Lauryl gallate produced skin irritation but did not produce skin sensitization. The property of skin irritation might be of importance to workers engaged in the production and handling of lauryl gallate. There is no evidence to indicate that ingestion of slight amounts, such as might be encountered in edible fats and oils protected against oxidation, would produce harmful effects.

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