Propyl Gallate-8/25/72

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REPORT

ACCESSION NO. 24

PROPYL GALLATE
CAS. REG. NO. 000121799

RETURN TO:
GRAS REVIEW BRANCH
DIVISION OF PETITIONS PROCESSING
-FDA

Submitted to DHEW/Public Health Service/BF-335
Food and Drug Administration, CA-272
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PROPYL GALLATE

Summary: Toxicological Information

Propyl gallate is widely used as an antioxidant in a variety of foodstuffs, particularly those high in fats and oils. FDA regulations limits its use in such products to 0.02% based upon the fat or oil matters present in the product (FR 121.101). Use of propyl gallate has been cleared by Meat Inspection Division to retard rancidity development in rendered animal fat or in a combination of such fat and vegetable fat up to 0.01%, or a combination of propyl gallate, BHA, BHT, glycine, and NDGA up to 0.02%; in fresh pork sausage and dried meats, up to 0.01% based on fat content, and up to 0.01% in dried meats (FR July 9, 1965; FR June 13, 1967). However, general recognition of its safety for use as a flavoring was revoked by FDA April 9, 1970.

An acceptable daily intake for man of 0-0.5 mg/kg body weight was estimated by the Joint FAO/WHO Expert Committee on Food Additives (196). They also estimate that the level causing no significant toxicological effect in the rat is 100 mg/kg body weight per day.

Detailed metabolic pathways for PG have been suggested (196) although the fate of orally ingested material appears not to be fully determined as yet. Apparently, the ester is fully hydrolyzed in the body and from studies in the rat it has been established that the major metabolites appearing in the urine are gallic acid and 4-O-methyl gallic acid (45). This latter metabolite has also been demonstrated to conjugate with glucuronic acid (196).

Studies to establish the safety of PG for human use are based principally on the results obtained from feeding studies on the rat, with a minority of studies done on mice, guinea pigs and dogs. Although FDA permits its use in foods for human use, substantial disagreement seems to exist among investigators as to the relative toxicity of PG and to the wisdom of its use for human consumption.

The acute LD_{50} values for PG administered orally extend from 2500 (85) to 3800 (300) for the rat, and for the mouse from 1700 (208) to 3500 (246) mg/kg. For the rabbit the oral LD_{50} value is 2750 (131). The intra-peritoneal LD_{50} for the rat is 380 mg/kg (300).

The results of chronic feeding studies appear to be contradictory, particularly regarding the effect of PG on growth and survival rates of test animals. For example, rats, dogs and guinea pigs fed 0.117% PG for 1-2 years showed no adverse changes in gross appearance, growth, and reproduction as compared to the control animals, and indices associated with blood components and renal function appeared also to be normal (300). Similar results were reported in another set of studies with rats chronically fed a diet containing up to 0.004% PG (415).

Russian investigators have stated that indices of fertility and physical endurance as well as weight increments were unchanged in female albino rats fed a daily diet containing PG at 35x the daily normal intake level (407). Similarly, in feeding experiments extending for 2.5 months weight gain was normal in rats receiving in their diet high levels

of mixed antioxidants, eg., 100 mg. of BHA and 50 mg. of PG/ kg. of body weight/ day. During this experimental period fluctuations were observed in blood cholinesterase and catalase activity, and in blood glucose levels, but these parameters returned to normal after termination of the study (84, 158). In male and female rats fed PG at the 0.1% dietary level the serum cholesterol level remained unchanged during the experimental period (195).

In contrast are a number of studies with rats which report a marked decrease in weight increments and inadequate utilization of fats as a result of including PG in the diet at levels 1/5 and 1/10 the LD_{50} (208) and in another study at the 0.02% level (54). This last is paralleled by the finding that PG tends to inhibit the liberation of fatty acids from adipose tissue (315). In another investigation, daily administering 10 mg/kg PG and 20 mg/kg BHA increased the mortality of male rats and caused their sterility; but, in this instance, no changes were observed in the blood sugar level or in the activities of blood catalase and cholinesterase (85). Such adverse findings prompted several investigators to recommend that PG not be used as a fat anti-oxidant in foods for human consumption (54, 85, 209). However, despite these cautionary statements (by these Soviet investigators), PG continues to be one of the two permitted antioxidants used in the Soviet Union (84).

PROPYL GALLATE

Chemical Information

I. Nomenclature

A. Common name

propyl gallate

B. Chemical names

- gallic acid, propylester
- n-propyl gallate
- n-propylester of 3, 4, 5-trihydroxybenzoic acid

C. Trade names

- Progallin P (Merck Index. 8th Ed., 1968)
- Tenox PG (Eastman Chemical Co.)

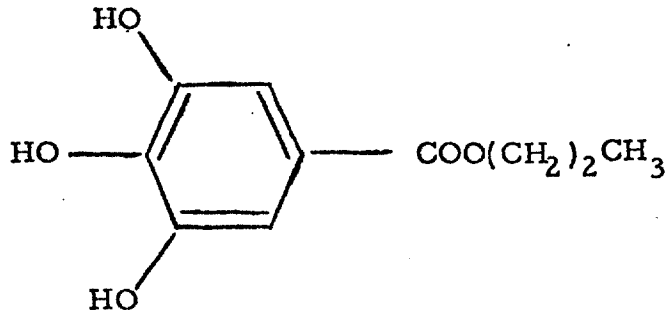
D. Chemical Abstracts Services Unique Registry Number

000121799

II. Empirical formula

$C_{10}H_{12}O_5$

III. Structural formula (Merck Index. 8th Ed. 1968)



IV. Molecular Weight

212.20 (Merck Index. 8th Ed. 1968)

V. Specifications

A. Chemical (124, 132).

- Purity: not less than 99% $C_{10}H_{12}O_5$

  " " " 98% "

  and not more than the equivalent of 102% $C_{10}H_{12}O_5$ after drying.

- Assay: not less than 98% and not more than the equivalent of 102 % of $C_{10}H_{12}O_5$ after drying.
- Melting range: between 146° and 148°
- Limits of Impurities

  Arsenic (as As). Not more than 3 parts per million (0.0003 %).

  Heavy metals (as Pb). Not more than 10 parts per million (0.001 %).

- Loss on Drying : not more than 0.5 %.
- Residue on Ignition: not more than 0.1 %.

B. Food Grade

No documented differentiation from chemical grade

VI. Description

A. General characteristics (124, 132, 309).

- A fine, white to nearly white, odorless powder having a slightly bitter taste. It is slightly soluble in water and freely soluble in alcohol and in ether.

- A white to creamy-white crystalline, odorless solid with a slightly bitter taste.
- Almost white, but commonly off-white or greyish microcrystalline powder or small crystals.
- Aqueous solutions of higher than 10 ppm concentration have a perceptibly bitter taste, or bitter-metallic taste and mouthfeel. The material is tasteless in the concentrations recommended for finished products.

B. Physical properties (124, 132, 309).

Crystals, mp 150°. Solubility in water at 25° = 0.35 g/100 ml; in alcohol at 25° = 103 g/100 g; in ether at 25° = 83 g/100 g; in cottonseed oil at 30° = 1.23 g/100 g; in lard at 45° = 1.14 g/100 g.

C. Stability (309).

It discolors in contact with Iron and upon prolonged exposure to air and daylight. The latter type of discoloration may not take place at the low concentration normally used.

VII. Analytical methods

Identification tests: (124)

A. Solubility

Water:	1 g is soluble in 300.0 ml.
Ethanol:	1 g is soluble in 3.5 ml.
Ether:	1 g is soluble in 3.5 ml.
Propylene glycol:	1 g is soluble in 3.5 ml.

B. Melting range is 146-148° after drying at 110° for 4 hours.

C. Dissolve about 0.5 g of propyl gallate in 10 ml. of N sodium hydroxide and boil for 30 minutes. Cool the mixture and carefully acidify with 2 N sulfuric acid. Filter off the precipitate, wash with a minimum amount of water and then dry at 110° for 2 hours. The melting point of the resulting gallic acid is about 240°, with decomposition.

D. Add 1 ml. of ammonium hydroxide to 5 ml. of a 1% ethanolic solution of propyl gallate. A pink to red color appears.

To distinguish among other antioxidants:

- TLC & Colorimetric (356).
- GLC (275).
- UV Spectra (275); luminescence (242).
- Colorimetric (395).

Identification in Foodstuffs:

- a general review of methods and problems associated with analysis of antioxidants in foods (395)
- in fats & oils (275, 356)

VIII. Occurence and levels found in:

A. Plants

(none)

B. Animals

(none)

C. Synthetics

(none)

D. Natural inorganic sources

(none)

PROPYL GALLATE

Biochemical Aspects

I. Breakdown

The major metabolite found in urine was 4-0-methyl-gallic acid. In addition, it appears that gallic acid itself, was one of the metabolites suggesting that cleavage of the ester-linkage had occurred (45).

II. Absorption-Distribution

(none)

III. Metabolism and Excretion

(none)

IV. Effects on Enzymes and Other Biochemical Parameters

(none)

V. Drug Interaction

(none)

VI. Consumer Exposure Information (NAS estimate)

As of 1970 the minimum amount of PG used for various purposes in the country by the food industry was estimated to be 123, 145 pounds.

PROPYL GALLATE

Biological Data

I. Acute Toxicity

Animal	Sex & No.	Route	Dosage mg/kg LD_0	LD_{50}	LD_{100}	Ref.
Mouse		oral		2000		42
"		oral		3500		246
"		oral	500	1700	2500	208
"	M&F	oral		2850 ± 0.26		131
Rat		oral	500	2600	3500	208
"	M&F	oral		3800		300
"	M&F	oral		3000 ± 0.25		131
"	M&F	i.p.		380		300
"	M	oral		2500		85
Hamster	M&F	oral		2480 ± 0.08		131
Rabbit	M&F	oral		2750 ± 0.18		131

II. Short Term Studies

A 1:2 mixture of PG and BHA was incorporated into the diets fed to rats. Over a 2.5 month period these animals received 100 mg. of BHA and 50 mg. of PG/kg body weight /day. The weight gain was normal during the experimental period. On the 20th day a decrease in the activity of blood cholinesterase and catalase was noted in the animals receiving the antioxidant, although at the end of the experimental period the activity

of the enzymes increased. Parallel changes were found in blood-glucose levels. Female rats appeared to be more sensitive to this antioxidant mix than were the male rats. The concentration of the antioxidant that was fed to these animals was 500 fold that deemed safe in the human diet. The LD_{50} of the 1:2 mixture for these animals was 2633 ± 140 mg/kg body weight (84).

In another study rats were fed a diet containing 0.002% or 0.004% PG. After 13 weeks some animals were sacrificed and the remaining ones placed on semi-starvation diets until death. Compared with the control group no differences were noted in water, protein, and fat utilization, or in survival time for the experimental animals (415).In a 1 to 3 month oral feeding study on rats, incorporation of PG in the diet did not effect any change in the development of enterokinase in the mucosa of the superior portion of the small intestine. Over this period of time no change was noted in the activity of the lipolytic enzymes secreted by the pancrease(210).

Chronic feeding studies were carried out on 92 female albino rats (46 control animals) in which the test animals received daily a mixture of food additives, including PG, which are found in commonly consumed foods, eg: cheese, flour, lard, etc. In the mixture, the level of each of the additives was 35 fold that normally taken in daily by the human. No harmful effects were noted as judged by weight increments, fertility, and physical endurance (407).

Adverse Findings:

1/5 & 1/10 of LD_{50} amounts of PG were included in the diets of rats and mice. A decrease in rate of growth was registered in all experimental animals and this was accompanied by reduced activities of blood catalase, peroxidase, and cholinesterase (208).

Daily administration of 20 mg/kg of BHA and 10 mg/kg of PG increased the mortality of male rats and caused their sterility. Over this period the level of ketone bodies in the blood decreased but blood sugar level and catalase and cholinesterase activities did not change (85).

Male albino rats were fed on a ration so that the daily intake was 2gms. of lard containing 4 mg. PG.. In those animals fed the diet containing PG, the assimilation of fat was somewhat lower than in the control animals (53).

Albino male rats were fed a diet consisting of sunflower seed oil, beef fat and mixed fat. Those animals ingesting this diet to which 0.02% PG had been added showed markedly lower weight gains and delayed development; and the nutritional value of the fats under examination tended to be lower. During food restriction, the animals which had previously ingested PG failed to adequately utilize the fat and protein resources any faster than did the control animals (54).

PG (as well as certain other antioxidants) inhibits the liberation of fatty acids from adipose tissue. This inhibiting effect was demonstrated by examination of the epididymis of Wistar rats starved for 16 hours. Adrenaline was present to function as an activator for fatty acid liberation(315).

In 126 pregnant control rats (not receiving PG in the diet) 40.8% had at term one or more recognizable resorption sites; 10.6% of implantations also terminated in resorption. However, fetal resorption rates were found to be substantially increased as a result of feeding PG to a group of pregnant test animals (409).

III. Long Term Studies

In studies with rats, dogs, guinea pigs fed a diet containing up to 0.117% PG for 1-2 years, no adverse changes were noted in : gross appearance, growth, reproduction (g. pigs), hemoglobin, erythrocyte and leukocyte levels , renal function (dogs). The histological findings on liver, kidneys, spleen, stomach, gonads, lungs, and heart were unremarkable. Larger amounts of PG (up to 2.34%) led to some growth inhibition and significantly lower hemoglobin values but no consistent pathological changes were noted. These investigators concluded that there were no detectable toxic effects due to PG when fed for prolonged periods even in amounts equal to 100x that which would be consumed if all the dietary fats were treated with the optimum amounts of PG necessary for effective antioxidant protection (300). Fed to rats (male and female) at a 0.1% level PG had no effect on the serum cholesterol level (195).

Rats were fed on bread containing 50x the normal concentration of PG, ClO_2, and BHA for 1 year. The bread represented 75% of the diet. There was no effect on the growth or mortality of the animals, nor on the organ weights and histopathology of the tissues. No evidence was forthcoming of synergistic action among these substances(158). Negative results were again obtained by the same authors for a similar study with male rats extending over a feeding period of 32 weeks (159).

Chronic studies on propyl gallate have also been conducted in guinea pigs and dogs. The guinea pigs were fed at a level of 0.011% of the antioxidant and were observed for one year. No detectable effect on the rate of growth was noted; no influence on reproduction was noted (300).

A group of 7 dogs was fed propyl gallate for 14 months in amounts similar to that fed the guinea pigs. The animals on the propyl gallate regimen showed no demonstrable effects and were similar in every respect to the 5 animals which served as controls (300).

Other observations on the biological effects of propyl gallate include:

- Studies with nematodes (phytohelminths) have shown that PG inhibits the activity of cathepsin as well as other enzymes containing SH-groups. However, in this species PG stimulates the synthesis of cellular enzymes and is without effect on the fermentation glands (287).

- PG was also observed to inhibit the contractile response of smooth muscle (of the guinea pig illium) by the action of bradykinesis. It is hypothesized in this instance that PG acts as a competitive inhibitor (320, 321).

- Intra-peritoneal (?) administration of PG to mice with Ehrlich ascites tumor and hepatoma retarded the biosynthesis of proteins and RNA and consequently inhibited the tumor growth (151). Similarly, PG suppressed oxidative phosphorylation and respiration in liver mitochondria and in solid hepatoma in mice (371).

- PG fed to Vitamin E deficient rats reduced the incidence of congenital abnormalities in offspring, although its action is not as effective as other anti-oxidants (221).

• PG was found to inhibit sulcal caries in rats by approximately 50% when incorporated in the diet at a 0.5% level (199).

IV. Special Studies

(none)

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Bibliography

1) Abbot, J. and R. Waite. 1962. The effect of antioxidants on the keeping quality of whole milk powder. I. Flavones, gallates, butylhydroxyanisole and nordihydroquaiaretic acid. Jour. Dairy Res. 29(1):55-61.

2) Abildgaard-Elling, K. 1953. Pulverförmige Aufbereitung fettlöslicher Vitamine als Futterzusatz. Dan. Prior. 11/2.

3) Abramova, I. and I. D. Gadaskina. 1965. Inhibition of benzene oxidation by some antioxidants. Gigiena Truda i Prof. Zabolevaniya. 9(1):33-36.

(4) Afanas'ev, L. P., et al. 1963. Sensitization of tumor cells to the action of ionizing radiation by inhibitors of radical reactions. Dokl. Akad. Nauk SSSR. 148:1199-1201.

(5) Afanas'ev, G. G., et al. 1968. Enhancement of radiation action by antioxidants. Izv. Akad. Nauk SSSR, Ser. Biol. 3:333-344.

(6) Agatowa, A. I. and N. M. Emanuel. 1963. Anderung der Struktur und Aktivitat der Aldolase bei der Einwirkung von Propylgallat. Ber. Akad. Wiss. UdSSR. 153:204-206.

(7) Agatova, A. I., et al. 1963. Reaktionsmechanismus freier Radikale, die sich aus Inhibitoren radikaler Prozesse mit SH-Gruppen von Proteinen bilden. Doklady Akad. Nauk SSSR. 150:547-550.

(8) Agatova, A. I. and N. M. Emanuel. 1966. Wirkung des Propylgallates auf die SH- und S-S-Gruppen enhaltenden Fermente. Biochimija. 31:299-305.

(9) Agatova, A. I., et al. 1st 1966(Pub. 1968). Effect of polyphenols on enzymes. Fenol'nye Soedin. Ikh Biol. Funkts., Mater. Vses. Simp. 146-154.

(10) Ahmad, I. et al. 1960. Vergleichende Untersuchung von "Amla-Produkten" und synthetischen Antioxydantien fur Speisefette. Pakistan J. sci. Res. 12:71-73.

(11) Andrianov, V. T., et al. 1969. Mechanism of the "structural" protection of DNA protein by some protectors. Radiobiologiya. 9(2):302-304.

(12) Andrianov, V. T., et al. 1969. A new mechanism of action of radioprotective agents: the principle of structural protection. Quantitative evaluations of the characteristics and effects of the drugs. Radiobiol. Inform. Byull. 12:61-63.

(13) Anglin, C., et al. 1956. Determination of antioxidants in edible fats. Jour. Agric. and Food Chem. 4(12):1018-1022.

(14) Antila, V., et al. 1965. The keeping quality of butterfat when stored dry. Finnish J. Dairy Sci. 25:21-30.

(15) Arenson, S.W. 1950. Shortenings for frying and baking. Food Indust. 22(6):1015-1020.

(16) Astrup, H. 1964. Oxidation of a highly unsaturated herring oil. Chem. Ind. (London). 3:107.

(17) Bajaj, I., et al. 1970. Separation of alkyl gallates as complexes with cinchonine and strychnine. J. Chromatogr. 46(3):261-266.

(18) Balanescu, G., et al. 1957. Esters of gallic acid used as antioxidants to prevent rancidity of vegetable oils. Lucrarile Inst. Cercetari Alimentare. 1:97-124.

(19) Baraboi, V.A. and S.A. Bershtein. 1962. Polarographic activity of serum proteins in acute radiation sickness and the use of propyl gallate as a prophylactic. Ukr. Biokhim. Zh. 34(1):32-38.

(20) Baraboi, V.A. and B. Ya. Medovar. 1963. Antiradiation and antioxidation properties of some polyphenols. Ukr. Biokhim. Zh. 35(6):924-930.

(21) Baraboi, V.A. 1964. Radiation protective action of gallate in irradiation. Fiziol. Zh., Akad. Nauk Ukr. RSR. 10(1):89-93.

(22) Baraboi, V.A. 1969. Mechanism governing the radioprotective action of gallates. Radiobio. Inform. Byull. 12:89-91.

(23) Bard, M. and B. Berkowitz. 1967. Training police as specialists in family crisis intervention: A community psychology action program. Community Ment. Health J. 3(4):315-317.

(24) Basu, U.P. and S. Bhattacharya. 1949. Studies on vitamin A in solution. I. Stability. J. Indian Chem. Soc. 26:419-424.

(25) Basu, U.P. and S. Bhattacharya. 1949. Studies on vitamin A in solution. II. Mode of action of the antioxidant. J. Indian Chem. Soc. 26:459-466.

(26) Basu, U.P. and S. Bhattacharya. 1949. Stability of vitamin A. Science and Culture. 14:481-482.

(27) Basu, U.P. and S. Bhattacharya. 1950. The keeping properties of vitamin A in solution. Indian Pharmacist. 5:172-174.

(28) Beal, R.E., et al. 1958. Pilot-plant preparation of edible safflower oil. Jour. Amer. Oil Chemists Soc. 35(2):97-99.

(29) Belgium. 1968. Current food additives legislation. Food and Agriculture Organization. (118)1.

(30) Bell, A., et al. 1956. Stabilization of fats and oils. U.S. Patent - No. 2,739,066. March 20.

(31) Bell, A., et al. 1957. Improvement in the stabilization of organic materials. Brit. 777,811. June 26.

(32) Belyaeva, N.I. 1967. Use of urea during the ensilage of corn and in combined fodder for milk cows. Sin. Azotistye Prep. Zhivotnovod. 48-52.

(33) Bentz, R.W., et al. 1952. Antioxidants and food preservation. Food Technol. 6(8):302-304.

(34) Berger, K.G., et al. 1960. Die Bestimmung chemischer Antioxydantien in Fetten nach Abtrennung durch Verteilungschromatographie. Analyst. 85:341-346.

(35) Bergstrom, S. 1958. Lipooxydasen. Acta polytechn. scand. Nr. 242. Chem. includ. Metallurgy Ser. 1:7-8.

(36) Berneis, K., et al. 1963. Der pro-oxydative Effekt tumorhemmender Methylhydrazin-Verbindungen. Helv. chim. Acta. 46:2157-2167.

(37) Beveridge, E.G. and W.B. Hugo. 1964. Resistenz von Gallussaure und deren Alkylestern gegen Bakterien, welche aromatische Ringstrukturen abbauen konnen. J. appl. Bacteriol. 27:304-311.

(38) Bhattacharya, S., et al. 1954. Vitamin A in solution. VI. Stability in relation to concentration and chemical forms of vitamin A and solvents. J. Indian Chem. Soc. 31:231-240.

(39) Bickoff, E.M. 1951. Phenolic antioxidants for carotene. Journ. Amer. Oil Chemists' Soc. 28(2):65-68.

(40) Blaricom, L.O. van. 1951. Retarding the loss of red color in cayenne pepper with oil antioxidants. Food Technol. 5(8):337-339.

(41) Blasi, F. 1966. Inhibition of synthesis of thyroxine from diiodotyrosine and diiodohydroxyphenylpyruvic acid by free-radical blocking agents. C.R. Soc. Biol. 160(7):1403-1408.

*(42) Boehm, E. and R. Williams. 1943. A study of the inhibiting actions of propyl gallate (normal propyl trihydroxy benzoate) and certain other trihydric phenols on the autoxidation of animal and vegetable oils. Quart. J. Pharm. Pharmacol. 16:232-243.

(43) Boehm, E. and R. Williams. 1943. Action of propyl gallate in the autoxidation of oils. Pharm. J. 151:53; Chemist Druggist. 140:146.

(44) Bonmassar, E., et al. 1968. Influence of vitamin E and propyl gallate on benzopyrene cancerogenesis in mice. Arch. Ital. Patol. Clin. Tumori. 11(1-2):245-250.

2*(45) Booth, A. N., et al. 1959. Die Umwandlung von Gallussaure und verwandten Verbindungen im Stoffwechsel. J. biol. Chemistry. 234:3014-3016.

(46) Borenstein, B. 1962. Stabilisieren von Carotin zum Farben von Lebensmitteln, bes. Puffmais. A. P. 3 039 877.

(47)Bratcher, S. and A. R. Kemmerer. Autoxidation and antioxidants in safflower oil. U.S. Dep. Agr., ARS No. 74-43, 53-7. 1967.

(48) Broitman, A. Ya. and V. E. Gavrilova. 1966. Intervention of synthetic antioxidants in enzymic oxidation systems. Toksikol. Vysokomol. Mater. Khim. Syr'ya Ikh Sin., Gos. Nauch.-Issled. Inst. Polim. Plast. Mass. 233-253.

(49) Brown, W. L. and M. L. Schmucker. 1963. Product to improve the color of meat. U.S. Appl. Sept. 26.

(50) Bruno, S. and G. Lupoli. 1960. New method for the determination of propyl gallate in fats. Farmaco(Prat). 15:419-426.

~~(51) Bruus~~gaard, A. , et al. 1970. Gallic salts in the pathologic state. Nord. Med. 84:1541-1542.

(52) Brshewskaja, K. M., et al. 1964. Freie Radikale bei der fermentativen Hydrolyse des Adenosintriphosphates. Biofizika. 9:500-501.

(52) Brzhevskaya, O. N., et al. 1966. Existence of free radicals during enzymic hydrolysis of ATP. Biofizika. 11(2):213-216.

3*(53) Budagyan, F. E. and L. I Smirnova. 1962. The influence of phenol antioxidants on the assimilation of alimentary fats. Voprosy Pitaniya. 21(2):47-50.

4*(54) Bukhman, N. D. 1962. The effect of propylgallate antioxidant on the nutritional value of fats. Vopr. Pitaniya. 21(1):68-72.

(55) Burlakova, E. B., et al. 1964. Antiradical activity and radioprotective properties of free-radical reaction inhibitors. Dokl. Akad. Nauk SSSR. 155(6):1398-1400.

(56) Burlakova, E. B., et al. 1965. Connection between radiation-protective and antitumor action of antioxidants. Dokl. Akad. Nauk SSSR. 164(4):934-936.

(57) Cantarelli, C. 1958. Gegen das Ranzigwerden von Olivenolen: Versuche mit Ascorbylpalmitat und anderen Antioxydantien. Olii minerali, Grassi Saponi, Colori Vernici. 35:281-289.

(58) Caspers, H. 1958. The influence of the ascending reticulary system of the brain stem on the spastic irritability of the cortex. II. Effect of narcosis. Zeitschr. ges. exptl. Med. 129(6):582-600.

(59) Cassidy, W. and A. J. Fisher. 1960. Die Bestimmung von Gallaten in Fetten fur die menschliche Ernahrung. Analyst. 85:295-297.

(60) Castell, C. H., et al. Rancidity in lean fish muscle. IV. Effect of NaCl and other salts. J. Fisheries Res. Board Can. 22(4):929-944. 1965.

(61) Castell, C. H., et al. 1966. Rancidity in lean fish muscle. V. Effect of amino acids. J. Fisheries Res. Board Can. 23(1):27-43.

(62) Cecil, S. R. and J. G. Woodroof. 1951. Butylated hydroxyanisole as an antioxidant for salted peanuts, salted pecans, and peanut butter. Georgia Agr. Exptl. Sta., Bull. No. 265:3-14.

(63) Cecil, S. R. and J. G. Woodroof. 1951. BHA ups shelf life of salted nuts. Food Indust. 23(2):81-84, 223-224.

(64) Cerutti, G. 1956. Maintaining the freshness of some dairy products. II. Butter stabilization. Latte. 30:267-268.

(65) Cerutti, G. 1957. The antioxidant action of ascorbic acid in edible fats. Olii minerali, grassi e saponi, colori e vernici. 34:41-45.

(66) Chahine, M. H. and F. A. El-Shobaki. 1963. The stability of oils and fatty foods. I. A comparative evaluation of several antioxidants in shark liver methyl esters. Grasas Aceites. 14(2):61-65.

(67) Chahine, M. H. and F. A. E.-Shobaki. 1963. The stability of oils and fatty foods. II. Factors affecting the stability of shark-liver oil. Grasas Aceites. 14(3):116-121.

(68) Chahine, M. H. and A. S. Radwan. 1964. The stability of oils and fatty foods. III. Factors affecting the stability of rice bran oil. Grasas Aceites. 15(3):129-140.

(69) Chahine, M. H. and F. A. El-Shobaki. 1966. The stability of oils and fatty foods. IV. The effect of added oleic acid and antioxidants on the oxidative stability of shark liver oil and of its methyl esters. Grasas Aceites. 17(6):195-200.

(70) Chang, I. and B. M. Watts. 1949. Antioxidants in the hemoglobin-catalyzed oxidation of unsaturated fats. Food Technol. 3:332-336.

(71) Chen, S. L. and E. J. Cooper. 1962. Herstellung von stabilisierter aktiver Trockenhefe. U. S. P. 3041249.

(72) Ch'en, S.-M., et al. 1958. Prevention of fat oxidation in dry mixed rations. Ying Yang Hsueh Pao 3, No. 1, 1-9.

(73) Ch'en, C.-H. 1961. Stability of ascorbic acid solution. J. Taiwan Pharm. Assoc. 13:2-9.

(74) Chenicek, J.A. 1954. Stabilization of edible fats and oils. U.S. 2,668,768. Feb. 9.

(75) Chenicek, J.A. 1954. Stabilisieren von Fetten und Olen. A.PP. 2 686 723 u. 2 686 724. Aug. 17.

(76) Chilson, W.H., et al. 1950. Use of propyl gallate to defer development of oxidized flavor in market milk. Jour. Dairy Sci. 33(2):924-927.

(77) Chipault, J.R. and G.R. Mizuno. 1966. Effect of Ionizing Radiations on Antioxidants in Fats. J. of Agri. and Food Chem. 14(3):221-224.

(78) Chomutow, B.L and N.A. Kulakowskaja. 1962. Quantitative Bestimmung von Butylhydroxyanisol, Propyl- und Octylgallat in Pflanzenolen. Ol- u. Fett-Ind. (UdSSR). 28(8):19-22.

(79) Ciegler, A., et al. 1961. Die mikrobiologische Produktion von Carotinoiden. Stabilisierung von β-Carotin in getrockneten Fermentationsprodukten. J. agric. Food Chem. 9:447-451.

(80) Cornell, D.G., et al. 1970. Partition coefficients of some antioxidants in butter oil-water model systems. J. Dairy Sci. 53(5):529-532.

(81) Curli, G. 1955. Mechanism of the action of antioxidants (in olive oil). Olearia. 9:160-162.

(82) Curli, G. 1955. Influence of extraneous substances of butter on the reaction of Tortelli-Jaffe. Latte. 29:537-538.

Dacre, J.C.

(83) Dal Brollo, F., et al. 1956. Antioxidants in pharmacy. Their action on some vegetal oils. II. Il Farmaco (Pavia) Ed. prat. 11:627-636.

5 *(84) Daniyalov, M.A. 1966. The effects of propyl gallate and butylhydroxyanisole, fat antioxidans, in animals. Vopr. Pitaniya. 25(3):76-80.

6 *(85) Daniyalov, M.A. 1966. Toxicity of a mixture containing butylhydroxyanisole and propyl gallate as antioxidation agents in alimentary fats. Vopr. Pitan. 25(6):47-51.

(86) Date, W.B., et al. 1958. Protection by jaggery against oxidative rancidity in sweets and in oil. Indian J. Appl. Chem. 21:165-172.

(87) Davidek, J. and J. Pokorny. 1961. Detection of antioxidants in fats with the aid of thin-layer chromatography on polyamide powder. Z. Lebensm.-Untersuch. u.-Forsch. 115:113-117.

(88) Dehority, B.A. 1959. Papierchromatographische Trennung einiger Antioxydantien. J. Chromatogr. (Amsterdam). 2:384-387.

(89) Del Pozo, A. and R. Salazar. 1962. Iron salts in the determination of alkyl gallates. Pharm. Acta Helv. 37:460-471.

(90) Deobald, H. J. and T. A. McLemore. 1964. The Effect of Temperature, Antioxidant, and Oxygen on the Stability of Precooked Dehydrated Sweet Potato Flakes. Food Technology. 18(5):145-148.

(91) Dessel, L. van, and J. Clement. 1969. Thin-layer chromatographic separation of antioxidants (in fats and oils). Z. Lebensm. - Unters. - Forsch. 139(3):146-149.

(92) Dorp, D. A. van. 1966. The biosynthesis of prostaglandins. Mem. Soc. Endocrinol. 14:39-46; comments 47, 75.

(93) Druckrey, H. 1957. Schutz vor Gefahrdung der Gesundheit durch Lebensmittlezusatze. Bericht uber die internationale Entwicklung, die Konferenzen in Rom 1956 und Ascona 1957. Dtsch. med. Wschr. 82:1310-1316.

(94) Dubinin, N. P. and V. K. Shcherbakov. 1964. Radiation protective compounds as mutagens and antimutagens. Radiobiologiya. 4(6):862-864.

(95) Dubinin, N. P., et al. 1966. Antimutagenic effect of antiradiation and antiblastomatous substances and regular characteristics of mutation by chromosomes. Vliyanie Ioniz. Izluch. Nasledstvennost, Akad. Nauk SSSR. 62-68.

(96) Dugan, L. R., Jr., et al. 1950. Butylated hydroxyanisole as an antioxidant for fats and foods made with fat. Food Technol. 4:457-460.

(97) Dugan, L. R., Jr. and L. Marx. 1953. Photoaktivierung und Farbbildung in mit Antioxydantien behandeltem Schmalz. J. Amer. Oil Chemists' Soc. 30:452-454.

(98) Di Luzio, N. R. 1964. Prevention of acute ethanol-induced fatty liver by the simultaneous administration of antioxidants. Life Sci. 3(2):113-118.

(99) Di Luzio, N. R. 1966. Mechanism of the acute ethanol-induced fatty liver and the modification of liver injury by antioxidants. Lab. Invest. 15(1):50-61; discussion 62-63.

(100) Dittrich, H. H. and E. Kerner. 1966. Fermentation inhibitory action of gallic acid esters. Z. Lebensm. -Untersuch. -Forsch. 129(6):364-369.

(101) Dul'tsina, S. M. , et al. 1966. In vitro experiments on kinetics of the tumor cell death influenced by chemotherapeutic agents. Vopr. Onkol. 12(9):60-66.

(102) Ebine, H., et al. 1957. Browning of miso surface. Shokuryo Kenkyusho Kenkyu Hokoku. 12:37-42.

(103) Elkhovskaya, E. S., et al. 1962. Reaction of propyl gallate with the virus of Rous sarcoma adsorbed on erythrocytes and stromas. Dokl. Akad. SSSR. 142:465-467.

(104) Elkhovskaya, E.S., et al. 1962. Comparative sensitivities to propylgallate of hepatoma and normal liver tissue. Doklady Akad. Nauk SSSR (Transl.).139(1/6):144-146.

(105) Emanuel, N. M. and L. P. Lipchina. 1958. Leucosis in mice and its development during interaction with inhibitors of chain oxidative processes. Doklady Akad. Nauk SSSR. 121:141-144.

(106) Emanuel, N. M., et al. 1959. Die selektive Unterdruckung der Aktivitat der oxydierend-reduzierend wirkenden Fermente in Geschwulstzellen bei Einwirkung von Inhibitoren der Kettenreaktionen. Ber. Akad. Wiss. (UdSSR). 124:1157-1159.

(107) Emanuel, N. M., et al. 1959. Selective decrease of the ribonucleic acid (RNA) content in tumor cells and their loss of transplantability after the in vitro interaction with inhibitors of chain reactions. Doklady Akad. Nauk SSSR. 125:411-413.

(108) Emanuel, N. M. and L. P. Lipchina. 1959. Loss of blastomogenic properties of sarcoma virus (Rous) after the action of propyl gallate. Doklady Akad. Nauk SSSR. 125:1148-1150.

(109) Emanuel, N. M. and T. E. Lipatowa. 1960. Verringerung der Zahl der freien Radikale in den Zellen des Ehrlichschen Ascites-Krebses bei Einwirkung von Propylgallat. Doklady Akad. Nauk SSSR (Ber. Akad. Wiss. UdSSR). 130:221-222.

(110) Emanuel, N. M. and E. A. Neifakh. 1960. Inhibition of glycolysis in cancer cells in the presence of propyl gallate. Doklady Akad. Nauk SSSR. 130:453-456.

(111) Emanuel, N. M., et al. 1960. Inhibition of biosynthesis of cell proteins of Yoshida ascitic hepatoma by action of inhibitors of free radical processes. Doklady Akad. Nauk SSSR. 134:1475-1477.

(112) Emanuel, N. M. und Je. A. Neifach. 1960. Verhinderung der Glykolyse in Krebszellen bei Einwirkung von Propylgallat. Doklady Akad. Nauk SSSR. (Ber. Akad. Wiss. UdSSR). 130:453-456.

(113) Emanuel, N. M., et al. 1960. Inhibition of radiation depolymerization of deoxyribonucleic acid (DNA) by inhibitors of free radical reactions. Doklady Akad. Nauk SSSR. 131:1451-1453.

(114) Emanuel, N. M. 1961. Inhibition of redox enzymes by inhibitors for free-radical processes. Proc. Intern. Congr. Biochem., 5th, Moscow, 4:58-65. (Pub. 1963).

(115)Emanuel, N. M. 1963. The role of free radicals in radiobiological processes and some new possibilities of developing agents protecting against radiation injury. Tr. Mosk. Obshchestva Ispytałelei Prirody, Otd. Biol. 7:73-83.

(116) Emanuel, N. M., et al. 1964. Suppression of protein biosynthesis in Ehrlich ascites cancer cells by chemical compounds of different classes. Biochem. Pharmacol. 13(2):241-247.

(117) Emanuel, N. M., et al. Physicochemical investigation of the radiation effects on DNA, proteins, and enzymes and the protective mechanism that operates in the presence of free radical inhibitors. Abhandl. Deut. Akad. Wiss. Berlin, Kl. Med. 1964(6):349-360.; Izv. Akad. Nauk SSSR, Otd. Khim. Nauk. 1963(1):173.

(118) Emanuel, N. M. 1965. Prospects for the kinetic study of biochemical processes in the rational chemotherapy of cancer. Vestn. Akad. Med. Nauk SSSR. 20(8):86-94.

(119) Emerson, J. A. and R. A. Greig. 1967. Extending the shelf life of frozen cisco (Coregonus Artedii) products through the use of water-soluble antioxidants. Fishery Industrial Research. 3(4):1-10.

(120) Epstein, S. S. , et al. 1967. Toxicity of antioxidants to Tetrahymena pyriformis. J. Protozool. 14(2):238-244.

(121) Ershoff, B. H. and C. W. Steers, Jr. 1960. Antioxidants and survival time of mice exposed to multiple sublethal doses of x-irradiation. Proc. Soc. Exptl. Biol. Med. 104:274-276.

(122) Esch, G. J. van. 1960. Suitability of a rapid test for carcinogenic properties of chemical compounds with the aid of a promoter substance. Verslag. Mededel. Betreffende Volksgezondheid. 186-189.

(123) Esh, G. C., et al. 1964. Untersuchungen uber die Stabilitat von Vitaminen in pharmazeutischen Zubereitungen. 1. Mitt. Einfluss von Tragersubstanzen auf flissige orale Multivitamin-Zubereitungen. Indian J. Pharmacy. 26:133-136.

7 *(124) FAO/WHO. 1967. Propyl Gallate. 3rd report. pp. 83-85.

(125) Federal Register 30. July 9, 1965. Miscellaneous amendments. 8674-8675.

(126) Federal Register 28. Sept. 5, 1963. Food additives. Hot-melt strippable food coatings. 9689-9690.

(127) Federal Register 27. May 9, 1962. Food additives. Chewing gum base. 4419-4420.

(128) Fenech, G., et al. 1956/1957. Einfluss einiger Antioxydantien auf die Konservierung des Vitamingehaltes in Leberol von Hexanchus griseus. Atti Soc. Peloritana Sci. fisiche mat. natur. 3:419-430.

(129) Feuer, G., et al. 1965. Liver response tests. VI. Application to a comparative study of food antioxidants and hepatotoxic agents. Food Cosmet. Toxicol. 3(3):457-469.

(130) Flores, H. and R.E. Morse. 1952. Deterioration of orange oil. Food Technol. 6:6-8.

8 *(131) Food and Drug Research Laboratories, Inc. Unpublished data.

9 *(132) Food Chemicals Codex. 1966. Propyl Gallate. pp. 568-569. National Academy of Sciences - National Research Council, Washington, D.C.

(133) Forgo, I., et al. 1970. Synthesis, physical-chemical properties and antioxidative effect of some gallic acid esters. I. Nature, mechanism of action and properties of antioxidants. Pharm. Acta Helv. 45:207-226.

(134) Forgo, I., et al. 1970. Synthesis, physical-chemical properties and antioxidative effect of some gallic acid esters. 2. Synthesis, purity test and quantitative determination. Pharm. Acta Helv. 45:227-236.

(135) Franzke, Cl., et al. 1966. The behavior of antioxidants in fat substrates containing heavy metals. Lebensm. Ind. 13(9):343-346.

(136) Fukuzumi, K. and N. Ikeda. 1969. Study on the effect of antioxidants in the autoxidation of methyl nonconjugated octadecadienoates. J. Amer. Oil Chem. Soc. 46(2):64-66.

(137) Funabashi, H. 1952. Antioxidants of lipide products. Koryo (Aromatics) No. 22. pp. 38-45.

(138) Gakichko, S.I., et al. 1968. About the sub-skin yellowing of frozen horse-mackerel. Rybnoe Khozyaystvo. 44(6):58-60.

(139) Gearhart, W. M. and B.N. Stuckey. 1955. The effect of various antioxidants on the keeping quality of yellow grease. Jour. Amer. Oil Chemists' Soc. 32(5):287-290.

(140) Gearhart, W. M. and B.N. Stuckey. 1955. A comparison of commercially used phenolic antioxidants in edible animal fats. Jour. Amer. Oil Chemists' Soc. 32:386-390.

(141) Gerschman, R., et al. 1954. Oxygen poisoning and x-irradiation: mechanism in common. Science. 119:623-626.

(142) Gershenzon, S., et al. 1959. Suppression of propagation of nuclear polyhedrosis virus of oak silkworm (Antherea pernyi). Doklady Akad. Nauk SSSR. 131:442-444.

(143) Gibson, P. 1958. Stabilisierung von Fetten, Ölen und Fettsäuren. A.P. 2 828 320.

(144) Goded y Mur, A. 1963. The oxidized flavor of milk; causes, measurement, correction. III. Anales Lactol. Quim. Agr. Zaragoza No. 6:1-22.

(145) Godkin, W. J., et al. 1951. Retardation of rancidity in pecans. Food Technol. 5:442-447.

(146) Golucki, Z. 1962. Stability of pharmacopeial and hardened rapeseed oil as tested by accelerated aging. 19(5):403-407.

(147) Golucki, Z. 1965. Einfluss von Antioxydantien und der Verpackung auf die Haltbarkeit von gehartetem Rapsol bei Raumtemperatur. Acta polon. pharmac. 22:255-258.

(148) Gonikberg, E. M., et al. 1967. Kinetic study of xanthine oxidation by xanthine oxidase in the presence of polyphenols. Mol Biol. 1(3): 373-380.

(149) Gonikberg, E. M., et al. 1967. Complex formation in the flavin mononucleotide-H--propylgallate system. Biofizika. 12:814-819.

(150) Gonzalez-Quijano, R. G. 1963. The preservation with antioxidants of a 50:50 mixture of olive and soybean oils stored in open cans. Grasas Aceites. 14:249-253.

(151) Gorbacheva, L. B., et al. 1966. Retardation of tumor growth and RNA biosynthesis by administration of phenolic compounds to tumor-bearing animals. Fenol'nye Soedin. Ikh Biol. Funkts., Mater. Vses. Simp., 1st (Pub. 1968). pp. 345-348.

(152) Gorgen, K. 1962. Der heutige Stand der industriellen Gefriertrocknung. Z. techn. Chem. 19:151-156.

(153) Gorodetskii, A. A., et al. 1961. Protective action of some inhibitors of the chain oxidative processes during the acute radiation syndrome. Radiobiol. 1:781-788.

(154) Gorodetskii, A. A., et al. 1961. Protective action of certain inhibitors of chain oxidative processes in the acute radiation sickness. Dopovidi Akad. Nauk Ukr. R. S. R. 812-815.

(155) Gorodetskii, A. A. and V. A. Baraboi. 1961. Chemoprophlaxis and chemotherapy of experimental acute radiation disease. Fiziol. Zhur., Akad. Nauk Ukr. R. S. R. 7:617-624.

(156) Gorodetskii, A. A. , et al. 1964. Protective action of certain inhibitors of chain oxidative processes in acute radiation sickness. Vopr. Biofiz. i Mekhanizma Deistviya Ionizir. Radiatsii Sb. pp. 159-162.

(157) Grace, W. R. & Co. 1966. Flexible packaging film. Neth. Appl. 6,605,779.

// * (158) Graham, W. D., et al. 1954. Chronic toxicity of bread additives to rats. J. Pharm. and Pharmacol. 6:534-545.

/2 * (159) Graham, W. D. and H. C. Grice. 1955. Chronic toxicity of bread additives to rats. II. J. Pharm. and Pharmacol. 7:126-134.

(160) Greene, B. E. 1969. Lipid oxidation and pigment changes in raw beef. J. Food Sci. 34(2):110-112.

(161) Greig, R. A. 1965. Technological investigations of pond-reared fish. 2. Extension of the shelf life of buffalofish products through use of antioxidants. Fish Ind. Res. 2(4):1-4.

(162) Grieg, R. A., et al. 1967. Extending the shelf life of frozen cisco (Coregonus Artedii) products through the use of water-soluble antioxidants. Fishery Industrial Research. 3(4):1-10.

(163) Hall, L. A. 1946/1950. Antioxydierendes Mutel. A. P. 2 511 802.

(164) Hall, L. A. 1947/1950. Stabilisieren von Olen und Fetten. A. P. 2 493 288.

(165) Hall, L. A. and L. L. Gershbein. 1945/1949. Antioxidant. U.S. Patent. 2,464,927.

(166) Hall, L. A. 1945/1949. Phospholipoid carrier for antioxidant (for fats and oils). U.S. Patent. 2,464,928.

(167) Hall, L. A. 1951/1958. Synergistische Antioxydantien. Can. P. 559 292.

(168) Hall, L. A. 1956. Antioxidant Composition. U.S. 2,772,170.

(169) Hall, L. A. 1956. Antioxidant compositions for edible fats and oils. U.S. 2,758, 931.

(170) Hall, L. A. and G. L. Clark. 1956. The detection of chemical reactions in complex mixtures of food antioxidants by infrared spectrometry. Food Technol. 10:384-386.

(171) Hall, L. A. 1961. Antioxidant composition. U.S. 2,981,628.

(172) Hammond, E. G. 1970. Stabilizing milk fat with antioxidants. American Dairy Review. 32(6):40-41, 43 & 76-77.

(173) Hanley, J. W., et al. 1953. Antioxidant treatment of bacon. Food Technol. 7:429-431.

(174) Hanning, F., et al. 1953. Antioxidants in the home preservation of foods. J. Home Econ. 45:660-662.

(175) Harrison, D. L., et al. 1953. Precooked frozen stews and Swiss steaks. Food Technol. 7:139-142.

(176) Heide, R. F. van der, et al. 1965. Identification of antioxidants in plastics. Chem. Weekblad. 61(38):440-443.

(177) Heide, R. ter. 1966. Thin-layer chromatography of antioxidants. Seifen Oele Fette Wachse. 92(24):890-891.

(178) Henderson, R., et al. 1960. Antioxidant cellophane. Modern Packaging. 34(4):127-130.

(179) Hida, M. 1958. Testverfahren fur Antioxydantien fur Vitamin A. 3 Mitt. Die Oxydation von Vitamin A durch Fette mit hoher Peroxidzahl. Vitamins (Kyoto). 15:387. (Vitamins. 16:144-148. 1959.)

(180) Higgins, J. W. and H. C. Black. 1944. Preliminary comparison of the stabilizing effect of several recently proposed antioxidants for edible fats and oils. Oil & Soap. 21:277-279.

(181) Hill, L. M., et al. 1969. Protection of milk fat with antioxidants. Journ. of Dairy Science. 52(6):888.

(182) Hirose, K., et al. 1956. Antibacterial property of antioxidants. Gifu Yakka Daigaku Kiyo No. 6:66.

(183) Hizon, R. P. and C. L. Huyck. 1956. Die Haltbarkeit von Mandel- und Maisol fur den Gebrauch in parenteralen Losungen. J. Amer. pharmac. Assoc., sci. Edit. 45:145-150.

(184) Hoffman, A. E., et al. 1954. Antioxidant for edible fats and oils. U.S. 2,683, 694.

(185) Horikawa, K. and Masuyama, S. 1969. Aliphatic hydroxycarboxylic acids as antioxidants. II. Influence of hydroxycarboxylic acids in dissolved oxygen in oil-in-water emulsion. Journ. of the Japanese Oil Chemists Soc. 18(11):808-812.

(186) Huber, R. and E. Schroeder. 1962. Antioxidants and survival rates of whole-body irradiated mice. Strahlentherapie. 119:308-315.

(187) Huber, R. 1963. The radioprotective effect of antioxidants. Radiobiol., Radiotherap. Fis. Med. 4:751-756.

(188) Ibaceta G., M. A. and B. J. Valladares. 1968. Preservation of sardine oil with antioxidants. Rev. Real Acad. Cienc. Exactas, Fis. Natur. Madrid. 62(4):843-849.

(189) Inoue, Y., et al. 1963. Manometric evaluation of antioxidants in a whale oil-hemoglobin system. Nippon Shokuhin Kogyo Gakkaishi. 10(10):399-401.

(190) Ishii, Y. 1952. Mechanism of the autoxication of fats. Yushi Kagaku Kyokaishi. 1, 84, 85-90.

(191) Ishii, R. and C. Horikawa. 1959. Faktoren, die die biologische Aktivitat von Carotin beeinflussen. 2. Mitt. Wirkung der Aminosauren, Proteine und Antioxydantien auf die Carotin-Stabilitat in synthetischen Magensaft. Vitamins (Kyoto). 16:687-689.

(192) Isupova, L.S. and V.S. Balabukha. 1963. Prevention of radiation-induced depolymerization of deoxyribonucleic acid (DNA) of rat liver by means of propyl gallate and 5-methoxytryptamine. Radiobiologiya. 3(2):256-258.

(193) Ivannik, B.P. and N.I. Ryabchenko. 1966. Mechanism of irradiation-induced degradation of DNA macromolecules in solutions. Radiobiologiya. 6(6):913-915.

(194) Janecke, H. 1957. Suitability of antioxidants for stabilization of pharmaceutical preparations. I. Adeps suillus. Arch. Pharm. 290:178-194.

13 *(195) Johnson, A.R. and F.R. Hewgill. 1961. The effect of the antioxidants, butylated hydroxyanisole, butylated hydroxy toluene and propyl gallate on growth, liver and serum lipids and serum sodium levels of the rat. Australian Joun. Exptl. Biol. and Med. Sci. 39(4):353-360.

14 *(196) Joint FAO/WHO Expert Committe on Food Additives. 1965. Specifications for Identity and Purity and Toxicological Evaluation of some Antimicrobials and Antioxidants. WHO/Food Add./24.65; FAO Nutr. Meet. Rep. Ser. No. 38A.

(197) Jonas, J. 1962. Thin-layer chromatographic separation of phenolic antioxidants (food additives). J. Pharm. Belg. 17(3-4): 103-110.

(198) Jonas, J. 1966. Analytic von Antioxydantien, die bei Speiseolen Anwendung finden. J. Pharmac. Belgique (N.S.). 21(48):3-66.

15 *(199) Jordan, H.V. , et al. 1961. Testing of antioxidants against experimental caries in rats. J. Dental Res. 40(5):878-883.

(200) Judina, L.I. 1963. Anwendung von Gallaten fur den Schutz des Fettes von Salzheringen gegen Oxydation. Fischerei-Wirtsch. (UdSSR). 39(6):78-83.

(201) Kahan, S. 1952. Bericht uber Antioxydantien in Olen, Fetten und Wachsen. Propylgallat. J. Assoc. off. agric. Chemists. 35:186-192.

(202) Kajimoto, G. 1960. Influence of rancid oil on cooking. I. Eiyo To Shokuryo. 12:385-390.

(203) Kakushkina, M. L., et al. 1966. Action of synthetic and natural inhibitors of lipid axidation on oleic acid (radiomimetic) hemolysis. Tr. Mosk. Obshchest. Ispyt. Prir. Otdel. Biol. 16:91-94.

(204) Kamath, G. G. and N. G. Magar. 1953. Role of antioxidants during stabilization of vitamin A in shark-liver oil. J. Indian Chem. Soc. 30:335-341.

(205) Kapadia, V. H. and N. G. Magar. 1954. Antioxidants during frying of fat. J. Indian Chem. Soc., Ind. & News Ed. 17:101-104.

(206) Kaplan, E. Ya. and V. V. Ogleznev. 1968. Effect of antioxidants on the resistance of an organism and on some functions during hypoxia. Probl. Kosm. Biol. 8:235-253.

(207) Karel, M., et al. 1965. Effects of added amino carboxylic compounds on the autoxidation of methyl linoleate in a freeze-dried model system. Presented at the 25th Annual Meeting, Institute of Food Technologists, Kansas City, Missouri, May 16-20. Paper No. 36.

16*(208) Karplyuk, I. A. 1959. Toxicologic characteristics of phenolic antioxidants of edible fats. Voprosy Pitaniya. 18(4):24-29.

17*(209) Karplyuk, I. A. 1962. Effect of phenolic antioxidants of nutritive fats on the animal organism. Tr. 2-oi(Vtoroi) Nauchn. Konf. po Vopr. Probl. Zhira v Pitanii, Leningrad. 318-325.

18*(210) Karplyuk, I. A. 1968. Study of the enzyme secretory function of the small intestine and pancreas mucosa in rats fed a diet containing phenol antioxidants. Vopr. Pitan. 27(2):21-26.

(211) Kato, T., et al. 1966. Behandlung von Olen mit phenolischen Substanzen. Bull. Nagoya Inst. Technol. 17:307-313.

(212) Katz, J. R. and M. A. Katz. 1952/1954. Speiseeispulver. A. P. 2 679 458.

(213) Kawashiro, I., et al. 1959. Studies on the detection of food adjuncts. II. Separation and identification of antioxidants by reversed-phase paper chromatography. Eisei Shikensho Hokoku. 77:175-177

(214) Kenyon, E. M. and B. E. Proctor. 1951. Effect of antioxidants on orange oil. Food Res. 16(5):365-371.

(215) Khan, N. A. 1961. Enzymic oxidation. IV. Antioxidants and lipoxidase - catalyzed oxidation of the linoleate system. Pakistan J. Sci. Res. 13:49-51.

(216)Khomutov, B. I. and N. A. Kulakovskaya. 1962. Spectrophotometric determination of antioxidants in fats and oils. Maslob.-Zhir. Prom. 28(5):13-18.

(217) Khristoferzen, G.S. 1965. Antioxidant properties of propyl gallate and butylhydroxytoluene. Izv. Vysshikh Uchebn. Zavedenii, Pishchevaya Tekhnol. 2:86-88.

(218) Kihara, Y. and T. Inoue. 1962. The estimation of rancidity for cereal and vegetable foods. II. Rancidity of potato chips. Kaseigaku Zasshi. 13:5-8.

(219) Kimura, K., et al. 1959. Untersuchungen an Tanninen. 8. Mitt. Einfluss von Tanninen auf die Cystein-Desulfhydrase-Aktivitat von Escherichia coli. Chem. pharmac. Bull. (Tokyo). 7:426-434.

(220) Kimura, K., et al. 1959. Untersuchungen an Tanninen. 9. Mitt. Wirkung von Tanninen und verwandten Verbinkungen auf die Tryptophanaseaktivitat von Escherichia coli. Chem. pharmac. Bull. (Tokyo). 7:531-533.

19 *(221) King, D.W. 1964. Comparative effects of certain antioxidants on gestational performance and teratogeny in vitamin E-deficient rats. J. Nutr. 83(2):123-132.

(222) Kinumaki, T. and K. Tabei. 1954. Stabilizing effect of commercial antioxidants on vitamin A concentrate. Bull. Japanese Soc. Sci. Fish. 20(6):539-550.

(223) Klose, A.A., et al. 1952. Use of antioxidants in the frozen storage of turkeys. Food Technol. 6(8): 308-311.

(224) Knorre, D.G., et al. 1959. Die Wirksamkeit von 3.5-Bis-(tert.-butyl)-4-hydroxytoluol und Propylgallat als Antioxydans fur Schweinefett. J. angew. Chem. 32:1359-1363.

(225) Knowles, M.B. and H.S. Pridgen. 1960. Acetins as solvents for antioxidants for fats and oils. U.S. 2,944,908.

(226) Kok, I.P. 1960. Effect of propyl gallate on the nucleic acid concentration in healthy and virus-infected pupae of Antheraea pernyi. Doklady Akad. Nauk SSSR. 130:1141-1143.

(227)Kolesnik, A.A. and N.A. Bazanov. 1960. Investigation of tannin substances and anthocyans in fresh and processed black currants. Izvest. Vysshikh Uchebn. Zavedenii, Pishchevaya Tekhnol. No. 6: 45-50.

(228) Komarik, S.L. and L.A. Hall. 1951. Curing process for bacon. U.S. 2,553,533.

(229) Korner, J. and H.P. Loomis. 1946. Stabilization of vitamin A and vitamin A-containing material. U.S. 2, 394, 456.

(230) Kotova, O.G. 1958. Efficiency of antioxidants for increasing the induction period of butterfat. Izvest. Vysshikh Uchebn. Zavedenii, Pishchevaya Tekhnol. No. 5:53-56.

(231) Kotova, O. 1959. Antioxidants for butterfat. Molochnaya Prom. 20(10):37-38.

(232) Kozlov, Yu. P. and A.I. Gorin. 1964. Effect of inhibitors of free-radical processes upon irradiated yeast cells. Nauchn. Dokl. Vysshei Shkoly, Biol. Nauki. 2:91-93.

(233) Kring, P. 1950. Investigations on the ability of some esters of gallic acid and of a few other substances to prevent rancidification of different fats. Dansk. Tidsskr. Farm. 24(8):211-227.

(234) Krishnamurthy, K., et al. 1957. The composition, storage, and nutritive value of palm oil. Food Sci. (Mysore). 6:248-252.

(235) Kruglyakova, K.E., et al. 1964. Radiational decomposition of deoxyribonucleic acid as an experimental model for evaluation of effectiveness of radiation-protection agents. Dokl. Akad. Nauk SSSR. 157(4):979-981.

(236) Kukushkina, G.V., et al. 1962. Differences in the character of inhibition of biosynthesis of protein in cancer cells by hydroxy aromatic compounds and alkylating agents. Dokl. Akad. Nauk SSSR. 147:1218-1219.

(237) Kukushkina, G.V., et al. 1966. Effect of phenolic compounds on the biosynthesis of protein and nucleic acids in cells of the human laryngeal cancer HEp-2. Vopr. Onkol. 12(5):54-56.

(238) Kukushkina, G.V., et al. 1966. Inhibition of biosynthesis of protein and nucleic acids by phenolic compounds in vivo. Vopr. Med. Khim. 12(5): 452-455.

(239) Kwapniewski, Z. and J. Sliwiok. 1965. Effect of sulfur amino acids on autoxidation of edible oils. Zeszyty Probl. Postepow Nauk Rolniczych. 53:365-367.

(240) Labuza, T.P., et al. 1969. Kinetics of linoleate oxidation in model systems. J. Amer. Oil Chem. Soc. 46(8):409-416.

(241)Laszlo, H. 1957. Extraneous substances added to foodstuffs. III. The detection in foodstuffs of commercially used synthetic phenolic antioxidants and their identification. Eng. e quim. (Rio de Janeiro). 9(5):1-5.

20 *(242) Latz, H.W., and R.J. Hurtubise. 1969. Luminescence analysis of food antioxidants; determination of propyl gallate in lard. Journ. Agr. Food Chem. 17(2):352-355.

(243) Lebedeva, Z. K. 1959. Increase in the stability of refined vegetable oils by the use of antioxidants. Masloboino-Zhirovaya Prom. 25(7):24-28.

(244) Lee, Shu-Chi. 1968. Detection of additives in foods. II. Thin-layer chromatographic determination of antioxidants. Hua Hsueh. 4:155-161.

(245) Lehman, A. J. 1950. Some toxicological reasons why certain chemicals may or may not be permitted as food additives. Assoc. Food & Drug Officials U.S., Quart. Bull. 14:82-98.

21 *(246) Lehman, A. J., et al. 1951. Pharmacological Evaluation of Antioxidants. Adv. Food Res. III. p. 200. Academic Press, Inc. N.Y., N.Y.

(247) Lemieszek-Chodorowska, K. 1965. Detection of synthetic antioxidants used for edible fat stabilization. Roczniki Panstwowego Zakladu Hig. 16(2):177-187.

(248)Lemieszek-Chodorowska, K. and A. Snycerski. 1969. Thin-layer chromatographic detection of phenolic antioxidants in edible fats and oils. Rocz. Panstw. Zakl. Hig. 20(3):261-266.

(249) Levitt, M., et al. 1967. A new class of tyrosine hydroxylase inhibitors and a simple assay of inhibition in vivo. Biochem. Pharmacol. 16(7):1313-1321.

(250) Lew, Y. T. and A. L. Tappel. 1956. Antioxidant and synergist inhibition of hematin-catalyzed oxidative fat rancidity. Food Technol. 10:285-289.

(251) Liljemark, A., et al. 1959. Improvement of the keeping quality of fresh fish by treatment with antioxidants. Fette, Seifen, Anstrichmittel. 61:465-468.

(252) Lineweaver, H., et al. 1952. Effect of antioxidant on rancidity development in frozen creamed turkey. Food Technol. 6:1-3.

(253) Lipchina, L. P., et al. 1960. Suppression of the mitotic activity of human cancer cells in tissue cultures by the action of free-radical reaction inhibitors. Doklady Akad. Nauk SSSR. 131:667-669.

(254) Lipkan, M. F., et al. 1962. Changes in nucleic acid content in the rat after X-irradiation and prophylactic administration of propylgallate. Ukrain. Biokhim. Zhur. 34(2):167-175.

(255) Lippitsch, M. 1961. Tannase in Aspergillus niger. Arch. Mikrobiol. 39:209-220.

(256) Lisanti, V. F. and B. Eichel. 1963. Antioxidant inhibition of experimentally induced caries in hamsters. J. Dental Res. 42(4): 1030-1035.

(257) Little, J.E. , et al. 1953. Ethyl gallate, a mycobacteria-specific antibiotic isolated from Haematoxylon campechianum. I. Isolation and chemical studies. Antibiotics & Chemotherapy. 3:183-191.

(258) Lundberg, W.O. and H.O. Halvorson. 1945. Observations on solubilities and other properties of several antioxidants in fats. Proc. Inst. Food Technologists. 1945:115-126.

(259) Lundberg, W.O. 1953. Antioxydantien und ihre Anwendung. Bakers Digest. 27(3):21-25.

(260) Lusikow, W.N. and Je. B. Burlakowa. 1964. Uber die Natur der thermischen Nachwirkung in rontgenbestrahlten Trypsinlosungen. Doklady Akad. Nauk SSSR. 155:1205-1208.

(261) Luzikov, V.N. 1965. Nature of thermal aftereffects in trypsin solutions irradiated with x-rays and ultraviolet light. Radiobiologiya. 5(1):11-16.

(262) Madhavakrishna, W. and S.M. Bose. 1962. The essential groups and the nature of crystalline tannase from divi-divi (Caesalpinia coriaria) pods. Seminar Vegetable Tannins, Papers, Madras. 83-98.

(263) Magoffin, J.E. and R.W. Bentz. 1949. The use of antioxidants in potato chipping. J. Am. Oil Chemists' Soc. 26:687-690.

(264) Mahon, J.H. and R.A. Chapman. 1951. Verhalten der Antioxydantien wahrend des Backprozesses und der Lagerung von "pie crust". Food Technol. 5:69.

(265) Mahon, J.H. and R.A. Chapman. 1951. Estimation of antioxidants in lard and shortening. Anal. Chem. 23:1116-1120.

(266) Mahon, J.H. and R.A. Chapman. 1953. The relative rates of destruction of propyl gallate (PG) and butylated hydroxyanisole (BHA) in oxidizing lard. Jour. Amer. Oil Chemists' Soc. 30(1):34-39.

(267) Mahon, J.H. and R.A. Chapman. 1954. Behavior of antioxidants during the baking and storage of pie crust. Jour. Amer. Oil. Chemists' Soc. 31(3):108-112.

(268) Marcuse, R. 1958. The occurrence of rancidity in salt herring. Fette, Seifen, Anstrichmittel. 60:482-487.

(269) Marcuse, R. and P.O. Fredriksson. 1969. Fat oxidation at low oxygen pressure:II. Kinetic studies on linoleic acid oxidation in emulsions in the presence of antioxidants. J. Amer. Oil Chem.Soc. 46(5):262-268.

(270) Markakis, P., et al. 1957. Quantitative aspects of strawberry-pigment degradation. Food Research. 22:117-130.

(271) Markuze, Z. 1967. Effects os some phenolic antioxidants on autoxidation of fats. Rocz. Panstw. Zakl. Hig. 18(1):45-48.

21a (272) Mathew, T.V. and S.N. Mitra. 1965. Separation and identification of antioxidants in oils and fats by thin-layer chromatography. Indian. J. Technol. 3(3):102.

(273) Mattil, K.F. and R.J. Sims. 1956. Stabilization of edible fats and oils. U.S. 2,759,829.

(274) Mayberry, M.G. 1949. Antioxidants for butter in confectionery. Confectioners J. November.

22 *(275) McCaulley, D.F., et al. 1967. The multidetermination of antioxidants in lard. J. Ass. Office. Anal. Chim. 50(2):243-250.

(276) McKillip, R.H. and R. Henderson. 1968. Transparent, flexible packaging material for food. Ger. 1,269,874.

(277) Mehta, A.C. and T.R. Seshadri. 1959. Flavonoids as antioxidants. J. Sci. Ind. Research (India). 18B:24-28.

(278) Mendoza-Galindo, S. 1951. Detecting rancidity. Antioxidant effect of nordihyroguaiaretic acid and of propyl gallate. Tesis quim., Univ. Chile. 3:23-36.

(279) Mihelic, F. 1963. Value of the thiobarbituric acid number in the investigation of vegetable edible oils and lard. Kem. Ind. (Zagreb): 12(3):147-150.

(280) Misumi, T. and K. Misumi. 1955. Soybean lecithin product for oxidation prevention emulsification, and prevention of off-flavor of foods. Japan. 3024.

(281) Mitchell, L.C. 1957. Separation and identification of four antioxidants, butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and nordihydroguaiaretic acid by paper chromatography. J. Assoc. Offic. Agr. Chemists. 40:909-915.

(282) Molinari, M. 1948. The autoxidation of fats, and the antioxidants with special reference to propyl gallate. Olii minerali grassie saponi, colori vernici. 25:70-72.

(283) Moore, R.N. and W.G. Bickford. 1952. Comparative evaluation of several antioxidants in edible fats. J. Am. Oil Chemists' Soc. 29:1-4.

(284) Mori, T., et al. 1952-1953. Antioxidant for vitamin A-enriched margarine. II. J. Japan. Soc. Food Nutrition (Eiyo to Shokuryo). 5:180-182.

(285) Morris, S.G., et al. 1952. Fractionation of animal fat glycerides by crystallization from acetone. An improved lard oil. J. Amer. Oil Chemists' Soc. 29:441-443.

(286) Musco, D.D. and W.V. Cruess. 1954. Deterioration of walnut meats. J. Agr. Food Chem. 2:520-523.

23 * (287) Myuge, S.G. 1968. Effect of propyl gallate on the extraintestinal digestion of phytohelminths. Tr. Gel'mintol. Lab., Akad. Nauk SSSR. 19:137-139.

(288) Naito, K. and T. Mori. 1953. Margarine enriched with vitamin A. II. The effects of milk components and some emulsifiers on vitamin A in margarine. J. Agr. Chem. Soc. Japan. 27:265-268.

(289) Naito, K. and T. Mori. 1953. Untersuchungen uber mit Vitamin A angereicherte Margarine. 3. Mitt. Wirkungen von wasserloslichen kolloiden Stoffen auf Vitamin A in Margarine. J. Agric. chem. Soc. Japan. 27:268-272.

(290) Natenzon, N.V. 1958. Test of certain antioxidants for preventing rusting of salted fish. Nauch.-Tekhnol. Byull. Polyar. Nauch.-Issledovatel. Inst. Morsk. Rybn. Khoz. i Okeanogr. 3:60-62.

(291) Naumann, U. 1959. A remark on the effect of propylgallate. Deut. Lebensm.-Rundschau. 55:66.

(292) Neifakh, E.A. 1962. Specific inhibition of activity of reduction-oxidation enzymes of glycolysis by inhibitors of free-radical reactions. Dokl. Akad. Nauk SSSR. 142:1405-1408.

(293) Newby, W.H. and H.L. Guidry. 1955. Preventing vaselation of unhydrogenated vegetable shortening. U.S. 2,726,159.

(294) Nichiforescu, E., et al. 1965. Stabilitat von Vitamin F. Farmacia (Bucuresti). 13:337-340.

(295) Nikolaeva, N.V., et al. 1962. Reduction of damage of deoxyribonucleic acid by radiation in the presence of propyl gallate. Dokl. Akad. Nauk SSSR. 142:713-715.

(296) Nowikawa, Ja. M. and W.I. Dubowik. 1961. Einfluss von Phenol und Sulfonamid auf die Stabilitat von Vitamin-A-Acetat in Sauerstoff. Izvest. Akad. Nauk BelorussSSR, Ser. fiz.-techn. Nauk. 4:91-95.

(297) Nugteren, D.H., et al. 1966. The enzymic conversion of all-cis-8, 11, 14-eicosatrienoic acid into prostaglandin E_1. Rec. Trav. Chim. 85(4):405-419.

(298) Olcott, H.S. and E. Einset. 1958. An antagonistic effect with antioxidants for unsaturated fats. J. Am. Oil Chemists' Soc. 35:159-160.

(299) Olin Mathieson Chemical Corp. 1968. Inhibition of oxidation. West German Patent. 1 269 874.

24*(300) Orten, J. M., et al. 1948. Studies on the toxicity of propyl gallate and of antioxidant mixtures containing propyl gallate. Food Technol. 2(4):308-316.

(301) Ozawa, Y., et al. 1953. Studies on the oxidation mechanism in emulsified fat or oil. I. The influence of milk constituents on oxidation in margarine. Bull. Natl. Inst. Agr. Sci., Japan, Ser. G. 28:67-74.

(302) Paquot, C. and J. Mercier. 1963. Experimentelle Ergebnisse bei der Fettoxydation. 2. Mitt. Antioxydantien. Rev. franc. Corps gras. 10:337-340.

(303) Parkhomenko, I. M. 1963. Effect of some radioprotectors under γ-irradiation of the cells of cynomolgus monkey heart in vitro. Radiobiologiya. 3(3):467-471.

(304) Parodi, P. W. 1965. The use of 2,2-diphenyl-1-picrylhydrazyl for measuring antioxidant properties of butterfat. Australian J. Dairy Technol. 20(1):6-9.

(305) Pasich, J. and T. Przadka. 1960. The value of some antioxidants for the preservation of arachis oil. Dissertationes Pharm. 12:237-243.

(306) Pasich, J. 1962. An evaluation of the usefulness of some substances with antioxidant properties for the conservation of peanut oil. Dissertationes Pharmaceut. 14(1):109-114.

(307) Pelewina, I. I. and L. P. Liptschina. 1961. Wirkung der Inhibitoren von Reaktionen freier Radikale auf die Aktivitat von Fermenten des Succinoxydasesystems. Doklaky Akad. Nauk SSSR. 140:1199-2000.

(308) Perepletchik, R. R. and T. A. Korduban. 1967. The use of antioxidants in the storage of codliver oil. Tr. Vses. Nauch.-Issled. Inst. Morsk. Ryb. Khoz. Okeanogr. 63:50-68.

25*(309) Perfume and Flavor Chemicals. 1969. Item 2710. Steffan Arctander, Publ. Montclair, N. J.

(310) Phillips, M. A. and R. D. Hinkel. 1957. Determination of 2,6-ditert-butyl-p-cresol in edible fats by ultraviolet spectrophotometry. Jour. Agric. and Food Chem. 5(5): 379-384.

(311) Pietrzyk, C. 1962. Einfluss der Konzentration von Inhibitoren auf die Geschwindigkeit der Oxydation mancher Pflanzenole. Roczniki Technol. Chem. Zywnosci. 9:81-98.

(312) Pietrzyk, C. 1962. Prooxidant action of propyl gallate during the autoxidation process of soybean oil and of rapeseed oil. Roczniki Technol. Chem. Zywnosci. 9:29-46.

(313) Pietrzyk, C. 1965. The influence of the concentration of phenolic antioxidants on the rate of oxidation of lard. Roczniki Technol. i Chem. Zywnosci. 11:65-75.

(314) Pisarevskii, A.N., et al. 1968. Protection of nucleoproteins from ionizing radiation. Radiobiologiya. 8(3):339-346.

26* (315) Placer, Z., et al. 1965. Effect of antioxidants on the liberation of fatty acids from adipose tissue. Nahrung. 9(1):155-156. Not Here

(316) Pohle, W.D., et al. 1962. Ein Vergleich verschiedener analytischer Methoden zur Bestimmung der relativen Stabilitat von Fetten und Olen gegen Oxydation. J. Amer. Oil Chemists' Soc. 39:226-229.

(317) Pokorny, V. and G. Esmansky. 1958. Lagerung von ausgelassenem Schweinefett unter Benutzung von Propylgallat. Prumysl Potravin. 9:92-95.

(318) Pokorny, J., et al. 1961. Studies on the stability of fats and its influence. IV. The effect of Chlorella on the stability of fatty foods and fodder. Sb. Vysoke Skoly Chem.-Technol. Praze, Potravinarske Technol. 5(3):153-159.

(319) Pokorny, J., et al. 1963. Untersuchungen uber die Fettstabilitat und ihre Beeinflussung. 7. Mitt. Stabilisierung der als Zusatze zu Geflugelfutter geeigneten Fette. Sbornik Vysoke Skoly chem.-technol. Praze, Potravinarska Technol. 7:103-112.

27* (320) Posati, L.P., et al. 1969. Inhibition of bradykinin activity by gallates and other antioxidants. Abstracts of Papers. American Chemical Society. 158:AGFD 40. Not Here

28* (321) Posati, L.P., et al. 1970. Inhibition of bradykinin by gallates. J. Agr. Food Chem. 18(4):632-635.

(322) Postnikova, M.S., et al. 1969. Study of propyl gallate radical reactions in DNA irradiated frozen solutions in the presence of oxygen by the EPR method. Biofizika. 14(5):927-930.

(323) Pozza, F. and G. Verza. 1960. Influence of propyl esters of gallic acid on the mortality from x-radiation. Boll. Soc. Ital. Biol. Sper. 36:1120-1123.

(324) Privett, O.S. and C. Nickell. 1962. Determination of structure of unsaturated fatty acids via reductive ozonolysis. J. Am. Oil Chemists' Soc. 39:414-419.

(325) Pruss, G.M. and N.S. Novitskii. 1967. Protein metabolism in the intact myocardium during myocardial infarction. Vop. Med. Khim. 13(6):584-587.

(326) Rabending, G. and H. Klepel. 1971. Photoconvulsive and photomyoclonic reactions : Age dependent genetically determined variations of enhanced photosensitivity. Neuropadiatrie. 2(2):164-172.

(327) Ramamurti, K. and B.N. Banerjee. 1948. Studies on Indian edible oils. Groundnut oil. Indian Jour. Med. Res. 36(4):371-386.

(328) Ramanujam R.A. and C.P. Anantakrishnan. 1958. Stabilitat von Tocopherol in Fetten wahrend Lagerung und Erhitzung. Indian J. Dairy Sci. 11:179-184.

(329)Rao, M.K.G. and K.T. Achaya. 1967. Role of tocopherol as an antioxidant in safflower oil. Fette, Seifen, Anstrichm. 69(10): 711-714.

(330) Raven, R.W. 1968. Recent concepts of cancer prevention(human). Progress in Experimental Tumor Research. 10:133-162.

(331) Rhee, I.S. and B.M Watts. 1966. Effect of antioxidants on lipoxidase activity in model systems and peas (Pisum Sativum) slurries. Journal of Food Science. 31(5):669-674.

(332) Rich, S. and J.G. Horsfall. 1954. Relation of polyphenol oxidases to fungi toxicity. Proc. Natl. Acad. Sci. U.S. 40:139-145.

(333) ~~Robertson~~, J.M. 1952/1956. Antioxydans fur Fette, Wachse u. Lebensmittel. A.P. 2 741 563.

(334) Robinson, H.M. 1956. Stabilization of fats in candy. II. The importance of free fatty acids. Mfg. Confectioner with Intern. Confectioner. 36:21.

(335) Roesler, H. and K. Schlogel. 1969. Testing the effectiveness of different antioxidants in processed cream cheese. Deutsche Molkerei-Zeitung. 90(32):1645-1647.

(336) Romanskaya, I.L. 1959. Influence of synthetic antioxidants on the stability of butterfat and butter. Vysshikh Ucheb. Zavedenii, Pishchevaya Tekhnol. 4:73-76.

(337) Romanskaya, N.N. and A.N Valeeva. 1962. Effect of synthetic antioxidants on the stability of milk fat during long storage. Tr. Frunzensk. Politekhn. Inst. 7:17-21.

(338) Rosenwald, R.H. 1955. Stabilization of organic compounds. U.S. 2,721,804.

(339) Roy, B.R. 1957. Stabilitat von Vitamin A in mit Vanitin angereichertem Vanaspati. J. sci. ind. Res., Sect. C. 16:236-240.

(340) Rutkowski, A., et al. 1962. Preservation of goose fat with synthetic and natural antioxidants. Zeszyty Nauk Wyzszej Szkoly Rolniczej-Olsztynie. 13(3):387-396.

(341) Rutkowski, A., et al. 1963. Suitability of some quantitative and qualitative methods for determination of antioxidants in fats. Roczniki Panstwowego Zakladu Hig. 14(4):361-370.

(342) Ryuzo, T., et al. 1969. Food additives. XIII. Detection for some antioxidants in foods. Eisei Kagaku. 15(2):77-83.

(343) Sabalitschka, Th. 1931. Synthetisch Studien uber die Beziehung zwischen chemischer Konstitution und antimikrobischer Wirkung. XI. Mit H. Tietz. Zwei-und dreifach hydroxylierte oder oxalkylierte Benzoesauren und deren Ester. X. vgl. C. II. 271.

(344) Sabalitschka, B. and E. Bohm. 1938. Antioxydantien fur Fette und Ole, auch fur ather. It. P. 502 301.

(345) Sahasrabudhe, M.R. 1953. Comparative evaluation of antioxidants for groundnut oil and its hydrogenated products. J. Sci. Ind. Research (India). 12B:63-67.

(346) Sahasrabudhe, M.R., et al. 1954. Effect of shortening consistency and added antioxidants on the keeping quality of biscuits. J. Sci. Ind. Research (India). 13B:521-524.

29 *(347) Sahasrabudhe, M.R. 1964. Application of thin-layer chromatography to the quantitative estimation of antioxidants: butylated hydroxyanisole, butylated hydroxytoluene, propylgallate, and nordihydroguaiaretic acid (in lard). J. Ass. Offic. Agr. Chem. 47(5):888-893.

(348) Sair, L. and L.A. Hall. 1951. The use of antioxidants in deep fat frying. Food Technol. 5(2):69-73.

(349) Sakamura, S., et al. 1965. In Eierfrucht (Solanum melangena L.) vorkommende Anthocyanase und Anthocyane. 3. Mitt. Oxydative Entfarbung des Anthocyans durch Polyphenoloxydase. Agric. biol. Chem. (Tokyo). 29:181-190.

(350) Salaschko, M. 1959. Der Einfluss synthetischer Antioxydantien auf die Bestandigken von Milchfett und Butter. Milch-Ind. 20(2):25-26.

(351) Salazar Macian, R. 1963. Ferrous salts in the colorimetric determination of alkyl gallates. II. An.Real Acad. Farm. 29(1/2): 17-29.

(352) Sandell, E. and B. Spross. 1950. The stabilization of lard. Svensk Farm. Tidskr. 54(4):61-73.

(353) Ssapeshinkski, I.I. and N.M. Emanuel. 1960. Energetische Niveaus metastabiler Zustande biologischer Objekte und Wirkungsmechanismus einiger Radioschutzstoffe. Dokl. Akad. Nauk SSSR (Ber. Akad. Wiss. der UdSSR). 132:1441-1443.

(354) Sapezhinskii, I. I. and E. G. Dontsova. 1967. Reaction of serum albumin and glycyltryptophan free radicals with radioprotective substances. Biofizika. 12(5):794-798.

(355) Saruya, K., et al. 1954. The protection method of marine products from their deterioration due to the oxidation of oil. I. Applicability of antioxidant to salted and dried saury. Bull. Japan. Soc. Sci. Fisheries. 20:58-65.

30* (356) Scheidt, S. A. and H. W. Conroy. 1966. Detection of propyl gallate, nordihydroguaiaretic acid, butylated hydroxytoluene, and butylated hydroxyanisole in fats and oils by thin layer chromatography. J. Ass. Offic. Anal. Chem. 49(4):807-809.

(357) Schon, R. and P. Venker. 1960. The action of propyl gallate on the glycolysis of tumor and normal tissue. Acta Biol. et Med. Ger. 5:97-99.

(358) Schroder, E., et al. 1960. Elektronenresonanz-Untersuchungen uber die Reaktion einiger Strahlenschutzstoffe mit freien Radikalen. Strahlentherapie. 112:457-461.

(359) Schroder, E. and H. G. Thom. 1961. Paramagnetische Elektronen-spinresonanz-Untersuchungen uber die Wirkung einiger Strahlen-schutzstoffe in Modellsystemen. Arch. Sci. (Geneve). 14(166):197-200.

(360) Schwien, W. G. and H. W. Conroy. 1965. Qualitative analysis of propyl gallate, nordihydroguairaretic acid, butylated hydroxyanisole, and butylated hydroxytoluene in fats and oils. J. Ass. Offic. Agr. Chem. 48(3):489-492.

(361) Seamer, J. and F. C. Chesterman. 1967. A survey of disease in laboratory animals. Lab Anim. 1(2):117-139.

(362) Sedlacek, B. A. J., et al. 1959. Methods for the determination of propyl gallate in lard. Prumysl Potravin. 10:594-597.

(363) Sedlacek, B. A. J. 1959. Eine neue semimikro-komplexmetrische Methode zur Bestimmung von Propylgallat in Fetten. Z. Lebensmittel-Unters. u. -Forsch. 111:108-111.

(364) Sedlacek, B. A. J. 1963. A new paper chromatographic method for the separation and identification of synthetic antioxidants. Fette, Seifen, Anstrichmittel. 65:915-919.

(365) Sedlacek, B. 1964. Determination of antioxidants and their chromatographic separation. Vopr. Pitaniya. 23(4):8-13.

(366) Sedlacek, B. 1966. Changes in lard resulting from the addition of antioxidants during heating. Nahrung. 10(2):155-163.

(367) Sedlacek, B. A. J. 1968. Use of certain antioxidants in the stabilization of oils. I. Initial and other nonvolatile products of autoxidation. Nahrung. 12(7):721-725.

(368) Sedlacek, B. A. J. 1968. Use of certain antioxidants in the stabilization of oils. II. Volatile products formed during autoxidation. Nahrung. 12(7):727-730.

(369) Sedlacek, B. A. J. 1968. Application of antioxidants for improving the storage properties of shortenings. I. Primary nonvolatile products of autoxidation. Fette, Seifen, Anstrichm. 70(10):795-801.

(370) Seher, A. 1958. The qualitative analysis of antioxidants. Fette, Seifen, Anstrichmittel. 60:1144-1153.

31 *(371) Semenova, L. P., et al. 1965. Suppression by propyl gallate of the oxidative phosphorylation and respiration in mitochondria of the liver and of solid hepatoma of mice. Dokl. Akad. Nauk SSSR. 163(3):774-776. Not Here

(372) Sengupta, A. and S. P. Mehta. 1965. Autoxydative Veranderungen linolensaurereicher Ole in Emulsionen. Indian J. Technol. 3:254-257.

(373) Shaidorov, Yu. I., et al. 1968. Changes of propyl gallate radioprotective action during proton and gamma-irradiation of potatoes. Kosm. Biol. Med. 2(1): 18-21.

(374) Shedek, M. Ss., et al. 1963. Stabilisierung der Butter durch Antioxydantien. I. Mitt. Antioxydantien fur kontinuierlich hergestellte Butter. Hochschulnachr. (Krasnodar), Nahrungsmittel-Technol. 6:55-58.

(375) Shedek, M. Ss., et al. 1963. Stabilisierung der Butter durch Antioxydantien. 2. Mitt. Einfluss von Antioxydantien auf die Lagerfestigkeit der kontinuierlich hergestellten Butter bei langfristiger Lagerung. Hochschulnachr. (Krasnodar), Nahrungsmittel-Technol. 6:59-63.

(376) Sherwin, E. R. and J. W. Thompson. 1966. Tertiary-butylhydroquinone - An antioxidant for fats and oils and fat-containing foods. Presented at the 26th Annual meeting, Inst. of Food Technologists, Portland, Ore., May 22-27. (Paper 11).

(377) Sherwin, E. R. and B. M. Luckadoo. 1970. Studies on antioxidant treatments of crude vegetable oils. J. Amer. Oil Chem. Soc. 47(1): 19-23.

(378) Siedler, A. J. and B. S. Schweigert. 1954. Der Einfluss des Zusatzes von stabilisiertem tierischem Fett auf die Haltbarkeit von Vitamin A in der Nahrung. J. agric. Food Chem. 2:193-195.

(379) Siegfried, B. and R. Schneider. 1953. Preservation of fats and oils. Pharm. Acta Helv. 28:131-139.

(380) Sims, R. J. and L. Hilfman. 1956. Stabilisierung von tierischen Nahrungsfetten wahrend des Ausschmelzens. J. Amer. Oil Chemists' Soc. 33:381-383.

(381) Sims, R. J. 1970. Fat antioxidants. U.S. Patent. 3 497 531.

(382) Sinel'nikova, V. N. 1965. Leaf morphology of Chilean forms of Solanum tuberosum. Trudy Prikladnoi Bot Benet Selek. 37(3):37-50.

(383) Sokkary, A. M. E. and M. H. Zaki. 1956. The use of some antioxidants in samna (Butteroil). Ann. Agric. Sci. 1(2):33-48.

(384) Spetsig, L.-O. 1954. A comparison between some polyphenolic antioxidants in a fatwater system. Svensk Kem. Tidskr. 65(4):65-69.

(385) Spirichev, V. B. and N. V. Blazheevich. 1968. The hemolytic action of vitamin D_2. Formation of lipoperoxides. Vop. Med. Khim. 14(4):371-375.

(386) Spirichev, V. B. and N. V. Blazhevich. 1969. Mechanism of the toxic action of vitamin D. Int. Z. Vitaminforsch. 39(1):30-36.

(387) Stawowczyk, A. 1960. Untersuchung der Wirkung des Gallussaureathyl-und -propylesters auf die Bestandigkeit von Rapsol. Acta polon. pharmac. 17:229-236.

(388) Steinkamp, G. 1956. Inhibition of rancidity of refined olive oil by treatment with citric acid. Grasasy aceites. 7:89-93.

(389) Stirton, A. J., et al. 1945. Oxygen absorption of methyl esters of fat acids, and the effect of antioxidants. Oil and Soap. 22(4):81-83.

(390) Stoloff, L. S., et al. 1948. Curb mackerel fillet rancidity with new dip-and-coat technique. Food Inds. 20:1130-1132, 1258.

(391) Strelkov, R. B. and L. F. Semenov. 1966. Radioprotective properties of antioxidants. Radiobiologiya. 6(4):578-579.

(392) Stuckey. 1954. Antioxidants in candy and candy packaging materials. Mfg. Confectioner. 34(6):47.

(393) Stuckey, B. N. 1955. Increasing shelf life of cereals with phenolic antioxidants. Food Technol. 9:585-587.

(394) Stuckey, B. N. and W. M. Gearhart. 1957. Trihydroxybutyrophenone - A food grade antioxidant. Food Technol. 11(12):676-679.

32 *(395) Stuckey, B. N. and C. E. Osborne. 1965. A review of antioxidant analysis in food products. J. of the American Oil Chemists' Society. 42(3):228-232.

(396) Sun, C. -H. 1960. The effect of certain antioxidants on the stability of dry milk. Izvest. Timiryazev. Sel'skokhoz. Akad. 2:121-124.

(397) Swarthout, D. M., et al. 1958. Effect of moisture and antioxidant treatment on shelled English walnuts. Food Technol. 12(11):599-601.

(398) Szilas, E. and G. Palik. 1959. Storage of packed foods. III. Improvement of the shelf-life of margarine by antioxidants. Budapesti Muszaki Egyetem Elelmiszerkemiai Tanszekenek Kozlemenyei. 16-20.

(399) Takeshita, R., et al. 1969. Separation and detection of gallic acid and its alkyl esters by polyamide thin-layer chromatography. Journ. Chromatogr. 45:269-277.

(400) Talyzin, F. F. and L. B. Yurkova. 1968. Change in the nucleic acid level in mouse tissues caused by the venom of Vipera lebetina, antiserum, and propyl gallate. Tr. Univ. Druzhby Nar. 38:171-175.

(401) Tamsma, A. , et al. 1963. Factors related to the flavor stability during storage of foam-dried whole milk. III. Effect of antioxidants. J. Dairy Sci. 46:114-119.

(402) Tanaka, K. 1967. Protective effects of antioxidants against fat rancidity caused by oxidation of fats of frozen fatty whale meat and Pacific saury. Reito. 42(8):618-625.

(403) Tao, W. -H. 1961. Effect of antioxidant on amino acid composition of dry milk proteins. Sb. Stud. Nauchn. -Issled. Rabot Mosk. Sel'skokhoz. Akad. 10:383-385.

(404) Tappel, A. L. , et al. 1953. Effect of temperature and antioxidants upon the lipoxidase-catalyzed oxidation of sodium linoleate. Arch. Biochem. and Biophys. 42(2):293-304.

(405) Tappel, A. L. 1954. Studies of the mechanism of vitamin E action. II. Inhibition of unsaturated fatty acid oxidation catalyzed by hematin compounds. Arch. Biochem. and Biophys. 50(2):473-485.

(406) Tappel, A. L. 1954. Zunehmende Verwendung von Antioxydantien. Food Engng. 26(6):73-75, 133-134.

33 *(407) Tarjan, R., et al. 1965. Effects produced on warm-blooded animals by admixtures and adjuvants contained in foodstuffs of Hungarian origin. Vopr. Pitaniya. 24(5):11-17.

(408) Tarr, H. L. A. 1947. Control of rancidity in fish flesh. I. Chemical antioxidants. Jour. Fish. Res. Bd. Canada.7(3):137-154.

34*(409) Telford, I. R., et al. 1962. Fetal resorption in the rat as influenced by certain antioxidants. Amer. Jour. Anat. 110(1):29-36.

(410) Terrier, J. and J. Deshusses. 1949. Investigation and determination of propyl gallate (progalline P) in edible fats. Mitt. Lebensm. Hyg. 40:221-226.

(411) Ter-Vartanyan, L. S. and N. M. Emanuel. 1962. Inactivation of lactic dehydrogenase by free radicals as inhibitors from radical processes. Dokl. Akad. NaukSSSR. 143:1215-1218.

(412) Thompson, D. T., et al. 1965. Certain organic substances and their effects on the incidence of dental caries in the cotton rat. J. Dental Res. 44(3):596-599.

(413) Thompson, J. W. and E. R. Sherwin. 1966. Investigation of antioxidants for polyunsaturated edible oils. J. Amer. Oil Chemists' Soc. 43(12): 683-686.

(414) Tollenaar, F. D. 1957 (Pub. 1963). Prevention of rancidity in edible oils and fats with special reference to the use of antioxidants. Proc. Pacific Sci. Congr., Pacific Aci. Assoc., 9th Bangkok, Thailand. 5:92-103.

35*(415) Tonkelaar, E. M. den, et al. 1968. The influence of propyl gallate and α-tocopherol on the survival time of rats during fasting. Food Cosmet. Toxicol. 6(1):25-31.

(416) Toyama, K. and K. Saruya. 1955. The protection method of marine products from their deterioration due to the oxidation of oil. IV. Application of some antioxidants to salting of salmon. Bull. Japan. Soc. Sci. Fisheries. 21:248-252.

(417) Toyama, K, et al. 1960. Applicability of antioxidants to marine products 1)-5). Jour. Tokyo Univ. Fish. 46(1/2): 107-131.

(418) Toyama, K. 1962. Reaction mechanism for antioxidants applied to marine products. I. Relation between the efficacy of antioxidant and the degree of unsaturation of substrate oil. Jour. Tokyo Univ. Fish. 48(1):111-118.

(419) Toyama, K. 1962. Reaction mechanism for antioxidants applied to marine products. II. An experimental examination of the relation between the efficacy of antioxidant and the degree of unsaturation of substrate fatty acid. Jour. Tokyo Univ. Fish. 48(1):119-125.

(420) Tsen, C. C. and I. Hlynka. 1962. The role of lipids in oxidation of doughs. Cereal Chem. 39:209-219.

(421) Tye, B.S. 1970. Antioxidants improve food stability. Chemical Processing. 16(5):28-32.

(422) Tyler, A. 1954. Chemical agents and poisonous metals in food and water. Roy. Sanit. Inst. J. 74:985-992, discussion:993-994.

(423) Ueno, R., et al. Konservierungsmittel. U.S.P. 3 097 131:1965. Brit. P. 940 379:1960/1963.

(424) Ugazio, G. and M.V. Torrielli. 1968. Action of propyl-gallate in hepatic steatosis induced by carbon tetrachloride. Boll. Soc. Ital. Biol. Sper. 44(14):1166-1169.

(425) Ugazio, G. and M.V. Torrielli. 1969. Effect of propyl gallate on carbon tetrachloride-induced fatty liver. Biochem. Pharmacol. 18(9):2271-2274.

(426) Uprety, C.M.C., et al. 1963. Stabilization of vitamins in pharmaceutical preparations. VII. Influence of some stabilizers on the stabilities of vitamins B_1 and C. Indian J. Technol. 1:391-394.

(427) Uprety, M.C. and B. Revis. 1964. Elevated temperature studies on stability of ascorbic acid in certain fruit juice and aqueous vehicles. J. Pharm. Sci. 53(10):1248-1251.

(428) Valeyeva, A.N. and M.I. Goryayev. 1967. Influence of antioxidants on stability of melted butter under different conditions of storage. Pishchevaya Tekhnologiya. 6:23-25.

(429) Valeyeva, A.N. and M.I. Goryayev. 1967. Fatty acids of drawn butter stabilized by antioxidants after prolonged storage. Pishchevaya Tekhnologiya. 1968(1):41-44.

(430) Vartanyan, L.S., et al. 1964. Effect of propyl gallate on kinetic constants of the reaction of enzymic reduction of sodium pyruvate. Dokl. Akad. Nauk SSSR. 154(1):223-225.

(431) Vartanyan, L.S., et al. 1964. Kinetics of lactic dehydrogenase inactivation with free-radical products of propyl gallate autoxidation. Izv. Akad. Nauk SSSR, Ser. Khim. 10:1742-1748.

(432) Vigneron, P.Y. and P. Spicht. 1970. Spectrophotometric determination of antioxidants in oils. Revue Francaise des Corps Gras. 17(5):295-302.

(433) Vos, H.J., et al. 1957. Die quantitative Bestimmung der Antioxydantien Propyl-, Octyl- und Dodecylgallat in Olen und Fetten. Analyst 82:362-367.

(434) Voss, J. and W. Muenker. 1966. Application of antioxidants and bactericides in cold storage of herring filets. Fischereiforschung. 4(4):73-84.

(435) Wachs, W. and L. Gassmann. 1970. Determination of gallic acid esters by gas chromatography. Deutsche Lebensmittel-Rundschau. 66(2):37-38.

(436) Waginaire, L. 1963. Autoxidation of fatty substances. Antioxidant test. Gattefosse-SFPA Bull. Tech. 1963(60):51-58.

(437) Walser, R., et al. 1961/1965. Futtermittelmischung. D.A.S. 1 202 625.

(438) Wang, R.T. and S.S. Chou. 1969. Polyamide chromatography of antioxidants. J. Chromatogr. 43(4):522-523.

(439) Wartanjan, L.Ss, et al. 1964. Kinetik der Inaktivierung der Lacticodehydrogenase durch die Radikale der Autoxydation des Propylgallates. Izvest. Akad. Nauk SSSR, Ser. chim. 10:1742-1748.

(440) Wartanjan, L.Ss. and N.M. Emanuel. 1962. Inaktivierung der Lacticodehydrase durch die aus Inhibitoren der Radikalprozesse entstehenden freien Radikale. Doklady Akad. Nauk SSSR. 143:1215-1218.

(441) Wartanjan, L.Ss., et al. Einfluss des Propylgallates auf die kinetischen Konstanten der fermentativen Reduktion des Natriumpyruvats. Doklady Akad. Nauk SSSR.

(442) Weir, C.E., et al. 1960. Einige Faktoren, welche die Stabilitat von Fett in tiefgekuhlten Teigen beeinflussen. Food Res. 25:120-126.

(443) Whetsel, K.B., et al. 1955. Determination of butylated hydroxyanisole and propyl gallate in food antioxidants. J. Am. Oil Chemists' Soc. 32:493-496.

(444) Wieren, W.H. van and J.C. Langberg. 1961/1966. Making soft unripened curd cheeses. U.S. 3,232,768.

(445) Wilson, J.B. 1958. Analysis of flavors and nonalcoholic beverages. J. Assoc. Offic. Agr. Chemists. 41:611-612.

(446) Winkelman, A. 1966. Shelf-life of oils and fats. Pharm. Weekbl. 101:833-841.

(447) Wisniewski, W. and Z. Golucki. 1965. Effect of phenolic and synergetic mixtures on the chemical stability of hydrogenated soybean oil and palm oil. Farm. Polska. 21(12):439-443.

(448) Wurziger, J. and E. Lindemann. 1958. Zum schnellen Nachweis von Antioxydantien im Schweineschmalz. Fleischwirtschaft. 10:405-407.

(449) Wyatt, C.J. and E.A. Day. 1965. Evaluation of antioxidants in deodorized and nondeodorized butteroil stored at 30°. J. Dairy Sci. 48(6):682-686.

(450) Yamada, H. and H. Haga. 1956. Antioxidation of fishes. I. Salmon. Repts. Japan Marine Products Co. Research Lab. No. 7:1-4.

(451) Yudina, L. I. 1962. Use of antioxidants for prevention of oxidation of fat in Atlantic salt herring. Tr. Vses. Nauchn.-Issled. Inst. Morsk. Rybn. Khoz. i Okeanogr. 45:32-35.

(452) Zalewski, S. and H. Karpinski. 1964. Antioxidant action of ascorbic acid during autoxidation of animal fats. Przemysl Spozywczy. 18(3): 27-33.

(453) Zalewski, S. 1966. Activity of antioxidants as influenced by the degree of unsaturation and oxidation of animal fats. Roczniki Technol. Chem. Zywnosci. 12:167-187.

(454) Zalicka, B. 1967. Studies on continuous cultures of pediococci. Acta Microbiol.Pol. 16(2):181-187.

(455) Zathurecky, L. and G. Somoskeoy. 1961. Bewertung der Wirkung von Antioxydantien auf die Haltbarkeit der Salbengrundlagen mit tierischen und pflanzlichen Fetten unter Anwendung der beschleunigten Methode des aktiven Sauerstoffes. Ceskoslov. Farmac. 10:497-506.

(456) Zhedek, M.S., et al. 1963. Stabilization of butter with antioxidants. I. Investigation of antioxidants for continuous-process butter. Izvest. Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhnol. 6:55-58.

(457) Zhedek, M.S., et al. 1963. Stabilization of butter with antioxidants. 2. Effect of antioxidants on the stability of continuous-process butter upon prolonged storage. Izvest. Vysshikh Uchebnykh Zavedenii Pishchevaya Technol. 6:59-63.

(458) Zhil'tsova, V.M., et al. 1966. Kinetics of DNA transformation under the influence of ultraviolet irradiation. Biofizika. 11(3):398-405.

Supplement:

(459) Alvarez Pino, M.I., et al. 1969. A comparative study of colorimetric and chromatographic methods for investigation of phenolic antioxidants in edible fats. Grasas y Aceites. 20(3):129-132.

(460) Pulgar, A.Y. 1953. Antioxidant substances applied to fats. Univ. Chile, fac. quim. y farm., Tesis quim. farm. 5:247-264.

(461) Van Ness, L.W. 1964/1968. Antioxidant compositions for edible fats and oils. U.S. 3,390,098.

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6. If the powder is a mixture of both species of senna it will be possible to find many cells giving a palisade ratio above 10 and many giving a value below 5·0.

I particularly wish to thank Dr. T. E. Wallis, at whose suggestion this work was undertaken, for his continued interest; and for his assistance in providing authentic samples of powder.

REFERENCES

1. Wallis and Forsdike, *Quart. J. Pharm. Pharmacol.*, 1938, 11, 700.
2. Saber, *ibid.*, 1934, 7, 161.
3. Rowson, *ibid.*, 1943, 16, 24.

A STUDY OF THE INHIBITING ACTIONS OF PROPYL GALLATE (NORMAL PROPYL TRIHYDROXY BENZOATE) AND CERTAIN OTHER TRIHYDRIC PHENOLS ON THE AUTOXIDATION OF ANIMAL AND VEGETABLE OILS

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Received 21st June, 1943

Many organic compounds containing olefinic linkages oxidise spontaneously on exposure to atmospheric conditions. The presence of such linkages in fats and oils, therefore, renders these complex organic mixtures susceptible to atmospheric oxidation. When this oxidation process reaches a sufficiently advanced stage the oil is said to be rancid and assumes an unpleasant taste and odour. But, in addition to these objectionable properties, the vitamin content, and hence the nutritive value, of the oil has been shown to decrease with the oxidation of the latter.[1,2,3,4]

These oxidation processes exhibit the characteristics of autocatalytic reactions; an initial slow period at almost constant velocity is followed by a second stage, during which the velocity of reaction increases in a logarithmic manner to a maximum value. A graphic illustration showing the progress of this autoxidative reaction in an oil is given in Figure 1.

The development of rancidity is known to be considerably retarded by the addition of minute quantities of certain compounds to the autoxidisable substance. A wide variety of chemical substances exhibit this antioxygenic property, e.g. certain inorganic acids, phenols, substituted aromatic amines, aliphatic acids, etc., and these compounds are generally referred to as antioxidants. Hydroquinone, catechol, pyrogallol and 1:2:4-hydroxyhydroquinone, are perhaps the best known members of the phenolic group of antioxidants. In the case of phenols, although no definite relationship between antioxidant properties and chemical constitution has yet been established, marked antioxygenic activity seems to be associated with the presence of two or more hydroxyl groups in certain positions of the aromatic nucleus. Thus phenol, resorcinol and phloroglucinol, show no marked retarding influence on autoxidative reactions

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whilst catechol, hydroquinone and pyrogallol are active inhibitors. Gallic acid and its esters belong to this phenolic group of antioxidants. The marked inhibiting action of gallic acid and ethyl gallate on the autoxidation of both animal and vegetable oils has already been demonstrated by Golumbic and Mattill.[5] For the past four years we have also been engaged in an investigation of the antioxidant properties of gallic acid and certain of its esters, and the antioxidative powers of these substances have been compared with those of certain well known antioxidants. From the practical and commercial standpoint, e.g. ease of solubility at relatively low temperatures, acidity and colour, and from the point of view of general effectiveness, normal propyl gallate is in our experience the most outstanding antioxidant of this class of compounds.

In this publication we have confined our remarks to points which are of particular interest in pharmaceutical and medical work. For an antioxidant to be of general use in pharmaceutical preparations it is most desirable for it to possess the following properties:—(1) An effective inhibiting action on the autoxidation of fats and oils. (2) It should be odourless and tasteless and it should not discolour the preparation to which it is added. (3) It should be as nearly neutral in reaction as possible. (4) It should be easily and definitely soluble in oils so that its concentration can be varied to meet the particular conditions to which the oil might be subjected and so that concentrated solutions of the antioxidant in oils can be prepared, which can be subsequently diluted as desired. (5) It must be pharmacologically safe and must not be strongly toxic to animal tissue.

The results embodied in this paper indicate how nearly normal propyl gallate fulfils all these requirements.

PROPYL GALLATE

(Normal Propyl 3:4:5-Trihydroxy Benzoate)

Pure anhydrous propyl gallate is a white-creamy, odourless, solid melting at 146° to 148° C. The *p*H values of aqueous solutions of the ester at different concentrations are shown in the second column of Table I; columns 3 and 4 of this table refer to the *p*H values of aqueous solutions of pyrogallol and gallic acid respectively.

TABLE I

*p*H VALUES OF AQUEOUS SOLUTIONS OF PROPYL GALLATE, PYROGALLOL AND GALLIC ACID AT VARIOUS CONCENTRATIONS

Concentration per cent.	*p*H Values		
	Propyl gallate	Pyrogallol	Gallic acid
0·05	6·3	5·7	3·5
0·10	5·9	5·6	3·4
0·20	5·7	5·6	3·2

The solubilities of the ester in different oils, expressed as percentages w/w, are as follows:—almond oil, 0·8 at 70° C.; cod-liver oil, 0·8 at 80° C.; cottonseed oil, 0·8 at 80° C.; arachis oil, 0·8 at 80° C.; linseed oil, 0·8 at 70° C.; olive oil, 0·8 at 80° C.; sesame oil, 0·8 at 80° C.; coconut oil, 0·8 at 70° C.; castor oil, 1·0 at 70° C.; soya bean oil, 1·0 at 80° C.; cocoa butter,

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0·2 at 45° C. The ester remains dissolved at these concentrations even after cooling down to room temperature. In comparison with pyrogallol and gallic acid, propyl gallate dissolves more quickly in oils than pyrogallol while gallic acid is difficultly soluble and has a comparatively low solubility in most oils.

Experimental

With few exceptions, all the tests herein described have been carried out under ordinary atmospheric conditions. In some instances the onset of rancidity has been accelerated by increasing the temperature, increasing the surface area of the oil exposed, or by subjecting the oil to ultra violet radiation.

The requisite amount of the antioxidant was weighed accurately and placed in a known weight of oil. For liquid oils and certain of the solid fats, solution of the antioxidant was brought about by warming for a short time in an air chamber at 70° C. In the case of some solid fats the antioxidant was incorporated by triturating in a mortar.

The autoxidative reactions of the various oils, which we have examined, have been followed iodimetrically. It is well known that samples of oxidised fat liberate iodine from an acid solution of potassium iodide. This fact has been made the basis of several quantitative methods which have been proposed for the determination of the degree of autoxidative rancidity in oils. Results obtained by these methods have usually been expressed as "peroxide numbers"; we prefer to express our results as "active oxygen numbers" (A.O.N.) because we are of the opinion that peroxides are not the sole primary products of the oxidation of oils, but are probably accompanied by other oxidation products, which are capable of liberating iodine from acidified potassium iodide solution. The active oxygen number may be defined as the number of milligram equivalents of the primary oxidation products, which liberate iodine from an acid solution of potassium iodide, and is given by the expression:—

$$\text{A.O.N.} = \frac{(\text{Actual titration} - \text{Blank}) \times \text{Factor of Thiosulphate} \times 2}{\text{Weight of the oil}}$$

The iodimetric method is undoubtedly the best method of comparing samples of the same oil, which have been subjected to similar conditions of storage, for it is sensitive, generally applicable and fairly easy to reproduce. We have used a modification of the Wheeler method.[6]

About 0·5 gm. of a sample of the oil to be examined is weighed out accurately into a clean, dry, conical flask. The oil is dissolved in 10 mils of a solution of chloroform in glacial acetic acid (ratio 1 : 2 by volume). To this solution is added 1 mil of a saturated solution of potassium iodide and then the flask is allowed to stand in the dark for three minutes, the time being indicated on a stop watch. After this interval, the flask is removed from the dark chamber, 10 mils of distilled water is added and the mixture is titrated against N/500 thiosulphate solution. The thiosulphate solution is run in until the yellow colour of the liberated iodine is nearly discharged. Then 3 drops of a 1 per cent. starch solution are added and the titration is continued until the solution is colourless, which marks the end-point.

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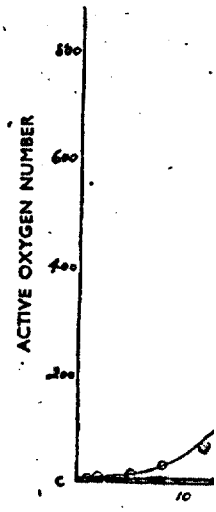

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The oils were well stirred to ensure thorough mixing before removing a sample. The thiosulphate solution was restandardised each day against a standard solution of potassium iodate. Before each series of determinations a blank estimation was carried out using only 10 mils of the glacial acetic acid-chloroform mixture. The apparatus used throughout was cleaned with a strong solution of chromic acid and rinsed thoroughly several times with warm water and finally with distilled water. It was then dried in an air chamber at 110° C.

A Comparison of the Antioxygenic Activities of Propyl Gallate, Gallic Acid and Pyrogallol

We have compared the effectiveness of propyl gallate as an antioxidant with that of other well-known and effective phenolic inhibitors, viz. gallic acid and pyrogallol in lard, and arachis oil (Tables II, III and IV).

The inhibiting effects of the three substances at a concentration of 0·05 per cent. are represented diagrammatically in Figure 1.

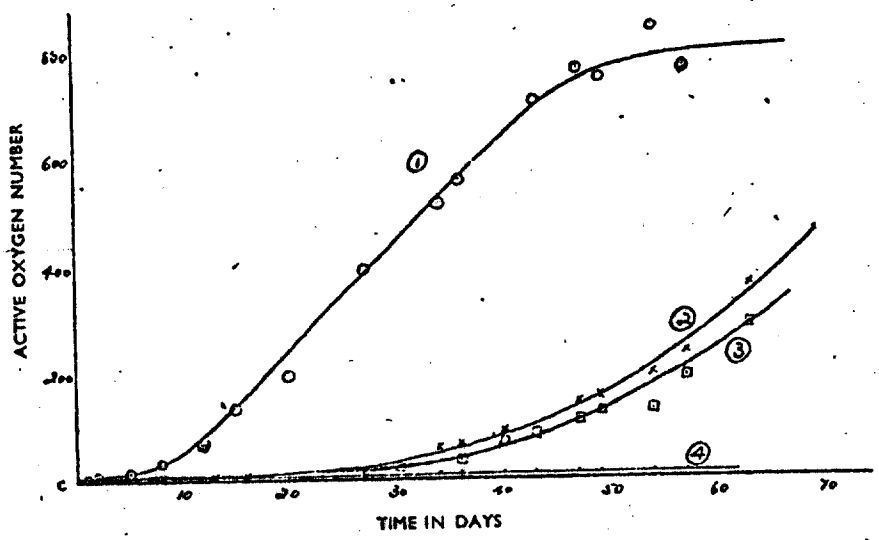

Fig. 1.—The autoxidation of lard and the inhibiting actions of gallic acid, propyl gallate and pyrogallol. (1) control, (2) 0·05 % gallic acid, (3) 0·05 % propyl gallate, (4) 0·05 % pyrogallol.

Samples of lard containing pyrogallol were brown, whereas those containing propyl gallate and gallic acid were perfectly white.

The results indicate that for equal concentrations of these antioxidants the order of effectiveness is:—pyrogallol > propyl gallate > gallic acid.

In general, combinations of propyl gallate and gallic acid, and pyrogallol and gallic acid, show no marked increase in protective influence, certainly no increase comparable with the "synergistic effect" displayed by certain mixtures of "phenolic" and "acidic" antioxidants. This indicates that, although gallic acid, pyrogallol and esters of gallic acid exhibit the

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dual characteristics of the "acid" and "phenolic" type inhibitors,[5] addition of pyrogallol or the ester to an animal fat containing gallic acid does not cause the latter to change its rôle to that of an acidic type inhibitor.

Propylene glycol neither accelerates nor retards the autoxidation of lard. We have found this liquid to be an excellent solvent for these antioxidants, and by using such solutions we were certain of achieving a uniform distribution of the inhibitor throughout the fat substrate. But the results given in Table II indicate that trituration of these solid antioxidants in a mortar is a sufficiently adequate means of introducing the antioxidant into the fat.

TABLE II

LARD

The antioxidants were incorporated with the lard by trituration in a mortar at room temperature.

Conditions:—15 gm. samples of lard were stored in open 4-oz. wide-mouth bottles at room temperature (23° to 25° C.). After twenty-five days the samples were exposed for eight hours each day to ultra-violet light over a period of about six weeks.

Active oxygen number of the freshly prepared lard, 5.

Concentration of antioxidant		Active oxygen number after							
		2 days	8 days	16 days	27 days	34 days	40 days	47 days	57 days
Pyrogallol	0·05 per cent.	5	5	5	5	7	10	11	13
Propyl gallate ..	0·05 "	5	6	13	15	27	69	108	189
Gallic acid	0·05 "	4	7	7	18	60	90	141	233
Gallic acid	0·01 "								
Pyrogallol	0·05 "	6	7	6	5	7	9	10	15
Gallic acid	0·01 "								
Propyl gallate ..	0·05 "	3	6	5	10	11	41	60	107
Propyl gallate ..	*0·05 "	3	6	6	14	19	20	35	136
Gallic acid	*0·05 "	8	8	8	21	45	74	142	338
Gallic acid	*0·01 "								
Propyl gallate ..	0·05 "	7	8	10	20	30	35	86	101
Propylene glycol ..	0·4 "	5	28	83	352	566	634	768	504
Control	..	9	30	161	395	517	692	767	770

* The requisite amount of a 20 per cent. solution of the antioxidant in propylene glycol (w/v) was incorporated with the fat in these instances.

Owing to the very low solubility of gallic acid in this oil we have had to compare its antioxygenic activity in this case with that of higher concentrations of propyl gallate and pyrogallol. We observed that fine particles separated from the samples to which the requisite amount of a 20 per cent. solution of gallic acid in propylene glycol had been added. This fact indicates very definitely that the solubility of gallic acid in this oil is less than 0·01 per cent. Nevertheless, the results demonstrate that even this small, indefinite amount of gallic acid dissolved by the oil exerts an inhibiting action on the autoxidative process. Again, samples of the oil containing pyrogallol were badly discoloured, whereas those containing gallic acid and propyl gallate retained the natural colour of the oil. Of the three substances, pyrogallol again proved to be the most effective inhibitor. Figure 2 is a graphic representation of the autoxidation of arachis oil contained in Petrie dishes and also shows the inhibiting actions of pyrogallol, gallic acid and propyl gallate on this autoxidative process.

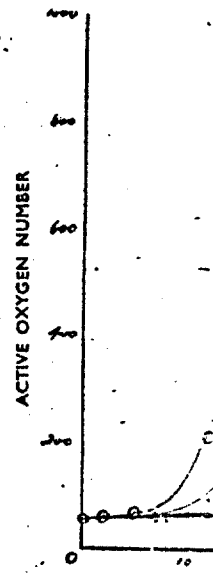

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TABLE III

Arachis Oil

Samples of the oil containing the requisite amounts of the antioxidants were heated for one and a half hours at 70° C. Gallic acid did not dissolve completely and pyrogallol dissolved more slowly than propyl gallate.

Conditions:—For the first twenty-three days, the samples which were contained in open, 4-oz., wide-mouth glass bottles were maintained at room temperature (23° to 25° C.) in ordinary daylight. After this period they were exposed to ultra-violet light for eight hours each day.

Original active oxygen number of the oil, 55.

Concentration of antioxidant		Active oxygen number after				
		7 days	21 days	34 days	41 days	49 days
Pyrogallol	0·05 per cent.	52	60	58	66	72
Propyl gallate ..	0·05 "	55	62	63	81	166
Gallic acid	0·01 "	67	81	145	260	333
Pyrogallol	0·05 "	51	58	61	66	72
Gallic acid	0·01 "					
Propyl gallate ..	0·05 "	55	62	72	91	197
Gallic acid	0·01 "					
Propyl gallate ..	*0·05 "	60	67	71	92	164
Gallic acid	*0·01 "	59	83	109	164	234
Propyl gallate ..	*0·05 "	63	73	79	100	104
Gallic acid	0·01 "					
Propylene glycol ..	0·4 "	71	153	295	497	497
Control	..	74	219	460	600	740

* The requisite amount of a 20 per cent. (w/v) solution of the antioxidant in propylene glycol was added to the oil in these instances.

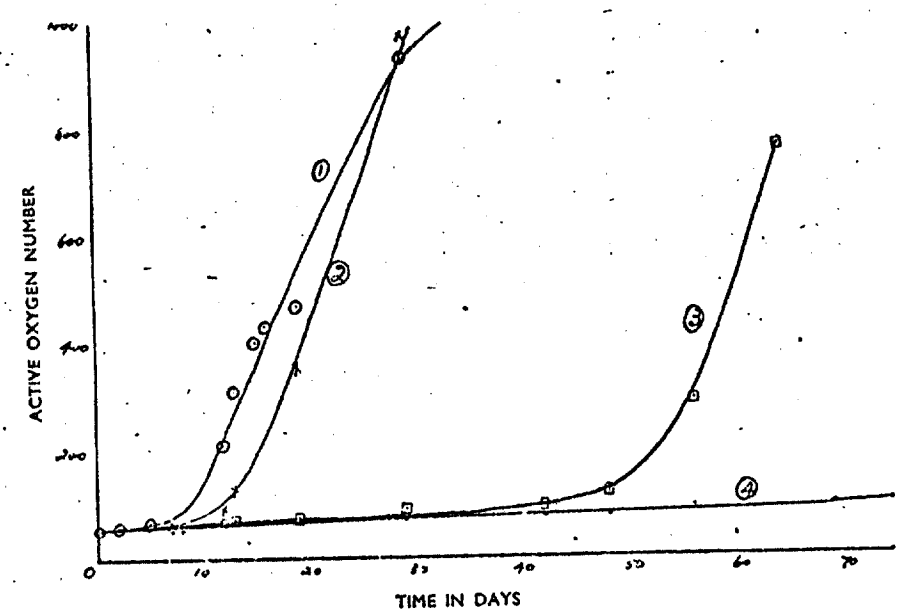

Fig. 2.—The autoxidation of arachis oil and the inhibiting actions of gallic acid, propyl gallate and pyrogallol, (1) control, (2) 0·01 % gallic acid, (3) 0·05 % propyl gallate, (4) 0·05 % pyrogallol.

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Taking into account that pyrogallol is one of the most effective phenolic antioxidants known,[7,8] this graph shows very clearly the profound inhibiting action exerted by propyl gallate on the autoxidation of arachis

TABLE IV

ARACHIS OIL

5 gm. of the oils given in the previous table were placed in petrie dishes after nine days in order to accelerate the autoxidation. The oils were kept at room temperature (23° to 25° C.)

Original active oxygen number of the oil, 55.

Concentration of antioxidant		Active oxygen number after						
		12 days	15 days	19 days	29 days	42 days	48 days	56 days
Pyrogallol	0·05 per cent.	63	66	68	72	76	78	88
Propyl gallate	0·05 "	67	63	75	87	97	120	201
Gallic acid	0·01 "	93	193	355	973	—	—	—
Pyrogallol	0·05 "							
Gallic acid	0·01 "	66	67	69	74	78	86	92
Propyl gallate	0·05 "							
Gallic acid	0·01 "	71	74	80	95	114	129	202
Propyl gallate	*0·05 "							
Gallic acid	*0·01 "	80	87	85	126	860	—	—
Propyl gallate	*0·05 "	135	265	432	1070	—	—	—
Gallic acid	0·01 "							
Propylene glycol	0·4 "	84	90	89	100	115	131	143
Control		207	412	626	1021	—	—	—
		209	401	468	930	1096		

* Requisite amount of a 20 per cent. (w/v) solution of the antioxidant in propylene glycol was added to the oil in these instances.

oil. The results also show that propylene glycol has no influence on the autoxidation. Combinations of the ester and acid, and phenol and acid, show no marked increases in antioxidant power.

A COMPARISON OF THE INHIBITING ACTIONS OF PROPYL GALLATE, GALLIC ACID, PYROGALLOL AND SIAM BENZOIN ON THE AUTOXIDATION OF LARD

The British Pharmacopœia and the British Pharmaceutical Codex recommend the use of 3 per cent. of Sumatra benzoin for the protection of lard against rapid autoxidation, while the United States Pharmacopœia XII specifies the use of 1 per cent. of Siam benzoin. We have compared the retarding influence of Siam benzoin at 0·1 per cent., 1 per cent. and 3 per cent. concentration with that of propyl gallate, gallic acid and pyrogallol at 0·1 per cent. concentration. Table V summarises the results obtained.

The sample containing pyrogallol was brown, while samples containing Siam benzoin were creamy-yellow; the colours of these samples deepened as the oxidation progressed. Furthermore, lard containing Siam benzoin possessed a pronounced, though pleasant, aromatic odour.

As can be seen from the table, gallic acid is relatively ineffective for this specimen of lard under the conditions used in this experiment. With increasing concentration of Siam benzoin, lard becomes increasingly more stable toward autoxidation, but the use of 3 per cent. of Siam benzoin does not show much advantage over 1 per cent. of benzoin; 0·1 per cent. of Siam benzoin is not very effective. This confirms the work of Husa and Riley[9] who have also shown that 1 per cent. of Siam benzoin affords an

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Gallic acid ..	0·1 "
Siam benzoin	0·1 "
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excellent and adequate protection for lard against autoxidative rancidity. The protective influence of 1 per cent. and 3 per cent. of Siam benzoin lies between that of 0·1 per cent. of pyrogallol and 0·1 per cent. of propyl gallate. Brindle and Pedley[10] have shown that the protective action of 1 per cent. of Siam benzoin in lard is greater than that of 3 per cent. of Sumatra benzoin. Thus, the inhibition afforded by 0·1 per cent. of propyl gallate is almost equal to that induced by ten times as much Siam benzoin and probably greater than that afforded by thirty times as much Sumatra benzoin.

TABLE V

LARD

The antioxidants were incorporated with the fat by trituration in a mortar.

Conditions:—These samples were contained in open Petri dishes so as to expose a large surface of the fat to the air. The dishes were kept at room temperature (23° to 25° C.) and exposed to ultra-violet light for five hours each day.

Active oxygen number of the freshly prepared lard, 4.

Concentration of antioxidant		Active oxygen number after								
		3 days	5 days	9 days	11 days	13 days	18 days	20 days	23 days	26 days
Pyrogallol ..	0·1 per cent.	4	7	8	8	9	12	15	16	19
Propyl gallate	0·1 "	5	14	21	33	32	65	101	115	200
Gallic acid ..	0·1 "	43	83	142	162	208	308	313	336	404
Siam benzoin	0·1 "	20	46	109	131	173	324	354	390	471
Siam benzoin	1·0 "	8	15	19	20	24	30	34	35	58
Siam benzoin	3 "	7	13	15	16	20	27	29	34	38
Control	.. "	40	91	221	250	310	432	430	468	528

The conditions under which this experiment was conducted are somewhat more drastic than the ordinary conditions of storage, but it should be pointed out that where a greater inhibition than that afforded by 0·1 per cent. of propyl gallate is required it can be achieved by increasing the concentration of the antioxidant. For lard stored under ordinary conditions, a concentration of 0·05 per cent. of propyl gallate provides adequate protection.

THE INHIBITING EFFECT OF METHYL AND PROPYL GALLATES ON THE AUTOXIDATION OF SUET

Methyl and ethyl gallates, like the parent acid, are difficultly and

TABLE VI

SUET

Original active oxygen number of the fat, 4.

Concentration of antioxidant		Active oxygen number after				
		3 days	24 days	30 days	37 days	44 days
Methyl gallate ..	0·025 per cent.	4	9	12	12	14
Propyl gallate ..	0·025 "	4	8	13	12	14
Control	"	4	10	23	47	123

sparingly soluble in most fats and oils, but they both possess antioxidant properties. The results quoted in Table VI show the protective influences of the methyl and propyl esters on the autoxidation of suet.

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After one hour's heating at 70° C. the propyl ester was completely, while the methyl ester was only partly, soluble. After a further one and a half hours' heating at this temperature the samples were stored at room temperature (20° to 25° C.) in open glass containers.

To illustrate the general effectiveness of propyl gallate as an antioxidant for animal and vegetable oils, experiments have been carried out with representative samples of oils of both classes. We have examined the antioxidative influence of this ester in highly unsaturated oils, quick drying oils, stable oils, whose stability arises from the presence of natural inhibitols, solid vegetable oils, and others. The results shown in Tables VII and VIII show that propyl gallate exerts a considerable protective

TABLE VII

ACTIVE OXYGEN NUMBERS OF OILS AFTER STORAGE IN OPEN PETRI DISHES AT ROOM TEMPERATURE (25° C.)

Effect of propyl gallate

Concentration of propyl gallate per cent.	Control	0·05	0·075	0·10	0·20	0·30
Linseed oil, original active oxygen number, 8; ester dissolved by heating at 80° to 85° C. for ten minutes.						
4 days	36	26	25	24	23	
6 "	47	41	36	30	31	
7 "	57	37	39	38	35	
11 "	62	45	45	44	42	
16 "	80	46	46	45	44	
Cod-liver oil, original active oxygen number, 25; ester dissolved by heating at 70° C. for seven minutes.						
2 days	66		38	37	35	34
8 "	480		91	42	37	34
35 "	1108		182	77	45	36

influence on every oil which we have examined. From the tables it can be seen that with increasing concentrations of the ester the oils become increasingly more stable, but there is a certain optimum concentration which provides sufficient protection for the oil. The increased protection afforded by concentrations higher than this optimum is often so slight as not to merit the use of such high concentrations of the antioxidant, nor, indeed are they necessary for the protection of oils under normal conditions of storage.

PHARMACOLOGICAL ACTIONS OF PYROGALLOL, GALLIC ACID AND PROPYL GALLATE

The toxic nature of pyrogallol precludes the wide use of this substance in pharmaceutical preparations. If absorbed, pyrogallol is toxic to red blood cells and the kidney. The corpuscles are fragmented and large amounts of methaemoglobin are formed. Renal injury is manifested by the presence of albumen, red blood cells and casts in the urine. Concentrated solutions acting on blood *in vitro* (not in the body) produce a peculiar insoluble substance, hemogallol. Although it has been employed in ointment form in the treatment of ringworm, psoriasis, lupus erythematous and other skin disorders, fatal poisoning can occur, simply by application of the drug to the intact skin. It is, therefore, inadvisable to use this drug in cases where an extensive area of skin requires medication.[11]

ACTIVE OXYGEN NUMBERS

The propyl gallate was disso
stored in ½-oz. glass bottles, fi
25° C.)

Concentration of propyl gallate per cent.	Cont
Soya bean oil, original active oxygen	
24 days	1
30 "	2
93 "	6
Cottonseed oil, original active oxygen	
24 days	2
30 "	3
120 "	15
Almond oil, original active oxygen	
8 days	7
22 "	13
101 "	4
Cod-liver oil, original active oxygen	
30 days	1
48 "	2
102 "	5
Coconut oil, original active oxygen	
28 days	
45 "	
240 "	
Linseed oil, original active oxygen	
20 days	
44 "	
380 "	
Palm oil.	
30 days	
47 "	
130 "	

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TABLE VIII

ACTIVE OXYGEN NUMBERS OF OILS AFTER STORAGE IN CLOSED CONTAINERS

Effect of propyl gallate

The propyl gallate was dissolved by warming the oil at 70° C. The samples were stored in ½-oz. glass bottles, fitted with Bakelite caps, at room temperature (23° to 25° C.)

Concentration of propyl gallate per cent.	Control	0·01	0·05	0·10	0·15	0·20
Soya bean oil, original active oxygen number, 2.						
24 days	16	3	3	3		
39 ,, ...	28	7	7	7		
93 ,, ...	65	14	11	10		
Cottonseed oil, original active oxygen number, 3.						
24 days	21	10	7	6	5	4
39 ,, ...	34	15	9	9	8	9
120 ,, ...	152	115	22	20	17	17
Almond oil, original active oxygen number, 30.						
8 days	77	42	31	30	32	32
22 ,, ...	130	57	32	32	33	33
101 ,, ...	441	131	40	40	35	34
Cod-liver oil, original active oxygen number, 53.						
30 days	158		94	68	62	60
48 ,, ...	228		102	74	68	66
102 ,, ...	508		512	114	80	68
Coconut oil, original active oxygen number, 0.						
28 days	2	1	1	1	1	
45 ,, ...	2	1	1	1	1	
240 ,, ...	28	8	5	3	3	
Linseed oil, original active oxygen number, 12.						
29 days	44	42	23	23	23	
44 ,, ...	67	60	30	27	27	
380 ,, ...	102	100	86	36	35	
Palm oil.						
30 days	25	20	13	9	9	
47 ,, ...	38	26	21	9	10	
130 ,, ...	177	170	200	10	12	

An investigation of the pharmacological action of propyl gallate, pyrogallol and gallic acid is still in progress, and it is hoped that a comprehensive survey of their properties will be ready for publication in the near future. Appended to this paper is a report which was recently received from the Pharmacological Laboratories of the College of the Pharmaceutical Society (University of London). The authors wish to express their thanks to Dr. Coward and Dr. Dyer, for permission to publish these results.

In addition to this pharmacological report we would like to record that 0·5 gm. of propyl gallate was administered orally to one of the authors of this paper on six consecutive days. An examination of the urine during this period, and for a further six days, showed that no albumen was present; abnormal sedimental contents were not observed, there being a complete absence of red blood corpuscles and casts of any kind.

There is, therefore, every reason to believe that this ester will prove a safe and harmless substance for internal and external use and, we hope, a valuable antioxidant for use in medicinal and pharmaceutical preparations.

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APPENDIX

Preliminary Report on the Toxicity of Normal Propyl Gallate

Preliminary "acute" tests were carried out to investigate the toxicity by the oral route on mice of normal propyl gallate.

1. Propylene glycol was recommended as a suitable solvent. To check its non-toxicity in mice, control experiments were made in which 0·25, 0·5 and 1·0 mil/25 gm. mouse of propylene glycol were given by stomach tube overnight to groups of mice from which food had been removed. Results appear in Table I.

TABLE I

Propylene glycol

Oral toxicity to Mice

Group	Number of mice	Dose per 25 gm. of mouse	Dose calculated per gm. body weight of mouse	Mice killed		
				1 hour	24 hours	48 hours
1	10	0·25 mil	0·01 mil	None	None	None
2	10	0·5 ,,	0·02 ,,	None	None	None
3	12	1·0 ,,	0·04 ,,	8 (narcotised)	6 dead 3 others affected	1 dead 1 other affected

Observation: Evidently 0·02 Ml/G is a safe dose as solvent.

2. Subsequently, a 10 per cent. w/v solution of normal propyl gallate in propylene glycol was made and administered to groups of mice as before. Observations were again made at frequent intervals and again after 24, 48 and 96 hours. Results are given in Table II, for three different dose-levels.

TABLE II

Normal propyl trihydroxybenzoate (in propylene glycol 10 per cent. w/v)

Oral toxicity to Mice

Group	Number of mice	Dose (actual) Vol. (mils) per 25 gm. mouse	Ester mgm. per gm. of mouse	Effect Mortality of mice		
				1 hour	24 hours	48 hours
1	10	0·25 of 10 per cent. solution	1·0	0/10	2/10	3/10
2	10	0·25 of 20 ,, ,,	2·0	1/10	6/10	6/10
3	6	0·5 of 20 ,, ,,	4·0	3/6	5/6	5/6

Observation: The LD50 is (approx.) 2·0 mgm./gm. of mouse.

3. Control tests were simultaneously made on mice with pyrogallol in propylene glycol (10 per cent. w/v). Results are shown in Table III.

TABLE III

Pyrogallol in propylene glycol 10 per cent.

Oral toxicity to Mice

Group	No. mice	Dose (actual) Vol. (mils) per 25 gm. mouse	Pyrogallol mgm./gm. of mouse	Effect Mortality of mice		
				1 hour	24 hours	48 hours
1	6	0·25 mil of 10 per cent. solution	1·0 mgm.	1/6	3/6	3/6
2	6	0·25 mil of 20 per cent. solution	2·0 mgm.	3/6	5/6	5/6

Observation: The LD50 is (approx.) 1·0 mgm./gm. of mouse.

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1. Fridericia, *J. biol. Chem.*,
2. Mattill, *J. Amer. med. Ass*
3. Cummings and Mattill, *J*
4. Norris, Heuser and Wilg
5. Golumbic and Mattill, *Oil*
6. Wheeler, *Oil and Soap*, 1
7. Mattill, *J. biol. Chem.*, 1
8. Olcott, *J. Amer. chem. So*
9. Husa and Riley, *J. Amer.*
10. Brindle and Pedley, *Qua*
11. Goodman and Gilman, "*P*
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4. *Skin Tests.* Neither propylene glycol itself nor the 10 per cent. w/v solution of normal propyl gallate produced any untoward local lesions on the skin of shaven guinea pigs, whereas 10 per cent. w/v solution of pyrogallol in propylene glycol caused severe erythema in 24 hours.

Two human subjects suffered no untoward skin effects as a result of leaving a 10 per cent. solution of normal propyl gallate in propylene glycol in contact with the skin of the back of the hand for 24 hours.

5. *Experiments in Progress.* The following experiments are in progress and will be reported upon later in the year:—

(*a*) *Rabbits.* Stomach tube administration of propylene glycol and solutions therein of normal propyl gallate, pyrogallol and gallic acid.

(*b*) *Rats.* Chronic tests on the above substances are in progress in order to observe any metabolic and hæmatopoietic effects. These experiments require prolonged periods of time and the results will be reported upon in the early autumn.

Conclusions of Preliminary Investigations (tentative)

(1) Normal propyl trihydroxybenzoate is less toxic than pyrogallol when administered orally to mice (acute tests).

(2) There are no acute skin effects observable when a 10 per cent. solution of normal propyl gallate in propylene glycol is left in contact with shaven guinea pigs' skin for forty-eight hours, or the human skin for twenty-four hours.

This contrasts favourably with the erythematous effect produced under similar conditions by a 10 per cent. solution of pyrogallol in propylene glycol.

References

1. Fridericia, *J. biol. Chem.*, 1924, 62, 471.
2. Mattill, *J. Amer. med. Ass.*, 1927, 89, 1505.
3. Cummings and Mattill, *J. Nutrit.*, 1931, 3, 421.
4. Norris, Houser and Wilgus, *Mem. Correll. Agric. Exp. Sta.*, 1929, 126.
5. Columbic and Mattill, *Oil and Soap*, 1942, 19, 145.
6. Wheeler, *Oil and Soap*, 1932, 9, 89.
7. Mattill, *J. biol. Chem.*, 1931, 90, 141
8. Olcott, *J. Amer. chem. Soc.*, 1934, 56, 2492
9. Husa and Riley, *J. Amer. pharm. Ass.*, 1934, 23, 544.
10. Brindle and Pedley, *Quart. J. Pharm. Pharmacol.*, 1942, 15, 389.
11. Goodman and Gilman, "*Pharmacological Basis of Therapeutics*," 833; Sollmann, "*Manual of Pharmacology*," 658.

CARDAMOM: A STUDY OF ITS SCLERENCHYMATOUS LAYER

By J. W. Fairbairn

(*From the Pharmacognosy Research Laboratory of the College of the Pharmaceutical Society of Great Britain*)

Received 21st June, 1943

Several varieties of cardamom fruits occur in commerce; these varieties can be distinguished with reasonable accuracy by the characters of their pericarps. If, however, the seeds occur separated from their pericarps the

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3. Наиболее эффективным ингибитором процесса окисления при хранении свиного жира, облученного в дозах 1 000 000 и 1 500 000 рад, является бутилоксианизол. Бутилокситолуол в этих условиях не оказывает ингибирующего действия.

ЛИТЕРАТУРА

Карплюк И. А. Вопр. питания, 1960, № 1, стр. 67. — Коробкина Г. С. Вопр. питания, 1954, т. 13, № 5, стр. 33. — Лебедова З. К. Маслобойно-жировая аром., 1958, № 4, стр. 12. — Эмануэль Н. М., Кнорре Д. Г., Лясковская Ю. Н. и др. Мясная индустрия СССР, 1955, № 6, стр. 47. — Anglin C., Mahon J. H., Chapman R. A., J. agric. Food Chem., 1956, v. 4, p. 1018. — Astrack A., Sorbye O., Brasch A. et al., Food Res., 1952, v. 17, p. 571. — Caldwell H. M., Glidden M. A., Kelley G. C. et al., Ibid., 1960, v. 25, p. 139. — Filer L. J., Mattil K. F., Longenecker H. E., Oil and Soap, 1944, v. 21, p. 289. — Golumbic C., Ibid., 1943, v. 20, p. 105. — Hannan R. S. Research on the Science and Technology of Food Preservation by Ionizing Radiations. New York, 1956. — Hougham D. F., Food Technol., 1960, v. 14, p. 170. — Huber W., Brasch A., Waly A., Ibid., 1953, v. 7, p. 109. — Kraybill H. R., Dugan L. R., J. agric. Food Chem., 1954, v. 2, p. 81. — Lundberg W. O., Dockstader W. B., Halvorson H. O., J. Am. Oil. chem. Soc., 1947, v. 24, p. 89. — Mahon J. H., Chapman R. A., Analyt. Chem., 1951, v. 23, p. 1120. — Idem, J. Am. Oil. chem. Soc., 1953, v. 30, p. 34. — Idem, Ibid., 1954, v. 31, p. 108. — Polister B. H., Mead J. F., J. agric. Food Chem., 1954, v. 2, p. 199. — Rutkowski A., Roczn Zakl. Hig. (Warsz.), 1952, стр. 89. — Tarladgis B. G., Younathan M. T., Watts B. M., Food Technol., 1959, v. 13, p. 635. — Tollenaar F. D., Vos H. J., Fette, Seifen, Anstrichmittel, 1956, Bd. 58, S. 112.

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THE EFFECT OF GAMMA-RAYS AND THERMAL PROCESSING ON THE DESTRUCTION OF ANTIOXIDANTS IN LARD

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Summary

There is evidence about thermal destruction of butyloxyanisole and butyloxytoluene in lard and also about dissociation of these antioxidants, depending upon the dose of gamma-irradiation.

The action of heat on lard results in the accumulation of peroxides and in simultaneous dissociation of antioxidants. An increased dose of gamma-irradiation tends to accelerate this process.

ВЛИЯНИЕ АНТИОКИСЛИТЕЛЕЙ ПРОПИЛГАЛЛАТА НА ПИЩЕВУЮ ЦЕННОСТЬ ЖИРА

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Жиры, будучи малостойкими продуктами, при хранении быстро подвергаются порче. Окисленные жиры, как известно, теряют свою пищевую ценность (Н. Д. Бухман, К. М. Левкович; 1953; Н. А. Запольская, Э. Ф. Соловьева, 1953; Б. И. Кадыков, 1959), а в ряде случаев могут оказаться даже токсичными (Creach, 1955; Johnson и Sakuragi, 1956, Kaunitz, Slanetz, Johnson, 1956).

В целях предохранения жиров от окисления прибегают к химическому способу замедления порчи жиров, заключающемуся в применении антиокислителей.

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Эффективность различных антиокислителей с технологической точки зрения неодинакова и зависит, с одной стороны, от природы антиокислителя, а с другой — от вида жира. Как указывает Tollenaar и Vos (1956), в пищевой промышленности ряда зарубежных стран широко используются синтетические антиоксиданты (бутилоксианизол, бутилокситолуол, пропилгаллат). В СССР антиокислители применяют с большой осторожностью после тщательной и всесторонней апробации их. В последние годы во Всесоюзном научно-исследовательском институте жиров занимаются технологической проверкой ряда синтетических антиокислителей. По данным этого института, наиболее эффективным антиоксидантом, значительно повышающим стойкость различных жиров при хранении (при добавлении его в количестве 0,01—0,02%), является пропилгаллат. Для внедрения в жировую промышленность указанного антиокислителя наряду с технологической характеристикой его необходимо в первую очередь решить вопрос о токсичности, а затем и биологической ценности его. Токсикологическую характеристику пропилгаллата всесторонне изучали на кафедре гигиены питания Центрального института усовершенствования врачей (Н. А. Карплюк, 1959). Одновременно в Институте радиационной гигиены приступили к изучению влияния пропилгаллата на пищевую ценность жира. Последняя, по данным Б. И. Кадыкова (1959), Н. Д. Бухман (1959), Н. А. Запольской (1959), Э. Ф. Романовской (1959), М. А. Погорелко (1959), Л. И. Язевой (1959) и др., значительно изменяется от добавления к жиру малых количеств различных веществ (витаминов, эмульгаторов и пр.). В связи с этим можно было предположить, что и пропилгаллат окажет влияние на характеристику жира, меняя весь ход переваривания, всасывания и дальнейшего использования жира в организме.

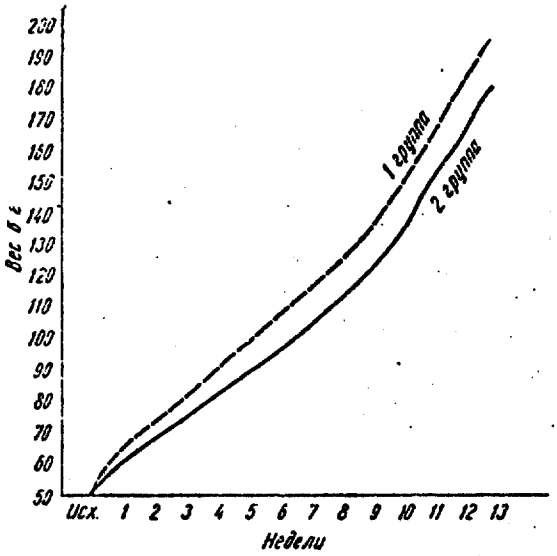

Рис. 1. Изменение веса крыс первой и второй групп.

Изучению подвергалось рафинированное подсолнечное масло, говяжий жир и комбижир. К каждому жиру было добавлено по 0,02% пропилгаллата (концентрация, которая, по данным зарубежных авторов, считается безвредной).

Для решения поставленной задачи было использовано 6 групп белых крыс-самцов, по 35 крыс в каждой группе. Всех животных содержали длительное время ($3^1/_2$ месяца) на так называемом синтетическом питании. В состав диеты входили картофельный крахмал (52% калорийности), казеин (18% калорийности) и тот или иной изучаемый жир (30% по калорийности). Кроме того, все животные получали солевую смесь, дрожжи и рыбий жир и в неограниченном количестве воду. Отдельные группы животных различались тем, что получали жир без антиокислителя и с антиокислителем. Так, крысы первой группы получали в диете говяжий жир, второй группы — тот же жир с 0,02% пропилгаллата, третьей и четвертой группы — соответственно комбижир и комбижир с 0,02% пропилгаллата, пятой и шестой группы — соответственно рафинированное подсолнечное масло и то же масло с добавлением 0,02% пропилгаллата.

В процессах опыта наблюдали за весом и развитием молодых крыс, за выживаемостью животных и использованием ими своих резервных веществ в неблагоприятные для организма моменты, в данном случае — при частичном голодании. По истечении $3^1/_2$ месяцев содержания животных на указанной диете забивали по 5 крыс каждой группы. В их органах и тканях определяли содержание белка и жира (в среднем на

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одну крысу) по методике Бухман и Левкович (1953). Остальных животных всех групп переводили на частичное голодание (безжировая диета и уменьшение калорийности рациона в 4—5 раз по сравнению с исходной), на котором и содержали их до момента гибели. В тканях и органах погибших крыс также определяли содержание белка и жира.

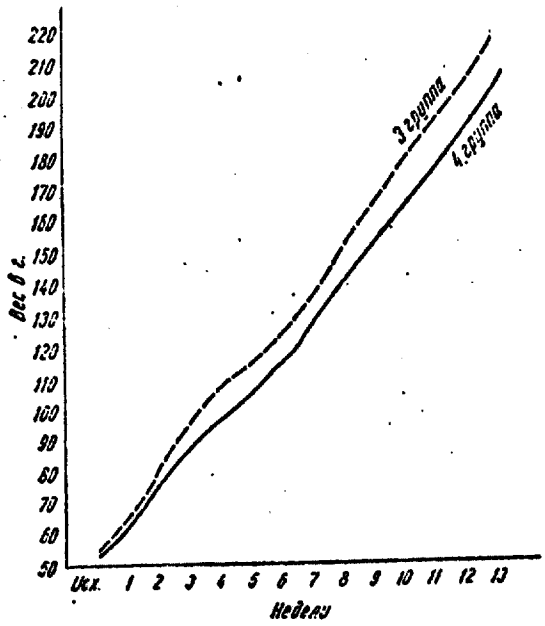

Рис. 2. Изменение веса крыс третьей и четвертой групп.

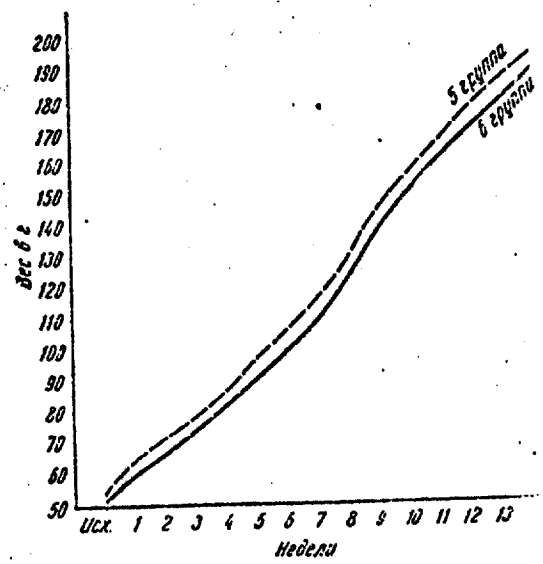

Рис. 3. Изменение веса крыс пятой и шестой групп.

Биологическое наблюдение показало, что у молодых животных всех групп, получавших жир с пропилгаллатом, несколько задерживался рост, снижалась прибавка веса. Так, из рис. 1 видно, что привес крыс, получавших говяжий жир, составил за период наблюдения 143 г, а привес животных, получавших тот же жир с антиокислителем, — 126 г.

Крысы, содержавшиеся на комбижире, прибавили в весе 159 г, а крысы, получавшие тот же жир с антиокислителем, — 142 г (рис. 2) Меньшее различие в приросте веса наблюдалось при питании животных рафинированным подсолнечным маслом с антиокислителем и без него (рис. 3).

Аналогичные данные о привесе крыс были получены И. А. Карплюк (1959) при скармливании животным свиного жира с добавлением к нему пропилгаллата.

Наблюдения в период частичного голодания животных показали, что все крысы, получавшие в рационе пропилгаллат, были заметно слабее и средняя продолжительность их жизни была значительно меньшей, чем тех животных, которые получали чистые жиры (табл. 1).

Как указано выше, при решении вопроса о биологической ценности жира с антиокислителем нас интересовало, в какой степени полно используются резервные вещества в организме животных отдельных групп в период частичного голодания.

Как видно из табл. 2, у всех животных, получавших в рационе жир с добавлением пропилгаллата, процент использования резервных веществ в организме, особенно белка, заметно снижался.

Из приведенных данных следует, что животные, получавшие обычные жиры, выдерживали частичное голодание в течение большего про-

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Таблица 1

Средняя продолжительность жизни животных различных групп в период частичного голодания

Группа животных	Средняя продолжительность жизни (в днях)
Первая — рацион с говяжьим жиром	27,0
Вторая — рацион с тем же жиром и 0,02% антиокислителя	13,6
Третья — рацион с комбижиром	28,5
Четвертая — рацион с тем же жиром и 0,02% антиокислителя	16,0
Пятая — рацион с рафинированным подсолнечным маслом	25,0
Шестая — рацион с тем же маслом и 0,02% антиокислителя	14,5

Таблица 2

Использование резервных веществ организма у различных групп животных в период частичного голодания

Группа крыс	Содержание жира		Использование жира (в %)	Содержание белка		Использование белка (в %)
	к концу нормального питания	к моменту гибели		к концу нормального питания	к моменту гибели	
	(в среднем на 1 крысу)			(в среднем на 1 крысу)		
Первая—рацион с говяжьим жиром	25,9	1,0	95,9	36,5	22,3	38,3
Вторая—рацион с тем же жиром и 0,02% антиокислителя	18,7	1,1	93,3	32,1	24,6	23,4
Третья—рацион с комбижиром	34,4	1,0	97,0	37,9	26,2	30,8
Четвертая — рацион с тем же жиром и 0,02% антиокислителя	33,6	1,3	96,0	35,7	29,3	18,4
Пятая — рацион с рафинированным подсолнечным маслом	25,9	1,3	95,0	37,2	25,8	33,6
Шестая—рацион с тем же маслом и 0,02% антиокислителя	18,1	1,3	92,8	37,6	28,4	24,5

межутка времени. При этом они медленнее, следовательно экономнее, расходовали свои тканевые белки. Однако благодаря большей длительности жизни к моменту гибели у них расходовалось больше белка и степень использования его была выше.

Животные же, получавшие жиры с добавлением антиокислителя, оказались менее устойчивыми к частичному голоданию. Гибель их наступала при меньшей степени использования тканевых жиров и белка, чем у контрольных животных.

Из предыдущих наших работ известно, что менее совершенное использование белка всегда сопровождается повышением окислительного коэффициента мочи, указывающего на накопление в организме большого количества токсических, недоокисленных продуктов межуточного обмена (Н. Д. Бухман, 1959). На основании данных настоящей работы можно высказать предположение, что наличие в диете крыс антиокислителя вызывает снижение окислительных процессов в их организме. И. А. Карплюк (1959) в своих выводах так же указывает, что фенольные антиокислители, к которым относится и пропилгаллат, пони-

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жают уровень окислительных процессов в организме и далеко не безразличны для организма животных.

Проведенная работа вновь показывает, что наличие в изучавшихся жирах (говяжий жир, комбижир и рафинированное подсолнечное масло) 0,02% пропилгаллата значительно снижает их биологическую ценность.

Мы считаем, что использование пропилгаллата как антиоксиданта в жировой промышленности нецелесообразно. Весьма желательно в качестве антиокислителей применять биологически полноценные вещества. В настоящее время в литературе появились указания, что хорошими антиоксидантами являются: пиридоксин (Sakuragi, Kummerow, 1958), фосфатиды, аскорбиновая кислота и витамин Р (Н. В. Новотельнов, М. Т. Головкина, Э. Р. Ставрова, 1959).

ЛИТЕРАТУРА

Бухман Н. Д., Левкович К. М. Вопр. питания, 1953, № 3, стр. 49 — Бухман Н. Д. Труды 1-й научной сессии по вопросу: «Проблема жира в питании». Л., 1959, стр. 124. — Запольская Н. А., Соловьева Э. Ф. Вопр. питания, 1953, № 4, стр. 47. — Запольская Н. А. Труды 1-й научной сессии по вопросу «Проблема жира в питании». Л., 1959, стр. 179. — Кадыков Б. И. Труды 1-й научной сессии по вопросу «Проблема жира в питании». Л., 1959, стр. 3. — Карплюк И. А. Вопр. питания, 1959, № 14, стр. 24. — Новотельнов Н. В., Головкина М. Т., Ставрова Э. Р. Научн. докл. Высшей школы, биол. науки, 1959, № 1, стр. 137. — Погорелко М. А. Труды 1-й научной сессии по вопросу «Проблема жира в питании». Л., 1959, стр. 214. — Романовская Э. Ф. Там же, стр. 67. — Язева Л. И. Труды 1-й научной сессии по вопросу «Проблема жира в питании». Л., 1959, стр. 210. — Creach M. P., Oléagineux, 1955, v. 3, p. 161. — Johnson O. C., Sakuragi T., J. Am. Oil. Chem. Soc., 1956, v. 33, p. 433. — Kaunitz H., Slanetz C., Johnson R. E., Ibid., p. 630. — Sakuragi T., Kummerow F., Ibid., 1958, v. 35, p. 401. — Tollenaar E. D., Vos H. J., J. Fette, Seifen, Austrichmittell, 1956, v. 58, p. 412.

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THE EFFECT OF PROPYLGALLATE ANTIOXIDANT UPON THE NUTRITIONAL VALUE OF FATS

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Summary

The object of the work is to elucidate the influence exerted by propylgallate antioxidant on the nutritional value of fat. Investigation was conducted on growing albino male rats, kept on synthetic dietary. Additions to fats (refined sunflower seed oil, beef fat, mixed fat) of propylgallate amounting to 0.02% tended to lower the nutritional value of the fats under test — trial. Animals, which were given fat with additions of antioxidant, showed markedly lower gains of weight and delayed development. At the time of partial fasting they failed to adequatly utilize their fat and protein resources and succumbed at an earlier date. Basing upon these data the author holds it inexpedient to make use of propylgallate as an antoxidant in fat-producing industry.

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УДК 613.268+613.288]:664.3.033.5

ВЛИЯНИЕ АНТИОКИСЛИТЕЛЕЙ ПИЩЕВЫХ ЖИРОВ ПРОПИЛГАЛЛАТА И БУТИЛОКСИАНИЗОЛА НА ЖИВОТНЫЙ ОРГАНИЗМ

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Жиры и содержащие их продукты подвергаются быстрой порче главным образом из-за окисления жиров даже при хранении их при температуре ниже 0°. Окисляются в первую очередь высоконепредельные жирные кислоты и жирорастворимые витамины с большой физиологической активностью.

Появляются работы, свидетельствующие о токсическом действии ряда продуктов окисления, таких, как перекиси, альдегиды, кетоны, полимеры. Это и объясняет заинтересованность, которая проявляется к процессам, происходящим в жирах, и способам их защиты от порчи.

Одним из наиболее эффективных способов защиты жиров от окисления является химический. За последнее время для этой цели предложено множество соединений и их сочетаний, получивших названия антиоксидантов или антиокислителей. Однако многие из них не отвечают строгим требованиям врачей и органов санитарного надзора. Иллюстрацией к изложенному выше могут служить цифры, приведенные Крейбилом и Дуганом [1]: только в США за 10 лет (с 1944 по 1954 г.) было выдано 600 патентов на предложения веществ и их сочетаний в качестве антиокислителей пищевых жиров. Антиокислители и различные их комбинации применяются для добавления в пищевые продукты более чем в 20 странах. Во многих из них, таких, как США, ЮАР, Финляндия, Швеция, Югославия, Канада, используется значительное количество различных антиокислителей (до 25 наименований). В Советском Союзе разрешено применение в пищевых продуктах двух антиокислителей: бутилоксианизола и бутилокситолуола.

Многие антиокислители могут усиливать свой эффект в сочетании с другими веществами. Этот синергизм проявляется и в смесях различных антиокислителей. Исследуя различные смеси антиокислителей, Герхарт и Стакей [2] пришли к выводу, что наилучшей является комбинация бутилоксианизола (БОА) и пропилгаллата (ПГ). Н. Эмануэль, Д. Кнорре, Ю. Лясковская и В. Пиульская [3] показали, что наибольший синергетический эффект ПГ проявляет в концентрации 0,005%, а БОА — в 0,01%. Крейбил и Дуган [1] нашли, что если к 0,01% БОА прибавить 0,003% ПГ и 0,002% лимонной кислоты, то стабильность исследуемого жира увеличится вдвое по сравнению с жиром, содержащим один БОА, и в 13 раз по сравнению с контрольным жиром без антиокислителя.

Возможность добавлять меньшее количество антиокислителей (благодаря их синергизму) весьма заманчива. И. Смородинцев считает, что целесообразнее добавлять как можно меньшее количество фенольных антиокислителей, но усиливать их действие введением одного или нескольких синергистов.

Грахам и соавторы [4] изучали смесь ТЕНОКС-2, состоящую из 20% БОА, 6% ПГ, 4% лимонной кислоты и 70% пропиленгликоля (как растворителя). Двум группам крыс скармливали дозы ТЕНОКС-2, увеличенные в сравнении с возможным попаданием в орган[illegible] человека в 6 и 300 раз. Было выявлено, что комбинация БОА и ПГ [illegible]зывала токсического действия на животных, наблюдение за которыми велось в течение года. Смесь ТЕНОКС-2 разрешена к применению в США для стабилизации рыбьего жира, а также жира в свином мясе и мясе индеек.

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Опубликованные в зарубежной литературе данные о токсичности смеси антиокислителей БОА и ПГ недостаточны, так как, согласно представленным материалам, наблюдалось действие смеси лишь на некоторые общебиологические показатели.

В связи с изложенным выше, а также потому, что в СССР токсичность смеси не изучалась, для полной токсикологической характеристики смеси антиокислителей нами проведены острые, подострые и хронические опыты.

Целью острых опытов было определение LD_{50} смеси.

Затравку проводили раствором антиокислителей (соотношение БОА к ПГ — 2 : 1). Затравочная смесь состояла из 20% БОА, 10% ПГ и 70% подсолнечного масла в качестве растворителя. Затравку осуществляли на 7 группах белых крысцев (по 10 животных в каждой группе, средний вес животных 100—120 г) путем введения смеси антиокислителей непосредственно в желудок через металлический зонд. LD_{50} была рассчитана по методу Миллера и Тейтнера[1]. Результаты представлены на рис. 1.

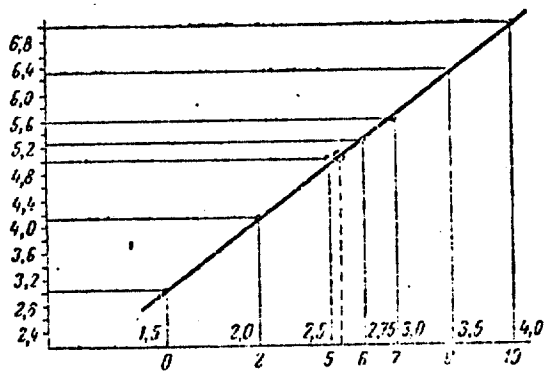

Рис. 1. Определение LD_{50} методом пробит-анализа. На оси ординат — линейные выражения пробитов, на оси абсцисс сверху — испытываемые дозы (в г/кг), снизу — полученный результат (число погибших животных).

О — графическое выражение LD_{50} смеси антиокислителей.

Полученная LD_{50} для смеси антиокислителей равна 2633±140 мг/кг; она оказалась между LD_{50} БОА (2950 мг/кг) и ПГ (2500 мг/кг). Эти данные показали, что смесь БОА и ПГ в острых опытах не проявляет синергетического действия и что смесь БОА и ПГ может быть причислена к слаботоксичным веществам.

Подострые опыты были проведены на молодых белых крысах-самцах и самках весом 100—120 г. В опыте было 40 животных (20 самцов и 20 самок). Цель подострых опытов — изучение и выбор наиболее чувствительных тестов для проведения хронических опытов.

Скармливание проводили в течение 2½ месяцев. Смесь антиокислителей давали в свином топленом жире с молотым кормовым брикетом № 1. Количество антиокислителей аггравировали в 500 раз по сравнению с количеством, которое может попасть в организм человека: БОА — 100 мг/кг; ПГ — 50 мг/кг.

В течение опыта ежедневно проводились наблюдения за взаимосвязью между поеданием корма и внешним видом животных опытной и контрольной групп, вес определялся через каждые 10 дней.

Исходя из того, что антиокислители в живом организме, возможно, оказывают влияние на процессы окисления, мы исследовали вакат-кислород крови, а также активность каталазы и холинэстеразы крови.

Прерывая окислительные процессы в жирах или тормозя их, антиокислители могли повлиять на жировой обмен в организме подопытных животных, поэтому мы изучали количество кетоновых тел в крови. Кроме того, исследованию были подвергнуты диастаза мочи и сахар крови.

Животные находились в опыте от 60 до 70 дней. Исследования проводились на 20-й, 40-й, 60-й день затравки. При обработке материала за статистически достоверные результаты принимались те, в которых $P < 0,05$.

На протяжении всего опыта у животных не было отмечено изменений в поведении, внешнем виде и поедании корма. Подопытные и контрольные животные одинаково хорошо прибавляли в весе. На 20-й день затравки несколько снижалась активность холинэстеразы ($P < 0,02$). В дальнейшем, видимо, происходила адаптация организма к введению

[1] Цит. Л. М. Беленький. Элементы количественной оценки фармакологического эффекта. Л., 1963, стр. 63.

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антиокислителей, и разница между животными опытной и контрольной групп нивелировалась (табл. 1). Такой эффект был выявлен только у самок. У самцов разница между контролем и опытом не была отмечена.

Исследования каталазной активности показали, что антиокислители вначале ингибируют действие этого фермента (на 20-й день), а затем

Таблица 1

Активность холинэстеразы крови

Животные	Дни исследования	Контроль			Опыт			M_0-M	m_{M_0-M}	t	P
		n	M	m_M	n_0	M_0	m_M				
Самцы	До опыта	10	1,22	0,024	10	1,23	0,02	0,01	0,032	0,31	>0,5
	20	10	1,26	0,02	10	1,26	0,015	0	0,022	0	—
	40	10	1,27	0,02	10	1,27	0,012	0	0,016	0	—
	60	10	1,32	0,02	10	1,34	0,014	0,02	0,026	0,79	>0,25
Самки	До опыта	10	1,30	0,03	10	1,31	0,007	0,01	0,03	0,3	>0,5
	20	10	1,33	0,007	10	1,30	0,003	0,03	0,011	2,5	<0,02
	40	10	1,29	0,01	10	1,29	0,007	0	0,012	0	—
	60	10	1,34	0,03	10	1,35	0,033	0,01	0,043	0,25	>0,5

его активность возрастает (на 40-й день). Эти сдвиги также отмечены только у самок (рис. 2).

Аналогичную динамику действия мы обнаружили в отношении сахара крови: вначале происходит уменьшение количества сахара, а затем

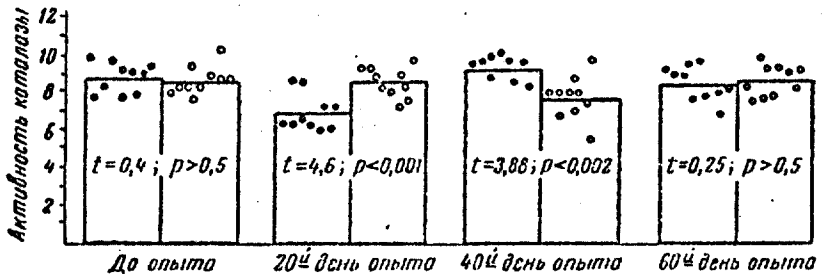

Рис. 2. Активность каталазы, выраженная в показателе каталазы (самки).

Темные кружки — опыт; светлые кружки — контроль; сплошная линия — средняя величина.

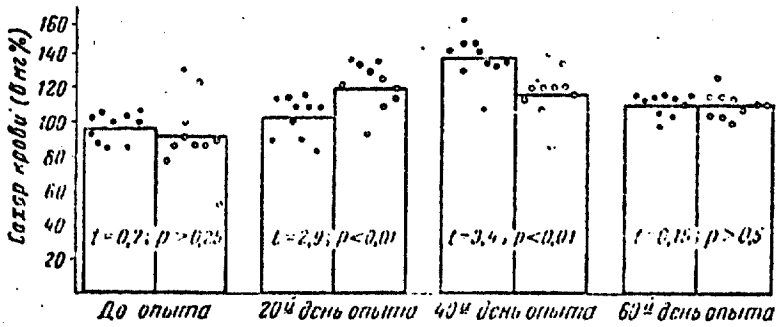

Рис. 3. Сахар крови (самцы). Условные обозначения те же, что на рис. 2.

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увеличение (рис. 3). Эти изменения наблюдались только у самцов. У самок разницы между опытом и контролем не было.

На 40-й день затравки нами было обнаружено статистически достоверное увеличение кетоновых тел у самцов ($P<0,02$) и статистически достоверное уменьшение кетоновых тел у самок ($P<0,02$). Эти данные представлены в табл. 2. Изменений в вакат-кислороде крови не было отмечено.

Таблица 2

Кетоновые тела крови

Животные	Дни исследования	Контроль			Опыт			$M-M_0$	m_{M_0-M}	t	P
		n	M	m_M	n_0	M_0	m_M				
Самцы	До опыта	10	9,02	1,87	10	10,04	1,7	1,02	2,62	0,4	>0,5
	20	10	8,35	1,77	10	11,56	0,999	3,21	2,02	1,57	>0,1
	40	10	5,94	1,30	10	14,90	3,03	8,96	3,32	2,66	<0,02
	60	10	8,53	1,70	10	10,40	0,92	1,87	1,90	0,9	>0,25
Самки	До опыта	10	7,68	0,50	10	8,09	0,60	0,41	0,81	0,2	>0,5
	20	10	6,63	0,919	10	7,27	0,26	0,64	1,24	0,51	>0,5
	40	10	10,00	1,16	10	6,27	0,90	3,73	1,43	2,6	<0,02
	60	10	7,86	0,81	10	8,60	0,40	0,74	0,91	0,81	>0,25

Антиокислители также не оказали влияния на поджелудочную железу: исследования диастазной активности мочи и активности липазы непосредственно в поджелудочной железе не показали разницы между животными контрольной и опытной групп.

После опытного периода всех животных забивали обезглавливанием. Проведенные исследования гликогена печени не выявили различий между контролем и опытом. Отношение веса печени к весу тела показало тенденцию к увеличению этого отношения у опытных животных ($P<0,1$).

Выводы

1. БОА и ПГ в отдельности, а также смесь этих антиокислителей не относятся к сильнотоксичным веществам.

2. В действии смеси антиокислителей БОА и ПГ на организм в острых опытах не проявляется ни синергизм, ни антагонизм.

3. Большие концентрации смеси антиокислителей (БОА — 100 мг/кг и ПГ — 50 мг/кг) не безразличны для организма. Они влияют на активность каталазы и холинэстеразы крови, количество кетоновых тел и сахара крови, что свидетельствует об их отрицательном действии на организм.

4. Самки белых крыс оказались более чувствительными к введению смеси антиокислителей, чем самцы.

5. Для разрешения применения антиокислителей БОА и ПГ (в смеси) в пищевых жирах и жиросодержащих продуктах необходимо проведение исследований в условиях хронической затравки животных, что и является предметом исследования в настоящее время.

ЛИТЕРАТУРА

1. Kraybill H. R., Dugan L. R., J. Agr. Food Chem., 1954, v. 2, p. 81.— 2. Gearhart W. M., Stuckey B. N., J. Am. Oil Chem. Soc., 1955, v. 32, p. 386.— 3. Эмануэль Н., Кнорре Д., Лясковская Ю. и др. Мясн. индустр. СССР, 1958, № 2, с. 52.— 4. Graham W. D., Feed H., J. Pharm. Pharmacol., 1954, v. 6, p. 543.

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THE EFFECT PRODUCED BY PROPYLGALLATE AND BUTYLOXYANISOL — ALIMENTARY FATS ANTIOXYDANTS — ON THE ANIMAL ORGANISM

M. A. Daniyalov (Moscow)

Summary

Acute and subacute experiments were instituted to study the toxicity of mixed antioxydants — propylgallate and butyloxyanisol (taken in proportion of 1:2). The LD_{50} of the mixture was found to equal 2633±140 mg/kg. In subacute tests the animals were given a mixture of antioxydants aggravated 300-fold as compared to a possible penetration into the human organism (butyloxyanisol — 100 mg/kg, propylgallate — 50 mg/kg). The mixture of the studied antioxydants produced a negative effect of the blood enzymes—catalase and cholinesterase, as well as upon the ketone bodies and blood sugar.

УДК 614.31:[63+631.42]:615.778.3-099-014.3+614.7:
615.778.3-014.1

ПРИМЕНЕНИЕ ТОНКОСЛОЙНОЙ ХРОМАТОГРАФИИ ДЛЯ ОПРЕДЕЛЕНИЯ ГЕПТАХЛОРА, ДДТ И ЛИНДАНА В РАСТИТЕЛЬНОМ МАТЕРИАЛЕ И ПОЧВЕ

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В нашей стране для борьбы с вредителями сельскохозяйственных культур широко применяются различные инсектициды, в том числе и хлорорганические. Известно, что многие хлорорганические инсектициды обладают резко выраженными кумулятивными свойствами, т. е. опасны с точки зрения хронического отравления ими. В связи с этим большую актуальность приобретает определение этих ядохимикатов в пищевых продуктах и почве. Однако установить наличие их в биологических объектах очень трудно из-за отсутствия приемлемых методов очистки экстракта от веществ, мешающих определению инсектицидов.

В недавно появившейся работе Эббота и Томпсона [1] указывается способ очистки пестицидов на пластинке, имеющей форму трапеции, в тонком слое сорбента силикагель — кизельгур. Быстрота, эффективность и простота выполнения побудили нас применить тонкослойную хроматографию для очистки экстракта, содержащего гептахлор, ДДТ и линдан. Сорбентом служил силикагель марки «КСК». Очистку экстракта осуществляли на стеклянной пластинке трапециевидной формы. Преимуществом такой формы пластинки является то, что наряду с очисткой она позволяет и концентрировать инсектициды. Из неокрашенных зон силикагеля инсектицид элюировали н-гексаном.

В элюате представлялась возможность определять содержание инсектицидов общеизвестными методами (хроматографическим, колориметрическим, спектрофотометрическим и др.).

Приготовление пластинок с закрепленным слоем сорбента

Силикагель марки «КСК» размалывают на шаровой мельнице или растирают в ступке. Размолотый силикагель обрабатывают соляной кислотой (1 : 1); через сутки кислоту декантируют и силикагель заливают свежей порцией кислоты. Эту операцию

повторяют до тех пор,
гель отмывают дистил
тем дважды промывае
ванной водой (до ней
ют метанолом при нагр
силикагель подсушива
верстий 0,04—0,06 мм.
ликагеля с величиной
фракцию, но с величин
при 120°, затем охлаж
товленный силикагель
Слой силикагеля
ристого кальция (20%
ра осадок промывают
в течение 48 часов при
Для очистки обр
следующих размеров:
5 см. На пластинку н
готовленную из расчет
стинки. Пластинку рас
был толще (до 300 мк
в термостат на 1 час и
Разделение веще
рые наносят силикагел
силикагеля и 3 г гипса
ют в склянке с притер
вынимают, при этом н
ния хлороформа (чер
жат (сорбентом вниз)
вируют 10 мин. в сушн

Экстракция инсект

Навеску иссле
количеством безво
бу и заливают пе
бы навеска была
встряхивания на
отделяют от расти
сы или почвы. Ос
промывают петр
ром. Слитый в одн
ракт концентрируе
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края (рис. 1).
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3. Скармливание прогретого хлопкового масла приводит к понижению содержания непредельных жирных кислот в сыворотке крови (за исключением линоленовой кислоты) и повышению их содержания в печени (кроме той же кислоты).

ЛИТЕРАТУРА

1. Исмаилов М. Н. Вопр. питания, 1963, № 5, с. 34.—2. Он же. Там же, 1964, № 6, с. 62.—3. David T., William H., Leo T. et al., J. Am. Oil Chem. Soc., 1961, v. 38, p. 253.—4. Holman R. T., Hayes H., Analyt. Chem., 1958, v. 30, p. 1422.—5. Леутский К. М., Любович Е. И. Докл. АН СССР, 1954, т. 96, № 2, с. 341.

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EFFECTS PRODUCED BY COTTON SEED OIL AND MUTTON FAT, SUBJECTED TO HEAT TREATMENT, ON THE LEVEL OF CERTAIN FATTY ACIDS IN THE BLOOD AND LIVER OF EXPERIMENTAL ANIMALS

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Summary

Protracted experiments were conducted on growing albino male rats to study the action of qualitatively different fats upon the polyunsaturated fatty acids content in the blood serum and the liver. As distinct from the mutton fat the cotton seed oil was found to raise the level of polyunsaturated fatty acids in the blood serum of rats. The liver of rats receiving cotton seed oil contained 2.5—5 times as much of unsaturated fatty acids as that of rats kept on a mutton-fat diet. Feeding on a heated cotton seed oil results in the reduced level of polyunsaturated fatty acids in the blood serum and in the rise of their content in the liver of rats (exept for linolenic acid).

УДК 613.291 : [613.268+613.288]

ГИГИЕНИЧЕСКАЯ ОЦЕНКА СМЕСИ АНТИОКИСЛИТЕЛЕЙ ПИЩЕВЫХ ЖИРОВ БУТИЛОКСИАНИЗОЛА И ПРОПИЛГАЛЛАТА

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Проблема предохранения жиров от порчи имеет большое народнохозяйственное значение. В связи с тем, что даже при относительно низких температурах происходит порча жиров (главным образом в результате окисления), сроки их хранения ограничиваются несколькими месяцами. При окислении изменяются не только органолептические показатели (вкус, цвет, запах жиров), но они утрачивают и ценные составные части — жирорастворимые витамины и высоконепредельные жирные кислоты, подвергающиеся окислению в первую очередь.

Помимо того, экспериментальные работы ряда авторов [1, 2, 3, 4] свидетельствуют о токсическом действии продуктов окисления жиров — перекисей, альдегидов, кетонов, полимеров. Некоторые работы говорят о том, что гретые жиры, так же как и окисленные, могут вызывать развитие опухолей в организме [5, 6, 7].

В последние годы для предохранения жиров от окисления предложены химические соединения, получившие название антиоксидантов или

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антиокислителей. Это — химические вещества, способные прерывать цепную реакцию окисления в жирах; добавляются они в небольшом количестве (0,01—0,02%).

Исследуя различные смеси антиокислителей, gearhart и stuckey [8] пришли к заключению, что наиболее эффективной является смесь пропилгаллата и бутилоксианизола; эта смесь в 2 раза эффективнее, чем каждый компонент в отдельности. Н. Эмануэль с соавторами [9] установили, что наибольший синергический эффект эти вещества дают в концентрациях: бутилоксианизол — 0,01% и пропилгаллат — 0,005%.

В СССР для стабилизации пищевых жиров разрешено применение бутилокситолуола и бутилоксианизола. Пропилгаллат признан небезвредным для организма и к применению в продуктах питания не рекомендован [10]. Мы в исследованиях применяли этот антиокислитель в дозах в 4 раза меньших, чем дозы, оказывавшие вредное действие.

Для изучения токсичности смеси антиокислителей бутилоксианизола и пропилгаллата нами были проведены острый, подострый и хронический эксперименты.

Целью острых опытов являлось определение $ЛД_{50}$ смеси антиокислителей бутилоксианизола и пропилгаллата в соотношении 2 : 1. Целью подострых опытов было выявление наиболее чувствительных тестов для использования их в хронических опытах.

При выборе тестов мы исходили из результатов работ, проводимых на нашей кафедре, допуска, что антиокислители и в живом организме действуют на окислительно-восстановительные процессы и жировой обмен.

В острых опытах, проводившихся на белых крысах-самцах весом 100—130 *г*, обнаружено, что смесь антиокислителей бутилоксианизола и пропилгаллата не проявляла ни синергического, ни антагонического действия. $ЛД_{50}$ смеси антиокислителей, определявшаяся методом пробит-анализа [11], оказалась равной 2633±140 *мг/кг*. Она находилась между $ЛД_{50}$ бутилоксианизола (2950 *мг/кг*) и пропилгаллата (2500 *мг/кг*).

В подострых опытах смесь антиокислителей бутилоксианизола и пропилгаллата (бутилоксианизола 100 *мг/кг*, пропилгаллата 50 *мг/кг*) вводили с аггравацией в 500 раз по сравнению с количеством, которое может попасть в организм человека, что приводило к изменению содержания в крови кетоновых тел, каталазы и холинэстеразы, а также сахара.

В хронических опытах, проводившихся на белых крысах-самцах и самках, выясняли возможность использования смеси антиокислителей для стабилизации пищевых жиров.

В процессе исследования мы определяли вес животных (вначале через каждые 10 дней, а затем, когда прибавление стало незначительным, через каждый месяц), активность каталазы в крови по методике Баха и Зубковой (12), активность холинэстеразы в крови по методике Хестрина в модификации Киевского института труда и профзаболеваний, количественное содержание сахара в крови по методу Хагедорна и Иенсена [13]. Кроме того, выясняли содержание кетоновых тел в крови и белковых фракций в сыворотке крови. Ежедневно проводили наблюдения за внешним видом, поведением животных и поедаемостью корма. Опыт ставили на молодых, растущих животных весом 120±20 *г* — 3 группах самцов (1-я, 2-я, 3-я) и 3 группах самок (4-я, 5-я и 6-я) по 10 животных в каждой. Животным 1-й и 4-й группы антиокислители (бутилоксианизол и пропилгаллат) давали в 100-кратной аггравации (бутилоксианизола 20 *мг/кг*, пропилгаллата 10 *мг/кг*) по сравнению с количеством, которое может попасть в организм человека; животным 2-й и 5-й группы смесь антиокислителей вводили в 10-кратной аггравации (бутилоксианизола 2 *мг/кг*; пропилгаллата 1 *мг/кг*); 3-я и 6-я группа служила контролем. Антиокислители давали в свином топленом жире с кормовым брикетом. Исследования проводили до начала затравки и на 3, 6 и 9-м месяце затравки.

При биохимических исследованиях на 3-м и 6-м месяце эксперимента разницы между контрольными и подопытными животными не выявлено.

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На 6-м месяце животных обеих групп соединили для получения 2-го поколения. Оказалось, что крысы, получавшие с кормом антиокислители, к воспроизведению потомства не способны: из 5 животных 4-й группы, подвергавшихся воздействию антиокислителей в 100-кратной аггравации, ни у одного не было потомства; из 5 самок 5-й группы, получавших антиокислители в 10-кратной аггравации, потомство появилось только у одной, тогда как среди контрольных самок приплод был у трех (см. таблицу).

Результаты эксперимента по получению потомства

Первая подсадка					
антиокислители				контроль	
100-кратная аггравация		10-кратная аггравация		№ животного	количество приплода
№ животного	количество приплода	№ животного	количество приплода		
31	Нет	41	4	51	Обнаружены остатки крысят
33	»	43	Нет	53	То же
35	»	45	»	55	Нет
37	»	47	»	57	»
39	»	49	»	59	7
Вторая подсадка					
32	Нет	42	2	52	8
34	»	44	Нет	54	Нет
36	»	46	»	56	8
38	»	48	»	58	2
40	»	50	»	60	1

Для подтверждения результатов эксперимент был проведен повторно на других животных. При повторной подсадке из 5 крыс, получавших смесь антиокислителей в 100-кратной аггравации, ни одна не имела потомства; из 5 животных, которым антиокислители вводили в 10-кратной аггравации, приплод был только у одной; среди контрольных животных потомство появилось у 4 (см. таблицу). Повторная подсадка подтвердила, что животные, получавшие с кормом антиокислители, к воспроизведению потомства не способны.

На 9-м месяце затравки нами был проведен очередной цикл биохимических исследований. Выявлено статистически достоверное уменьшение количества кетоновых тел в крови: $P<0{,}001$ у самцов и 0,01 (у самок). Статистически достоверными считали результаты, в которых вероятность ошибки P была меньше или равнялась 0,05 (см. рисунок). Изменений других биохимических тестов нами не выявлено.

Процент гибели среди крыс, получавших антиокислители, был выше, чем в контроле; так, из 20 животных, которым вводили с кормом антиокислители в 100-кратной аггравации, к 9-му месяцу затравки осталось в живых 14; из 20 животных, получавших антиокислители в 10-кратной аггравации, — 18; между тем в контрольных группах из 20 крыс осталось в живых 19.

Таким образом, смесь антиокислителей бутилоксианизола и пропилгаллата оказалась токсичной для крыс и в связи с этим не должна быть допущена для стабилизации пищевых жиров.

Выводы

1. Смесь антиокислителей бутилоксианизола и пропилгаллата в 100-кратной аггравации при длительном скармливании ее крысам увеличивает смертность подопытных животных по сравнению с контролем.

2. Введение подопытным животным указанной смеси антиокислителей вызывает у животных бесплодие.

3. Бутилоксианизол и пропилгаллат при воздействии на организм крыс приводят к снижению количества кетоновых тел в крови. Измене-

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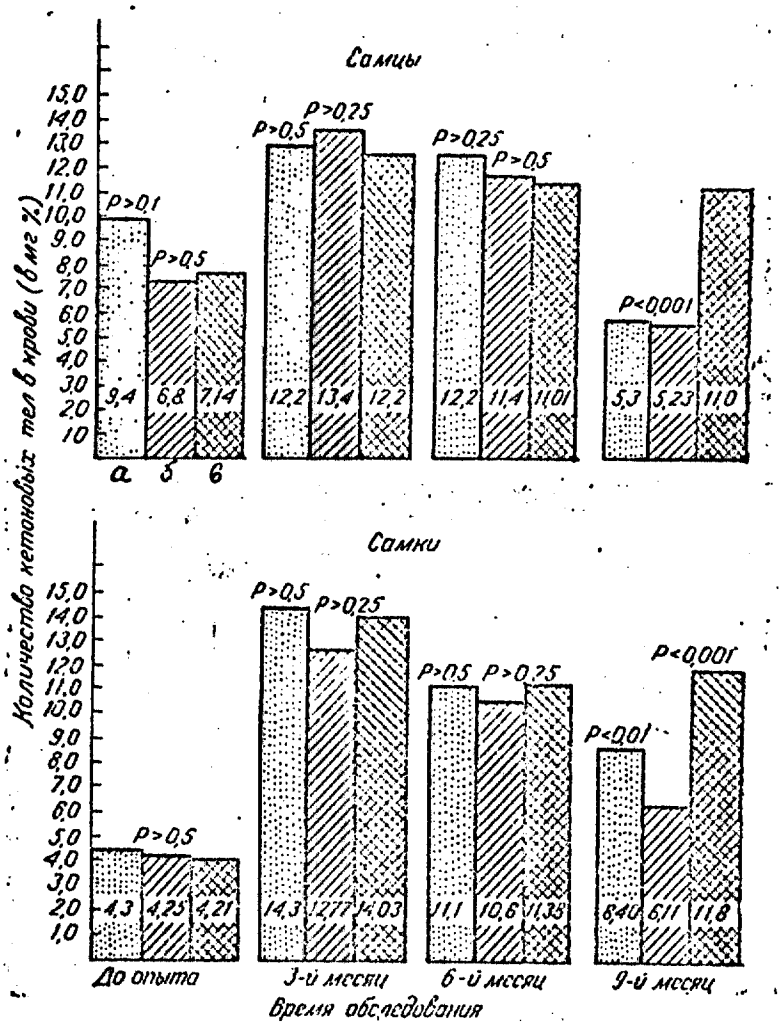

Изменение содержания кетоновых тел в крови подопытных и контрольных крыс.

Обозначения: *а* — животные, получавшие антиокислители в 100-кратной аггравации; *б* — животные, получавшие антиокислители в 10-кратной аггравации; *в* — контроль (не получавшие антиокислителей). Цифры внутри столбцов обозначают средние величины полученных результатов. *P* — вероятность ошибки.

ний в содержании сахара, а также активности ферментов (каталаза и холинэстераза) в крови не наблюдалось.

4. Смесь антиокислителей бутилоксианизола и пропилгаллата является токсичной для организма и не может применяться для стабилизации пищевых жиров.

ЛИТЕРАТУРА

1. Кадыков Б. И. Тезисы докл. 5-й Научной сессии Украинск. научно-исслед. ин-та питания. Киев, 1958, ч. 1, с. 19.—2. Кудряшов Б. А., Агатов П. А. Гин. и акуш., 1935, № 6, с. 1.—3. Dangouman A., Berlureau F., Debruyne H., Rev. franç. corps gras., 1957, v. 4, p. 541.—4. Holman R. T., Greenberg S. L., J. Am. Oil Chem. Soc., 1958, v. 35, p. 707.—5. Beck S., Brit. J. exp. Path., 1941, v. 22, p. 299.—6. Beck S., Kirby A. H. M., Peacock P. R., Cancer Res., 1945, v. 5, p. 135.—7. Kirby A. H. M., Ibid., p. 129.—8. Gearhart W. M., Stuckey B. N., J. Am. Oil Chem. Soc., 1955, v. 32, p. 386.—9. Эмануэль Н., Кнорре Д., Лясковская Ю. и др. Мясная индустрия СССР, 1958, № 2, с. 52.—10. Карплюк

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И. А. Влияние антиокислителей пищевых жиров (бутилоксианизола, бутилокситолуола и пропилгаллата) на животный организм и их гигиеническая оценка. Дисс. канд. М., 1960.—11. Беленький М. Л. Элементы количественной оценки фармакологического эффекта. Л., 1963, с. 63.—12. Балаховский С. Д., Балаховский И. С. Методы химического анализа крови. М., 1953, с. 592.—13. Травина О. В. Руководство по биохимическим исследованиям. М., 1955, с. 171.

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HYGIENIC ASSESSMENT OF A MIXTURE CONTAINING ANTIOXIDIZERS OF ALIMENTARY FATS — BUTYLOXYANISOLE AND PROPYL GALLATE

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Summary

A mixture comprising butyloxyanisole and propyl gallate — antioxidizers of alimentary fats — was studied for its effect on the organism after long-term administration. Experiments were carried out on albino rats. The mixture of antioxydizers caused a greater destruction of animals, while the surviving ones lost the ability to reproduce offsprings.

The obtained data point to the toxic action of the discussed mixture and to the impossibility of employing it as an antioxidizer for alimentary fats.

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ИССЛЕДОВАНИЕ СТОЙКОСТИ НЕКОТОРЫХ РАСТИТЕЛЬНЫХ ЖИРОВ, ИСПОЛЬЗУЕМЫХ ДЛЯ ФРИТЮРНОЙ ЖАРКИ

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В общественном питании и торговле ежегодно реализуется не менее 2 млрд. порций кулинарных изделий, приготавливаемых обжариванием во фритюре. Сюда относятся пирожки жареные, пончики, гарнирный картофель, хрустящий картофель, рыба, овощи и другие продукты. Они находят широкое распространение ввиду их высоких вкусовых достоинств и питательности.

Для удовлетворения растущего спроса на эти изделия организовано централизованное поточно-механизированное производство некоторых из них на предприятиях промышленного типа. Техника и технология производства обжаренных во фритюре продуктов быстро развиваются на основе применения полуфабрикатов. Однако в процессе длительного и многократного использования фритюрных жиров последние претерпевают физико-химические превращения [1—4], в результате которых их пищевая и биологическая ценность существенно уменьшается или даже полностью исчезает [5]. Кроме того, в таких жирах возможно образование веществ, не безвредных для здоровья человека [2].

Эти обстоятельства приводят к образованию больших отходов (неиспользуемых в пище остатков) фритюрных жиров, что удорожает кулинарное производство и сдерживает его развитие. Вот почему подбор наиболее термостойких жиров или их смесей, а также оптимальной технологии использования этих продуктов в качестве фритюра является важной экономической и гигиенической задачей.

Part of
FAO/WHO Third Report - Specifications for Identity and Purity
of Food Additives, Food and Agriculture Organization, Rome, 1967.
pp83-85.

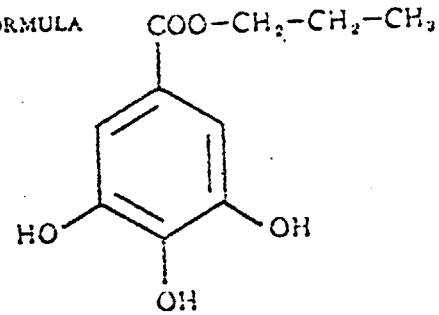
9. PROPYL GALLATE

SYNONYMS

CHEMICAL NAME Propyl gallate; *n*-propyl ester of 3,4,5-trihydroxybenzoic acid

EMPIRICAL FORMULA $C_{10}H_{12}O_5$

STRUCTURAL FORMULA



MOLECULAR WEIGHT 212.21

DEFINITION

Propyl gallate contains not less than 99% of $C_{10}H_{12}O_5$ after drying at 110° for 4 hours and conforms to the following specifications.

DESCRIPTION

Propyl gallate is a white to creamy-white crystalline, odorless solid with a slightly bitter taste.

IDENTIFICATION TESTS

A. *Solubility*

Water:	1 g is soluble in	300.0 ml.
Ethanol:	1 g is soluble in	3.5 ml.
Ether:	1 g is soluble in	3.5 ml.
Propylene glycol:	1 g is soluble in	3.5 ml.

B. Melting range is 146-148° after drying at 110° for 4 hours.

C. Dissolve about 0.5 g of propyl gallate in 10 ml of *N* sodium hydroxide and boil for 30 minutes. Cool the mixture and carefully acidify with 2 *N* sulfuric acid. Filter off the precipitate, wash with a minimum amount of water and then dry at 110° for 2 hours. The melting point of the resulting gallic acid is about 240°, with decomposition.

D. Add 1 ml of ammonium hydroxide to 5 ml of a 1% ethanolic solution of propyl gallate. A pink to red color appears.

PURITY TESTS

Chlorinated organic compounds: Dissolve 1.0 g of propyl gallate in 10 ml of 0.1 *N* sodium hydroxide. Acidify with nitric acid and filter off the precipitate. Mix the precipitate with 2 g of calcium carbonate, dry the mixture and then ignite. Take up the ignition residue in 20 ml of dilute nitric acid T.S. and filter. Mix the solution with 0.5 ml of 0.1 *N* silver nitrate. The turbidity should be not more than that obtained in a similar volume by addition of 0.5 ml of 0.1 *N* silver nitrate and 0.2 ml of 0.1 *N* hydrochloric acid, corresponding to not more than 100 mg/kg as chlorine.

Free acid: To a mixture of 50 ml of carbon dioxide-free water and 50 ml of acetone, then add 5 drops of bromocresol green T.S. and titrate with 0.005 *N* hydrochloric acid to match a buffer (pH 5.0) T.S. containing the same amount of indicator. Dissolve about 0.400 g of propyl gallate in 50 ml of acetone and add 50 ml of carbon dioxide-free water, 5 drops of bromo cresol green T.S. and the amount of 0.005 *N* hydrochloric acid found in the preliminary test to bring the solvent to pH 5.0. Titrate the solution back to pH 5.0 with 0.05 *N* sodium hydroxide, matching against the buffer (pH 5.0) T.S. The free acidity calculated as gallic acid (9.407 mg of gallic acid is equivalent to 1 ml of 0.05 *N* NaOH) should be not more than 0.5%.

Loss on drying: Not more than 0.5% after drying for 4 hours at 110°. (Ventilation during drying is advisable).

Sulfated ash: Not more than 0.05%.

Arsenic: Not more than 3 mg/kg.

Lead: Not more than 10 mg/kg.

ASSAY

Spectrophotometric method: Prepare an ethanolic solution of a dried sample of propyl gallate containing 5 μg/ml and determine the extinction at 218 and 276 mμ.

$E^{1\%}_{1\,cm}$ 218 mμ 1240-1255; $E^{1\%}_{1\,cm}$ 276 mμ 500-505;

Calculation:

$$\frac{E^{1\%}_{1\,cm} \text{ of sample}}{E^{1\%}_{1\,cm} \text{ of a pure standard reference specimen}} \times 100 = \%\ \text{propyl gallate}$$

Bismuth nitrate precipitation: Weigh about 0.200 g of a dried sample of propyl gallate into a 400-ml beaker. Add 150 ml of water and heat to boiling. Then with constant and vigorous stirring add 50 ml of bismuth nitrate T.S. (I). Continue stirring for a few minutes more until precipitation is complete, then allow the solution to cool to room temperature. Filter the yellow precipitate on a sintered glass crucible, wash it first with cold 0.05 *N* nitric acid and then with ice cold water, until free from acid. Dry the crucible with its contents at 110° to constant weight.

Calculation:

$$\frac{\text{weight of precipitate} \times 48.63}{\text{weight of sample}} = \%\ \text{propyl gallate}$$

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The Metabolic Fate of Gallic Acid and Related Compounds

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The discovery in this laboratory that 3,4-dihydroxyphenyl compounds such as caffeic acid and 3-(3,4-dihydroxyphenyl)-L-alanine undergo *O*-methylation as well as dehydroxylation in the animal body (1) has been confirmed by others (2–4). We are not aware of any reports of monohydroxyphenyl compounds undergoing either of these metabolic changes. The question is raised as to what the metabolic fate would be of trihydroxyphenyl compounds such as gallic (3,4,5-trihydroxybenzoic) and 2,3,4-trihydroxybenzoic acids.

According to a report by Tompsett (5), the pyrogallol detected in human urine was probably derived from gallic acid by decarboxylation in the alimentary tract. In a subsequent report this worker presented evidence that oral ingestion of tannic acid by man leads to an increased excretion of both 3,4-dihydroxyphenolic and 3-methoxy-4-hydroxyphenolic compounds (4).

Interest in the metabolic fate of gallic acid also stems from its natural occurrence in foods, the use of propyl gallate as an antioxidant in fats and oils, and the earlier use of tannic acid in the treatment of burns (6).

In the present study it will be shown that *O*-methylation of gallic acid and 2,3,4-trihydroxybenzoic acid accounts for the major metabolites in the urine of rats and rabbits ingesting these compounds.

EXPERIMENTAL

Methods—The collection of the urine and the paper chromatographic procedures used for the detection of the urinary phenolic metabolites have been previously described (7). The purified diet fed to the rats and the semipurified diet fed to rabbits have also been described previously (8).

Materials—Gallic acid, pyrogallol, syringic acid, *n*-propyl gallate, tannic acid, and 2,3,4-trihydroxybenzoic acid were purchased from commercial sources. The 4-*O*-methyl gallic acid was prepared according to the method of Schöpf and Winterhalder (9). The 2-*O*-methyl pyrogallol and 3-methoxy-2,4-dihydroxybenzoic acid were prepared according to Geissman and Mojé (10). The lauryl gallate used in these experiments was kindly supplied by the Animal Fats Laboratory, Eastern Utilization Research and Development Division, Philadelphia, Pennsylvania. We are indebted to Dr. Leonard Jurd of the Fruit and Vegetable Chemistry Laboratory at Pasadena, California, for a sample of 3-*O*-methyl gallic acid. 3-Hydroxy-4,5-dimethoxybenzoic acid was prepared by treating gallic acid with 3 mole equivalents of diazomethane. The product which was extracted from the ethereal solution with 4% aqueous sodium hydroxide was hydrolyzed, and the resulting acid recrystallized from water giving a preparation melting at 195–196° (corrected). Fischer *et al.* (11) reported a value of 197–198°. All compounds were checked chromatographically for purity.

RESULTS

Rat Experiments—A diet containing 0.5% of gallic acid was fed to adult albino rats. The urine, collected without the addition of acid in the receiving flask, was normal in color and volume. Two-dimensional paper chromatograms of ether extracts of the acidified urine revealed the presence of one major metabolite in addition to gallic acid itself. The metabolite gave a stable yellow-orange color when sprayed with diazotized sulfanilic acid-sodium carbonate which did not turn black as would be expected if a catechol structure had been involved. Dehydroxylation was ruled out since the R_F values were not in agreement with those of either 3,4- or 3,5-dihydroxybenzoic acid. The presence of a carboxyl group was indicated by the fact that the metabolite was extractable with ether from an acid solution but not from an alkaline solution. These observations suggested the possibility that the metabolite was 4-*O*-methyl gallic acid. When this compound was synthesized its characteristics were identical with those of the urinary metabolite as judged by R_F values, behavior under ultraviolet light and color after spraying with diazotized sulfanilic acid followed by sodium carbonate (Table I). The isolation of this compound from urine is described under rabbit experiments.

Small amounts of a second metabolite of gallic acid were also detected on the two-dimensional paper chromatogram of the ether extract of the urine. When the ether-extracted urine was hydrolyzed (2 N HCl reflux for 1 hour), then extracted into ether and chromatographed, an additional amount of this minor metabolite was obtained. Since gallic acid and 4-*O*-methyl gallic acid are stable to acid hydrolysis under these conditions, it may be concluded that some of this metabolite was present in the urine as a conjugate. The chromatographic properties of this compound after hydrolysis were identical with those of 2-*O*-methyl pyrogallol (Table I).

When gallic acid was given to rats by stomach tube (100 mg per rat) the results were similar to those obtained when the compound was ingested in the diet.

In order to by-pass the gastrointestinal tract and avoid effects caused by intestinal microorganisms, rats were given gallic acid by intraperitoneal injection (100 mg per rat). Chromatographic examination of the urine revealed the presence of 4-*O*-methyl gallic acid as well as gallic acid itself. However, an additional metabolite with the same chromatographic behavior as pyrogallol

TABLE I

Chromatographic behavior of trihydroxyphenolic derivatives

Compound	R_F		Appearance under ultraviolet	Color with diazotized sulfanilic acid
	$CHCl_3$-acetic-H_2O	20% KCl		
Gallic acid	0.00	0.45	Deep blue	Green, then darkens
4-*O*-Methyl gallic acid	0.16	0.56	Deep blue	Yellow-orange
2,3,4-Trihydroxy-benzoic acid	0.13	0.41	Blue	Darkens
3-Methoxy-2,4-dihydroxybenzoic acid	0.65	0.55	Blue	Yellow-orange
3-*O*-Methyl gallic acid	0.20	0.43	Deep blue	Reddish, then darkens
3,4-Dimethoxy-5-hydroxybenzoic acid	0.76	0.58	Deep blue	Yellow-orange
Syringic acid	0.84	0.40	Deep blue	Red
Pyrogallol	0.14	0.71	Absorbs	Darkens
2-*O*-Methyl pyrogallol	0.61	0.79	Absorbs	Yellow-orange
Tannic acid	0.00	0.26	Absorbs	Darkens slightly
Propyl gallate	0.33	0.40	Deep blue	Darkens slightly
Lauryl gallate	0.82	0.00	Absorbs	Darkens slightly

was clearly evident (Table I). A trace of 2-*O*-methyl pyrogallol was also present. Pyrogallol was subsequently isolated from the urine of rabbits receiving gallic acid in the diet.

Additional evidence of *O*-methylation of a trihydroxyphenyl compound was obtained when rats were given 2,3,4-trihydroxybenzoic acid by stomach tube (100 mg per rat). Some of the administered compound was found in the urine, along with the major metabolite which was identified chromatographically as 3-methoxy-2,4-dihydroxybenzoic acid (Table I).

When rats were given 4-*O*-methyl gallic acid, the only spots on the chromatogram other than those accounted for in control urine were the compound given, and a conjugate area which on hydrolysis was converted to 4-*O*-methyl gallic acid. The excretion of a dimethoxy derivative of 4-*O*-methyl gallic acid by *O*-methylation of either the number 3 or 5 hydroxyl group was not observed. However, when rats were given 3-*O*-methyl gallic acid (100 mg per rat by stomach tube), two dimethoxy derivatives were readily detected in the urine. These were identified chromatographically as 3,5-dimethoxy-4-hydroxybenzoic (syringic) acid and 3,4-dimethoxy-5-hydroxybenzoic acid (Table I).

The metabolism *in vivo* of esters of gallic acid was also studied with the use of propyl and lauryl gallate. Each of these compounds was given to rats by stomach tube (100 mg per rat). The major metabolite in each case was 4-*O*-methyl gallic acid. In addition, an area on the chromatograms corresponding to gallic acid was clearly evident, indicating cleavage of the ester linkages of propyl and lauryl gallate had taken place after oral ingestion.

The administration of tannic acid to rats by stomach tube (100 mg per rat) yielded the same results as obtained when gallic acid was administered, in that 4-*O*-methyl gallic acid was the major metabolite excreted. An area corresponding to gallic acid was also located on the same chromatogram. No other metabolites including 3,4-dihydroxyphenolic and 3-methoxy-4-hydroxyphenolic compounds were detected. The sample of tannic acid used was not entirely free from gallic acid as judged by the two-dimensional chromatogram. However, the amount of 4-*O*-methyl gallic acid excreted could not be accounted for entirely by the small amount of gallic acid present in the tannic acid as an impurity.

When pyrogallol was given to rats by stomach tube (50 mg per rat), there was no evidence of pyrogallol *per se* or metabolites thereof in the urine.

Gallic acid was incubated with rat and rabbit liver slices in bicarbonate buffer (pH 7). The chromatogram of the ether extracts of the medium contained 4-*O*-methyl gallic acid. No other metabolites such as vanillic acid or protocatechuic acid were detected. Similarly, 3-methoxy-2,4-dihydroxybenzoic acid was formed from 2,3,4-trihydroxybenzoic acid. Thus the results *in vitro* and *in vivo* are in agreement.

Semiquantitative data on the excretion of 4-*O*-methyl gallic acid were obtained by a comparison of appropriate dilutions of the hydrolyzed urine of rats receiving gallic acid by stomach tube with known amounts of authentic 4-*O*-methyl gallic acid. After a 100-mg dose of gallic acid per rat by stomach tube or by intraperitoneal injection, approximately 25% of the gallic acid given (23 mg) was excreted as the methylated derivative. When the amount of gallic acid given was reduced to 10 mg per rat, nearly 50% (4.6 mg) was excreted as 4-*O*-methyl gallic acid.

Rabbit Experiments—After adjustment to the special diet, New Zealand white rabbits weighing approximately 2 kg were fed this diet containing 0.5% gallic acid. Chromatograms of the ether extracts of the acidified urine contained 4-*O*-methyl gallic acid, pyrogallol, and an additional area on the chromatogram suspected to be a conjugate. When this area was cut from an unsprayed chromatogram and hydrolyzed (2 N HCl reflux for 1 hour), the ether extract of the hydrolysate was found to contain pyrogallol and 4-*O*-methyl gallic acid. As mentioned earlier, neither gallic acid nor 4-*O*-methyl gallic acid are decarboxylated under these conditions of acid hydrolysis. When the aqueous phase of the rabbit urine (urine minus ether solubles) was similarly hydrolyzed, both pyrogallol and traces of 2-*O*-methyl pyrogallol were readily detected on the chromatogram.

The chromatographic evidence for the identification of 4-*O*-methyl gallic acid and pyrogallol was confirmed by their isolation from rabbit urine after feeding a diet containing 0.5% gallic acid. The urine was collected in acid (HCl) and extracted with ether in a liquid-liquid extractor for 24 hours. The ether extract was evaporated and the residue dissolved in a small volume of water made slightly alkaline with sodium bicarbonate. This aqueous solution was extracted with ether; pyrogallol was crystallized from this extract. The alkaline aqueous phase was then acidified, extracted exhaustively with carbon tetrachloride and with chloroform. These extracts were discarded. Then the aqueous phase was extracted with ether. This ether extract was chromatographed on several sheets of Whatman No. 1 filter

paper with chloroform-acetic acid-water (2:1:1; lower phase). The appropriate bands corresponding to 4-*O*-methyl gallic acid were eluted and the eluate rechromatographed on several sheets of paper with 20% KCl. Once again the appropriate bands were cut out and eluted. The eluate was reconstituted in a small volume of water, filtered over charcoal and Celite and crystals of 4-*O*-methyl gallic acid appeared upon standing. The two isolated metabolites had the same crystallographic measurements as synthetic pyrogallol and 4-*O*-methyl gallic acid respectively.

DISCUSSION

The major urinary metabolites of gallic, 2,3,4-trihydroxybenzoic, and of 3-methoxy-4,5-dihydroxybenzoic acids are shown in Fig. 1. Additional metabolites may eventually be identified, particularly if other methods of detection are used. The results clearly indicate that *O*-methylation and decarboxylation are the reactions involved in the metabolic conversions of the administered compounds. It is interesting to note that the middle hydroxyl group of both gallic acid and 2,3,4-trihydroxybenzoic acid becomes methylated. This group is *para* to the carboxyl group in the case of gallic acid and *meta* in the case of 2,3,4-trihydroxybenzoic acid. This selective *O*-methylation prevents the formation from either compound of the potential *ortho* dihydroxy (catechol) configurations. Had *O*-methylation taken place on either of the outer hydroxyl groups, then a catechol configuration such as 3-*O*-methyl gallic acid, for example, would have been formed. In this case, further metabolic change leading to the expenditure of another methyl group would be expected, on the basis of previous experience with 3,4-dihydroxy phenolic acids (1). This prompted us to study the metabolic fate of 3-*O*-methyl gallic acid. As predicted, *O*-methylation of a second hydroxyl group occurred as evidenced by the appearance in the urine of two dimethoxy derivatives: syringic acid and 3,4-dimethoxy-5-hydroxybenzoic acid. On the other hand, when 4-*O*-methyl gallic acid, which does not contain a catechol structure, was fed, no further methylation was detected. It is tempting to generalize from these results that *O*-methylation is a preferred mechanism for the metabolism of acids containing a catechol nucleus.

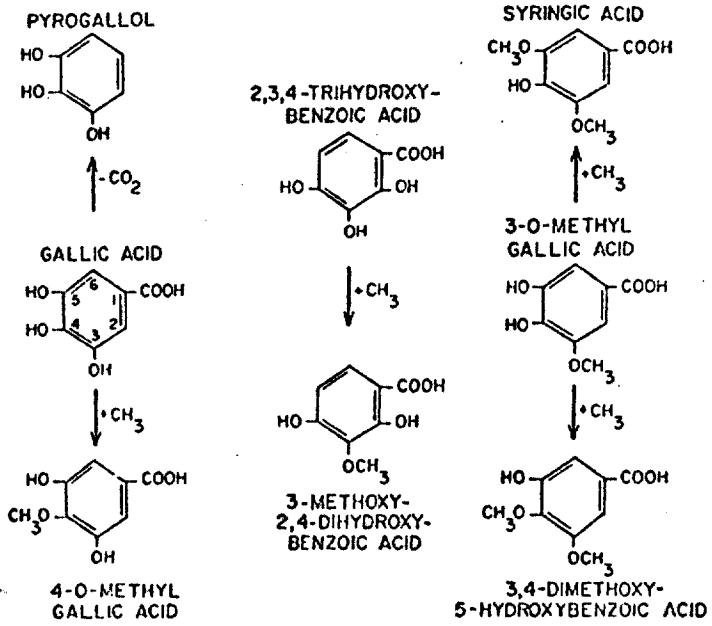

FIG. 1. Chemical structures of major urinary metabolites identified after feeding gallic acid, 2,3,4-trihydroxybenzoic acid, and 3-*O*-methyl gallic acid.

The observation that as much as 25% of the administered gallic acid is methylated would be expected to bring about an increased dietary requirement for methyl donors. This aspect of the metabolism of gallic acid is currently being investigated.

None of the hydroxyl groups of gallic acid or tannic acid were removed (dehydroxylated). The paper chromatographic system used here would have detected such a reaction if it had occurred. In this respect our results with rats and rabbits differ from those obtained by Tompsett (4) with human beings since dehydroxylation would have to be involved to account for the reported excretion of 3,4-dihydroxy- and 3-methoxy-4-hydroxybenzoic acids after the ingestion of tannic acid. There is agreement concerning the excretion of pyrogallol by man (4) as well as by rats and rabbits. The fact that pyrogallol was found in the urine of rats after the intraperitoneal injection of gallic acid indicates that decarboxylation is not dependent on passage through the gastrointestinal tract.

The use of lauryl and propyl gallate as food additives (antioxidants) is currently being practiced. Studies on the chronic toxicity of lauryl gallate did not indicate any deleterious effects when dietary levels up to 0.5% were fed to rats (12). In view of our finding of a major pathway for the metabolism and excretion of gallic acid by means of *O*-methylation, it is understandable that symptoms of choline deficiency would not be expected as long as the diet contains an adequate reserve of methyl donors.

SUMMARY

O-Methylation resulting in the formation of 4-*O*-methyl gallic acid accounts for the major metabolite in the urine of rats or rabbits ingesting gallic acid, propyl gallate, lauryl gallate, or tannic acid. Decarboxylation accounts for a second metabolite identified as pyrogallol in the urine of rats receiving gallic acid by intraperitoneal injection or rabbits receiving gallic acid in the diet. 3-Methoxy-2,4-dihydroxybenzoic acid was the major urinary metabolite of rats given 2,3,4-trihydroxybenzoic acid. Syringic acid and 3,4-dimethoxy-5-hydroxybenzoic acid were identified as urinary metabolites of rats receiving 3-*O*-methyl gallic acid.

REFERENCES

1. DeEds, F., Booth, A. N., and Jones, F. T., *J. Biol. Chem.*, 225, 615 (1957).
2. Shaw, K. N. F., McMillan, A., and Armstrong, M. D., *J. Biol. Chem.*, 226, 255 (1957).
3. Shaw, K. N. F., and Trevarthen, J., *Nature*, 182, 797 (1958).
4. Tompsett, S. L., *J. Pharm. and Pharmacol.*, 11, 32 (1959).
5. Tompsett, S. L., *J. Pharm. and Pharmacol.*, 10, 157 (1958).
6. Robinson, H. J., and Graessle, O. E., *J. Pharm. Exptl. Therap.*, 77, 63 (1943).
7. Booth, A. N., Emerson, O. H., Jones, F. T., and DeEds, F., *J. Biol. Chem.*, 229, 51 (1957).
8. Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., Jones, F. T., and DeEds, F., *J. Biol. Chem.*, 234, 946 (1959).
9. Schöpf, C., and Winterhalder, L., *Ann. Chem. Liebigs*, 544, 62 (1940).
10. Geissman, T. A., and Mojé, W. J., *J. Am. Chem. Soc.*, 73, 5765 (1951).
11. Fischer, E., Bergmann, M., and Lipschitz, W., *Ber. deut. chem. Ges.*, 51, 45 (1918).
12. Allen, S. C., and DeEds, F., *J. Am. Oil Chemist's Soc.*, 28, 304 (1951).

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CEREBROCORTICAL DYNAMICS IN PATIENTS AFFECTED WITH OBESITY AT VARIOUS STAGES OF TREATMENT

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Summary

An attempt was undertaken to educe the mode of cerebrocortical activity changes in patients afflicted with obesity occurring at individual stages of their treatment, inclusive low caloric diet days. At the terminal phase of the treatment, against the background of general tendency towards improved cerebrocortical dynamics, the examined patients showed individual instances of its disturbance, following introduction of low caloric diet days. Immediately after a high-protein diet day (the ration consisting of meat or curds) the higher nervous activity either tends to improve, or remains without any appreciable changes; two successive high-protein diet days provoke in some of the patients derangement of the closing function in the cerebral cortex, or impaired mobility of nervous processes. Sharp deterioration of the cerebrocortical activity could be noted after apple-diet days, particularly with two successive ones. In addition to this a reduced excitability of the large hemispheres cortex was marked after each low-caloric diet day.

ВЛИЯНИЕ ФЕНОЛЬНЫХ АНТИОКИСЛИТЕЛЕЙ НА УСВОЯЕМОСТЬ ТОПЛЕНОГО СВИНОГО ЖИРА

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За последние годы в отечественной и зарубежной литературе опубликован ряд работ по исследованию различных антиокислителей, предотвращающих порчу жиров. У нас в этом направлении имеются работы Н. М. Эмануэля и др., показавших значительный эффект фенольных антиокислителей: бутилокснанизола, бутилокситолуола и эфиров галловой кислоты. Оказалось, что при добавлении их в небольших количествах (0,01—0,02%) в жир задерживаются окислительные процессы и удлиняются сроки хранения последнего.

Вопрос о возможности использования бутилокснанизола, бутилокситолуола и пропилгаллата в качестве антиокислителей пищевых жиров с гигиенических позиций специально изучался И. А. Карплюк (1959, 1960). Ею исследовались как органолептические свойства жира, содержащего упомянутые антиокислители, так и влияние на организм животных (собаки и белые крысы) при длительном скармливании. На основании проведенных исследований И. А. Карплюк пришла к выводу, что полностью безвредными является бутилокснанизол и бутилокситолуол, но не пропилгаллат.

Исходя из того, что упомянутые соединения задерживают окислительные процессы в жирах, мы сочли интересным изучить возможность их влияния на усвояемость жира. В литературе данных по этому вопросу мы не нашли.

В качестве объекта исследования был взят свежий топленый свиной жир, в который добавляли бутилокситолуол, бутилокснанизол и пропилгаллат из расчета 0,2 г на 100 г жира.

Опыты были поставлены на 10 взрослых белых крысах-самцах одного поколения, всего 5 опытов продолжительностью по 7 дней. Крысам скармливали рацион, состоящий из сухого концентрированного корма с добавлением 2 г свиного жира — в 2 контрольных опытах без антиокислителей, в 3 остальных жир добавляли с упомянутыми антиокислителями. Количество антиокислителей в 2 г жира составляло 4 мг. Таким образом, крысы в течение 7-дневного периода опыта получали 14 г свиного жира и по 28 мг бутилокситолуола, бутилокснанизола и пропилгаллата.

s urged to investigate its copyright status.

Для равномерного распределения добавляемого в рацион топленого свиного жира и исключения возможности разбрасывания крысами пищи сухой концентрированный корм размельчали, тщательно смешивали с жиром, смачивали водой и скатывали в виде шариков, которые и скармливали животным.

Для обеспечения учета количества несъеденного корма и выделенного кала всех крыс содержали в отдельных клетках следующей конструкции. Пол клетки, на котором находились крысы, состоял из стеклянных трубочек, неплотно прилегающих друг к другу. Под трубочками на расстоянии 5 см находилась металлическая сетка, на которую падали кал и крупные остатки корма; более мелкие частички пищи задерживались на нижележащей сетке, обтянутой капроновой тканью (расстояние между второй и третьей сеткой было 2,5 см).

В целях приспособления животных к условиям опыта предварительно, в течение 2 недель их содержали в приготовленных для эксперимента клетках и давали указанный выше корм со свиным жиром, но без антиокислителей. Для отделения одного опытного периода от другого в корм в конце опыта добавляли кармин (0,03 г). Окрашенный кармином кал отбрасывали и в опытах не учитывали.

Между опытами, когда скармливали жир с антиокислителями, имелся промежуток в 4—5 дней. В эти дни крыс пересаживали в обычные клетки и рацион их был таким же, как и в контроле. Сбор кала и остатков корма в эти дни не производился. В течение 7-дневного опытного периода выделенные от каждой крысы кал и остатки несъеденной пищи ежедневно собирали и подсушивали.

Собранные и подсушенные за 7 дней остатки корма доводили до абсолютно сухого состояния, а затем производили расчет фактически съеденного корма и содержащегося в нем жира на сухое вещество. Собранный за 7 дней кал измельчали и из его средней навески 5 г извлекали жир по методу Сокслета. После этого рассчитывалось количество выделенного с калом жира за весь опытный период на абсолютно сухое вещество. В сухом концентрированном корме определялось содержание жира по методу Сокслета и количество влаги.

Опыты показали, что поедаемость корма крысами была различной. Плохая поедаемость у крыс наблюдалась в опытах, в которых крысам скармливали рацион с добавлением в жир бутилокситолуола и особенно пропилгаллата. Неохотно крысы поедали корм и в заключительном контрольном опыте.

Плохую поедаемость корма мы склонны объяснить прежде всего возможным влиянием высокой температуры воздуха. В июне и первой половине июля, когда проводились опыты, средняя суточная температура воздуха колебалась в пределах 21—22°. В помещении вивария, где содержались крысы и где вентиляция была недостаточной, температура воздуха днем достигала 28°.

Возможно также, что у крыс к концу опытов могла появиться приедаемость к корму, ибо в рацион добавлялось относительно большое количество жира. Поскольку поедаемость корма крысами была различной, для сравнения полученных данных нами рассчитывалось относительное количество неусвоенного жира:

$$X = \frac{б \cdot 100}{a},$$

где a — количество фактически съеденного крысами жира в граммах; $б$ — количество жира, выделенного с калом, в граммах; X — количество неусвоенного жира в процентах. Оказалось, что в обоих контрольных опытах (в первом — в начале работы и во втором — в конце) при ежедневном скармливании крысам 2 г жира без антиокислителей среднее количество неусвоенного жира составило 5,3%. При добавлении к 2 г жира 4 мг антиокислителей среднее количество неусвоенного жира соответственно составляло для бутилокситолуола 6,1%, для бутилоксианизола — 5,8%, для пропилгаллата — 6,2%. Таким образом, по сравнению с контрольными опытами усвояемость жира с антиокислителями оказалась несколько пониженной. Рисунок иллюстрирует влияние антиокислителей на усвояемость жиров.

Как видно на рисунке, количество неусвоенного жира в последнем контрольном опыте находилось в тех же пределах, что и в первом контрольном опыте. По существу усвояемость жира в первом и последнем

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контрольных опытах была почти одинаковой у всех 10 крыс несмотря на то, что в последнем контрольном опыте крысы неохотно поедали корм.

При добавлении в жир пропилгаллата наряду с плохой поедаемостью корма у 8 крыс отмечается и более высокий процент неусвоенного жира. При добавлении в жир бутилокситолуола и бутилоксианизола также наблюдается повышенное количество неусвоенного жира: в первом случае — у 6 крыс, во втором — у 5 (по сравнению с контрольными опытами). Очевидно, плохая поедаемость корма и пониженная усвояемость жира с антиокислителями связываются не столько с внешними условиями окружающей среды и возможной приедаемостью, сколько с действием антиокислителей на процесс усвоения.

Влияние антиокислителей на усвояемость пищевых жиров.
1 и *5* — контроль; *2* — бутилокситолуол; *3* — бутилоксианизол; *4* — пропилгаллат.

Следует отметить, что в течение всего опытного периода ($2^1/_2$ месяца) внешний вид крыс и их поведение были нормальными; животные очень быстро привыкли к новым клеткам и опытному режиму. К концу опытов все крысы прибавили в весе от 30 до 100 г.

Считаем необходимым отметить, что жир, экстрагированный из кала животных, полученного за контрольные периоды и периоды с антиокислителями, отличался между собой, а именно: жир, не содержащий антиокислителей, имел желтоватый цвет и обычный, свойственный крысиному калу, запах. Жир легко смывался со стенок колбы при споласкивании ее мыльным раствором. Жир, содержащий бутилокситолуол, бутилоксианизол и пропилгаллат, имел темный красноватый цвет и слабый ароматический запах, трудно смывался со стенок колбы даже при споласкивании ее щелочным раствором. Изменение цвета и запаха кала объясняется, очевидно, тем, что все использованные в нашей работе антиокислители являются производными фенольного ряда. Общеизвестно, что обыкновенный фенол под влиянием кислорода воздуха превращается в фенохинон, имеющий красный цвет. При попадании в желудочно-кишечный тракт как топленый свиной жир, так и, возможно, антиокислители подвергаются гидролизу. В результате гидролиза от жира отщепляются свободные жирные кислоты — олеиновая и др., а от антиокислителей спирты — бутиловый, метиловый и пропиловый. Это дает возможность образования сложных эфиров, которые имеют как бы фруктовый запах.

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Выводы

1. При ежедневном скармливании белым крысам 2 г жира с добавлением 4 мг бутилокситолуола, бутилоксианизола и пропилгаллата усвояемость жира несколько снижается. Наиболее четкие данные получены при скармливании жира с пропилгаллатом.

2. Вопрос о причине несколько пониженной усвояемости пищевых жиров с антиокислителями нуждается в дальнейшем изучении.

ЛИТЕРАТУРА

Карплюк И. А. Вопр. питания, 1959, № 4, стр. 24. — Карплюк И. А. Влияние антиокислителей пищевых жиров на животный организм и их гигиеническая оценка. Автореф. дисс. канд. М., 1960. — Эмануэль Н. М. и др. Мясная индустрия, 1958, № 2, стр. 52.

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THE INFLUENCE OF PHENOL ANTIOXIDANTS ON THE ASSIMILATION OF ALIMENTARY FATS

F. E. Budagyan, L. I. Smirnova, (Moscow)

Summary

The work deals with the study of the effect produced by three phenol antioxidants on the assimilation of fat. Under research was fresh rendered lard to which butyloxytoluene, butyloxyanisol and propylgaliate were added at the rate of 0.2 gm per 100 gm of fat.

Experiments involving assimilation (absorption) were staged on male albino rats of one generation. They were fed on a ration which included dry concentrated food with addition of 2 gm of fat containing 4 mg of the above-cited antioxidants.

As compared with control experiments the assimilation of fats containing antioxidants (especially propylgallate) proved to be somewhat lower.

О ВОДОПОГЛОЩАЕМОСТИ И СТУДНЕОБРАЗОВАНИИ МЫШЕЧНОЙ ТКАНИ РЫБ И УБОЙНЫХ ТЕПЛОКРОВНЫХ ЖИВОТНЫХ

Ф. С. Околов и *С. Х. Николов*

Из кафедры общей гигиены (зав. — проф. Ф. С. Околов) Кубанского медицинского института, Краснодар

Как известно, методы коллоидной химии, разработанные для характеристики отдельных веществ, за последние годы получили широкое применение при изучении сложных природных объектов, в том числе и пищевых продуктов.

Авторы настоящего исследования задались целью изучить некоторые показатели отношения к воде цельной мышечной ткани. При этом имелось в виду, что цельный естественный белковый комплекс имеет свои особые свойства, а не является простой суммой составляющих его частей.

Водопоглощение изучалось в условиях измельчения мышц при заданном количестве воды при помощи так называемого микроизмельчителя тканей, способного развивать скорость от 3000 до 5000 оборотов ножа в минуту. За меру водопоглощения было принято наибольшее количество воды, способное поглотиться определенным весом раздробленных мышц, с образованием при этом густого неперемещающегося студня и не имеющего над собой или внутри себя остатков несвязанной воды. Эта величина была названа относительной водопоглощаемостью, а вес мяса принят за единицу.

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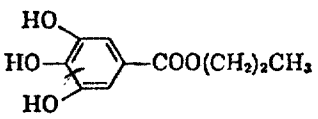
Food Chemicals Codex, First Edition, (1966). Publication 1406. National Academy of Sciences - National Research Council. Washington, D. C. pp 568-569

PROPYL GALLATE

Gallic Acid, Propyl Ester



$C_{10}H_{12}O_5$ Mol. wt. 212.20

DESCRIPTION

A fine, white to nearly white, odorless powder having a slightly bitter taste. It is slightly soluble in water and freely soluble in alcohol and in ether.

IDENTIFICATION

Dissolve about 500
solution for 30 minut
diluted sulfuric acid
amount of water, and
tained melts at about

SPECIFICATIONS

Assay. Not less thar
102 per cent of $C_{10}H_{1}$

Melting range. Bet

Limits of Impuritie

Arsenic (as As). N
Heavy metals (as
per cent).
Loss on drying. N
Residue on ignitic

TESTS

Assay. Transfer al
and accurately weighe
water, and heat to bo
50 ml. of bismuth nitr
cipitation is complete
tared sintered glass cr
300), and dry at 110° t
so obtained, multiplied

Melting range. I
page 775, after drying

Arsenic. A *Sample*
pounds meets the requ

Heavy metals. Pr
Method II under the *I*
ion (Pb) in the control

Loss on drying, pa

Residue on ignitic
general method.

Packaging and stora

Functional use in fo

IDENTIFICATION

Dissolve about 500 mg. in 10 ml. of sodium hydroxide T.S., boil the solution for 30 minutes, and cool. Carefully acidify the mixture with diluted sulfuric acid T.S., filter, wash the precipitate with a minimum amount of water, and dry at 110° for 2 hours. The gallic acid so obtained melts at about 240° with decomposition (see page 775).

SPECIFICATIONS

Assay. Not less than 98 per cent and not more than the equivalent of 102 per cent of $C_{10}H_{12}O_5$ after drying.

Melting range. Between 146° and 148°.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 per cent).

Loss on drying. Not more than 0.5 per cent.

Residue on ignition. Not more than 0.1 per cent.

TESTS

Assay. Transfer about 200 mg., previously dried at 110° for 4 hours and accurately weighed, to a 400-ml. beaker, dissolve it in 150 ml. of water, and heat to boiling. With constant and vigorous stirring, add 50 ml. of bismuth nitrate T.S., continue stirring and heating until precipitation is complete, and cool. Filter the yellow precipitate on a tared sintered glass crucible, wash it with cold dilute nitric acid (1 in 300), and dry at 110° to constant weight. The weight of the precipitate so obtained, multiplied by 0.4866, represents its equivalent of $C_{10}H_{12}O_5$.

Melting range. Determine as directed in the general procedure, page 775, after drying at 110° for 4 hours.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 720.

Heavy metals. Prepare and test a 2-gram sample as directed in *Method II* under the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Loss on drying, page 774. Dry at 110° for 4 hours.

Residue on ignition, page 786. Ignite 1 gram as directed in the general method.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Antioxidant.

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CHRONIC TOXICITY OF BREAD ADDITIVES TO RATS

By W. Donald Graham, H. Teed and H. C. Grice

From the Food and Drug Laboratory, Department of National Health and Welfare Ottawa, Canada

Received March 29, 1954

Currently there is much interest in the use of "chemical" additives in the food industry. Probably a considerable proportion of this interest stems from the report of Sir Edward Mellanby in 1946[1] that running fits had been induced in dogs by including in their diets flour that had been bleached and improved by treatment with nitrogen trichloride. The offending agent was finally isolated and found to be a derivative of methionine, methionine sulphoximine.[2] As a result of these discoveries several countries, Canada included, switched to the use of chlorine dioxide as a flour improving and bleaching agent. More recently, a great many compounds have been investigated in an effort to establish whether they might be added to food with a reasonable surety that no harm would result. At the time this investigation was planned a great deal of interest was being shown in the additives used in bread manufacture. Of the many compounds intentionally added to bread, chlorine dioxide, sodium propionate, the antioxidants *n*-propyl gallate and butylated hydroxyanisole, and the emulsifier polyoxyethylene (8) monostearate* were selected for study. The number of compounds which could be tested in a chronic toxicity trial was limited by the facilities available and it was considered that these 4 additives were most deserving of study.

Most investigations of this nature have been made by adding such compounds singly, in abnormally large amounts, to otherwise normal experimental diets. This may result in unequal concentrations of proteins, vitamins, minerals, and calories among the diets and the difficulty of providing adequate controls under these conditions is obvious. It appeared that a sounder method of checking for chronic toxicity would be to incorporate the additives in bread at abnormally high levels and, maintaining the levels of the nutritive elements as close to those of the control diets as possible, to give this bread as a major part of a good experimental diet. It was decided to reverse the usual procedure and to check the toxicity of the additives in combination rather than singly since the toxicity of the finished bread was of major concern.

In view of the popular "100-fold margin of safety"[3] or "margin of uncertainty" as it is rather aptly termed by Oser[1], it was decided to incorporate the additives under test in bread at 50 times the normal concentration and to give the finished bread as 75 per cent. of the experimental diet. It was considered that giving this amount would correspond to over 100 times the usual rate of intake for man.

The seven diets used are shown in Table I. The emulsifier, polyoxyethylene (8) monostearate, and the mould inhibitor, sodium propionate,

* The use of this material in bread had not been prohibited in Canada at the time these investigations were started.

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were taken as approximately equivalent, on a caloric basis, to carbohydrate and additions of these ingredients were made at the expense of glucose. On this basis all 6 bread diets were approximately isocalorific and were as near alike in all respects, except for the additives under test, as could be achieved.

Diet I contained bread made to an essentially normal formula with the exception that the glucose content was elevated to permit substitution of high levels of emulsifier and mould inhibitor in other diets. Diet II contained all 4 test ingredients at 50 times the level used in diet I. With the flour and antioxidants this was accomplished conveniently with little weight change in the diet ingredients. Diets III, IV, V and VI were similar to diet II with the exception that one ingredient in each, emulsifier, propionate, antioxidants, or chlorine dioxide, respectively, was reduced to the control concentration. Diet VII consisted of a commercial cubed diet* ground to a consistency similar to that of the other diets. The basal diet used as 25 per cent. of diets I to VI supplied adequate supplementary amounts of all the required vitamins as well as high quality protein, bulk, minerals, and fat.

Technical difficulties were encountered in the preparation of the various breads, which are referred to here by the same numbers as the corresponding diets. Those mixes which contained the high concentration of propionate showed very poor yeast action. Increasing the quantity of yeast had little beneficial effect. To overcome this difficulty to some extent the dry propionate was incorporated only into the surface of the dough so that fair yeast action in the bulk of the material was obtained. With this modification of the mixing scheme it was possible to produce breads which had been proofed approximately equivalent lengths of time. As might be expected, loaf volume and texture on numbers II, III, V and VI were poor. To a lesser extent bread I was inferior to normal quality because of the inhibitory effect of the high glucose content on yeast action. Bread IV was excessively friable and open textured.

After baking, the loaves were wrapped and stored in the refrigerator for short periods before being thinly sliced. The slices were then placed on edge in a special drier and dried in a forced draft hot air oven at 70° C. The drying process usually took 3 to 5 hours, and, for those loaves high in propionate, was accompanied, as was the baking, by a strong odour of propionate. Subsequently the dried bread was ground in a hammer mill and stored in sealed metal containers in the refrigerator (4° C.) until required for use. This dried, ground bread was rarely kept for more than 1 month. Diets were mixed at intervals of no greater than 2 weeks and were kept in closed cans in the refrigerator except during feeding periods.

A preliminary feeding trial of 3 weeks' duration indicated that there was little difference in the acceptability of the several diets to weanling rats. None of the ingredients was acutely toxic at the concentrations under test. Accordingly, a large scale experiment was started. A group of 182 male albino rats of Wistar strain ranging in age from 27 to 41 days

* Master Fox Cubes, Toronto Elevators, Toronto.

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TABLE I

DIET COMPOSITIONS

Part A. 75 per cent. of the diet	Diet						
Ingredient	I	II	III	IV	V	VI	VII
Commercial bread flour[1]	70·5	—	—	—	—	70·5	GROUND MASTER
Bread flour 50 × ClO_2[1]	—	70·5	70·5	70·5	70·5	—	
Commercial lard[2]	1·8	—	—	—	1·8	—	
Lard 50 × Antioxidants[2]	—	1·8	1·8	1·8	—	1·8	
Sodium propionate[3]	0·1	5·0	5·0	0·1	5·0	5·0	
Polyoxyethylene (8) monostearate[4]	0·3	15·0	0·3	15·0	15·0	15·0	
Cerelose (glucose)	20·4	0·8	15·5	5·7	0·8	0·8	
Salt (NaCl)	1·8	as I	as I	as I	as I	as I	
Skim milk powder	2·2	"	"	"	"	"	
Malt flour	0·5	"	"	"	"	"	
Yeast food[5]	0·2	"	"	"	"	"	
Wytase[6]	0·9	"	"	"	"	"	
Yeast	1·3	"	"	"	"	"	
	100·0	100·0	100·0	100·0	100·0	100·0	

Part B. 25 per cent. of the diet	Diet						
Ingredient	I	II	III	IV	V	VI	
Casein	60·0	as I	as I	as I	as I	as I	FOX CUBES
Alphacel[7]	9·4	"	"	"	"	"	
Corn oil	12·8	"	"	"	"	"	
Mineral mix (a)	12·0	"	"	"	"	"	
Vitamins and excipient (b)	5·8	"	"	"	"	"	
	100·0	100·0	100·0	100·0	100·0	100·0	

(a)—Minerals—the 12·0 g. of mineral mix present in 100 g. of basal diet were made up as follows—

KCl	1·580	Fe PO_4	0·195
KH_2PO_4	4·100	$KAl(SO_4)\cdot 12H_2O$	0·001
$Ca_3(PO_4)_2$	1·970	$CuSo_4\cdot 5H_2O$	0·080
Mg SO_4	1·190	NaF	0·076
$MnSO_4\cdot H_2O$	0·027	KI	0·001

(b)—Vitamins—the 5·8 g. of vitamins and excipient in 100 g. of basal diet were made up as follows—

Thiamin hydrochloride	0·0020		
Riboflavin	0·0040	Menadione	0·0004
Calcium pantothenate	0·0080	E concentrate*	0·0560
Pyridoxine	0·0020	A and D concentrate†	0·1600
Nicotinamide	0·0080	Corn oil	4·2830
l-Inositol	0·0300		
Choline chloride	0·4000		
Folic acid	0·0008		
p-Aminobenzoic acid	0·0040		
Liver fraction‡	0·8000		

* Vitamin E concentrate, 350 mg. of *dα*-tocopheryl acetate equivalent per g.
† Navitol, 65000 A, 13000 D per g.
‡ Wilson's Liver fraction L.

NOTES

1. Chlorine dioxide.—Canadian number one patent flour was treated with 0·3 g. of chlorine dioxide per barrel (196 lb.) to yield commercial bread flour and with 15·0 g. of chlorine dioxide per barrel to yield high ClO_2 flour.
2. Antioxidants.—Tenox II, a commercial preparation containing 20 per cent. butylated hydroxyanisole, 6 per cent. *n*-propylgallate, 4 per cent. citric acid and 70 per cent. propylene glycol was used at the rates of 0·05 per cent. in commercial lard and 2·5 per cent. in high antioxidant lard.
3. Propionate.—The commercial sodium propionate sold by Du Pont de Nemours and Co., under the trade name Mycoban was used.
4. Polyoxyethylene monostearate.—The material sold by Atlas Powder Co. as polyoxyethylene (8) monostearate under the trade name Myrj 45 was used.
5. Yeast food.—The commercial material sold under the name RKD by Standard Brands was used.
6. Wytase.—This material is sold by J. R. Short Canadian Mills Limited, and is used commercially to improve whiteness.
7. Alphacel is a commercial cellulose supplying bulk without nutritive value.

and in weight from 41 to 91 g. was divided into 7 sub-groups by arranging the rats in order of decreasing weight and assigning consecutive lots of 7 rats by random selection to the sub-groups. The 182 female rats

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aged 25 to 41 days and weighing 36 to 86 g. were grouped in a similar manner. The oldest rats of each sex had been maintained on diet VII from weaning at 22 days of age until placed on test. The 14 groups of rats were housed in cages with wire screen floors. Each cage held 1 group (26 rats). Food and water were allowed *ad libitum*. For the first 3 weeks, feed consumption and body weight were recorded twice weekly; thereafter the measurements were made once a week. Cages were moved in a regular rotation on the racks so that all spent approximately equal intervals at the 4 different levels above the floor. Mortality was recorded and the probable cause of death (autopsy) was noted for those rats which died and were found before autolysis was too far advanced.

At 13 and 26 weeks, 3 rats of each sex from each diet were chosen by random selection and killed. Tissue samples from the bladder, small intestine, spleen, stomach, pancreas, adrenal, kidney, liver, heart, lung, thyroid, brain, and either testicle or ovary were preserved in formalin-saline fixative and reserved for histopathological examination. At the 52nd week all surviving animals were killed and the heart, liver, spleen and both kidneys were weighed. In addition, the usual tissue samples were reserved for histopathological examination. Gross abnormalities which were observed were recorded during this work. It should be noted that the rats were group fed and that the food consumption figures include wastage. Every effort was made to keep wastage at a minimum and there is no reason to believe, on the basis of observations made, that any particular group wasted more food than other groups.

Results

The mean body weights, at weekly intervals, of the rats in the 14 groups are presented graphically in Figures 1A (males) and 1B (females). The mean body weights of the survivors on each diet at 52 weeks and the standard error of the means are shown for males, females, and the sexes combined in Table II.

TABLE II

Final weight of survivors at 52 weeks

Weight in grams ± standard error

	Sexes combined		Males		Females	
Diet	Mean wt.	No.	Mean wt.	No.	Mean wt.	No.
I	248 ± 8·8	16	284 ± 9·8	6	226 ± 5·9	10
II	262 ± 10·8	17	288 ± 17·9	8	238 ± 6·9	9
III	266 ± 6·7	18	290 ± 7·5	8	251 ± 6·7	10
IV	251 ± 12·5	18	303 ± 25·7	6	226 ± 5·7	12
V	260 ± 9·4	22	295 ± 10·8	10	231 ± 7·5	12
VI	278 ± 16·7	14	333 ± 12·8	7	224 ± 6·8	7
VII	244 ± 7·8	19	266 ± 11·7	8	229 ± 7·7	11

The commercial diet (VII) was approximately equivalent to the bread diets in supporting the growth of female rats. Female rats on diet III surpassed in growth those on diet VII toward the end of the experiment. For male rats, with their more rapid growth, diet VII was inferior to

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bread diet I in the earlier weeks of the experiment. At 10 weeks the mean weight of male rats on diet I was significantly greater than that of male rats on diet VII. At the end of the 52nd week this difference had disappeared and the mean body weight of male rats on diet VII was not significantly lower than that of male rats on diet I or on all 6 bread diets combined.

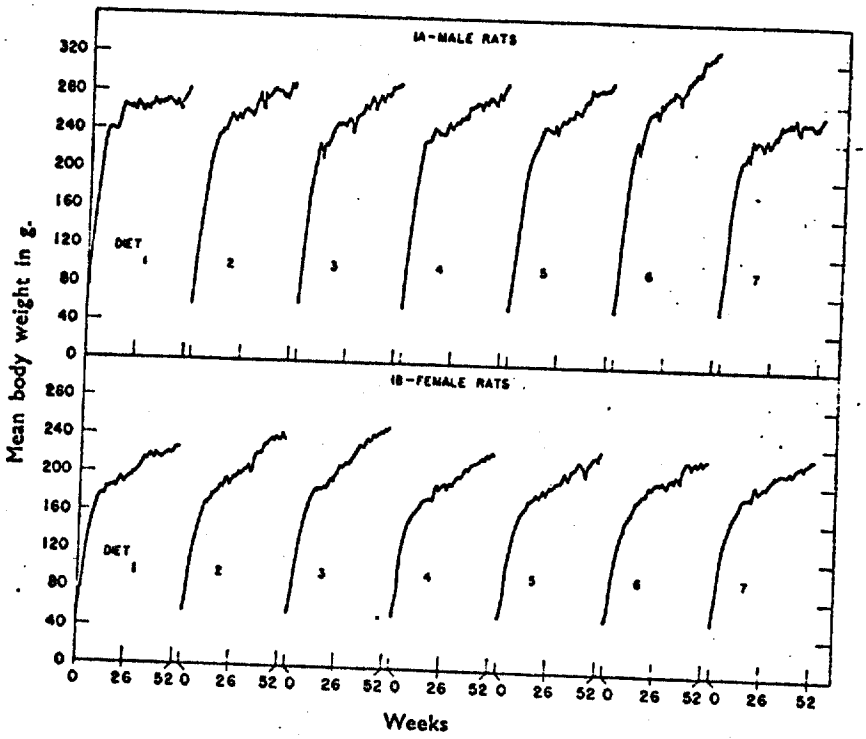

FIG. 1.

The final weights recorded in Table II were subjected to an analysis of variance based on the statistical methods of Snedecor[5] for disproportionate groups with significant interaction. The results for the commercial diet (VII) were excluded from these and subsequent calculations since the rats on this diet were included only to illustrate the usual growth, mortality, and pathology of animals from the laboratory colony. The results of the statistical analysis shown in Table III indicate that although sex had a significant effect on final weight, as was to be expected, diet was without significant influence.

The significant interaction apparently arose from the fact that male rats on the 4 diets high in polyoxyethylene (8) monostearate had a higher mean weight than those on the 2 diets low in this ingredient and female rats on the 4 diets high in chlorine dioxide had a higher mean weight than those on the 2 diets low in chlorine dioxide. These differences however were below the 5 per cent. point level of significance when the

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"t" test was applied to the appropriate means. Since the effects of diet were not significant it may be concluded that none of the additives tested, in the amounts and manner used, had any deleterious effect on the growth of male or female rats during one year.

TABLE III

ANALYSIS OF VARIANCE OF FINAL WEIGHT DATA

Main effect	D.F.	Mean square	F
Sex	1	109908	47**
Diet	5	951	<1
Diet × sex ..	5	2346	2·56*
Error	93	919	

* significant at P = 0·05 ** significant at P = 0·01

Cumulative food consumption per rat per day over the 52-week period is shown in Figure 2A. It is readily observed that the commercial diet was eaten to a greater extent than the other diets, particularly in the case of the male rats, where significantly larger amounts of the diet VII were consumed. The cumulative food efficiency data, shown in Figure 2B, were plotted in a log relationship with time and straight lines were fitted

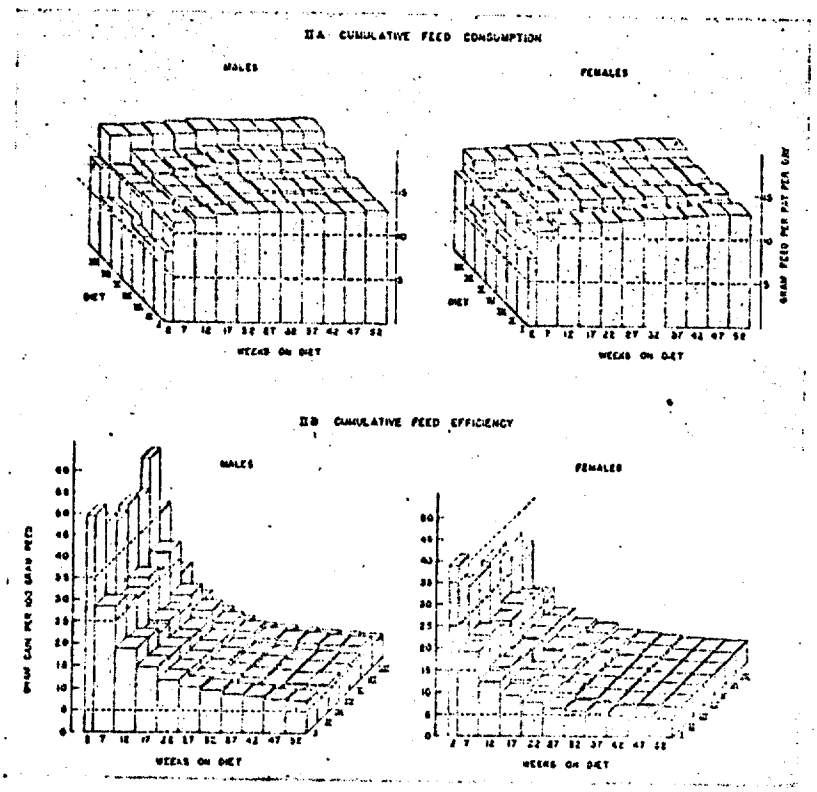

FIG. 2.

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TABLE IV

A. HISTOPATHOLOGICAL FINDINGS

	Sex													
	Male							Female						
Diet	I	II	III	IV	V	VI	VII	I	II	III	IV	V	VI	VII
Number of rats examined*	12	14	14	12	16	13	14	16	15	16	18	18	13	17
Normal rats	9	7	10	9	11	8	10	13	7	8	10	14	9	14
Brain liquefaction necrosis (embolus)	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Brain inflammation (cerebromeningitis)	—	—	—	—	—	1	—	—	—	—	—	—	—	—
Lungs—respiratory infection	1	2	2	1	1	1	2	2	—	3	1	—	2	2
Heart—fatty degeneration	—	—	—	—	—	—	1	—	—	—	—	—	—	—
Liver—cloudy swelling, fatty degeneration	—	1	—	—	—	1	1	—	2	—	—	—	—	—
Liver—excessive deposits of haemosiderin	—	—	—	—	—	—	—	—	—	—	2	—	—	—
Spleen—excessive deposits of haemosiderin	—	—	—	—	—	—	—	—	—	—	2	—	—	—
Stomach—inflammation of submucosa	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Intestine—erosion	—	—	—	—	—	—	—	1	—	1	2	—	—	—
Kidney—degeneration of tubules	—	—	—	—	1	—	—	—	1	—	2	1	2	—
Kidney—glomerulonephritis	—	—	—	—	—	—	—	—	2	2	—	—	—	—
Kidney—hydronephrosis	—	—	—	—	—	—	—	—	1	1	—	1	—	—
Adrenal—cloudy swelling, necrosis in zona fasiculata	—	—	—	—	1	1	—	—	1	—	—	1	—	—
Bladder—parasite Trichosomoides crassicauda	2	5	2	3	3	1	1	—	1	3	—	1	2	2
Testicle—degeneration	—	—	1	—	—	—	—	—	—	—	—	—	—	—

* 3 rats were killed at 13 weeks, 3 at 26 weeks, and the remainder at 52 weeks.

B. MORTALITY DATA

Died on test	14	12	12	14	10	13	12	10	11	10	8	8	13	9
Undiagnosed	5	4	9	7	6	9	8	7	9	5	4	7	8	4
Respiratory infection	8	6	3	7	4	3	3	1	2	5	4	1	2	5
Middle ear disease	1	2	—	—	—	1	1	2	—	—	—	—	1	—
Tumor—fibroadenoma	—	—	—	—	—	—	—	—	—	—	—	—	2	—

to the points by the method of least squares. These lines were not significantly different for female rats but for the males the food efficiency on diet VII was significantly less than that on diet I.

The sections from the tissues of the 208 rats were stained with hæmatoxylin and eosin and examined microscopically. The group to which the various rats belonged was unknown to the pathologist (H.C.G.) at the time the tissues were examined. The histopathological findings are presented in Table IVA and the results have been summarised by the pathologist as follows:

"The tubular degeneration and perhaps the glomerulonephritis seen mainly in the kidneys of female rats are the only histopathological findings considered to be significant. The tubular degeneration is, in all probability, due to a toxic substance. The lack of coexisting glomerular change is remarkable as the glomerulus is usually affected before the tubules. It may be that there is a tubular specificity of the injurious agent or that there is an inherent weakness of the tubular epithelium in these particular rats. The former supposition is more probable. The hydronephrosis in each case was unilateral. Dilatation of the kidney pelvis may be caused by concretions, parasites, or tumors in the urinary bladder or it may be congenital. Concretions were not found in any of the bladders, there were no tumors present in the bladders of any of the animals examined, and there was no evidence of congenital atresia of the ureters. While parasites were not observed in the bladders of rats suffering from hydronephrosis, these could have been missed in histological

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cross section (4 microns) and the known incidence of such parasites is sufficient to attribute the hydronephrosis to bladder parasites."

The significant kidney changes in female rats were found on diets II, III, IV and V. While the incidence is low, it should be noted that these were the rats receiving flour treated with the high concentration of chlorine dioxide. Tubular damage from propyl gallate has been demonstrated by Orten, Kuyper and Smith[6], and this may have been a contributing factor. Again, the incidence is so low that the distribution may be fortuitous.

The overall mortality data are tabulated in Table IVB. It will be noted that a considerable number of deaths occurred for which no explanation is given. These deaths occurred at night and by the time the bodies

TABLE V

EFFECT OF DIET ON THE WEIGHT OF VARIOUS ORGANS
Mean tissue weights in mg. per g. of rat

Diet	Number	Liver	Right kidney	Left kidney	Heart	Spleen
A. Males						
I	6	32·7	3·56	3·55	3·96	2·82
II	8	31·0	3·66	3·83	3·81	2·51
III	8	32·5	3·72	3·71	3·65	2·71
IV	6	37·4	3·38	3·31	3·22	2·16
V	10	34·0	3·60	3·51	3·42	2·64
VI	7	34·2	3·47	3·35	3·38	2·59
VII	8	34·1	3·41	3·19	3·84	2·67
Over-all standard deviation		4·6	0·43	0·42	0·40	0·38
B. Females						
I	10	35·0	4·10	4·07	4·48	3·47
II	9	39·5	4·95	5·06	4·29	3·31
III	10	41·5	4·82	4·81	4·70	4·01
IV	12	37·3	4·28	4·24	4·10	4·03
V	12	38·0	5·19	5·02	4·68	3·65
VI	7	35·6	4·46	4·61	4·47	3·43
VII	11	38·4	4·02	4·06	4·08	3·59
Over-all standard deviation		4·5	0·81	0·67	0·42	0·85

TABLE VI

ANALYSES OF DATA ON TISSUE WEIGHTS

Main effect	D.F.	Liver		Right kidney		Left kidney		Heart		Spleen	
		m.s.	F.	m.s.	F.	m.s.	F.	m.s.	F.	m.s.	F.
Sex	1	3088	15*	15932	49**	16934	61**	11508	46**	25473	33**
Diet	5	212	1·0	618	2·1	714	2·6	299	1·2	361	<1·0
Sex × diet ..	5	203	1·4	324	<1·0	276	2·1	248	3·4**	765	1·5
Error	93	147		397		133		73		493	

* significant at P = 0·05 ** significant at P = 0·01

were retrieved in the morning autolysis was too far advanced to allow conclusive autopsy findings. It may be stated with a considerable degree of confidence that most of these deaths were due to respiratory infection. A χ^2 analysis of the data revealed no significant influence of diet on mortality.

The mean weights of the livers, kidneys, hearts, and spleens of the rats which survived to the time of sacrifice at 52 weeks are listed in Table V.

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For statistical analysis the data in Table V were transformed into percentages of the mean weight for each tissue. The resulting data were analysed by Snedecor's methods for disproportionate subclass numbers. The results of these analyses are presented in Table VI.

As might be expected, sex had a significant influence on the weight of the tissues examined. Since the sex × diet interaction was significant in one instance and in 4 of 5 instances the mean square for interaction was of appreciable magnitude these interactions were used in the calculation of the F for diet and sex. In no instance did diet have a significant influence on tissue weight.

Discussion

Propionate.

Very few experimental data on the toxicity of sodium propionate are available. In a 3-week trial Harshbarger[7] fed concentrations of 1 and 3 per cent. sodium or calcium propionate in the diets of rats. No effect on weight gains was observed. Heseltine[8] reported that sodium propionate had a weak antihistamine action about 1/7·5 that of diphenhydramine. Daily oral doses of 6 g. to men led to the production of a fairly alkaline urine but no appreciable diuresis, catharsis, or other effects.

These findings are confirmed and extended by the present data which show that at concentrations of 5 per cent. in bread ingredients before baking, a finished product is obtained which exerts no discernible toxicity when fed as 3/4 of the diet of rats for 1 year; this in spite of the added stress of the presence of 3 other additives at high concentration in the diet.

Antioxidants.

n-Propyl gallate is a fat-soluble ester of the widely-occurring natural antioxidant gallic acid. It has been fairly intensively investigated by Orten *et al.*[6], who found that concentrations of 1·17 per cent. in the diet of male rats for 6 months caused growth depression, a decrease in hæmoglobin levels, and some damage to the kidney tubules. Guinea-pigs receiving 0·011 per cent. in the diet for 1 year and dogs receiving similar amounts for 14 months showed no ill-effects. Lehman, Fitzhugh, Nelson and Woodard[9] found this antioxidant even less toxic and, in rats, detected no growth inhibition at 1 per cent. of the diet of males or females. At 5 per cent. of the diet growth depression occurred in male rats, there was some increase in mortality over the 2-year feeding period, and there was evidence of patchy hyperplasia of the proventriculus. Allen and De Eds[10] and Van Sluis[11] reported very low toxicity for lauryl gallate given to rats.

Wilder and Kraybill[12] reported that butylated hydroxyanisole (the commercial material is usually a mixture of the 2- and 3-tertiary butyl esters) when given to rats for periods up to 21 months at concentrations up to 0·06 per cent. of the ration caused no toxic effects. At 0·12 per cent. palatability of the ration was lowered but no pathology was detected. Evidence accumulated by the U.S. Food and Drug scientists[13] indicated that this antioxidant was safe when used in the usual concentrations.

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The amounts of the several ingredients of the antioxidant mixture fed in the various diets is shown in Table VII.

In the amounts in which they occurred there is no reason to suspect any direct toxic action from the propylene glycol or the citric acid. The high level of butylated hydroxyanisole was about 1/10 that found to cause no harmful effects and the high level of *n*-propyl gallate was about 1/50 of the maximum non-harmful amount. Even the combination of these 2 failed to exert any significant influence on the health of the rats.

TABLE VII

ANTIOXIDANT AND PROPYLENE GLYCOL CONTENT OF DIETS I–VI

	Diets I and V	Diets II, III, IV and VI
	mg./kg.	mg./kg.
Butylated hydroxyanisole ..	1·35	67·50
n-Propyl gallate	0·405	20·25
Citric acid	0·27	13·50
Propylene glycol carrier ..	4·725	236·25

Polyoxyethylene (8) *Monostearate.*

The literature on the toxicity of polyoxyethylene (8) monostearate is rather considerable. The chief conclusion reached after a careful scrutiny of these data is that, under certain conditions, polyoxyethylene (8) monostearate in fairly large amounts in the diet may cause harmful effects[14,15,16,17]. Whether or not these certain conditions are of such a nature as to lend practical significance to the observed toxicity is a matter of argument. The literature is too voluminous to review in detail here. Suffice to say that loss in weight, diarrhœa, bladder stones, etc., have been observed in rats fed diets containing 5 per cent. or more of this emulsifier. Other investigators have not demonstrated any harmful effects at concentrations up to 5 per cent. in the diet[18]. To quote Frazer[19] "Grossly excessive amounts of polyoxyethylene monostearate—some workers have used as much as 15 per cent. of the diet—may produce some effects. Some of the results of these experiments are not convincing and in any case they have little relevance to the normal dietary use of these materials."

Culver, Wilcox, Jones and Rose[20] have shown that in humans receiving polyoxyethylene (40) monostearate the polyoxyethylene was recovered almost completely in the urine and fæces, that this portion of the molecule was recovered free of combined fatty acid, and that there was no evidence of storage.

In the experiments reported here, polyoxyethylene (8) monostearate occurred as 0·3 or 15·0 per cent. of the breads which made up 75 per cent. of the diet. Under these conditions and in spite of 2 or 3 additional dietary stresses, no toxic effect of this material was observed.

Chlorine Dioxide.

The toxicity of chlorine dioxide-treated flour has been the subject of several investigations. Arnold[21] gave dogs, as 80 per cent. of their diet, flour treated with 5 g. of chlorine dioxide per 100 lb. for periods up to 28 days without detecting any ill-effect. Newell, Gershoff, Suckle,

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Gilson, Erikson and Elvehjem[22], carried out similar test giving 60 per cent. flour in the ration with a maximum chlorine dioxide treatment of 4 g. per 100 lb. and continuing the trials for 13 weeks. The results of these studies on dogs were negative. Similar studies on rabbits (6 weeks) monkeys (5·5 months) and rats (5 weeks) also gave negative results. A group of 13 humans receiving .55 g. of chlorine dioxide-treated wheat gluten (20 g. of ClO_2 per 100 lb. of gluten) daily for 6 weeks showed no detectable injury. Nakamura and Morriss[23] gave to dogs flour treated with high concentrations of chlorine dioxide for prolonged periods without seeing any evidence of canine hysteria. With the exception of the last experiment most of the studies were of short duration and no long-term studies on rats have been reported.

In the present investigation it has been shown that rats receiving a diet containing 75 per cent. of bread made from flour treated with 15 g. of chlorine dioxide per barrel (196 lb.), 50 times the normal rate of treatment, for 1 year from weaning age exhibited no signs of toxic effect as evidenced by growth, mortality, gross observation, organ weights or histopathological examination of the tissues.

Summary

1. Bread containing 50 times the normal concentration of chlorine dioxide, propyl gallate and butylated hydroxyanisole, polyoxyethylene (8) monostearate, or sodium propionate as 75 per cent. of the diet for 1 year did not harmfully affect growth or mortality of rats.

2. The high concentrations of polyoxyethylene (8) monostearate, sodium propionate, antioxidants and chlorine dioxide had no detectable effect on organ weights or on histopathology of the tissues.

3. No evidence was obtained that the simultaneous presence of high concentrations of more than one potential toxicant in the diet led to any synergistic action.

The authors wish to thank Mr. Robert Van Burek and Wallace and Tiernan, Ltd., for the treatment of the flour with chlorine dioxide, the Tennessee Eastman Corporation for supplies of the antioxidant Tenox II, the J. R. Short Canadian Mills, Ltd., for a supply of Wytase, and the Lake of the Woods Milling Co. for a quantity of malt flour. They are deeply indebted to Miss E. Connell for technical assistance in the histopathological work. The laborious tasks of mixing and baking the experimental breads was carried out by Miss Margaret Lock and Miss Dorothea Tripp of the Nutrition Division of this Department. The authors express their gratitude for this assistance and for the interest and advice of Dr. L. B. Pett, Dr. L. I. Pugsley, Mr. M. G. Allmark, and Dr. J. A. Campbell of this Department and of Mr. N. Gridgeman of the National Research Council.

References

1. Mellanby, *Brit. med. J.*, 1946, 2, 885.
2. Bentley, McDermott, Pace, Whitehead and Moran, *Nature, Lond.*, 1950, 165, 150.

3. Lehman,
Cosmet
4. Oser, *Che*
5. Snedecor,
6. Orten, K
7. Harshbar
8. Heseltine,
9. Lehman,
10. Allen and
11. Van Sluis,
12. Wilder an
Institute
13. Lehman,
14. Schweigert
15. Wang, Me
16. Sherman,
17. Harris, Sh
18. Krantz, Te
Food Pr
Printing
19. Frazer, *Pro*
20. Culver, Wi
21. Arnold, *Ce*
22. Newell, Ga
1949, 26,
23. Nakamura

ention is drawn to the possibility that this text may be copyrighted. The reade
urged to investigate its copyright status.

3. Lehman, Lang, Woodard, Draize, Fitzhugh and Nelson, *Food, Drug and Cosmetic Law Quarterly*, 1949, 4, 412.
4. Oser, *Chem. Engng News*, 1951, 29, 2808.
5. Snedecor, *Statistical Methods*, 4th Ed. Iowa State College Press, Iowa, 1946.
6. Orten, Kuyper and Smith, *Food Technol.*, 1948, 2, 308.
7. Harshbarger, *J. Dairy Sci.*, 1942, 25, 169.
8. Heseltine, *J. Pharm. Pharmacol.*, 1952, 4, 120.
9. Lehman, Fitzhugh, Nelson and Woodard, *Adv. Food Res.*, 1951, 3, 197.
10. Allen and De Eds, *J. Amer. Oil Chem. Soc.*, 1951, 28, 304.
11. Van Sluis, *Food Manufact.*, 1951, 26, 99.
12. Wilder and Kraybill, Summary of toxicity studies on BHA. American Meat Institute Foundation, Chicago, Ill.
13. Lehman, *Bull. Assoc. Food and Drug Officials of the U.S.A.*, 1950, 14, 82.
14. Schweigert, McBride and Carlson, *Proc. Soc. exp. Biol., N.Y.*, 1950, 73, 427.
15. Wang, McBride and Schweigert, *ibid.*, 1950, 75, 342.
16. Sherman, Harris and Jetter, *Fed. Proc.*, 1950, 9, 361, 370.
17. Harris, Sherman and Jetter, *Arch. Biochem. Biophys.*, 1953, 34, 249.
18. Krantz, Testimony before the Delaney Committee reported in Chemicals in Food Products, Hearings, 81st Congress second session. U.S. Government Printing Office, Washington, 1951.
19. Frazer, *Proc. Roy. Soc. Med.*, 1952, 45, 681.
20. Culver, Wilcox, Jones and Rose, *J. Pharmacol.*, 1951, 103, 377.
21. Arnold, *Cereal Chem.*, 1949, 26, 46.
22. Newell, Gershoff, Suckle, Gilson, Erickson and Elvehjem, *Cereal Chem.*, 1949, 26, 160.
23. Nakamura and Morris, *ibid.*, 1949, 26, 501.

CHRONIC TOXICITY OF BREAD ADDITIVES TO RATS. PART II

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In an earlier investigation[1] of the chronic toxicity of certain bread additives the ingredients tested (chlorine dioxide, polyoxyethylene (8) monostearate, sodium propionate and a mixture of propyl gallate and butylated hydroxyanisole) were incorporated in bread at 50 times the normal concentration and used for 52 weeks at .75 per cent. of the diet of rats. Under the conditions of the experiment no adverse effects were noted and these results were at variance with some of the experimental evidence obtained by other investigators, particularly in regard to the polyoxyethylene (8) monostearate[2,3,4,5]. Possibly this difference was due to experimental technique. It was of interest, therefore, to retest the bread ingredients previously studied, with the difference that they were not mixed with water, baked into bread, and the bread dried and ground but were added directly to the basal ration.

Diet I contained the ingredients of an essentially normal bread (75 per cent.) added to a basal medium supplying minerals, vitamins, protein, fat, and bulk (25 per cent.). In diet II, flour treated with 50 times the normal amount of chlorine dioxide, lard containing 50 times the usual level of antioxidants, and sodium propionate and polyoxyethylene (8) monostearate each at 50 times the usual concentration were incorporated in the bread ingredient portion of the diet. Diets III, IV, V and VI were similar to diet II, except that one ingredient in each, respectively emulsifier, mould inhibitor, antioxidants, or flour improver, was reduced to the normal level. Diet VII was a commercial cubed ration ground to a consistency similar to that of the other diets. Diets were mixed at intervals of no greater than 2 weeks and were kept in closed cans in the refrigerator. The details of diet composition for convenience are reproduced from the previous publication[1] in Table I.

A group of 210 Wistar strain male albino rats ranging in age from 23 to 36 days and in weight from 35 to 79 g. was divided into 14 sub-groups by arranging them in descending order of weight and randomly assigning the rats in successive lots of 14 to the 14 sub-groups. These sub-groups, each consisting of 15 animals, were assigned to the 7 diets yielding two series, called for convenience replicates A and B, and numbered according to the number of the diet given. The oldest rats had been maintained on diet VII from weaning at 22 days of age until placed on test. The 14 groups of rats were housed in cages with wire screen floors. Each cage held 1 group (15 rats). Cages were moved in a regular rotation on the racks so that all spent approximately equal intervals at the 4 different levels above the floor. Food and water were permitted *ad libitum*. Records of weekly weight, food consumption and mortality

TABLE I

Diet compositions

Part A.—75 per cent. of the diet.

Ingredient	Diet I	II	III	IV	V	VI	VII
Commercial bread flour[1]	70·5	—	—	—	—	70·5	GROUND MASTER
Bread flour 50 × ClO_2[1]	—	70·5	70·5	70·5	70·5	—	
Commercial lard[2]	1·8	—	—	—	1·8	—	
Lard 50 × Antioxidants[2]	—	1·8	1·8	1·8	—	1·8	
Sodium propionate[3]	0·1	5·0	5·0	0·1	5·0	5·0	
Polyoxyethylene (8) monostearate[4]	0·3	15·0	0·3	15·0	15·0	15·0	
Cerelose (glucose)	20·4	0·8	15·5	5·7	0·8	0·8	
Salt (NaCl)	1·8	as I	as I	as I	as I	as I	
Skim milk powder	2·2	"	"	"	"	"	
Malt flour	0·5	"	"	"	"	"	
Yeast food[5]	0·2	"	"	"	"	"	
Wytase[6]	0·9	"	"	"	"	"	
Yeast	1·3	"	"	"	"	"	
	100·0	100·0	100·0	100·0	100·0	100·0	

B.—25 per cent. of the diet.

Ingredient	Diet I	II	III	IV	V	VI	
Casein	60·0	as I	as I	as I	as I	as I	FOX CUBES
Alphacel[7]	9·4	"	"	"	"	"	
Corn oil	12·8	"	"	"	"	"	
Mineral mix[a]	12·0	"	"	"	"	"	
Vitamins and excipient[b]	5·8	"	"	"	"	"	
	100·0	100·0	100·0	100·0	100·0	100·0	

[a] Minerals—the 12·0 g. of mineral mix present in 100 g. of basal diet were made up as follows:—

KCl	1·580	$FePO_4$	0·195
KH_2PO_4	4·100	$KAl(SO_4)_2.12H_2O$	0·001
$Ca_3(PO_4)_2$	1·970	$CuSO_4.5H_2O$	0·080
$MgSO_4$	1·190	NaF	0·076
$MnSO_4.H_2O$	0·027	KI	0·001

[b] Vitamins—the 5·8 g. of vitamins and excipient in 100 g. of basal diet were made up as follows:—

Thiamine hydrochloride	0·0020 g.	Folic acid	0·0008 g.
Riboflavine	0·0040 g.	*p*-Aminobenzoic acid	0·0040 g.
Calcium pantothenate	0·0080 g.	Liver fraction‡	0·8000 g.
Pyridoxine	0·0020 g.	Menadione	0·0004 g.
Nicotinamide	0·0080 g.	E concentrate*	0·0560 g.
i-Inositol	0·0800 g.	A and D concentrate†	0·1600 g.
Choline chloride	0·4000 g.	Corn oil	4·2830 g.

* Vitamin E concentrate, 350 mg. of *d*, α-tocopheryl acetate equivalent per g.
† Navitol, 65000 A, 13000 D per g.
‡ Wilson's Liver fraction L.

NOTES

[1] Chlorine dioxide.—Canadian No. 1 patent flour was treated with 0·3 g. of chlorine dioxide per barrel (196 lb.) to yield commercial bread flour and with 15·0 g. of chlorine dioxide per barrel to yield high ClO_2 flour.

[2] Antioxidants.—Tenox II, a commercial preparation containing 20 per cent. of butylated hydroxyanisole, 6 per cent. of *n*-propyl gallate, 4 per cent. of citric acid and 70 per cent. of propylene glycol was used at the rates of 0·05 per cent. in commercial lard and 2·5 per cent. in high antioxidant lard.

[3] Propionate.—The commercial propionate sold by Du Pont de Nemours and Co., under the trade name Mycoban was used.

[4] Polyoxyethylene monostearate.—The material sold by Atlas Powder Co. as polyoxyethylene (8) monostearate under the trade name Myri 45 was used.

[5] Yeast food.—The commercial material sold under the name RKD by Standard Brands was used.

[6] Wytase.—This material is sold by J. R. Short Canadian Mills, Ltd., and is used commercially to improve whiteness.

[7] Alphacel is a commercial cellulose supplying bulk without nutritive value.

were kept. Where the condition of the carcass warranted it, detailed autopsies were performed on animals that died on test; in other instances advanced autolytic changes precluded accurate decision as to the cause of death.

After 16 weeks on the diets 2 rats selected from each group by random choice were killed and a number of tissues were preserved for histological

study. Blood samples from each of these rats were analysed for hæmoglobin content by a slight modification of the pyridine hæmochromogen method of Rimington[6]. After 32 weeks on the diet only 3 rats survived in group II A and the experiment was terminated. All surviving rats were killed and the liver, left kidney, heart and spleen were weighed. Tissue samples were reserved for histological examination and subsequently sections from cerebellum, cerebrum, myocardium, trachea, œsophagus, thyroid, salivary gland, thymus, peri-bronchiolar lymph gland, lung, stomach, small intestine, liver, spleen, adrenal, pancreas, kidney, bladder and testis were studied.

Results

The mean body weights at intervals during the 32-week feeding trial of the rats in the 14 groups are presented graphically in Figure 1. The mean body weights of the survivors on each diet and the standard errors of the means are shown for replicates A and B and for the replicates combined in Table II.

TABLE II

Final weight of survivors at 32 weeks
Weight in g. ± standard error

Diet	Replicates combined		Replicate A		Replicate B	
	Mean weight	Number	Mean weight	Number	Mean weight	Number
I	296 ± 7·4	16	297 ± 12·4	7	295 ± 9·6	9
II	258 ± 7·4	13	257 ± 21·8	3	259 ± 13·2	10
III	268 ± 10·3	15	277 ± 13·5	7	260 ± 15·4	8
IV	281 ± 9·4	21	273 ± 14·2	11	290 ± 10·9	10
V	252 ± 7·0	21	247 ± 10·0	11	258 ± 9·9	10
VI	263 ± 6·5	18	255 ± 10·0	10	274 ± 7·0	8
VII	292 ± 8·1	19	305 ± 16·2	7	283 ± 8·4	12

TABLE III

Analysis of variance of final weight data

Main effect	DF	Mean square	F
Replicates	1	335	1
Diet	5	4,345	4·4*
Replicates X diet	5	792	1
Error	111	984	—

* Significant at P = 0·01.

Growth was depressed, particularly during the first few weeks on diets II, III, V and VI, i.e. on those diets containing the high level of sodium propionate. A detailed examination of the data revealed that the total weight gain subsequent to the first 4 weeks on the diet was approximately the same for each diet and the apparent depression of the final weights on these 4 diets high in propionate was the result of the curtailed growth during the first few weeks. Growth rates based on weight changes between the 5th and 32nd weeks were not significantly influenced by diet. Growth on diet I was very similar to that on diet VII throughout the test.

Statistical analysis of the final weight data for diets I to VI using Snedecor's methods for disproportionate groups[7] revealed a significant

(P = 0·01) influence of diet on final weight (Table III). The effect was mainly that of the high level of sodium propionate, for growth on diet IV was not significantly depressed.

Cumulative feed consumption per rat per day over the 32-week period is shown in Figure 2. As in the previous study[1] the rats ate more of the stock diet (VII) than of any of the test diets. In this experiment, feed consumption figures for all diets were higher than the corresponding

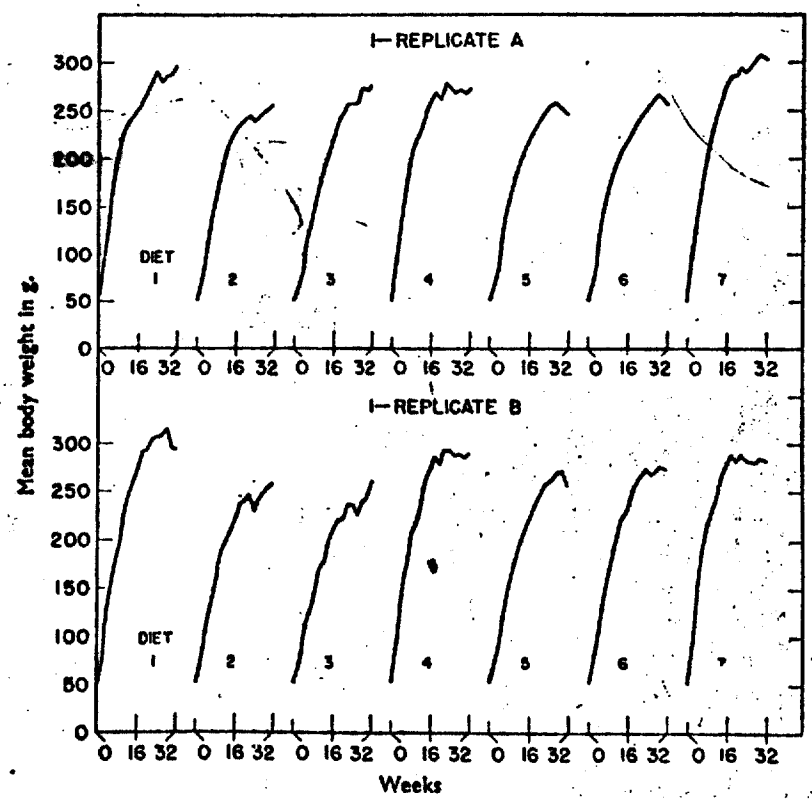

FIG. 1. Mean body weight of the rats in the 14 groups.

figures from the earlier data by an average of some 8·5 per cent. Mean body weights overall were only 3 per cent. higher. These differences are reflected in the generally poorer feed efficiency also depicted graphically in Figure 2. The striking effect of diet which may be observed here is the very poor feed efficiency during the first weeks on diets II, III, V and VI, particularly on diet III.

Curtailed growth, slightly lower feed consumption, and markedly lowered feed efficiency on diets II, III, V and VI during the early weeks indicate that sodium propionate fed at high levels to the young rat had a deleterious effect. That this effect was not solely due to lack of palatability of the diet is obvious from the fact that feed efficiency was much

more seriously affected than was feed consumption. Although the data are not amenable to statistical assessment, they suggest that polyoxyethylene (8) monostearate at high levels in conjunction with high sodium

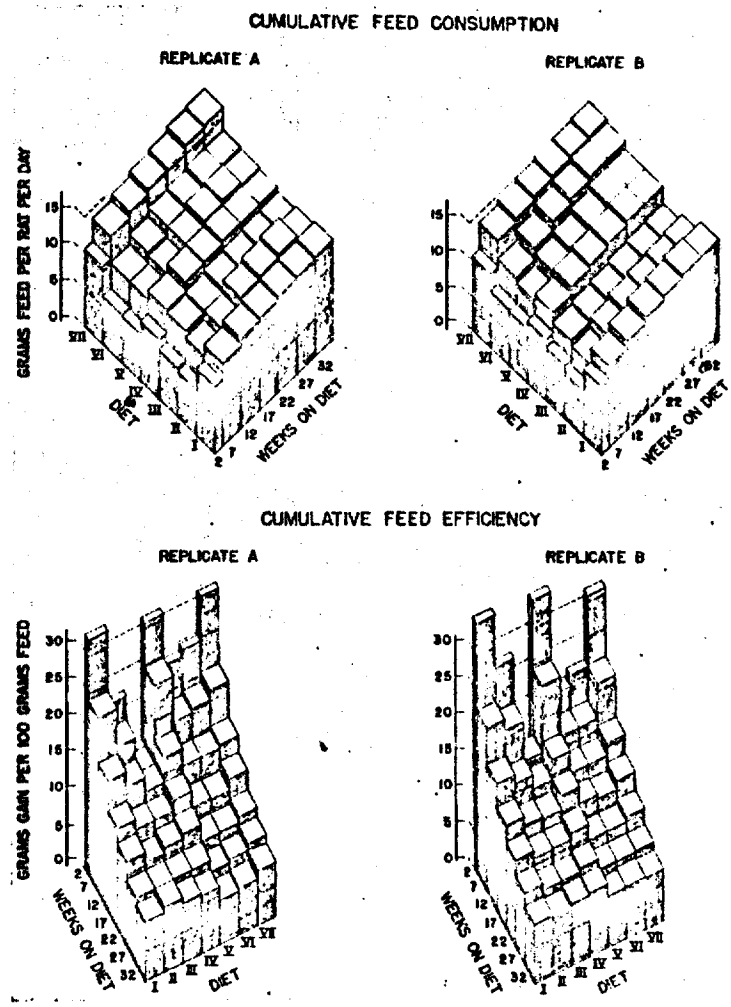

FIG. 2. Cumulative feed consumption per rat per day over the 32-week period.

propionate (diets II, V and VI) increased the efficiency with which the high propionate diet was utilised by young rats (compare diet III). Apparently the rats became acclimatised to the high propionate diet, for growth rate, feed consumption and feed efficiency approached the control levels within a few weeks.

The hæmoglobin levels in the blood of the 28 rats killed at the end of 16 weeks were all within the normal range for rats in this colony.

Sections from the tissues of 151 rats were stained with hæmotoxylin and eosin and examined microscopically. As in the previous investigation[1] the groups to which the individual rats belonged were unknown

TABLE IV

HISTOPATHOLOGICAL FINDINGS

	Replicate													
	A							B						
Diet	I	II	III	IV	V	VI	VII	I	II	III	IV	V	VI	VII
Number of rats examined*	9	5	9	13	13	12	9	11	12	10	12	12	10	14
Normal	4	2	4	2	0	1	1	5	4	1	5	1	1	3
Trachea—inflammation	–	–	–	–	5	2	–	2	–	1	–	4	1	–
Lung—respiratory infection	2	1	–	5	10	7	6	2	1	1	3	8	7	6
Lung—papilloma of bronchus	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Thymus—inflammation	–	–	1	–	–	1	–	–	–	–	–	–	–	–
Stomach—gastritis	–	–	1	–	–	–	–	–	2	–	–	–	–	–
Stomach—erosion	–	–	–	–	–	–	–	–	1	–	–	1	–	–
Adrenal—congestion	–	–	–	1	–	–	–	–	–	1	–	–	–	–
Kidney—tubal necrosis	–	–	–	–	1	–	–	–	–	–	–	–	–	–
Kidney—nephritis	–	1	–	1	1	–	–	–	–	–	–	1	–	–
Kidney—congestion	–	–	–	–	–	–	–	–	–	1	–	1	–	–
Liver—cloudy swelling	–	–	1	2	1	–	–	–	–	–	2	–	–	–
Liver—venous congestion	–	–	–	1	–	–	–	–	1	–	–	–	–	–
Pancreas—fatty degeneration of acinar tissue	–	–	–	–	–	–	–	–	–	–	–	–	1	–
Intestine—enteritis	–	–	1	–	1	–	–	–	1	–	–	–	–	–
Intestine—papilloma	–	–	–	–	–	–	–	–	–	1	–	–	–	–
Testis—reduced spermatogenesis	–	–	–	–	–	–	–	1	–	–	–	–	–	–
Bladder—parasitic cysts	4	3	5	8	5	6	8	5	5	9	6	4	6	8

* 2 rats were killed at 16 weeks, the remainder at 32 weeks.

MORTALITY DATA

Died on test	6	10	6	2	2	3	6	4	3	5	3	3	5	1
Autopsy	3	4	5	2	0	2	3	2	1	4	2	3	4	0
Undiagnosed	3	6	1	0	2	1	3	2	2	1	1	0	1	1
Respiratory infection	1	4	2	–	–	2	1	1	1	4	2	3	3	–
Middle ear disease	2	–	3	1	–	–	–	1	–	–	–	–	2	–
Encephalitis } Probable extension from middle ear infection	2	–	4	–	–	–	–	–	–	–	–	–	–	–
Meningitis } Probable extension from middle ear infection	–	–	1	1	–	–	–	1	–	–	–	–	2	–
Pericarditis	–	–	1	–	–	–	1	–	–	–	–	–	1	–
No visible lesion	–	–	–	1	–	–	1	–	–	–	–	–	–	–

to the pathologist at the time the tissues were studied. The histopathological findings are presented in Table IV and have been summarised by the pathologist as follows:

Findings in the cerebrum, cerebellum, myocardium, œsophagus, thyroid, salivary gland, thymus, small intestine, spleen, adrenal, pancreas, stomach and testis were negative or of no significance. Most of the lungs exhibited some changes due to pneumonia, including emphysema, bronchiectasis and atelectasis, although only acute and sub-acute cases have been listed in Table IV. The predominant bronchopneumonia invariably was accompanied by mild chronic inflammatory changes in the trachea. The two instances in which congestion and polymorphonuclear infiltration of the peribronchiolar lymph nodes were observed probably were the result of infection from the lungs. While cloudy swelling of the liver is the first sign of a degenerative process caused by

bacterial or chemical toxins, the few cases noted here were very mild and of doubtful significance. The incidence of kidney abnormality is similar to that reported previously[1], and again these abnormalities were observed in rats on diets II, III, IV and V, those diets high in chlorine dioxide. The incidence of the kidney changes again is too low to warrant any conclusion. The lung changes, which are of bacterial origin, and the bladder parasites (*Trichosomoides crassicauda*) occur normally in this rat colony. From a histopathological standpoint the tissues were essentially negative as far as consistent changes due to a dietary toxicant are concerned.

TABLE V

EFFECT OF DIET ON THE WEIGHT OF VARIOUS ORGANS
Mean tissue weight in mg./100 g. of rat

Diet	Number	Liver	Left kidney	Heart	Spleen
Replicate A—					
I	7	35·4	3·94	3·79	3·26
II	3	38·4	4·07	3·73	3·20
III	7	33·2	3·87	3·46	3·19
IV	11	36·0	3·76	3·54	2·71
V	11	35·7	4·05	3·65	2·85
VI	10	31·7	4·13	3·59	2·84
VII	7	36·3	3·56	3·39	2·69
Overall standard deviation		4·1	0·39	0·33	0·55
Replicate B—					
I	9	27·3	3·66	3·39	3·04
II	10	32·9	3·86	3·62	2·71
III	8	34·3	4·21	3·53	2·94
IV	10	30·0	3·48	3·49	2·68
V	10	32·4	3·83	3·65	2·62
VI	8	32·2	3·91	3·60	2·45
VII	12	30·9	3·56	3·73	2·74
Overall standard deviation		4·5	0·40	0·37	0·45

The mortality data also are presented in Table IV. Those deaths for which no explanation is given occurred at night and autolysis was too far advanced to permit a valid diagnosis. As in the previous study[1], it is confidently believed that most of these deaths may be attributed to respiratory infection. The high mortality in group II A appears to have been due to a particularly severe outbreak of respiratory infection in this cage. It is possible, of course, that the high level of all 4 potential toxicants in diet II predisposed these rats to infection, but the lack of abnormally high mortality in the corresponding group II B argues against such a possibility. In general, it may be concluded that the variations in mortality were of no significance in so far as they might reflect toxicity of the diet.

The mean weights of the livers, left kidneys, hearts and spleens of the rats surviving until killed after 32 weeks on the diets are presented in Table V. For statistical analysis the data summarised in Table V were transformed into percentage of the mean weight for each tissue and analysed by Snedecor's methods for analysis of variance with disproportionate subclass numbers[7]. The results of these analyses are presented in Table VI. Since the diet X replicate interaction was significant in one instance, and was of appreciable magnitude in the other 3 cases, these interactions were used in the calculation of F for diet and replicate. On

this basis spleen weights were significantly different (P = 0·05) between replicates. No explanation of this finding can be offered: it is suggested that this may be one of the 5 out of 100 cases where the difference is due to chance.

TABLE VI

ANALYSIS OF VARIANCE ON TISSUE WEIGHT DATA

Main effect	D.F.	Liver		Left kidney		Heart		Spleen	
		M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.
Replicates ..	1	2,005	3·6	252	1·1	250	1·1	3,000	6·7*
Diet	5	314	< 1·0	470	2·1	102	< 1·0	742	1·7
Diet X replicates	5	560	4·1*	221	2·1	229	2·5	447	1·8
Error	111	137	—	107	—	90	—	249	—

* Significant at P = 0·05.

DISCUSSION

The transient growth-depressing effect of a high level of sodium propionate in the diet of young rats in this experiment is contrasted with the lack of influence of this compound in the previous study[1]. As was noted at the time, baking the bread and later drying it resulted in a strong odour of propionate and it is probable that the final concentration of propionate in the diets of the previous study was considerably reduced. Since the present diets were unheated, little or no propionate was lost and the material at this higher level in the diet exerted an early growth-depressing effect. Obviously since normal rates of growth were resumed after a few weeks and no pathology attributable to propionate poisoning was detected, this effect of propionate cannot be regarded as too serious. In the human dietary, propionate is used almost entirely in baked goods and it is likely that the baking process would materially reduce any potential hazard.

In agreement with the previous findings, the high levels of the antioxidants butylated hydroxyanisole and propyl gallate, of the emulsifier polyoxyethylene (8) monostearate and of the flour improver chlorine dioxide had no detectable deleterious effect on the growth of rats over a 32-week feeding period. In spite of the change in experimental method, 11·25 per cent. of polyoxyethylene (8) monostearate in the diet failed to exert deleterious effects.

SUMMARY

1. Bread ingredients containing 50 times the normal concentration of chlorine dioxide, propyl gallate and butylated hydroxyanisole, or polyoxyethylene (8) monostearate given as 75 per cent. of the diet for 32 weeks did not harmfully affect growth or mortality of male rats. Sodium propionate at the same high level caused a depression of growth during the first few weeks of feeding, but did not influence mortality.

2. The high concentration of polyoxyethylene (8) monostearate, sodium propionate, antioxidants and chlorine dioxide had no detectable effect on hæmoglobin levels in the blood, on organ weights, or on histopathology of the tissues.

3. No evidence was obtained that the simultaneous presence of a high concentration of more than one potential toxicant in the diet led to any synergistic action.

The authors wish to thank Mr. Robert Van Burck and Wallace and Tiernan, Ltd., for the treatment of the flour with chlorine dioxide, the Tennessee Eastman Corporation for supplies of the antioxidant Tenox II, the J. R. Short Canadian Mills, Ltd., for a supply of Wytase, and the Lake of the Woods Milling Co. for a quantity of malt flour. They are indebted to Miss E. Connell and Mr. H. Teed for technical assistance, and to Dr. D. G. Chapman for the hæmoglobin determinations.

References

1. Graham, Teed and Grice, *J. Pharm. Pharmacol.*, 1954, **6**, 534.
2. Schweigert, McBride and Carlson, *Proc. Soc. exp. Biol. N.Y.*, 1950, **73**, 427.
3. Wang, McBride and Schweigert, *ibid.*, 1950, **75**, 342.
4. Sherman, Harris and Jetter, *Fed. Proc.*, 1950, **9**, 361, 370.
5. Harris, Sherman and Jetter, *Arch. Biochem. Biophys.*, 1953, **34**, 249.
6. Rimington, *Brit. med. J.*, 1942, **1**, 177.
7. Snedecor, *Statistical Methods*, 4th Ed., Iowa State College Press, Iowa, 1946.

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THE EFFECT OF THE ANTIOXIDANTS, BUTYLATED HYDROXY ANISOLE, BUTYLATED HYDROXY TOLUENE AND PROPYL GALLATE ON GROWTH, LIVER AND SERUM LIPIDS AND SERUM SODIUM LEVELS OF THE RAT[1]

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SUMMARY.

Confirmation of our previous finding that there are greater hazards in the use of butylated hydroxy toluene (BHT) than in that of butylated hydroxy anisole (BHA) as antioxidants for use in human foodstuffs was obtained. BHA and propyl gallate (PG) met one of the requirements for safety as a food additive. A sex difference in the effect of BHT was noted; BHT increased the relative weight of the male adrenal and had a greater deleterious effect on the growth rate of the male than of the female rat. A straight line relationship existed between the dietary level of BHT and the serum cholesterol level. When fed at the level of 0·1 p.c. of the diet, BHA increased the level of total serum cholesterol but no further elevations occurred at higher dosages. There was a relatively greater increase in the amount of serum free cholesterol than of ester cholesterol when BHA or BHT were fed. PG had no effect on the serum cholesterol levels. BHT increased both free and esterified adrenal cholesterol, a greater increase being noted in the latter. Although BHA and BHT produced increases in the size of the male adrenal none of the marked histological changes recorded by other workers using lethal doses of these antioxidants in the rabbit were apparent. The antioxidants had no effect on the concentration in the liver of the total and esterified cholesterol, total lipid or on the composition of the polyunsaturated fatty acids. However, as the liver weight was increased by BHA or BHT the absolute lipid content of the liver was increased.

INTRODUCTION.

It has been shown (Day, Johnson, O'Halloran and Schwartz, 1959) that the ingestion of the antioxidant butylated hydroxy toluene[4] (BHT) by rats elevates the serum cholesterol and phospholipid levels and in addition (Brown, Johnson

[1] This work was financed by the Department of the Army, as part of the research programme administered by the Army Food Science Establishment, Melbourne, Victoria, Australia.

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[4] Tenox BHT 2:6 di-t-butyl-p-cresol.

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and O'Halloran, 1959) when BHT was fed in conjunction with a 20 p.c. lard supplement to the stock diet of the rat it did not conform to the generally accepted criteria (World Health Organisation, 1958) for safety as an additive to foodstuffs for human consumption. On the other hand, the antioxidant butylated hydroxy anisole[5] (BHA) in a 10 p.c. lard supplement conformed to these criteria.

Denz and Llaurado (1957) attributed the death of rabbits given large doses of BHA and BHT to an excessive loss of electrolytes, especially sodium, in the urine which results in increased urinary aldosterone excretion with depletion of lipid in the zona glomerulosa of the adrenal.

The present study was undertaken to compare the effects at different dietary levels in the rat of BHA, BHT and a third antioxidant, propyl gallate[6] (PG), in a 20 p.c. lard supplement to the stock diet on the growth, liver and adrenal weights, liver, adrenal and serum cholesterol levels, polyunsaturated fatty acid composition of the total liver lipid, serum sodium and histological appearance of the adrenal.

Experimental.

The stock diet, the method of preparation of the experimental diets and the general procedure for the care of the animals have been described previously (Johnson, O'Halloran and Hewgill, 1958). The stock diet in the present work was supplemented with 20 p.c. lard to which were added increasing amounts of antioxidant so that the diets contained 0, 0·1, 0·2, 0·3, 0·4, 0·5 p.c. (dry weight) antioxidant. The antioxidant was added at the expense of 0·5 p.c. cellulose in the control diet, i.e., the 0 p.c. antioxidant level.

Litters of the Norway Hooded strain of rat were culled so that the litters contained three males and three females. At weaning (28 days) a litter was randomly allocated to one of the dietary concentrations of BHT. Two litters were placed on each experimental diet, the animals being caged in pairs with the sexes segregated. This procedure was repeated with the two remaining antioxidants.

Six weeks later the rats were fasted overnight, anaesthetized with ether and blood was withdrawn by cardiac puncture. The animals were killed by further anaesthetization. The liver was removed, dried with filter paper, and weighed. The left adrenal was removed and weighed after any extraneous tissue had been dissected away. The right adrenal was fixed in 10 p.c. formol saline.

Ethanol-ether (3:1) extracts of the sera were made. The liver lipids were extracted with petroleum ether (BP 40-60° C.) in a Waring Blendor according to the method of Thompson, Ganguly and Kon (1949). The left adrenals of the males and females of one litter were separately pooled and extracted for two hours in a micro-soxhlet apparatus using a 5:1 ethanol-ether solvent.

The total cholesterol content of the lipid extracts was determined by the method of Zlatkis, Zak and Boyle (1953). The free cholesterol was precipitated as the digitonide which was centrifuged off, washed with acetone and analysed by the method of Zlatkis *et al.* (1953). The ester cholesterol was calculated by difference.

The total lipid content of the liver extracts was determined gravimetrically and the polyunsaturated fatty acid composition of the total liver lipids was determined by a modi-

[5] Tenox BHA approximately 85 p.c. 3-t-butyl-4-hydroxy anisole and 15 p.c. 2-t-butyl-4-hydroxy anisole.
[6] Naarden PG.

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fication of the method of Davenport, Birch and Ryan (1956) using the potassium t-butoxide reagent.

Serum sodium was estimated by flame photometry. Some haemolysis occurred during the collection of the blood so that no attempt was made to estimate serum potassium.

Haemtoxylin-eosin and sudan IV staining were carried out on sections of the right adrenals.

RESULTS.

Weight and histological studies.

The effect of six weeks' feeding of the three antioxidants on the weight of the rat, expressed logarithmically, is shown in Fig. 1. The experimental design introduced a genetic effect which led to some variations between litters; despite this, as shown previously (Brown *et al.*, 1959), BHT significantly reduced the growth rate of the rats, especially the males. BHA and PG had no significant

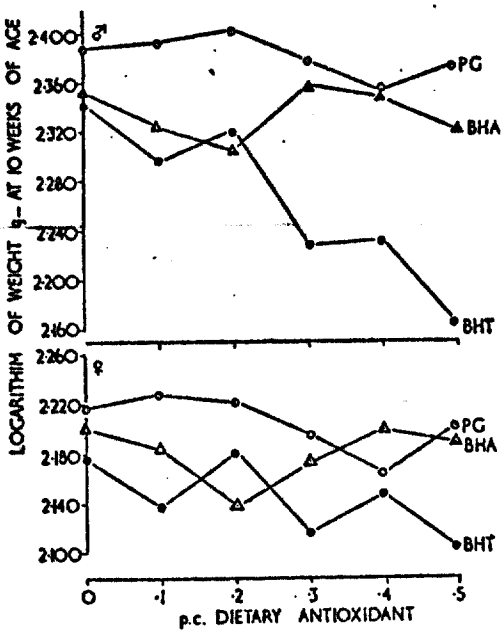

Fig. 1. The effect of six weeks' ingestion of a diet containing various levels of three antioxidants on the log. weight of rats placed on the diets at weaning.
1. Points shown are means of six observations.
2. L.S.D. ($p = 0{\cdot}05$) is the least significant difference for comparison of treatment means (error mean square on 24 degrees of freedom). BHT = 0·054, BHA = 0·036, PG = 0·029.

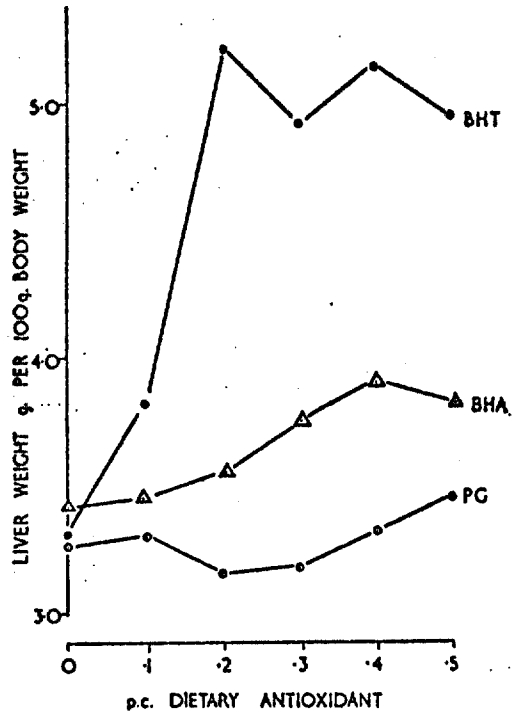

Fig. 2. The effect of three antioxidants on the relative liver weight of the rat.
1. Points shown are means of 12 observations, six male and six female.
2. L.S.D. ($p = 0{\cdot}05$). BHT = 0·444, BHA = 0·225, PG = 0·160.

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effect on the growth rate of the animals even when 0·5 p.c. was present in the diet.

BHA and BHT produced increases in the liver weight-body weight ratio, while PG had no effect (Fig. 2). With BHT the relative increases were more marked and the absolute weight of the liver was increased.

BHA and BHT produced increases in the weight of the male left adrenal relatively to the body weight (Fig. 3) but had no consistent effect on the female left adrenal. PG had no effect on the relative weight of either the male or female adrenal.

No histological changes attributable to the treatments could be detected in the adrenal. There was no depletion of lipid staining of the zona glomerulosa or any increase in cytoplasmic basophilia as shown by Denz and Llaurado (1957) in the adrenals of rabbits given large doses of BHA or BHT.

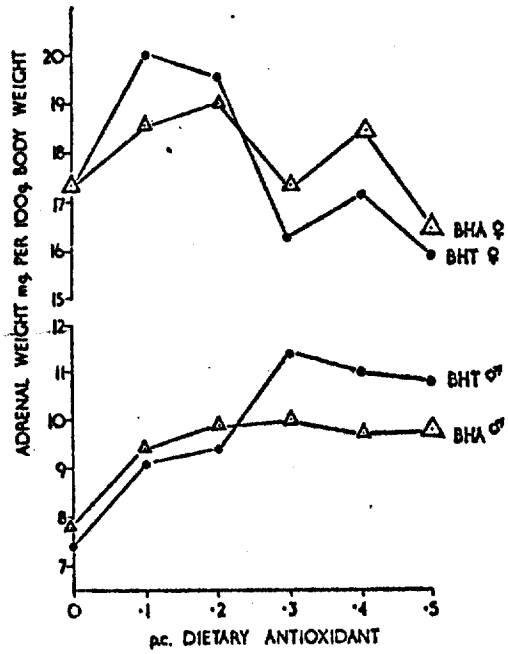

Fig. 3. The effect of BHA and BHT on the relative left adrenal weight of the rat.
1. Points are means of six observations.
2. L.S.D. ($p = 0·05$). BHT = 3·37, BHA = 1·57.

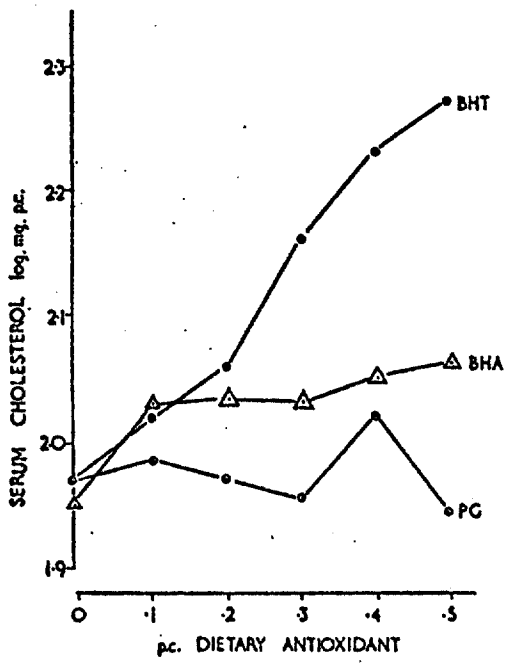

Fig. 4. The effect of three antioxidants on the log. serum cholesterol levels of the rat.
1. Points are means of 12 observations.
2. L.S.D. ($p = 0·05$). BHT = 0·049, BHA = 0·041, PG = 0·039.

Chemical Determinations.

Sera.

The effect of the three antioxidants on the mean serum cholesterol levels, expressed logarithmically, of both male and female rats is shown in Fig. 4. The level of serum cholesterol was directly proportional to the level of dietary BHT.

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The mathematical expression of the straight line response between these two variables was $y = 1 \cdot 9557 + 0 \cdot 6214x$. BHA also produced a significant increase in the level of serum cholesterol but this effect was not further increased significantly by increasing the dietary level of BHA above 0·1 p.c. PG did not have any significant effect on the serum cholesterol, with the exception of a diet containing 0·4 p.c. PG. This inconsistent effect was produced by the abnormally high level of serum cholesterol in three male rats of one litter.

The increase in total serum cholesterol produced by BHA and BHT was a result of increases in both the free and esterified fractions. However, as there was a greater increase in the free cholesterol fraction the percent. of esterified cholesterol in the total serum cholesterol fell (Fig. 5). PG had no effect on this value.

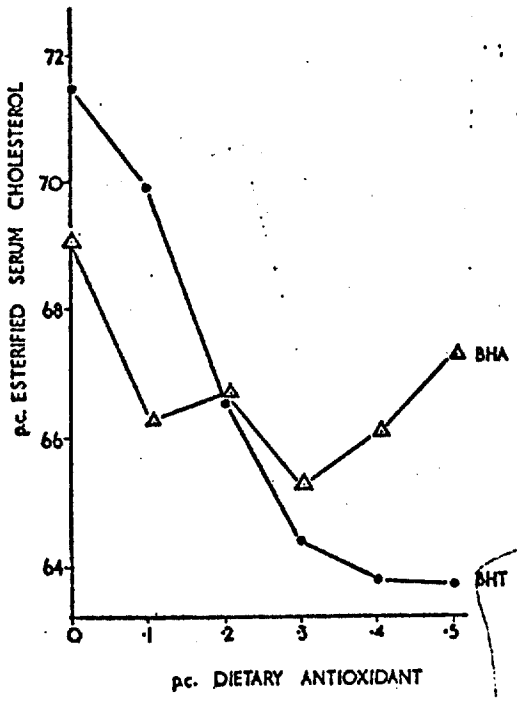

Fig. 5. The effect of BHT and BHA on the percent. esterified serum cholesterol.
1. Points are means of 12 observations.
2. L.S.D. ($p = 0 \cdot 05$). BHT = 2·5, BHA = 1·8.

The serum level of sodium was not affected by the inclusion of the antioxidants in the diet.

Adrenal cholesterol.

BHT produced a significant increase in the concentration of total adrenal cholesterol and there was a strong trend towards an increase in the percent. esterified adrenal cholesterol with increased dietary level of BHT (Figs. 6 and 7). BHA and PG had no significant effect on either of these constituents.

Liver lipids.

None of the antioxidants produced any significant changes in the concentration of the total or percent. esterified liver cholesterol, total liver lipid, or the $K_{1\,cm.}^{0.1\,p.c.}$ (extinction coefficient) value at the five wavelengths at which the polyunsaturated fatty acids are measured. However, as BHA and BHT produced increases in the relative weight of the liver, the absolute amounts of cholesterol and total lipid per rat liver were elevated.

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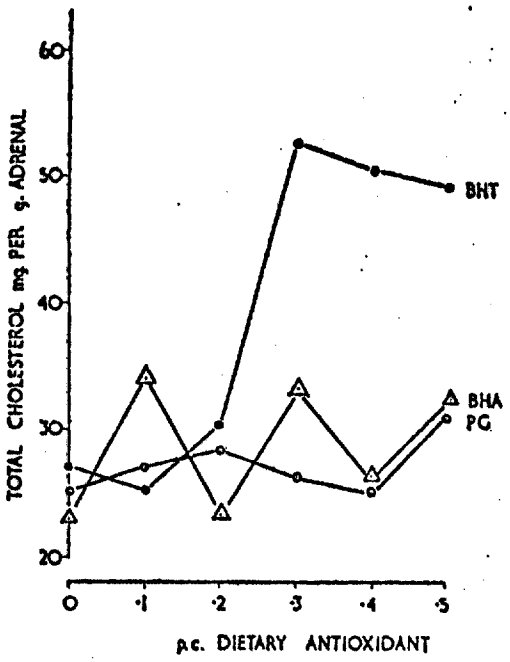

Fig. 6. The effect of three antioxidants on the concentration of total cholesterol of the rat left adrenal.
1. Points are means of four observations, being the pooled adrenals of three rats.
2. L.S.D. ($p = 0{\cdot}05$). BHT = 16·7, BHA = 27·2, PG = 10·9.

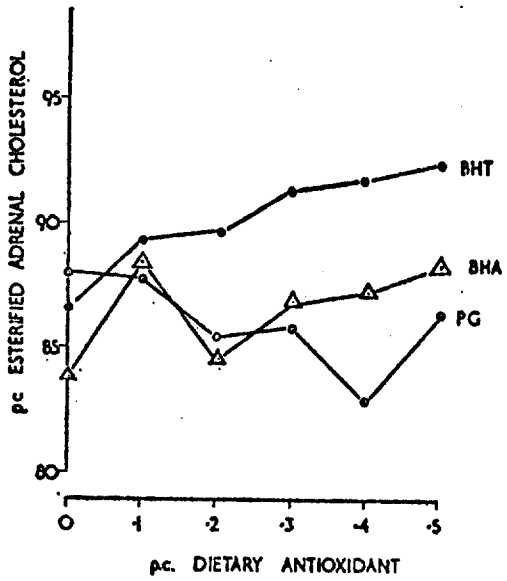

Fig. 7. The effect of three antioxidants on the percent. esterified adrenal cholesterol.
1. Points are means of four observations, being the pooled adrenals of three rats.
2. L.S.D. ($p = 0{\cdot}05$). BHT = 8·2, BHA = 8·3, PG = 5·3.

Urinary metabolites.

During food consumption studies the urine of the animals was absorbed on blotting paper placed in tin containers. The absorbed urine of the animals fed BHT and PG changed colour. That of the BHT animals turned bright pink and that of the PG animals turned purple. When the urine was collected in glass containers no such change occurred and these colours probably arose out of the metal catalysed oxidation of urinary metabolites of the two antioxidants (Dacre, 1961, and Booth *et al.*, 1959).

DISCUSSION.

The effects of BHA and BHT on the growth and liver weight of the rat confirm our previous finding (Brown *et al.*, 1959) that there is a greater hazard involved in using BHT as an additive for use in human foodstuffs than in using BHA. Neither BHA nor PG produced any significant deleterious effects on the growth rate of rats, giving confirmation to the work of Brown *et al.*

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(1959), Wilder and Kraybill (1948), Graham *et al.* (1954), Graham and Grice (1955) and Orten, Kuyper and Smith (1948). BHA and PG thus fulfil one of the requirements for safety for use in human foods.

In accordance with our previous findings (Brown *et al.*, 1959) a sex difference in the effect of BHT was noted. BHT had a greater deleterious effect on the growth of the male than of the female rat and BHT and BHA produced increases in the relative adrenal weight of the male but not the female. Differences in the antioxidant potency of the male and female hormones have previously been observed: Meyer and McShan (1948) and Schuler and Meier (1956) showed that the oestrogens, in contradistinction to the androgens, can act as antioxidants, while Hood *et al.* (1950) showed that the fat from chickens given oestrogen implants was more stable than the fat from control animals.

The rat chronically fed a diet containing relatively less BHA or BHT than the acute doses (1 gm. per day) given to the rabbit by Denz and Llaurado (1957) did not show the marked histological changes of the adrenal produced in that species. The increases in the relative weight of the adrenals in the case of male rats fed BHA and BHT may well be a reflection of the changes produced by the dose level of the antioxidants which led to the death of the rabbits.

The straight line relationship between dietary BHT and the serum cholesterol is indicative of a cause and effect relationship and suggests the possibility that dietary levels of BHT normally ingested by man would cause similar but greatly reduced effects on the serum cholesterol. The straight line relationship has also enabled us to use the maximum concentration of BHT, i.e., 0·5 p.c. of the diet, in our later experiments and relate our findings to more physiological levels of dietary BHT. The lack of any further elevation of serum cholesterol on increasing the dietary level of BHA above 0·1 p.c. of the diet cannot be easily explained. It may be related to the relative antioxidant potency or ease of absorption of BHT and BHA. It is unlikely that the effects of BHA and BHT can be attributed solely to their antioxidant properties, as PG produced no changes in any of the parameters measured.

The elevated levels of serum cholesterol and the increase in the absolute amount of liver and adrenal cholesterol produced by BHA and BHT could be accounted for by an increased rate of synthesis or decreased breakdown of cholesterol or variations in absorption or excretion. Evidence that the first explanation is more likely will be presented in two succeeding papers (Johnson and Reid; Johnson and Holdsworth, to be published).

Acknowledgments. The authors gratefully acknowledge the co-operation of Dr. C. J. Schwartz in the early phases of this work, of Mr. A. G. Constantine and Mr. K. M. Cellier of the Division of Mathematical Statistics, C.S.I.R.O., with the statistical analysis, and of Professor J. B. Cleland in histo-pathological examinations.

The authors also acknowledge the generous gifts of Tenox BHA and BHT supplied by Eastman Chemical Inc., U.S.A., and of Propyl Gallate by N.V. Chemische Fabriek, Naarden, Holland.

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REFERENCES.

Booth, A. N., Masri, Merle S., Robbins, Dorothy J., Emerson, O. H., Jones, Francis T., and De Eds Floyd (1959): J. biol. Chem., 234, p. 3014.

Brown, W. D., Johnson, A. R., and O'Halloran, M. W. (1959): Austral. J. exp. Biol., 37, p. 533.

Dacre, J. C. (1961): Biochem. J., 78, p. 758.

Davenport, J. B., Birch, A. J., and Ryan, A. J. (1956): Chem. and Ind., p. 136.

Day, A. J., Johnson, A. R., O'Halloran, M. W., and Schwartz, C. J. (1959): Austral. J. exp. Biol., 37, p. 295.

Denz, F. A., and Llaurado, J. G. (1957): Brit. J. exp. Path., 38, p. 515.

Graham, W. D., Teed, H., and Grice, H. C. (1954): J. Pharm. (Lond.), 6, p. 534.

Graham, W. D., and Grice, H. C. (1955): *Ibid.*, 7, p. 126.

Hood, M. P., Wheeler, R. S., and McGlamery, J. B. (1950): Poultry Sci., 29, p. 824.

Johnson, A. R., O'Halloran, M. W., and Hewgill, F. R. (1958): J. Amer. Oil Chemists' Soc., 35, p. 496.

Meyer, R. F., and McShan, W. H. (1948): Biological Antioxidants, Trans. 3rd Conf. Josiah Macy Jr, Foundation, p. 115.

Orten, J. M., Kuyper, A. C., and Smith, A. H. (1948): Food Technol., 2, p. 308.

Schuler, W., and Meier, R. (1956): Arch. expt. Pathol. Pharmakol., 228, p. 474.

Thompson, S. Y., Ganguly, J., and Kon, S. K. (1949): Brit. J. Nutrition, 3, p. 50.

Wilder, O. H. M., and Kraybill, H. R. (1948): Amer. Meat Institute Foundtn. Bulletin.

World Health Org. Techn. Rep. Ser. (1958): 144.

Zlatkis, A., Zak, B., and Boyle, A. J. (1953): J. lab. clin. Med., 41, p. 486.

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SPECIFICATIONS FOR IDENTITY AND PURITY AND TOXICOLOGICAL EVALUATION OF SOME ANTIMICROBIALS AND ANTIOXIDANTS

The content of this document
is the result of the deliberations
of the Joint FAO/WHO Expert Committee
on Food Additives
which met 8-17 December 1964[a]

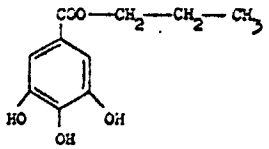
[a] Eighth Report of the Joint FAO/WHO Expert Committe on Food Additives, Wld Hlth Org. techn. Rep. Ser., 1965, 309; FAO Nutrition Meetings Report Series 1965, 38.

PROPYL GALLATE

CHEMICAL NAMES: Propyl gallate; n-propyl ester of 3,4,5-trihydroxybenzoic acid

EMPIRICAL FORMULA: $C_{10}H_{12}O_5$

STRUCTURAL FORMULA:



MOLECULAR WEIGHT: 212.21

DEFINITION: Propyl gallate contains not less than 99% of $C_{10}H_{12}O_5$ after drying at 110°C for 4 hours.

DESCRIPTION: A white to creamy-white, crystalline, odourless solid, with a slightly bitter taste. 1 g is soluble in 300 ml of water or in 3.5 ml of ethanol; freely soluble in fat.

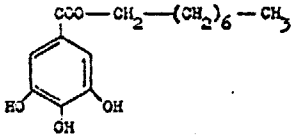
USE: As an antioxidant; more effective combined with citric acid as synergist.

OCTYL GALLATE

CHEMICAL NAME: Octyl gallate; n-octyl ester of 3,4,5-trihydroxybenzoic acid

EMPIRICAL FORMULA: $C_{15}H_{22}O_5$

STRUCTURAL FORMULA:



MOLECULAR WEIGHT: 282.34

DEFINITION: Octyl gallate contains not less than 98.5% of $C_{15}H_{22}O_5$ after drying at 60°C for 4 hours.

DESCRIPTION: A white to creamy-white, odourless solid, which may have a slightly bitter taste. 1 g is soluble in 2.5 ml of ethanol. It is insoluble in water but freely soluble in fats.

USE: As an antioxidant; more effective combined with citric acid as synergist.

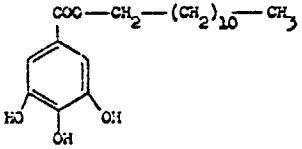
DODECYL GALLATE

CHEMICAL NAMES: Dodecyl gallate; n-dodecyl (or lauryl) ester of 3,4,5-trihydroxybenzoic acid

SYNONYM: Lauryl gallate

EMPIRICAL FORMULA: $C_{19}H_{30}O_5$

STRUCTURAL FORMULA:



MOLECULAR WEIGHT: 338.45

DEFINITION: Dodecyl gallate contains not less than 98.5% of $C_{19}H_{30}O_5$ after drying at 60°C for 4 hours.

DESCRIPTION: A white or creamy-white, odourless solid, which may have a slightly bitter taste. 1 g is soluble in 3.5 ml of ethanol. Insoluble in water but freely soluble in fats.

USE: As an antioxidant; more effective combined with citric acid as synergist.

Biological Data

Biochemical aspects

The available evidence indicates that the esters are hydrolysed in the body. Most of the gallic acid is converted into 4-O-methyl gallic acid. Free gallic acid or a conjugated derivative of 4-O-methyl gallic acid is excreted in the urine. Conjugation of the 4-O-methyl gallic acid with glucuronic acid was demonstrated.[1]

Rats fed a low-methionine, low-choline diet containing 1% gallic acid developed fatty livers which could be prevented by added choline or methionine.[2]

The detailed metabolic pathways for propyl gallate have been described.[3] There is no evidence to suggest that other esters of gallic acid differ greatly in their metabolism from the pattern described for propyl gallate.

Acute toxicity

	Animal	Route	LD_{50} (mg/kg body-weight)	Reference
Propyl gallate	Rat	oral	3800	4
	Rat	oral	3600	5
	Rat	intraperitoneal	380	4
	Mouse	oral	2000-3500	5
Octyl gallate	Rat	oral	4700	6
	Rat	intraperitoneal	60-80	7
Dodecyl gallate	Rat	oral	6500	6
	Rat	intraperitoneal	100-120	7

Short-term studies

Rat. Levels of propyl gallate of 1.2% and 2.3% in the diet caused interference with weight gain, the bitter taste of the gallate apparently making the diet unpalatable. The higher dose level caused some deaths (about 40%) during the first month; the survivors continued to eat the diet for 10-16 months and showed retarded growth, but no pathological lesions. The animals that died exhibited renal damage.[4]

Weanling rats were given diets containing 2.5% and 5% dodecyl gallate. All animals fed the smaller quantity were dead within 10 days, and all animals fed the larger quantity died within 7 days.[8]

Rats fed for 70 days on a diet containing 7% fat and 0.2% dodecyl gallate showed no effect on body-weight.[9]

Weanling rats were fed diets which contained 20% lard and 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% propyl gallate for 6 weeks. There was no effect on body-weight, liver weight, liver weight to body-weight ratio, left adrenal weight, total liver lipid, composition of liver polyunsaturated fatty acids, liver cholesterol, adrenal cholesterol or serum sodium.[10]

Propyl gallate was added to the dietary fat of weanling rats at levels of 0.02% in the fat for 13 weeks. The fat content of the diet accounted for 30% of its calorific value. There was a very slight inhibition of growth. The same rats were then placed on a partial starvation diet and kept until they died. The survival time of the animals which had received the propyl gallate was considerably reduced and the reduction in their total body protein was greater.[11]

Guinea-pig. Propyl gallate fed to guinea-pigs in groups of 20 at a level of 0.0117% in the diet for 14 months caused no detectable ill effects.[4]

Dog. A level of 0.0117% of propyl gallate in the diet was well tolerated by a group of 7 dogs over a period of 14 months.[4]

Pig. Diets containing 0.2% of propyl, octyl or dodecyl gallate were fed to pigs without demonstrable ill effect; no anaemia was observed.[12]

Long-term studies

Rat and mouse. A level of 5% propyl gallate in the diet in a 2-year chronic toxicity test on rats and mice gave rise to patchy hyperplasia in the proventiculus. At a level of 1% no difference was noted between test and control animals.[3]

Rats in groups of 10 males and 10 females were fed for 2 years on diets containing 0%, 0.00117%, 0.0117%, 0.117%, 1.17% and 2.34% of propyl gallate. The groups receiving 1.17% and 2.34% of propyl gallate showed stunted growth and evidence of renal damage. In the other groups, there was no detectable effect on haemoglobin, erythrocyte or leucocyte levels in the blood, or on the histological appearance of the organs examined.[4]

Propyl, octyl and dodecyl gallate were fed to rats at concentrations of 0.075%, 0.2% and 0.5% in the diet. Growth was affected only at the 0.5% level of dodecyl gallate; there was significant retardation, particularly in the second generation. At this level of dodecyl gallate, some litters were lost in the second generation because they were not sufficiently fed by the mothers. A slight hypochromic anaemia was noticed in the groups on diets containing 0.2% octyl and dodecyl gallate. No abnormalities were observed in the organs or tissues of the rats at autopsy.[12]

Young rats in groups of 12 males and 12 females were fed diets containing 7% fat and 0.2% octyl or dodecyl gallate. There was no significant difference between test and control animals over 3 generations.[6]

Comment on experimental studies reported

The acute and short-term toxicity studies reported are fairly extensive and cover a wide range of animal species. The results of the experiments involving partial starvation are of great interest and indicate a higher toxicity of propyl gallate than the other studies. Evaluation of these experiments is difficult, however, in the absence of comparable studies with other food additives. Several long-term studies have been made and the levels of feeding varied widely. There are some discrepancies in the results reported. However, the long-term studies on rats provide a basis for evaluation.

Evaluation

Level causing no significant toxicological effect in the rat

With one exception, in long-term studies in rats gallates caused no demonstrable ill effects when fed at a level of 0.2%; in one investigation, however, this level resulted in hypochromic anaemia. It seems likely that this may have been due to interference with iron absorption, but the cause was not established. Haematological effects were carefully examined in a number of other investigations and no abnormalities were seen. It seems probable, therefore, that this effect was related to the particular circumstances of one study and can be properly disregarded in arriving at the level that causes no significant effects in the rat. The level that causes no significant toxicological effect in the rat is 100 mg/kg body-weight per day.

Estimate of acceptable daily intakes for man

	mg/kg body-weight
Unconditional acceptance	0-0.2
Conditional acceptance	0.2-0.5

Comment

Attention should be given to the amounts of other phenolic antioxidants in the diet.

Further Work Considered Desirable

1. Long-term studies in other species than the rat, with special attention to possible interference with iron metabolism and haematopoiesis.

2. Metabolic studies in human subjects.

3. Further studies on the effects of stress on the toxicity of this and other food additives.

References

1. Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., Jones, F. T. & DeEds, F. (1959) J. biol. Chem., 234, 3014

2. Booth, A. N., Robbins, D. J. & DeEds, F. (1961) J. Nutr., 75, 104

3. Dacre, J. C. (1960) J. N.Z. Inst. Chem., 24, 161

4. Orten, J. M., Kuyper, A. C. & Smith, A. H. (1948) Food Techn., 2, 308

5. Lehman, A. J., Fitzhugh, O. G., Nelson, A. A. & Woodward, G. (1951) Advanc. Food Res., 2, 197

6. Sluis, K. J. H. van (1951) Food Manuf., 26, 99

7. Esch, G. J. van, Genderen, H. van (1954) Netherlands Institute of Public Health, Report No. 481

8. Allen, C. S. & DeEds, F. D. (1951) J. Amer. Oil Chem. Soc., 28, 304

9. Tollenaar, F. D. (1954) Fette u. Seifen, 56, 41

10. Johnson, A. R. & Hewgill, F. R. (1961) Aust. J. exp. Biol. med. Sci., 39, 353

11. Buckman, N. D. (1962) Vop. Pitan., 21, 68

12. Esch, G. J. van (1955) Voeding, 16, 683

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Testing of Antioxidants against Experimental Caries in Rats

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Demonstration of the caries-inhibiting potential of sodium metabisulfite[1] has been followed by subsequent investigations into certain other aspects of the chemical activities of this compound in an effort to clarify its mode of action. For example, two other carbonyl-binding compounds—dimedone and aminooxyacetic acid hemihydrochloride—were also found to inhibit rat caries.[2] The well-known antioxidant properties of bisulfite[3] have prompted the testing of a number of other common antioxidants, including some used commercially in foods, for their effect on experimental caries produced by a coarse corn-particle diet. Of additional interest in this respect are certain reports[4, 5] concerned with the effect of oxidizing agents on experimental caries and on acid production in saliva. However, the exact role of these compounds is not clear, since varying results have been reported. For example, Hein and Wisotsky[4] found that low levels of potassium permanganate had no effect, while Shapiro, Brat, and Ershoff[5] reported that hydrogen peroxide and other oxidizing agents, including zinc peroxide and sodium perborate, initiated caries in rats on a normally non-cariogenic diet.

EXPERIMENTAL METHODS

Animal experiments.—The procedure used in the present work for testing various compounds for their effect on sulcal caries has been previously described in detail.[1, 2] Each experimental group was composed of 24 weanling, female, Sprague-Dawley rats which were randomly distributed by threes in screen-bottom cages. The cariogenic diet employed was the coarse corn-particle diet of Stephan, Fitzgerald, McClure, Harris, and Jordan.[6] After an experimental period of 90 days the animals were sacrificed, and the molar dentition was scored for sulcal caries, using the method of Cox, Dodds, Dixon, and Matuschak.[7]

The first study was composed of three experimental groups and a control group. Groups 1 and 2 received 0.5 per cent ascorbic acid and isoascorbic acid, respectively, in the diet. The third group received the synergistic antioxidant combination[8] of α-tocopherol and ascorbic acid at 0.05 and 0.1 per cent, respectively. The α-tocopherol was first dissolved in ether and then blended into the diet. Ether was allowed to evaporate by spreading the diet out in shallow pans placed in the chemical hood.

A second animal experiment was composed of 6 experimental groups and a control

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group. Group 1 received butylated hydroxytoluene (BHT)* at 0.5 per cent, and group 2 received 0.5 per cent of BHT and citric acid (50-50). The citric acid acts as a metal chelator to enhance the activity of the antioxidant. The third group received 0.25 per cent citric acid as a control for group 2. The fourth and fifth groups received, respectively, *n*-propyl gallate and ethyl hydrocaffeate† each at 0.5 per cent. Group 6 received 0.4 per cent antioxidant salt‡ at the expense of the NaCl normally in the diet. Ethyl hydrocaffeate, a thick liquid, was first dissolved in propylene glycol and then blended into the diet. All other supplements were mixed into the diet as dry powders.

The third animal experiment was also composed of 6 experimental groups and a control group. The following compounds were all tested individually at 0.5 per cent in the diet: α-tocopherol acetate, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EMQ), rutin, hesperidin, butylated hydroxyanisole (BHA),§ and nordihydroguairetic acid (NDGA). Alpha tocopherol and EMQ were first dissolved in ether before blending into the diet as previously described. BHA was obtained in the form of 10-gm. pellets, which were ground to a fine powder before use.

Bacteriological experiments.—The antioxidants were also screened for inhibition of the growth, in vitro, of a variety of test bacteria representing types encountered in the oral cavity. The solubility in water of many of the antioxidants was quite low, and they could be brought into solution or stable suspension for testing only under special conditions. The limits of solubility generally determined the highest concentrations tested. Stock solutions of the compounds were prepared as follows: *n*-propyl gallate, 2 per cent in distilled water with the pH adjusted to 8.3; ethyl hydrocaffeate, 2 per cent solution in 10 per cent propylene glycol; antioxidant salt, 4 per cent in distilled water with 10 drops of 10 per cent HCl added; isoascorbic acid, 2 per cent in distilled water; α-tocopherol acetate, 0.5 gm. mixed with 5 gm. Tween 80 added dropwise with mixing up to a total of 100 ml. in warmed distilled water, 6 drops of 10 per cent HCl added to clarify the mixture; butylated hydroxytoluene, 0.5 gm. dissolved in 1 ml. 95 per cent ethyl alcohol, mixed with 6 gm. Tween 80 and made up to a total of 100 ml. with warmed distilled water, 6 drops of 10 per cent HCl added to clarify the mixture; BHA, prepared in the same way as BHT; EMQ, 1 gm. mixed with 0.5 gm. Tween 80 and made up to 200 ml. with distilled water; 4N HCl added drop-wise to clarify the mixture; NDGA, 0.5 gm. in 200 ml. distilled water with 10 drops of 10 per cent NaOH; rutin, 1.0 per cent in warmed distilled water with 10 per cent NaOH added dropwise to effect solution. All stock solutions were sterilized by filtration|| and added aseptically to the previously autoclaved medium. The details of testing, such as serial dilution of the antioxidants, media used, inoculum size, etc., are exactly the same as described previously.[1, 2] Appropriate controls were included for the various solubilizing agents employed. In tubes where no growth occurred the pH was determined, to make sure that it did not vary significantly from that of the basal medium.

* 2,6-Di-tert butyl *p*-cresol.

† Ethyl ester of 3,4-dihydroxy phenylpropionic acid.

‡ The percentage composition of antioxidant salt is butylated hydroxyanisole, 0.128; propyl gallate, 0.047; citric acid, 0.028; propylene glycol, 0.047; tricalcium phosphate, 1.5; sodium chloride to make 100 per cent.

§ 2-Tert butyl-4-hydroxyanisole.

|| Corning U-F sintered-glass filters were used for this purpose.

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RESULTS

The data for the three animal experiments are presented in Table 1. Only in the cases of propyl gallate in experiment 2 and EMQ in experiment 3 was there an appreciable reduction in the caries scores. Approximately a 50 per cent reduction was observed in each case. The mean caries scores in some of the groups were higher than the control scores. This was seen in the ascorbic acid, isoascorbic acid, BHT + citric acid, citric aid, rutin, and hesperidin groups. The caries reductions by propyl gallate and EMQ and the caries increase in the hesperidin group were found to be statistically significant at the 0.1 level. BHT and EMQ were apparently toxic at the levels employed. Because of unsatisfactory weight gains, the initial concentrations were reduced to half

TABLE 1

EFFECT OF ANTIOXIDANTS ON GROWTH AND SULCAL CARIES IN RATS WHEN TESTED ON DIET 585 FOR 90 DAYS

Expt. No.	Group	Compound	Conc. in Diet (Gm/kilo)	Av. Final Wt. (Gm.)	Av. Daily Food Intake (Gm.)	Survivor Ratio	Av. Carious Teeth	Av. Caries Surface	Av. Caries Score	S.E. (±)
I.....	1	Ascorbic acid	5	196.0	10.3	21/24	4.1	10.2	21.6	2.57
	2	Isoascorbic acid	5	193.0	10.0	21/24	3.9	9.5	20.4	2.84
	3	α-Tocopherol + ascorbic acid	0.5 and 1.0	204.2	9.9	24/24	3.6	7.7	15.1	2.04
	4	Control	.....	203.7	9.0	24/24	3.8	7.5	13.8	1.95
II....	1	Butylated hydroxytoluene (BHT)	5*	169.1	7.9	21/24	4.0	9.1	17.6	2.82
	2	BHT + citric acid (50/50)	5*	185.6	8.7	20/24	4.0	10.0	21.1	2.44
	3	Citric acid	2.5*	197.5	9.8	21/24	4.4	12.5	28.6	3.77
	4	n-Propyl gallate	5	178.7	9.5	23/24	3.0	5.2	9.7	1.76
	5	Ethyl hydrocaffeate	5†	196.9	9.6	21/24	4.5	8.3	15.6	1.96
	6	Antioxidant salt	0.4	196.1	9.5	22/24	3.6	9.3	17.0	2.65
	7	Control	.....	201.5	9.9	22/24	3.5	9.1	18.5	2.55
III...	1	α-Tocopherol	5	203.6	9.2	24/24	4.2	8.2	16.1	2.07
	2	1,2-Dihydro-6-ethoxy-2,2,4-trimethyl quinoline (EMQ)	5	169.7	9.4	19/24	2.9	4.7	7.2	1.91
	3	Rutin	5	194.1	8.1	22/24	5.1	11.5	23.8	2.71
	4	Hesperidin	5	201.6	9.0	22/24	4.4	10.3	22.5	2.61
	5	Butylated hydroxanisole (BHA)	5	193.2	8.4	24/24	3.9	8.5	19.5	4.38
	6	Nordihydrogualaretic acid (NDGA)	5	168.0	11.3	24/24	3.8	7.9	17.2	2.71
	7	Control	.....	206.6	9.3	23/24	4.1	8.0	14.8	1.62

* Reduced to half after 1 week.
† Reduced to half after 3 weeks.

after the first week for the groups receiving BHT alone and in combination with citric acid and after the third week for the EMQ group. The average final weight for animals in these groups was still considerably lower than the control animals. Feeding propyl gallate and NDGA at the levels employed also resulted in reduced weight gains. No excessive mortality was noted in any of the groups, with the possible exception of the EMQ group. The toxicity of this antioxidant has been more completely investigated by Wilson and DeEds.[9]

Characteristic stains were observed on the molar teeth of the rats fed propyl gallate. The exposed dentin on the occlusal surfaces of the cusps exhibited a brownish discoloration. In addition, the depths of the sulci, cracks in the enamel, and the gingival line on the molars in many cases were stained a deep black. This may be a consequence of the ability of propyl gallate to form dark precipitates with certain metals in the diet. Essen-

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tially the same picture, although to a much lesser degree, was seen in the group fed ethyl hydrocaffeate. Teeth from both groups were sectioned, and the staining appeared to be confined to the surface layers of the dentin. The dried skulls and teeth of animals from the hesperidin and rutin groups were examined under ultraviolet light. The characteristic induced bone fluorescence reported to result from quercetin ingestion[10] was not observed.

Only 4 of the 13 antioxidants and antioxidant preparations showed any appreciable inhibition of the test bacteria at the concentrations tested. Inhibitory levels of propyl

TABLE 2

INHIBITION OF SELECTED BACTERIA BY FOUR ANTIOXIDANTS*

Micro-organism	Strain No.	Propyl Gallate	E.M.Q.	Ethyl Hydrocaffeate	Nordihydro-guaiaretic Acid
Lactobacillus arabinosus	17-5	0.266	>0.10†	>0.40†	0.050
L. plantarum	8014	0.266	>0.10	>0.40	0.050
L. casei	7469	0.029	>0.10	>0.40	0.050
L. fermenti	36	0.088	>0.10	0.40	0.050
L. fermenti (rat)	NR77	0.266	>0.10	>0.40	0.050
L. acidophilus (rat)	42T	0.088	>0.10	>0.40	0.050
Candida albicans	358	0.266	0.033	0.40	0.05
C. albicans	394	0.266	0.033	0.40	0.05
C. pseudotropicalis	360	0.266	0.033	0.40	0.017
C. krusei	359	0.266	0.10	0.40	0.05
C. krusei	379	0.266	0.033	0.40	0.05
Neisseria catarrhalis	N6	0.0032	0.011	0.014	0.006
N. flava	N8	0.0032	0.10	0.014	0.017
Streptococcus salivarius	9756	0.266	>0.10	>0.40	0.017
S. salivarius	3	0.266	>0.10	>0.40	0.017
S. faecalis	R	0.266	>0.10	>0.40	0.017
S. mitis	2	0.266	>0.10	0.40	0.017
Streptococcus (rat)	FA-1	0.088	>0.10	>0.40	0.017
Streptococcus (rat)	3R-114	0.088	>0.10	>0.40	0.017
Staphylococcus aureus	13	0.088	0.033	0.40	0.017
Staphylococcus aureus	33	0.088	0.033	0.40	0.017
Gaffkya tetragena	10875	0.029	>0.10	0.13	0.006
G. tetragena	J113	0.029	>0.10	0.13	0.017
Micrococcus	19	0.088	>0.10	0.13	0.017
Micrococcus	6	0.088	>0.10	0.043	0.017
Leptotrichia	Sp.	0.029	0.0037	0.130	>0.05†
Leptotrichia	Sp.	0.029	0.011	0.130	>0.05

* Inhibitory levels are expressed as per cent antioxidant in the first tube which inhibited growth.
† Highest concentration tested.

gallate, EMQ, ethyl hydrocaffeate, and NDGA are shown in Table 2. It can be seen that EMQ and ethyl hydrocaffeate were only partially inhibitory to certain of the bacteria at the highest concentrations tested. With NDGA there was a difference in the relative sensitivities of streptococci and lactobacilli, the former being inhibited at a lower level than the latter. This antioxidant was reported to have no effect on bacterial counts in prepackaged meat.[11] The two staphylococci were more sensitive to EMQ and less sensitive to ethyl hydrocaffeate than were the gaffkyae and other micrococci. The neisseriae were generally more sensitive to these antioxidants than were the other bacteria. Isoascorbic acid at 0.266 per cent, α-tocopherol at 0.1 per cent, BHT at 0.1 per cent, and antioxidant salt at 0.4 per cent did not inhibit growth. BHA was generally ineffective at 0.2 per cent except for strains of leptotrichiae and neisseriae, which were inhibited at this concen-

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tration. Rutin was not inhibitory to any of the bacteria at concentrations up to the limit of its solubility of around 0.2 per cent. Naghski, Copley, and Couch[12] had previously reported the lack of antibacterial activity for rutin against various bacteria. Gustafsson[13] has reported the failure of glycosides of various flavonols to inhibit acid production from glucose in saliva while the aglycones were active. Naringenin was later shown to inhibit experimental caries in hamsters.[14]

DISCUSSION

The widespread use of antioxidants,[15] especially in foods, makes any knowledge of their physiologic effects of particular interest. In the present work these compounds were purposely tested at considerably higher concentrations than are normally used to stabilize foods. Even at these levels it seems apparent that antioxidants as a class do not possess any marked degree of caries-inhibiting properties under the conditions used for testing. Thus it would appear that the antioxidant properties of metabisulfite are not the primary cause of its inhibitory effect on experimental caries in rats. It is probably significant that the two antioxidants which showed some caries inhibition were also two of the four compounds which could be demonstrated to inhibit the growth of selected test bacteria. Thus it may be that any interference with caries development by these two agents was simply an antibacterial effect rather than any direct antioxidant effect in the oral cavity. However, the low solubilities of the antioxidants in general, except under the special conditions noted in the in vitro tests, make it difficult to interpret the relationship between bacterial inhibition in vitro and caries inhibition in vivo.

The work of Shapiro *et al.*[5] in which oxidizing agents were reported to induce dental caries on a non-cariogenic diet is difficult to evaluate in relation to the present experiments. These workers described a destruction of the exposed dentin at the tip of the molar cusps, proceeding inward until the pulp was involved. In the present work, caries was initiated in the molar sulci, penetrating the enamel before involving the dentin, and hence the two processes may not be comparable.

SUMMARY

A number of antioxidants were tested for their effect on sulcal caries in rats. Propyl gallate and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline inhibited caries by approximately 50 per cent when incorporated in the diet at 0.5 per cent level. The other antioxidants, including α-tocopherol, ascorbic acid, isoascorbic acid, butylated hydroxyanisole, ethyl hydrocaffeate, rutin, hesperidin, butylated hydroxytoluene, and nordihydroguaiaretic acid, did not inhibit caries. In fact, the caries scores of some groups were slightly increased. In the case of hesperidin, the increase was statistically significant. These compounds were generally not inhibitory to the growth of selected test bacteria under the conditions used for testing. Exceptions were propyl gallate, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, ethyl hydrocaffeate, and nordihydroguaiaretic acid.

Samples of antioxidants were obtained through the generosity of the following companies: 1,2-dihydro-6-ethoxy-2,2,4-trimethyl quinoline from the Monsanto Chemical Co., St. Louis, Mo.; isoascorbic acid from Sumner Chemical Co., New York, N.Y.; antioxidant salt from Diamond Crystal Salt Co., St. Clair, Mich.; butylated hydroxytoluene from McKesson and Robbins, Inc., New York, N.Y., and Hercules Powder Co., Wilmington, Del. Other antioxidants were purchased from commercial sources. Teeth were sectioned through the courtesy of Dr. Rachel H. Larson. The technical assistance of Mr. William L. Poole is acknowledged.

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REFERENCES

1. Jordan, H. V., Fitzgerald, R. J., and Bowler, A. E. Inhibition of Experimental Caries by Sodium Metabisulfite and Its Effect on the Growth and Metabolism of Selected Bacteria, *J. D. Res.*, **39**: 116, 1960.
2. Jordan, H. V., Fitzgerald, R. J., and Berger, N. D. Carbonyl-binding Compounds as Inhibitors of Experimental Caries in Rats, *J. D. Res.*, **40**:199, 1961.
3. Higuchi, T., and Schroeter, L. C. Reactivity of Bisulfite with a Number of Pharmaceuticals, *J. Am. Pharmacol. Assoc.*, **48**:535, 1959.
4. Hein, J. W., and Wisotzky, J. Effect of One Milleqiuvalent Potassium Permanganate Solution upon Experimental Caries in Male and Female Syrian Hamsters, *J. D. Res.*, **34**:756, 1955.
5. Shapiro, M., Brat, V., and Ershoff, J. H. Induction of Dental Caries and Pathological Changes in Periodontium of Rat with Hydrogen Peroxide and Other Oxidizing Agents, *J. D. Res.*, **39**:332, 1960.
6. Stephan, R. M., Fitzgerald, R. J., McClure, F. J., Harris, M. R., and Jordan, H. V. The Comparative Effects of Penicillin, Bacitracin, Chloromycetin, Aureomycin, and Streptomycin on Experimental Dental Caries and on Certain Oral Bacteria in Rats, *J. D. Res.*, **31**:421, 1952.
7. Cox, G. J., Dodds, M. L., Dixon, S. F., and Matuschak, M. C. Experimental Dental Caries. III. A System of Recording Corn-Meal Caries in Rats, *J. D. Res.*, **18**:469, 1939.
8. Hawk, P. B., Oser, B. L., and Summerson, W. H. *Practical Physiological Chemistry*, chap. 3, p. 104. 13th ed. New York: Blakiston Co., Inc., 1954.
9. Wilson, R. H., and DeEds, F. Toxicity Studies on the Antioxidant 6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, *Agr. and Food Chem.*, **1**:203, 1959.
10. Ribelin, W. E., Masri, M. S., and DeEds, F. Fluorescence of Bone after Quercetin Ingestion, *Proc. Soc. Exptl. Biol. Med.*, **103**:271, 1960.
11. Halleck, F. E., Ball, C. O., and Stier, E. F. Factors Affecting Quality of Prepackaged Meat. IV. Microbiological Studies. C. Effects of Initial Bacteria Count, Kind of Meat, Storage Time, Storage Temperature, Antioxidants and Antibiotics on the Rate of Bacterial Growth in Packaged Meat, *Food Technol.*, **12**:654, 1958.
12. Nagiski, J., Copley, M. J., and Couch, J. F. The Antibacterial Activity of Flavanols, *J. Bacteriol.*, **54**:34, 1947.
13. Gustafsson, B. E. Hämmande inverkan av flavoner och flavanoner på syrabildningen i saliv från karieskaktiva patienter, *Svensk Tandläkare-Tidskr.*, Vol. 45, Suppl., 1952.
14. Gustafsson, B. E., and Krasse, B. The Caries Reducing Effect of Naringenin and of Protamine in Hamsters, *Acta Odontol. Scand.*, **16**:355, 1958.
15. Kraybill, H. R., and Dugan, L. R., Jr. Antioxidants: New Developments for Food Use, *Agr. and Food Chem.*, **2**:81, 1954.

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ТОКСИКОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА ФЕНОЛЬНЫХ АНТИОКИСЛИТЕЛЕЙ ПИЩЕВЫХ ЖИРОВ (ОСТРЫЕ И ПОДОСТРЫЕ ОПЫТЫ)

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Жиры, являясь высокоценным продуктом питания, подвергаются быстрой порче, которая в основном обусловлена окислительными процессами. Окисление при низких температурах несколько замедляется, но не прекращается, поэтому даже в холодильнике жиры можно сохранять не дольше полугода. В процессе окисления жиры теряют свои вкусовые и питательные свойства и могут оказывать вредное действие (И. Смородинцев, 1947; Burr и Barues, 1943; Johnson, Perkins, Sugal и Kummerow, 1957, и др.).

Окончательно еще не выяснено, сами ли продукты окисления жира (перекиси, полимерные продукты) оказывают прямое вредное действие или при окислении жиров (при прогоркании) окисляются и инактивируются такие важные составные части рациона, как витамины A, D, E, K, пантотеновая кислота, пиридоксин, биотин, аскорбиновая кислота и жизненно необходимые ненасыщенные жирные кислоты (линолевая, линоленовая, арахидоновая). Патологические явления, развивающиеся при кормлении крыс прогоркшей экспериментальной диетой, возможно объясняются недостатком этих питательных веществ в рационе (Quackenbusch, 1945; Greenberg и Frazer, 1953; Witting, Hishida, Johnson и Kummerow, 1957).

Имеются также данные о том, что глубоко окисленные жиры обладают канцерогенным действием (Lavik и Baumann, 1941, 1943; Roffo, 1938). Спектр термически окисленного жира имеет сходство со спектром канцерогенных дериватов фенантрена (Roffo, 1938).

Одним из эффективных способов предохранения жиров от окисления является введение в жиры антиокислителей. Наибольший интерес проявляется к фенольным антиокислителям, как наиболее эффективным: бутилоксианизолу, бутилокситолуолу и эфирам галловой кислоты. В ряде стран эти антиокислители широко используются в пищевой промышленности (Tollenaar и Vos, 1956).

В настоящее время в Советском Союзе Всесоюзным научно-исследовательским институтом мясной промышленности и Всесоюзным научно-исследовательским институтом жиров предлагается вводить бутилоксианизол (BHA), бутилокситолуол (BHT) и пропилгаллат (PG) в топленые жиры и растительные масла. Антиокислители рекомендуется вводить в количестве 0,01—0,02%. При таком количестве бутилоксианизола или бутилокситолуола жиры повышают свою стойкость в 3—5 раз, а при добавлении галлатов — до 10 раз (Н. Эмануэль, Д. Кнорре, Ю. Лясковская и В. Пиульская, 1955, 1958). Органолептических изменений жира эти антиокислители в указанных количествах не вызывают.

Когда эти препараты были предложены для применения их в пищевой промышленности, встал вопрос об изучении их токсичности. Изучением токсичности бутилоксианизола занимались Lehmann, Fitzugh, Helson и Woodard (1951), Wilder, Ostby и Gregory (1956) и др. Takata, Nagata (1952), Deichmann, Clemmer, Rakoczy и Bianchine (1955) изучали бутилокситолуол. Токсичность галлатов определяли Boehm и Williams, (1943), Orten, Knyper и Smith (1948), Allen и De Eds (1951), Roy (1953), Sporn и Peretianu (1956). Работами вышеперечисленных авторов были установлены LD_{50} этих препаратов для мышей, крыс, кроликов, морских свинок, кошек, причем метод установления этой до-

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зы различен у каждого автора: одни устанавливали ее при подкожном введении, другие — при внутрибрюшинном, третьи — при энтеральном; вводились растворы различной концентрации. Понятно, что и полученные результаты отличаются друг от друга. При длительном введении (1—3 месяца) больших доз фенольных антиокислителей (около 1% от веса корма) авторами отмечалось нарушение роста и развития животных. Пропилгаллат при таких концентрациях приводил, кроме того, к уменьшению количества гемоглобина крови и к патологическим явлениям со стороны почек (появление в моче белка). Бутилоксианизол при концентрации 0,25 г на 1 кг веса у собак вызывал дегенеративные изменения паренхиматозной ткани печени. Скармливание высших галлатов (октил, додецил) самкам крыс в концентрации 0,5% от веса корма привело к нарушению лактации и оказывало вредное влияние на молодое поколение. Следует отметить, что все авторы в подострых опытах применяли очень ограниченное число тестов: вес животных, исследование крови и патоморфологические исследования органов. Только Deichmann и др. (1955), изучая токсичность бутилокситолуола, определяли протромбиновое время и измеряли кровяное давление. Нам кажется, что количество использованных авторами тестов недостаточно, чтобы судить о функциональном состоянии организма. В своих опытах мы пытались возможно шире исследовать различные стороны жизнедеятельности организма.

Для сравнительной токсикологической характеристики изучаемых препаратов (бутилоксианизол, бутилокситолуол, пропилгаллат) были поставлены острые опыты на крысах и мышах. В опыт были взяты растущие здоровые животные (самцы и самки). Антиокислители вводились утром, натощак, в растворе подсолнечного масла непосредственно в пищевод из шприца через металлический зонд, представляющий собой обыкновенную толстую инъекционную иглу с напаянной оливой на конце. Животные, оставшиеся в живых после введения препарата, находились под наблюдением в течение 20 дней. Животным вводили различные количества изучаемых препаратов (7—8 доз). Каждую дозу получало по 8 крыс и 10 мышей.

Проведя затравку животных различными дозами изучаемых препаратов, мы установили токсичность этих веществ от минимально токсической дозы до абсолютно смертельной. Затем, проведя принятое в

Таблица 1

Результаты определения токсических и смертельных доз изучаемых антиокислителей

Антиокислители	Крысы			Мыши	
	минимально токсическая доза в мг на 1 кг веса	LD_{50} мг на 1 кг веса	LD_{100} мг на 1 кг веса	LD_{50} мг на 1 кг веса	LD_{100} мг на 1 кг веса
Бутилоксианизол (BHA)	1 000	2 900	4 500	1 250	3 000
Бутилокситолуол (BHT)	500	2 450	3 500	2 000	2 500
Пропилгаллат (PG) . . .	500	2 600	3 500	1 700	2 500

токсикологии интегрирование по Беренсу (Н. С. Правдин, 1947; В. С. Шадурская, 1953), построили кривую смертности и по ней определили LD_{50} всех трех изучаемых антиокислителей для крыс и мышей. Результаты опытов представлены в табл. 1.

Из полученных данных видно, что токсичность этих препаратов очень близка друг к другу и что фенольные антиокислители сравнительно мало токсичны.

Подострые опыты были поставлены с целью выявления функциональных нарушений под влиянием изучаемых препаратов, в заведомо больших, аггравированных дозах (сравнительно с теми, которые могут

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попасть в организм человека). В случае обнаружения таких отклонений фиксировали внимание именно на этих тестах в хронических опытах при окончательном решении вопроса о возможности введения фенольных антиокислителей в пищевые жиры.

В подострых опытах использовались четыре группы крыс: первая группа получала в рационе жир с BHA в дозе $^1/_5$ от LD_{50}, т. е. 600 мг на 1 кг веса тела; вторая группа получала BHT, первоначально $^1/_5$ от LD_{50}, т. е. 500 мг на 1 кг веса; в дальнейшем эта доза была уменьшена до 250 мг на 1 кг веса тела из-за плохой поедаемости корма при более высоком содержании BHT; третья группа получала $^1/_5$ от LD_{50} PG — 500 мг на 1 кг веса тела; четвертая группа была контрольной.

Антиокислители вводились в свиной топленый жир, который примешивался к основному корму. Контрольная группа получала чистый лярд. Свиной топленый жир и препараты антиокислителей, необходимые для постановки опытов, были предоставлены нам Всесоюзным научно-исследовательским институтом мясной промышленности.

Каждая крыса содержалась в отдельной клетке, что позволяло следить за количеством съеденного корма и жира с антиокислителями.

Для наблюдения за функциональным состоянием подопытных животных мы остановились на следующих показателях.

1. Ежедневное наблюдение за животными.
2. Взвешивание животных один раз в 10 дней.
3. Проведение морфологического исследования крови, поскольку известно, что фенол и его производные часто вызывают патологию кровотворения. При морфологическом исследовании производился подсчет форменных элементов: эритроцитов, ретикулоцитов, тромбоцитов, лейкоцитов; определялось содержание гемоглобина и лейкоцитарная формула.
4. Исходя из того, что окислительные процессы лежат в основе большинства биохимических реакций организма и что антиокислители, возможно, и в живом организме оказывают влияние на процессы окисления, исследовалась активность окислительных ферментов, а именно каталазы и пероксидазы. Эти ферменты были выбраны еще и потому, что Tappel и Marr (1954), изучая in vitro действие пропилгаллата и нордигидрогвайаретовой кислоты (тоже фенольного антиокислителя) на 14 энзиматических систем, нашли, что они в концентрации 1×10^{-4} M тормозят деятельность каталазы и пероксидазы. Тормозящее действие фенольных соединений на каталазу показали также Goldacre и Galston (1953). Каталаза определялась по методу Баха и Зубковой и выражалась в виде показателя каталазы. Пероксидаза определялась по окислению индигокармина и выражалась во времени (секундах), необходимом для его окисления.
5. Исследовалась холинэстеразная активность сыворотки крови, которая, по мнению физиологов (К. М. Быков, 1949; Х. С. Коштоянц, 1946), тесно связана с функциональным состоянием нервной системы.
6. Исследовалось протромбиновое время (зависит от количества протромбина, синтезируемого в печени). Клиницисты признают ценность этого определения для выявления функциональных нарушений печени.

Все выбранные показатели проверялись у животных до опыта, а после дачи антиокислителей — каждые 2 недели. Животные находились на опыте от 68 до 82 дней. В конце опытного периода все животные забивались обезглавливанием. Производилось патологоанатомическое вскрытие и гистологическое исследование внутренних органов. Помимо патогистологического исследования, печень подвергалась химическому изучению: определялось содержание сухих веществ, количество гликогена, жира и липоидного фосфора для выявления возможного нарушения некоторых сторон углеводного и жирового обмена в этом органе.

За весь опытный период каждое подопытное животное получило следующее количество препаратов: BHA — от 5 до 7 г, BHT — от 2 до 4 г, PG — от 4 до 6 г.

Изменений в поведении и внешнем виде подопытных животных отмечено не было. Взвешивание животных показало, что до дачи антиокислителей животные всех групп одинаково хорошо прибавляли в весе. С началом же введения антиокислителей в рацион вес подопытных животных начал значительно отставать от веса контрольных (рис. 1). Отставание в весе животных подопытных групп особенно было выражено в первый месяц опыта и меньше — во второй месяц.

У животных, получавших в рационе BHA и BHT, было выявлено снижение активности каталазы крови. У контрольных крыс к концу опытного периода активность каталазы составляла 9,5—13,5 мг перекиси водорода, в группе BHA — 6,7—8,8 мг, в группе BHT к 43-му дню опыта — 6,1—8,8 мг; к 60-му дню активность несколько повысилась, но не достигла уровня каталазы контрольных животных. Пропилгаллат не вызвал изменений активности каталазы.

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Кровь всех трех подопытных групп животных показала понижение активности пероксидазы (количество секунд, идущее на окисление субстрата, увеличивалось). Активность пероксидазы крови контрольных животных за время опыта колебалась в пределах 56—86 секунд, в то время как в группе, получающей ВНА, она составила к 42-му дню 105—147 секунд и к концу опыта — 91—135 секунд. Группа животных, получавших ВНТ, показала время 83—116 секунд к концу опытного периода, а группа, получавшая пропилгаллат, — 84—110 секунд.

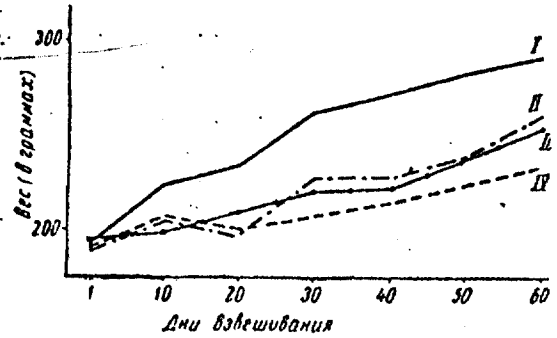

Рис. 1. Изменение веса крыс в течение опытного периода.

I — вес контрольных животных; *II* — вес животных, получавших пропилгаллат; *III* — бутилоксианизол; IV — бутилокситолуол.

Понизилась активность холинэстеразы у животных, в рацион которых примешивался жир с ВНТ и PG.

К концу опыта активность была следующей: у контрольных животных — 19,7—25,4% разрушенного ацетилхолинхлорида; в группе, получавшей ВНТ, — 10,4—16,3%; в группе, получавшей PG, — 13,2—16,3%; в группе, получавшей ВНА, не отличалась от контрольной. Каких-либо отклонений в морфологическом составе крови и при исследовании мочи подопытных животных отмечено не было. Протромбиновое время всех животных подопытных групп не отличалось от такового контрольной группы.

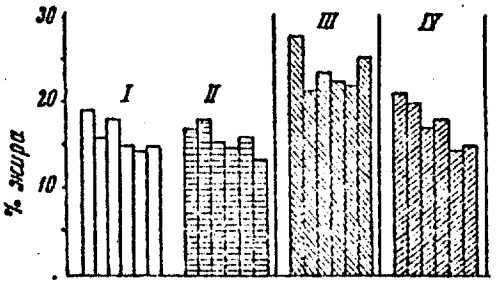

Рис. 2. Содержание жира в печени (на сухое вещество).

I — контрольная группа; *II* — животные, получавшие бутилоксианизол; *III* — получавшие бутилокситолуол; *IV* — получавшие пропилгаллат.

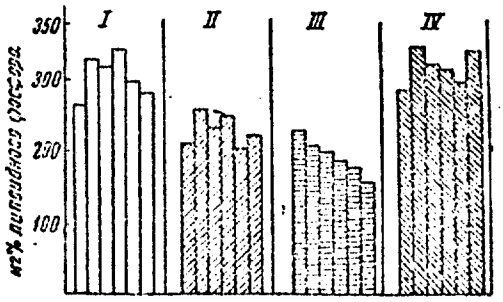

Рис. 3. Содержание липоидного фосфора в печени (на сухое вещество). Условные обозначение те же, что и на рис. 2.

При вскрытии животных грубых патологоанатомических изменений обнаружено не было. Вес печени крыс, получавших ВНА и ВНТ, был выше, чем контрольных, и группы, получавшей PG. При гистологическом исследовании внутренних органов (головного мозга, сердца, легких, желудка, двенадцатиперстной кишки, тонкого кишечника, слепой и ободочной кишки, печени, селезенки, почек, мочевого пузыря, половых органов) все три подопытные группы не дали сколько-нибудь значительных изменений сравнительно с контрольными животными. Только у одной крысы (№ 15) из группы пропилгаллата имелся отек стромы семенника с прекращением сперматогенеза. У другой крысы (№ 17) этой же группы ворсинки тонкого кишечника были отечны с резко расширенными лимфатическими капиллярами. Патоморфологические исследования были проведены в Отделе пищевой гигиены Института питания АМН СССР Ю. П. Кукелем.

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Содержание жира в печени было одинаковым в контрольной группе и в группах, получавших BHA и PG (от 14,6 до 20,6%). Печень же крыс, получавших BHT, содержала увеличенное количество жира (20,8—27,4%) (рис. 2). В то же время в печени этих животных было мало липоидного фосфора (рис. 3). Это указывает на то, что у животных, которые получали BHT, нарушены синтез фосфолипидов, процессы расщепления и вывода нейтрального жира из печени (правда, еще без гистологически выявляемой жировой инфильтрации печени). Несколько снижено содержание липоидного фосфора и у животных, получавших BHA.

Процент содержания сухих веществ и гликогена был приблизительно одинаков во всех четырех группах.

Проведенные острые и подострые опыты дают основание сделать следующие выводы.

1. Фенольные антиокислители (бутилоксианизол, бутилокситолуол, пропилгаллат) не относятся к сильнотоксичным веществам (LD_{50} больше 1000 мг на 1 кг веса тела), и токсичность их близка друг к другу.

2. Подострые опыты показали, что фенольные антиокислители, хотя и не вызвали гистологических изменений в органах при даче больших доз, являются далеко не безразличными веществами для организма животных:

а) подопытные крысы отставали в весе от контрольных; это указывает на то, что скармливаемые дозы препаратов вели к нарушению процессов ассимиляции в организме животных;

б) изучаемые антиокислители понижали уровень окислительных процессов в организме (понижение активности окислительных ферментов);

в) бутилокситолуол и пропилгаллат понижали активность холинэстеразы сыворотки крови;

г) наибольшую токсичность показал бутилокситолуол. Химические исследования печени выявили, что бутилокситолуол оказывает вредное воздействие на печень, нарушая процесс синтеза фосфолипидов, расщепления и вывода нейтрального жира из печени.

3. Для окончательного вывода о возможности введения фенольных антиокислителей в производственную практику в настоящее время ведутся хронические опыты с небольшими дозами препаратов. Это необходимо для того, чтобы полностью исключить возможное токсическое действие на человека жира, содержащего бутилоксианизол, бутилокситолуол и пропилгаллат.

ЛИТЕРАТУРА

Быков К. М. В кн.: Проблемы советской физиологии, биохимии, фармакологии. М., 1949, кн. 1, стр. 17.—Коштоянц Х. С., Турпаев Т. М. Докл. АН СССР, 1946, т. 54, № 2, стр. 181.—Правдин Н. С. Методика малой токсикологии промышленных ядов. М., 1947.—Смородинцев И. Мясная и молочная пром., 1947, № 9, стр. 12.—Шадурская В. С. Фармакол. и токсикол., 1953, № 3, стр. 48.—Эмануэль Н., Кнорре Д., Лясковская Ю. и др. Мясная индустрия, 1955, № 6, стр. 47.—Они же. Там же, 1958, № 2, стр. 52.—Allen Sh. C., De Eds F. J. Am. Oil Chem. Soc., 1951, v. 28, p. 304.—Boehm E., Williams R. Quart. J. pharm., 1943, v. 16, p. 232.—Burr G. O., Barnes R. H. Physiol. Rev., 1943, v. 23, p. 256.—Deichmann W. B., Clemmer J. J., Rakoczy R. a. oth. Arch. Industr. Health, 1955, v. 11, p. 93.—Goldacre P. L., Galston A. W. Arch. Biochem., 1953, v. 43, p. 169.—Greenberg S. M., Frazer A. C., J. Nutrition, 1953, v. 50, p. 421.—Johnson O. C., Perkins E., Sugal M. a. oth. J. Am. Oil. Chem. Soc., 1957, v. 34, p. 594.—Kaunitz H., Slanetz C., Johnson R. E. Ibid., 1956, v. 33, p. 630.—Lavik P. S., Baumann C. A. Cancer Res., 1941, v. 1, p. 181.—Idem. Ibid., J. 1943, v. 3, p. 749.—Lehmann A. J., Fitzugh O. G., Helson A. A. a. oth. Advances in Food Research, 1951, v. 3, p. 197.—Orten J. M., Knyper A. C., Smith A. H. Food Technology, 1948, N. 2, p. 308.—Quackenbush F. W. Oil and Soap, 1945, v. 22, N. 12, p. 336.—Roffo A. H. Bol. Inst. de med. exper. para el estud. y trat. d. cancer, 1938, v. 15, p. 837.—Roffo A. H. Ibid., 1938, v. 15, p. 407.—Roy B. R.

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Indian J. M. Res., 1953, v. 41, p. 201, 207. — Sporn A., Peretianu J. Igiena, 1956, v. 1, p. 52. — Takata R., Nagata T. Vitamins (Japan), 1952, v. 5, p. 203—205. — Tappel A. L., Marr A. G. J. Agricult. Food Chem., 1954, v. 2, p. 554. — Tollenaar E. D., Vos H. J. Fette, Seifen, Austrichmittel, 1956, v. 58, S. 112. — Wilder O. H. M., Ostby C., Gregory R. Publ. Am. Meat Inst. Foundation, 1956, Bull., N. 29, p. 38. — Witting L. A., Nishida T., Johnsohn O. C. a. oth. J. Am. Oil. Chem. Soc., 1957, v. 34, p. 421.

A TOXICOLOGIC CHARACTERISTICS OF PNEHOLIC ANTIOXIDANTS OF ALIMENTARY FATS

I. A. Karplyuk (Moscow)

Summary

In experiments stages for the study of acute toxicity phenolic antioxidants (butil-oxianisol, butyloxitoluol and propalgallate) minimal toxic doses of LD 50 and LD 100 have been determined for two kinds of animals viz.—mice and rats.

In subacute experiments lasting for 2.5 months with the preparation dosed in the amounts of 1/5—1/10 from LD 50—a delayed increase of the weight has been registered in all experimental animals, accompanied by a reduced ferment activity, viz.—catalases, perioxidases and cholinesterases. The butaloxitoluol produced disturbed fat metabolism in the liver.

Chronic experiments are being conducted at present to arrive at the final conclusion as to the possibility of introducing phenol antioxidants into the industrial practice.

ГИГИЕНИЧЕСКИЙ АНАЛИЗ ПИТАНИЯ УЧАСТНИКОВ ВСЕСОЮЗНЫХ СПАРТАКИАД УЧАЩИХСЯ ШКОЛ

Г. М. Краковяк

Из кафедры гигиены (зав.— доцент Я. М. Богданов) Государственного ордена Ленина и ордена Красного Знамени института физической культуры имени П. Ф. Лесгафта, Ленинград

В интересах укрепления здоровья и более широкого охвата детей спортом ежегодно (с 1954 г.) проводятся всесоюзные спартакиады учащихся школ.

В предшествовавших спартакиадам соревнованиях каждый год принимало участие свыше 10 миллионов школьников.

Питание юных спортсменов изучалось нами на I (1954 г., Ленинград), II (1955 г., Киев) и IV (1957 г., Рига) спартакиадах у 12 спортивных делегаций (1803 человека). Производилась гигиеническая оценка организации и режима питания, качественного и количественного состава пищи.

Полноценное питание имеет большое значение для роста и развития организма, выработки иммунитета, укрепления здоровья, повышения работоспособности.

У молодежи, занимающейся спортом, по сравнению со взрослыми обмен веществ относительно выше, поэтому затраченная энергия должна полностью восстанавливаться питанием (Г. М. Краковяк, 1956). Пища юного спортсмена должна быть более калорийной (75—80 калорий на 1 кг веса), чем у учащихся школ того же возраста (60—65 калорий на 1 кг веса), не занимающихся спортом. Она должна содержать 3—3,5 г белка на 1 кг веса при соотношении белков, жиров и углеводов 1 : 2 : 4, или 14% белков, 30% жиров и 56% углеводов суточной калорийности.

*(209) Karplyuk, I. A. 1962. Effect of phenolic antioxidants of nutritive fats on the animal organism. Tr. 2-oi(Vtoroi) Nauchn. Konf. po Vopr. Probl. Zhira v Pitanii, Leningrad. 318-325.

ВЛИЯНИЕ ФЕНОЛЬНЫХ АНТИОКИСЛИТЕЛЕЙ ПИЩЕВЫХ ЖИРОВ НА ЖИВОТНЫЙ ОРГАНИЗМ И ИХ ГИГИЕНИЧЕСКАЯ ОЦЕНКА

Аспирант И. А. КАРПЛЮК
(Центральный институт усовершенствования врачей, Москва)

Жиры, являясь высокоценным продуктом питания, подвергаются быстрой порче, которая в основном обусловлена окислительными процессами. Окисление при низких температурах несколько замедляется, но не прекращается, поэтому даже в условиях холодильника жиры можно сохранять не дольше полугода, что не может удовлетворить нужды пищевой промышленности.

В жирах при окислении в первую очередь разрушаются жирорастворимые витамины и полиненасыщенные жирные кислоты, необходимые для нормальной жизнедеятельности организма. Поэтому вопрос о сохранении жиров свежими в течение более длительного времени является также и гигиенической проблемой.

В последние годы предложено применять химические способы защиты жиров от порчи, которые заключаются в введении в жиры веществ, получивших название антиокислителей (антиоксидантов).

Окислительные процессы в жирах идут по типу цепных реакций. Механизм действия антиокислителей, по-видимому, заключается в их способности обрывать цепную реакцию, в результате чего процесс окисления резко замедляется.

Наиболее эффективными признаются фенольные антиокислители, а среди них: бутилоксианизол, бутилокситолуол и эфиры галловой кислоты. Для ингибирующего действия достаточно небольших количеств веществ (0,01—0,02%), что позволяет время хранения жиров увеличить в 4—10 раз.

В связи с предложением применять бутилоксианизол, бутилокситолуол и пропилгаллат для стабилизации топленых жиров и растительных масел, в Советском Союзе возник вопрос о безвредности и пригодности таких жиров для питания человека.

Отечественных работ по изучению токсических свойств бутилоксианизола, бутилокситолуола и пропилгаллата нет.

ИЯНИЕ ФЕНОЛЬНЫХ АНТИОКИСЛИТЕЛЕЙ ПИЩЕВЫХ ЖИРОВ НА ЖИВОТНЫЙ ОРГАНИЗМ И ИХ ГИГИЕНИЧЕСКАЯ ОЦЕНКА

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Отечественных работ по изучению токсических свойств бутил-
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Исследованием токсичности фенольных антиокислителей занимались в США, Англии, Голландии, Японии, Индии, Р[illegible].

Знакомство с этими работами показало, что LD 50 бутилоксианизола, бутилокситолуола и галлатов при их энтеральном введении является доза выше 1000 *мг/кг* веса животного, что свидетельствует о сравнительно слабой токсичности этих соединений.

При скармливании больших количеств (около 1% в пересчете на весь корм) этих фенольных антиокислителей в течение 1—3 месяцев авторами отмечалось нарушение роста и развития животных. Скармливание пропилгаллата вызвало уменьшение темпа [illegible] и морфологические изменения канальцевого аппарата почек. [illegible]ние октил- и додецилгаллатов в корм кормящих самок-крыс [illegible]водило к нарушению лактации и, как следствие этого, к худшему развитию молодняка.

Большинство исследователей, проводящих опыты по длительному скармливанию бутилоксианизола, бутилокситолуола и галлатов, приходят к выводу, что даже то количество антиокислителей, которое в 100—200 раз больше предлагаемого для введения в жиры количества антиокислителей, является безвредным. Однако выводы разных авторов о токсичности или безвредности соединений не всегда совпадают.

Леман с сотрудниками не отметили вредного действия для крыс пропилгаллата при скармливании его в дозе 1% в корме, в то время как Ортен, Кайзер и Смит считают эту концентрацию токсичной.

Рой утверждает, что на концентрацию этилгаллата в жире в количестве 1% животные не реагируют, тогда как Сворд и Перетиану при содержании 0,75% этилгаллата в жире диеты выявили расстройство роста подопытных животных, сокращение в использовании азота пищевого рациона. В таламусе и печени авторами были обнаружены дистрофические изменения.

Таким образом, имеющаяся литература по изучению токсических свойств бутилоксианизола, бутилокситолуола и пропилгаллата не дает достаточно полной сравнительной характеристики этих соединений.

В связи с разноречивостью литературных данных настоящая работа должна была дать ответ о возможности использования бутилоксианизола, бутилокситолуола и пропилгаллата для стабилизации пищевых жиров. Решалось это следующими путями.

1. Исследовались органолептические свойства изделий, изготовленных на жире, содержащем антиокислители.

2. Ставились биологические опыты на животных, включающие установление степени токсичности препаратов в острых опытах, изучение их токсичности в подострых опытах при скармливании больших доз и исследование возможного вредного действия на организм животных пищевого жира, содержащего бутилоксианизол, бутилокситолуол и пропилгаллат, при длительном скармливании.

вают подо[illegible] действие на течение окислительных процессов в живом организ[illegible].

При биохимических исследованиях печени обращают на себя внимание результаты исследований в группе бутилокситолуола. Животные этой группы имели по сравнению с контрольной группой больший вес печени, большее содержание в ней жира и уменьшенное количество липоидного фосфора (достоверность подтверждена статистически). По-видимому, у животных этой группы под влиянием введения в организм бутилокситолуола наступило расстройство в синтезе фосфолипидов и, как следствие этого, накопление жира в печени.

Несколько уменьшенным было содержание липоидного фосфора в печени крыс, получавших бутилоксианизол, хотя еще без задержки жира в этом органе.

Проведенные гистологические исследования внутренних органов не показали в тканях каких-либо изменений, которые можно было бы связать с введением в организм животных фенольных антиокислителей. Только у одной крысы, получавшей пропилгаллат, обнаружен отек стромы семенника с прекращением сперматогенеза.

Остальные исследуемые показатели, как морфологический состав крови, протромбиновое время, исследование мочи, не показали каких-либо изменений.

Наибольшее внимание в работе было уделено изучению возможного токсического действия пищевых жиров, содержащих антиокислители, в хронических опытах. Они проводились на двух видах животных: крысах и собаках, которые находились на опыте один год.

Как и в подострых опытах, животные были разбиты на четыре группы. Трем группам скармливался жир с одним из антиокислителей, четвертая группа служила контролем.

Поскольку по отношению к большинству ядов человек значительно чувствительнее, чем экспериментальные животные, мы в хронических опытах допускали некоторую аггразацию (в 10—20 раз, т. е. увеличение дозы по сравнению с той, которая может максимально попасть в организм человека с суточным рационом).

Неполноценное питание делает животных обычно более чувствительными к введению вредных агентов. Поэтому для еще большей аггразации опыта из кормового рациона крыс дважды в течение годичного опыта (на 7-м и 11-м месяцах) на 14 дней исключались молоко и мясо.

Считая, что вредное действие вещества может сказаться не на первом поколении, а на последующих, мы от основной подопытной группы крыс получили два поколения, которые по достижении месячного возраста отделялись от матерей и ставились на опыт. Крысы второго и третьего поколений находились на опыте по 6 месяцев.

Опыты по воспроизведению интересны тем, что при таких [illegible]знологических состояниях, как беремен[illegible] чувствительн[illegible] к ядам возрастает. Наблюдая самок в п[illegible] беременнос[illegible] лактации, мы могли проследить действие антиокислителей на [illegible]низм, когда к нему предъявляются повышенные требования.

Таким образом, возможное токсическое действие фенол[illegible] антиокислителей в хронических опытах изучалось на 104 кр[illegible] трех поколений и 8 собаках.

На протяжении всего опыта как крысы, так и собаки вне[illegible] выглядели здоровыми, были подвижны, активно реагировал[illegible] окружающее, имели хороший аппетит.

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Эти исследования показали, что скармливание сравни[illegible] небольших доз пропилгаллата повышает активность ферме[illegible] крови, что является нежелательным и должно рассматриваться [illegible] вредное действие препарата на организм подопытных живот[illegible].

С целью выявления возможных нарушений в функции пе[illegible] в отношении синтеза белков исследовалось общее содерж[illegible] белков в сыворотке крови, а также ставились флокуляцио[illegible] проба Таката и коагуляционная Вельтмана. Никаких измен[illegible] в количестве белков сыворотки, в белковых фракциях кр[illegible] электролитном пороге крови подопытных животных эти п[illegible] не уловили.

Исследования остаточного азота в крови собак также не[illegible]казали патологии в дезаминирующей и мочевинообразовате[illegible] функциях печени или нарушений в выделительной способ[illegible] почек.

Изучение показателей, характеризующих функциональное [illegible]стояние печени, таких, как содержание в ней гликогена, жира [illegible]пондного фосфора, не выявило нарушений в деятельности [illegible] органа у животных, получавших бутилоксианизол и бутилок[illegible]луол. Несколько иные результаты были получены в группе пр[illegible]галлата. При исследовании печени животных этой группы у [illegible] крыс отмечено уменьшение печени (вес менее 5 г), у трех

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Вес подопытных животных не отличался от веса животных контрольной группы. Даже в тот период, когда организм был искусственно ослаблен исключением из корма мяса и молока, изменение веса у подопытных животных не отличалось от такового контрольной группы.

С особым интересом мы подходили к исследованию ферментов крови, которые раньше других тестов реагировали на введение в организм животных больших количеств антиокислителей в подострых опытах. Эти исследования не выявили заметных отклонений у животных, получавших бутилоксианизол и бутилокситолуол. У крыс, которым скармливался пропилгаллат, иногда по группе в целом, иногда у отдельных животных удалось уловить повышение активности пероксидазы, сывороточной холинэстеразы у крыс первого поколения, пероксидазы — у животных второго поколения и каталазы — у третьего поколения.

Эти исследования показали, что скармливание сравнительно небольших доз пропилгаллата повышает активность ферментов крови, что является нежелательным и должно рассматриваться как вредное действие препарата на организм подопытных животных.

С целью выявления возможных нарушений в функции печени в отношении синтеза белков исследовалось общее содержание белков в сыворотке крови, а также ставились флокуляционная проба Таката и коагуляционная Вельтмана. Никаких изменений в количестве белков сыворотки, в белковых фракциях крови и электролитном пороге крови подопытных животных эти пробы не уловили.

Исследования остаточного азота в крови собак также не показали патологии в дезаминирующей и мочевинообразовательной функциях печени или нарушений в выделительной способности почек.

Изучение показателей, характеризующих функциональное состояние печени, таких, как содержание в ней гликогена, жира, липоидного фосфора, не выявило нарушений в деятельности этого органа у животных, получавших бутилоксианизол и бутилокситолуол. Несколько иные результаты были получены в группе пропилгаллата. При исследовании печени животных этой группы у двух крыс отмечено уменьшение печени (вес менее 5 *г*), у трех сни-

лено содержание гликогена и у трех — липоидного фосфора. Изменения печени в виде уменьшения ее веса, нарушения гликогенообразования или синтез фосфолипидов отмечено у 5 животных из 12, находящихся на опыте. Эти наблюдения указывают, что введение в рацион крыс жира с пропилгаллатом сказалось отрицательным образом на функции печени части подопытных животных. Подобных изменений у собак обнаружено не было.

Скармливание жира с антиокислителями не оказало влияния на воспроизведение потомства. Приплод принесли как подопытные, так и контрольные животные. Наблюдение за такими физиологическими показателями, как количество детенышей в помете, их вес при рождении, фиксирование дня появления резцов, шерстного покрова, открытия глаз, не выявило различий в развитии молодого поколения ни в одной из подопытных групп.

Шестимесячное скармливание жира с антиокислителями двум поколениям также не влияло на их общее состояние, вес и исследуемые показатели, за исключением изменений ферментативной активности крови, о которых уже говорилось.

Проведенные патологоанатомические и гистологические исследования внутренних органов не выявили каких-либо закономерных изменений, которые можно было бы считать следствием введения антиокислителей в организм животных, за исключением обнаруженных изменений в половой системе самцов. Атрофия в семенниках выявлена по одному животному из групп «бутилоксианизола, бутилокситолуола и контрольной». Среди крыс, получавших пропилгаллат, атрофия семенников отмечена у трех самцов. И хотя выявленные изменения могут быть связаны со случайным подбором животных с атрофическими процессами в семенниках в группе пропилгаллата, все-таки эти данные опять настораживают нас в отношении вредного действия пропилгаллата.

Таким образом, проведенные экспериментальные исследования по длительному скармливанию жира с фенольными антиокислителями трем поколениям крыс и собакам не показали вредного действия бутилоксианизола и бутилокситолуола на живой организм, тогда как пропилгаллат был небезразличным для подопытных животных.

Наши данные в отношении пропилгаллата перекликаются с только что закончившимися исследованиями проф. Будагяна и Смирновой, которые, изучая усвояемость пищевого жира, содержащего те же антиокислители, что и в наших опытах, показали, что усвояемость жира несколько снижается при включении в рацион жира с пропилгаллатом, тогда как введение в корм бутилоксианизола и бутилокситолуола не оказывало такого действия.

Проделанная работа позволяет сделать заключение о том, что введение бутилоксианизола и бутилокситолуола в пищевые жиры с целью замедления в них окислительных процессов не встречает возражений с гигиенических позиций, тогда как применение пропилгаллата не может быть разрешено.

Также следует отметить, что введение антиокислителей в жиры может быть разрешено на тех предприятиях, где лаборатории или соответствующие институты могут осуществлять повседневный контроль за количеством введенного препарата.

Внесение антиокислителей в жиры ни в коей мере не должно отразиться на проведении и изыскании новых мероприятий по предотвращению окисления жиров в процессе производства и хранения. В такой же степени, как и раньше, следует стремиться не допускать длительного хранения сырья, по возможности герметизировать процессы вытопки жира, хранение жира проводить при низкой температуре. Только эти мероприятия в сочетании с антиокислителями дадут возможность значительно повысить время хранения жиров без снижения их физиологической полноценности.

...ed to investigate its copyright status.

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ИЗУЧЕНИЕ ФЕРМЕНТОВЫДЕЛИТЕЛЬНОЙ ФУНКЦИИ СЛИЗИСТОЙ ОБОЛОЧКИ ТОНКОГО КИШЕЧНИКА И ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ У КРЫС, ПОЛУЧАВШИХ В РАЦИОНЕ ФЕНОЛЬНЫЕ АНТИОКИСЛИТЕЛИ

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Защита пищевых жиров и жирсодержащих продуктов от окисления путем внесения антиоксидантов находит все большее распространение и у нас, и за рубежом. Некоторые из предлагаемых антиокислителей изучены в токсикологическом аспекте и разрешены для использования в пищевой промышленности.

Вместе с тем многие стороны деятельности организма при введении антиокислителей с пищей еще не изучены. Вопрос о том, как влияет пища, содержащая антиокислители, на ферментативную деятельность желудочно-кишечного тракта, освещен недостаточно. Известно, что ферментовыделительная функция пищеварительных желез тесно связана с характером питания [1—4]; при изменении химического состава пищи железы способны тонко регулировать количество и состав вырабатываемых секретов [5].

Целью данного исследования явилось изучение влияния ряда антиокислителей фенольного ряда на активность энтерокиназы и щелочной фосфатазы в слизистой оболочке тонкой кишки, где их концентрация наиболее высока, а также на секрецию панкреатических ферментов (липазы и амилазы).

Было изучено 5 антиокислителей (бутилоксианизол, бутилокситолуол, этил-, пропил-, додецилгаллаты) и смесь антиокислителей (бутилоксианизол с пропилгаллатом).

В опыт было взято 160 белых крыс-самцов с начальным весом 80—90 г. Опыты проведены в 4 сериях. В I и II серии антиокислители скармливались в течение 1 месяца, в III и IV серии — 3 месяца. Всего в опыте было 12 опытных и 4 контрольные группы. Подопытные животные получали ежедневно 15 г пищевого кормового концентрата № 1 (пшеничная обойная мука, а также овсяная обойная и просяная мука, сухие пшеничные зародыши, очищенный от шелухи подсолнечный жмых, обезжиренная рыбная мука, обезжиренная сухая костная мука, кровяная мука, яичный порошок, снятое сухое молоко, гидролизные облученные дрожжи, меласса), 10 мл цельного молока, 3 г моркови и 1 г свиного топленого жира свежей вытопки с перекисным числом 0,006% йода. Приведенный рацион по калорийности составлял 20% белка, 25% жира, 55% углеводов.

В I и III серии опытов животным 1-й группы давали в рационе со свиным топленым жиром бутилоксианизол (БОА), 2-й группы — бутилокситолуол (БОТ), 3-й — додецилгаллат (ДГ); животные 4-й группы являлись контрольными (К). Во II и IV серии опытов животные 1-й группы получали в рационе этилгаллат (ЭГ), 2-й — пропилгаллат (ПГ), 3-й — смесь бутилоксианизола и пропилгаллата (БОА+ПГ); 4-я группа являлась контрольной (К). Соответствующие антиокислители включали в рацион определенной группы из расчета 4 мг на 1 кг веса тела животного (аггравация в 10 раз). Смесь БОА и ПГ скармливали в этой же дозе, но в соотношении 2 : 1 (наиболее эффективное соотношение для защиты пищевых жиров и продуктов от окисления). Животные контрольных групп получали тот же рацион, но включаемый в корм свиной топленый жир был свободен от антиокислителей. Разница между I и III, II и IV сериями заключалась только в продолжительности скармливания. Продолжительность опытов I и II серии 1 месяц, III и IV серии 3 месяца.

Активность энтерокиназы и щелочной фосфатазы определяли в соскобе со слизистой оболочки тонкого кишечника. Для этого после опыта животных забивали декапитацией, из верхнего отдела тонкого кишечника отделяли отрезок длиной 10 см. После отмывания кишечника от содержимого отшлифованной гранью предметного стекла делали соскоб со слизистой оболочки. Взвесив, его помещали в стеклянный гомогенизатор, где готовили водную вытяжку в разведении 1 : 20. Экстракция продолжалась 2 часа при комнатной температуре. Из полученного центрифугата (центрифугирование 10—15 мин. при 3000 об/мин) готовили разведение 1 : 1000, которое использовали для определения энтерокиназы и щелочной фосфатазы. Содержание энтерокиназы определяли по методу, предложенному Г. К. Шлыгиным, и выражали в условных единицах на 1 г веса.

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Активность щелочной фосфатазы определяли по методу, рекомендованному Л. С. Фоминой, и выражали также в условных единицах на 1 г веса. Ферментативную деятельность поджелудочной железы изучали путем определения содержания амилазы в крови и липазы в ткани поджелудочной железы. Амилазу исследовали методом Энгельгардта и Герчука у животных прижизненно до начала эксперимента, на 15, 30, 45—50 и 85—95-й день опытного периода и активность ее выражали в миллиграммах на 1 мл.

Содержание липазы определяли в ткани поджелудочной железы сразу же после декапитации и вскрытия брюшной полости животных. После извлечения железу взвешивали и растирали в ступке с дистиллированной водой (разведение 1:20) с добавлением мелко измельченного стекла. Экстракция продолжалась 30 мин. при комнатной температуре. После 5-минутного центрифугирования со скоростью 1500 об/мин готовили разведение 1:20 000, которое служило исходным для определения липазы по методу Г. К. Шлыгина, Л. С. Фоминой и З. М. Павловой. Активность липазы выражали в условных единицах на 1 г веса. Полученные результаты обрабатывали методом вариационной статистики.

В период опытов животные выглядели здоровыми, опрятными, съедали весь корм. Прибавка веса в опытных и контрольных группах осуществлялась практически одинаково.

В табл. 1 и 2 приведены результаты определения содержания энтерокиназы в слизистой оболочке тонкого кишечника при введении

Таблица 1

Активность энтерокиназы в соскобе со слизистой оболочки верхнего отдела тонкого кишечника у крыс, получавших в течение 1 месяца в рационе жир с антиокислителями (в *ед/г*)

Серия опытов	Группа животных	Антиокислитель	M	σ	m_M	t	P
I	1-я	Бутилоксианизол	2 750	700	300	0,38	>0,5
	2-я	Бутилокситолуол	2 600	600	250	0	1
	3-я	Додецилгаллат	2 600	600	250	0	1
	4-я	Контроль	2 600	600	250		
II	1-я	Этилгаллат	3 900	870	360	1,0	>0,25
	2-я	Пропилгаллат	4 000	1300	530	1,0	>0,25
	3-я	Бутилоксианизол + пропилгаллат	3 500	650	270	0,2	>0,5
	4-я	Контроль	3 400	880	360		

Таблица 2

Активность энтерокиназы в соскобе слизистой оболочки верхнего отдела тонкого кишечника у крыс, получавших в течение 3 месяцев в рационе жир с антиокислителями (в *ед/г*)

Серия опытов	Группа животных	Антиокислитель	M	σ	m_M	t	P
III	1-я	Бутилоксианизол	3 100	480	200	0	1
	2-я	Бутилокситолуол	2 500	320	130	2,5	0,05
	3-я	Додецилгаллат	2 700	610	260	1,2	0,25
	4-я	Контроль	3 100	480	200		
IV	1-я	Этилгаллат	3 100	800	330	0,2	>0,5
	2-я	Пропилгаллат	3 800	270	110	0,3	>0,5
	2а-я	Контроль ПГ	3 700	620	250		
	3-я	Бутилоксианизол + пропилгаллат	3 600	990	400	1,0	>0,25
	4-я	Контроль	3 160	360	150		

в течение 1 и 3 месяцев животным корма, содержащего жир с антиокислителями.

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Активность энтерокиназы в контрольных группах составляла 2600±300 ед/г (I серия) и 3400±360 ед/г (II серия). У животных, получавших антиокислители, активность энтерокиназы была приблизительно в тех же пределах: в I серии 2600—2750 ед/г, во II серии 3500—3900 ед/г. Несколько увеличена, по нашему мнению, активность энтерокиназы в группе животных, получавших ПГ (M=4000 ед/г), но в этой группе довольно велика разбросанность ряда ($\sigma=\pm1300$) и разница целиком по группе недостоверна ($P>0,25$).

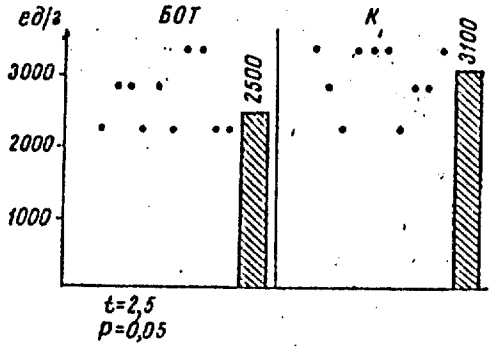

Рис. 1. Содержание энтерокиназы в соскобе со слизистой оболочки верхнего отдела тонкого кишечника у контрольных крыс и получавших в течение 3 месяцев жир с бутилокситолуолом.

БОТ — животные, получавшие жир с бутилокситолуолом; *К* — контрольные животные, получавшие чистый жир. Темные кружки — активность энтерокиназы у отдельных животных. Здесь и на рис. 2, 3: столбики с цифрами — средняя арифметическая (M) по каждой группе.

Трехмесячное введение в корм большинства изучаемых антиокислителей также не оказывало заметного влияния на содержание энтерокиназы. Статистически достоверное уменьшение количества этого фермента отмечено в группе животных, получавших БОТ (активность энтерокиназы в контрольной группе 3100±200 ед/г; в опытной группе 2500±130 ед/г). Несмотря на то что при статистической обработке получены достоверные результаты, можно считать, что большой разницы в активности фермента нет (рис. 1).

При определении активности щелочной фосфатазы в соскобе со слизистой оболочки тонкого кишечника при одномесячном скармливании антиокислителей не было обнаружено различий между опытными и контрольными группами животных (табл. 3).

Трехмесячное поступление антиокислителей в организм животных заметно не отразилось на активности щелочной фосфатазы в слизистой

Таблица 3

Активность щелочной фосфатазы в соскобе слизистой оболочки верхнего отдела тонкого кишечника у крыс, получавших в течение 1 месяца в рационе жир с антиокислителями (в *ед/г*)

Серия опытов	Группа животных	Антиокислитель	M	σ	m_M	t	P
I	1-я	Бутилоксианизол	33 800	15 500	6 300	1,7	>0,1
	2-я	Бутилокситолуол	39 700	11 100	4 500	1,1	>0,25
	3-я	Додецилгаллат	40 500	8 500	3 500	1,1	>0,25
	4-я	Контроль	46 800	10 650	4 350		
II	1-я	Этилгаллат	43 900	13 600	5 800	0,8	>0,25
	2-я	Пропилгаллат	41 100	15 000	6 100	1,2	0,25
	3-я	Бутилоксианизол + пропилгаллат	42 700	15 000	6 100	0,9	>0,25
	4-я	Контроль	48 400	3 640	1 500		

оболочке верхнего отдела тонкого кишечника (табл. 4). Только в группе животных, получавших ЭГ, мы отметили статистически достоверное понижение активности щелочной фосфатазы.

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Таблица 4

Активность щелочной фосфатазы в соскобе слизистой оболочки верхнего отдела тонкого кишечника у крыс, получавших в течение 3 месяцев в рационе жир с антиокислителями (в *ед/г*)

Серия опытов	Группа животных	Антиокислитель	*M*	σ	m_M	*t*	*P*
III	1-я	Бутилоксианизол	57 800	17 800	7 300	0,9	>0,25
	2-я	Бутилокситолуол	54 000	16 600	6 800	1,2	0,25
	3-я	Додецилгаллат	54 900	26 600	10 800	1	>0,25
	4-я	Контроль	70 800	30 800	12 200		
IV	1-я	Этилгаллат	24 700	10 400	4 250	2,2	0,05
	2-я	Пропилгаллат	46 000	8 100	3 300	0,12	>0,5
	2а-я	Контроль ПГ	46 900	16 800	6 850		
	3-я	Бутилоксианизол + пропилгаллат	45 200	18 000	7 350	0,2	>0,5
	4-я	Контроль	47 500	22 750	9 300		

Таким образом, месячное введение антиокислителей в организм животных не оказывало влияния на активность энтерокиназы и щелочной фосфатазы.

При 3-месячном введении антиокислителей только в группе животных, получавших ЭГ, было отмечено снижение активности этого фермента. Выявленная разница в активности энтерокиназы в группе БОТ вряд ли может быть принята во внимание, так как, исходя из представ-

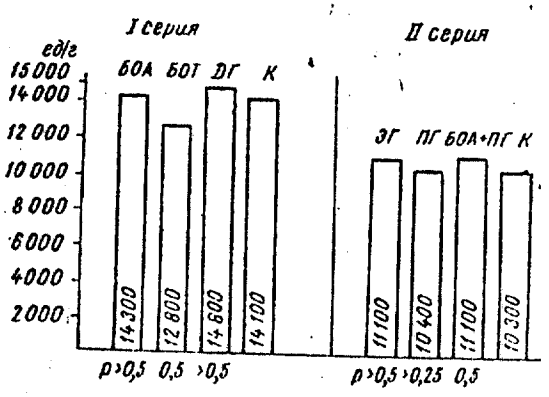

Рис. 2. Активность липазы ткани поджелудочной железы у крыс, в рацион которых в течение 1 месяца включали жир с антиокислителями.

ления о пределе физиологических колебаний активности этого фермента, мы не можем рассматривать эту разницу как существенную.

Проведенные исследования амилазы крови показали, что активность этого фермента, определенная в течение ряда дней подряд, у различных животных и даже у одних и тех же животных очень сильно варьирует. У контрольных животных, не подвергающихся возможному вредному влиянию антиокислителей, активность амилазы была от 60 до 180 мг/мл. Одна и та же крыса контрольной группы на протяжении нескольких дней давала следующие показатели амилазной активности крови: 62—90—180—83 мг/мл.

Во всех опытных группах предел колебаний у отдельных животных был также очень велик. В связи с этим мы считаем, что этот непостоянный в своей количественной характеристике показатель не представляет для нас особого интереса.

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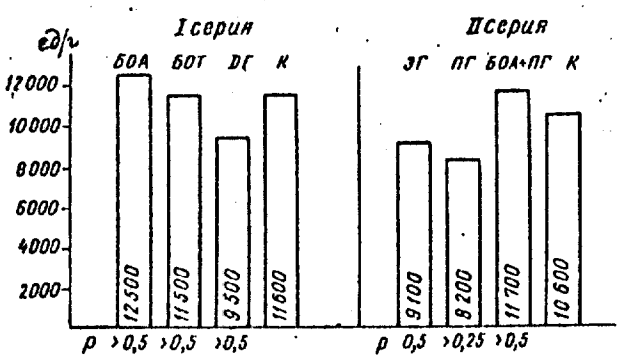

Рис. 3. Активность липазы ткани поджелудочной железы у крыс, получавших в течение 3 месяцев в рационе жир с антиокислителями.

На рис. 2 и 3 показаны результаты определения активности липазы в ткани поджелудочной железы при скармливании антиокислителей в течение 1 и 3 месяцев. Содержание липазы в отдельных группах дано в виде средних. Анализ этого материала говорит о том, что значительной разницы в опытных группах по сравнению с контролем нет, что подтверждается также результатами статистической обработки материала. Имеющаяся разница в некоторых группах должна рассматриваться как несущественная (во всех случаях $P>0,25$).

Ф. Е. Будагян и Л. И. Смирнова [6] показали, что включение в рацион животных антиокислителей ведет к некоторому уменьшению усвояемости пищевых веществ, особенно жиров (разница была не очень существенна — 0,5—0,9%). Авторы в своей работе не объяснили механизма возможного уменьшения усвояемости. При постановке данного исследования нам хотелось проследить, не является ли уменьшение всасывания пищевых веществ кишечной стенкой результатом нарушения секреторной функции пищеварительных желез. Как показали проведенные исследования, большинство изучаемых нами антиокислителей (БОА, ПГ, ДГ, БОА + ПГ), вводимых per os в течение 1 и 3 месяцев, не оказывает влияния на содержание энтерокиназы и щелочной фосфатазы в слизистой оболочке тонкого кишечника.

Отмечаемое в работе некоторое уменьшение активности щелочной фосфатазы у животных, получавших ЭГ, и энтерокиназы у животных, которым вводили БОТ, возможно, связано с тем, что включение в пищу этих антиокислителей, изменяя привычный химический состав пищи, рефлекторно приводит к компенсаторному изменению ферментовыделительной функции кишечника.

Содержание липазы в ткани поджелудочной железы не изменилось ни в одном из 12 опытных групп животных. Эти исследования позволили отвергнуть предположение о снижении усвояемости жира, содержащего антиокислители, за счет уменьшения секреции липолитического фермента поджелудочной железой.

Определение активности амилазы в крови у крыс показало очень большую вариабельность этого показателя во всех группах, в том числе и в контрольных. Мы полагаем, что показатель, дающий большие физиологические колебания, не может быть использован как чувствительный тест для изучения возможного токсического действия различных химических добавок на организм.

Выводы

1. Введение в рацион в течение 1 и 3 месяцев некоторых антиокислителей фенольного ряда не оказывало влияния на активность энтерокиназы в слизистой оболочке верхнего отдела тонкого кишечника белых крыс.

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2. Содержание щелочной фосфатазы в слизистой оболочке тонкого кишечника не было изменено у животных, получавших в течение 1 месяца с кормом антиокислители. Скармливание этилгаллата в течение 3 месяцев привело к некоторому уменьшению активности щелочной фосфатазы в слизистой оболочке тонкого кишечника. Остальные 5 изучаемых антиокислителей такого действия не оказывали.

3. Активность панкреатических ферментов (липазы и амилазы) существенно не изменялась при скармливании антиокислителей в течение 1 и 3 месяцев.

ЛИТЕРАТУРА

1. Шлыгин Г. К. Тер. арх., 1956, в. 1, с. 39. — 2. Он же. Вестн. АМН СССР, 1964, № 3, с. 21. — 3. Он же. Ферменты кишечника в норме и патологии. Л., 1967. — 4. Михлин С. Я. Лабор. дело, 1966, в. 10, с. 597. — 5. Бабушкина Л. М., Фомина Л. С., Фалтова Э. Физиол. ж. СССР, 1961, № 11, с. 1404. — 6. Будагян Ф. Е., Смирнова Л. И. Вопр. питания, 1962, № 2, с. 47.

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A STUDY OF THE ENZYME SECRETORY FUNCTION OF THE SMALL INTESTINE AND PANCREAS MUCOSA IN RATS FED ON A DIET CONTAINING PHENOL ANTIOXIDENTS

I. A. Karplyuk (Moscow)

Summary

Study was made of the action exerted by a series of phenol antioxidents (butyloxyanisol, butyloxytoluene, ethyl-, propyl-, dodethylgallates and a mixture of butyloxyanisol and propylgallate) introduced per os to rats during one and three months, on the activity of enterokinase and alkaline phosphatase in the small intestine mucosa, and also on the secretion of pancreatic enzymes (amylase and lipase).

The effected research did not show any changes in the development of enterokinase in the mucosa of the superior portion of the small intestine. A diminished production of phosphatase by the small intestine wall was noted in the group of animals receiving ethylgallate for 3 months. One and three months long feeding of antioxidents does not affect the activity of the lipolytic enzyme secreted by the pancreas.

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ЭКСКРЕЦИЯ НЕЙТРАЛЬНЫХ ЖИРОВ И СВОБОДНЫХ ЖИРНЫХ КИСЛОТ СТЕНКОЙ ТОНКОГО КИШЕЧНИКА В ПРОЦЕССЕ ПИЩЕВАРЕНИЯ У СОБАК

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Выделение различных веществ в полость пищеварительного тракта имеет большое физиологическое значение [1—5]. Значительный интерес представляет выделение различных липидов в пищеварительном тракте. Так, детально изучено выведение фосфолипидов с желчью [6—8], с кишечным соком [4—5, 9—12]. Полагают, что выведение фосфолипидов в полость пищеварительного тракта в какой-то степени обеспечивает печень липотропными факторами, особенно при их недостатке в пище, и является приспособительной реакцией, направленной на поддержание определенного уровня этих веществ в пищеварительных соках.

В значительно меньшей степени изучено выведение в пищеварительный тракт нейтральных жиров (НЖ) и свободных жирных кислот

Comparative Effects of Certain Antioxidants on Gestational Performance and Teratogeny in Vitamin E-deficient Rats [1,2]

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ABSTRACT In an effort to elucidate the mechanism of function of vitamin E, many substitutes with antioxidant activities have been used. Many of these non-tocopherol substances, when fed to vitamin E-deficient animals, acted like a tocopherol in preventing, ameliorating or relieving deficiency symptoms. Three of these substances, N, N'-diphenyl-p-phenylenediamine (DPPD), N-propyl gallate (NPG), and 1, 2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EMQ) were used to test their effects on the incidence of congenital malformations in vitamin E-deficient rats. It was found that all 3 antioxidants reduced the incidence of congenital abnormalities by promoting the production of normal young. Judged by the criteria of increase in the incidence of normal young with concurrent decrease in the incidence of resorption and the non-occurrence of congenital abnormalities in the groups fed the antioxidants in the rations at the 0.025% level in the absence of vitamin E, DPPD showed the most antioxidant potency. EMQ was the next, and NPG was the least potent of all. However, the gestational performances of the groups receiving either 2 or 10 mg of vitamin E in addition to the antioxidants at this level appears to indicate that EMQ was superior to DPPD. At 0.05% level, DPPD was shown to be superior to EMQ, as evidenced by the gestational performances of all groups of rats.

Thomas and Cheng (1) first reported congenital abnormalities associated with maternal vitamin E deficiency. Later experiments showed that normal young were produced after administration of a single dose of *d, l*-α-tocopheryl acetate to vitamin E-deficient rats on the fourth through the eighth day of gestation. When identical therapy was given on either the ninth, tenth, eleventh, or twelfth day of gestation, a considerable number of young showed a multiplicity of congenital malformations (2). A dose of vitamin on the tenth day of gestation produced the highest level of teratogeny. No noticeable shift of this level was observed, however, by increasing the therapeutic dosage of the vitamin from 1 to 4 mg,[3] but administration after the twelfth day resulted in resorption only.

Preliminary observations were made on the histological differences between the normal- and abnormal-term fetuses.[4] The distribution of glycogen, polymucosaccharides[5] and lipids[6] in the developing embryos was briefly reported. In addition, gross observations of abnormal embryos from the eleventh day of gestation onward were published (3). Arrested development characterized the 11-day-old embryos from the experimental group that had received a single dose of the vitamin on the tenth day of gestation. By the thirteenth day of gestation, exencephalus, when present, was clearly evident. Yet the incidence of congenital malformation was either reduced or eliminated by the administration of 4 mg progesterone or 2 μ estrone, continuously or by a single dose (4). The chemical determinations of the vitamin E content of a portion of the maternal and fetal tissues from the vitamin E-sufficient and vitamin E-deficient groups, and the

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[1] Supported by grant no. RG-4993, U. S. Public Health Service.

[2] Presented in part at the First Annual Meeting of the Teratology Society at Cincinnati, Ohio, [illegible]

[3] Cheng, D. W. 1954 A study of the [illegible] of teratogeny in vitamin E-deficient rats [illegible] ciated abnormalities in blood and tissues. Iowa State College J. Sci., 30: 340 (abstract).

[4] Cheng, D. W., and E. H. Thomas [illegible] logical changes in the abnormal rat fetuses [illegible] by maternal vitamin E deficiency. Anat. Rec. [illegible] 274 (abstract).

[5] Cheng, D. W., and J. Scranton 1959 [illegible] of glycogen in the developing vitamin [illegible] vitamin E-deficient and congenitally [illegible] bryes. Anat. Rec., 133: 367 (abstract).

[6] Cheng, D. W., and L. F. Chang [illegible] tion of lipid in the early vitamin E-sufficient [illegible] E-deficient and congenitally abnormal [illegible] placentas. Anat. Rec., 136: 312 (abstract).

TABLE 1
Composition of rations

Ration	Dextrin 41%	Lard 22%	Vitamin-free casein [1] 18%	Brewer's yeast [2] 5%	Salt mixture [3] 4%	Cod liver oil 2%	DPPD [4]			NPG [5]			EMQ [6]		
							% 0.025	% 0.050	% 0.075	% 0.025	% 0.10	% 0.40	% 0.0625	% 0.0125	% 0.025	% 0.050
Basal	+	+	+	+	+	+										
A	+	+	+	+	+	+	+									
B	+	+	+	+	+	+		+								
C	+	+	+	+	+	+			+							
D	+	+	+	+	+	+				+						
E	+	+	+	+	+	+					+					
F	+	+	+	+	+	+						+				
G	+	+	+	+	+	+							+			
H	+	+	+	+	+	+								+		
I	+	+	+	+	+	+									+	
K	+	+	+	+	+	+										+

[1] From Nutritional Biochemicals Corporation, Cleveland.
[2] Obtained from Pabst Brewing Company, Milwaukee, Wisconsin.
[3] USP XIV.
[4] DPPD indicates N,N'-diphenyl-*p*-phenylenediamine.
[5] NPG indicates N-propyl gallate.
[6] EMQ indicates 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyquin).

groups marginally deficient in vitamin E were reported (5). Also, sites of cholinesterase activity were briefly described.[7]

The data to be presented here deal with the effect of some chemical antioxidants, namely, N, N'-diphenyl-*p*-phenylenediamine (DPPD) N-propyl gallate (NPG) and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EMQ, also called ethoxyquin) on the gestational performance and teratogeny of vitamin E-deficient female rats. The investigation was, in a way, a further study of the effect of variation of ration on the incidence of teratogeny as reported previously by Cheng et al. (6).

MATERIALS AND METHODS

For this investigation weanling female albino rats (Holtzman) weighing about 40 g were used. From the time of arrival until termination of gestation, these rats were fed one of the 11 rations used in this series of experiments (table 1). The chemical antioxidant, DPPD or NPG, was added in 3 percentage levels to the basal ration. Four levels were used when EMQ was employed. The chemical was mixed with melted lard before being incorporated with the other dry ingredients of the ration. The ration was prepared 2 weeks in advance of use and stored in a cold room. A supply sufficient for 2 weeks was prepared each time.

The females, at maturity (175 g), were mated with proven males in a special mating cage. The presence of sperm in the vaginal smear was taken as evidence of mating. The day of this event was labeled as the zero day of gestation. After mating, the females were randomly assigned to one of the 3 subgroups. Those in the negative control subgroup received no additional supplement of vitamin E to their diet during gestation. Those in the positive control subgroup received 10 mg of dl-α-tocopheryl acetate in corn oil[8] administered by stomach tube at the rate of 2 mg a day for the first 5 days of gestation. Previous experiments demonstrated that this supplement provided an adequate positive control group (3). Those in the third experimental subgroup were given 2 mg of

[7] King, D. W., and M. Overturf 1963 Sites of cholinesterase activity in developing rat embryos. Anat. Rec., 145: 330 (abstract).
[8] Mazola, Corn Products Company, Argo, Illinois.

vitamin E on the tenth day of gestation. This supplement to the basal diet produced female rats that were marginally deficient in vitamin E, and were thus capable of giving birth to some abnormal young. Nine groups of rats each received DPPD and NPG, and 12 groups received EMQ. In addition, female rats fed the basal vitamin E-deficient ration were similarly divided into 3 subgroups, the negative control, the positive control, and the experimental group. The gestational performance of these 3 groups of rats served as reference standards for the corresponding groups fed other rations. Altogether, 485 rats were used in the series of experiments reported here.

On the twenty-first day of gestation the females were killed by etherization. While the rat was still breathing, the complete uterus was removed and incised. The dead and resorbed fetuses were noted, and the live ones were examined under the dissecting binocular microscope for the presence of congenital abnormalities. The term, "dead" fetus, denotes a fetus that was completely intact and of the same size as the live, term fetus, but was dead. The "resorbed" fetuses varied considerably in size, but the common denominator was the partial or complete resorption.

The criteria of a good gestational performance were the high incidence of normal young, the absence of abnormal young, and the low incidence of dead young, or resportion, or of both.

The data were treated by either Student's *t* or the chi-square tests to ascertain the significance of the difference.

RESULTS

1. *Experiments with DPPD.* When the rats fed the basal diet were examined, the negative controls showed 100% resorption, whereas the positive controls produced only normal young (table 2). The rats in the experimental group yielded normal young, abnormal young, dead fetuses and resorptions at 8.5, 15, 2 and 74.5%, respectively.

The gestational performances of the 3 groups of rats fed the basal ration containing 0.025% DPPD were unusual in many respects. The group receiving 0.025% DPPD and no additional vitamin E gave the best results, with 93.4% of normal young and 6.6% of resorption. In the group receiving 0.025% DPPD and 10 mg of vitamin E, the percentage of live normal young was reduced to 84, with a concomitant increase in the percentage of resorption to 16. The rats in the group

TABLE 2

Effects of N,N'-diphenyl-p-phenylenediamine (DPPD) on gestational performance of vitamin E-deficient rats

Supplement to basal vitamin E-deficient ration		Vitamin E given during gestation	No. rats bred	Live fetuses			Dead fetuses	
				Normal	Abnormal		Dead	Resorbed
	%	mg		% [1]	% [1]	% [2]	% [1]	% [1]
DPPD	0.025	0	17	93.4	0	0	0	6.6
DPPD	0.025	2	20	62.9	1.3	2.1	0	35.8
DPPD	0.025	10	12	84.0	0	0	0	16.0
DPPD	0.050	0	10	86.8	2.9	3.2	5.9	4.4
DPPD	0.050	2	21	86.3	0	0	1.9	11.8
DPPD	0.050	10	12	87.1	1.2	1.3	0	11.7
DPPD	0.075	0	9	80.0	0	0	0	20.0
DPPD	0.075	2	20	88.5	1.6	1.8	0.8	9.1
DPPD	0.075	10	9	86.3	0	0	0	13.7
None		0 [3]	24	0	0	0	0	100.0
None		2 [4]	20	8.5	15.0	63.8	2.0	74.5
None		10 [5]	19	100.0	0	0	0	0

[1] In terms of total number of implantation sites.
[2] In terms of total number of viable young.
[3] Negative control with basal E-deficient diet.
[4] Experimental group with basal E-deficient diet.
[5] Positive control with basal E-deficient diet.

receiving 0.025% DPPD and 2 mg of vitamin E gave birth to some abnormal young (1.3%), and yielded the lowest percentage of normal young (62.9%) with the highest incidence of resorption (35.8%). When these 3 groups were compared with one another, except for the significant difference between the negative control and the experimental groups concerning resorption, no other significant difference was observed between any 2 groups with respect to either normal young or resorption.

From the standpoint of teratogeny, the results from the 3 groups of rats fed the basal ration containing 0.05% DPPD were unexpected in that some of the rats from both the positive and the negative controls produced abnormal young, whereas the rats of the experimental group yielded none. The percentages of normal young were about the same in all 3 groups. Again, as in the rats fed the ration with 0.025% DPPD, the incidence of resorption was lowest in the negative group when compared with the other 2 groups. There was no significant difference between any 2 groups as far as the normal young and resorption were concerned.

The rats in the group receiving 0.075% DPPD in the absence of vitamin E gave the lowest percentage of normal young (80%) and the highest incidence of resorption (20%), compared with similar groups fed rations containing 0.025 an 0.05% DPPD. When vitamin E was give at both the 2- and 10-mg levels, its ben ficial effect was demonstrated by the i creased percentages of normal young (88.5 and 86.3%, respectively), with a co current decrease in resorption (to 9.1 an 13.7%, respectively). However, none the differences were significant. The a normal young occurred in the experime tal group only.

Compared with the negative group fe the basal ration, those groups that receive rations containing 0.025, 0.05 and 0.075 DPPD and no added vitamin E showe a significant increase in the incidence normal young with concomitant reducti in the incidence of resorption. On th whole, the experiments with DPPD showe that the percentage of abnormal young i the experimental groups was significant reduced because of the great increases i the incidence of normal young.

2. *Experiments with NPG.* As in ra receiving 0.05% DPPD without added vit min E, the occurrence of congenital a normalities was observed in the offsprin of the rats receiving 0.025% NPG and n vitamin E (table 3). With supplement tion of vitamin E, at either 2- or 10-m levels, to rats fed the ration containin 0.025% NPG, there was a significant i crease in the percentage of normal youn

TABLE 3

Effects of N-propyl gallate (NPG) on gestational performance of vitamin E-deficient female rats

Supplement to basal vitamin E-deficient ration		Vitamin E given during gestation	No. rats bred	Live fetuses			Dead fetuses	
				Normal	Abnormal		Dead	Resorbed
	%	mg		% [1]	% [1]	% [2]	% [1]	% [1]
N-propyl gallate	0.025	0	10	0	1.3	100	0	98.7
N-propyl gallate	0.025	2	22	18.7	0	0	2.4	78.9
N-propyl gallate	0.025	10	10	97.5	0	0	0	2.5
N-propyl gallate	0.10	0	10	13.6	0	0	1.2	85.2
N-propyl gallate	0.10	2	20	20.9	5.7	21.4	2.5	70.9
N-propyl gallate	0.10	10	10	90.5	0	0	0	9.5
N-propyl gallate	0.4	0	11	1.2	0	0	1.2	97.7
N-propyl gallate	0.4	2	21	49.3	4.2	7.8	0	46.5
N-propyl gallate	0.4	10	10	89.9	0	0	0	10.1
None		0	24	0	0	0	0	100.0
None		2	20	8.5	15.0	63.8	2.0	74.5
None		10	19	100.0	0	0	0	0

[1] In terms of total number of implantation sites.
[2] In terms of total number of viable young.

(from 0 to 18.7 or 97.5%, respectively), with a concurrent significant decrease in the percentage of resorption (from 98.7 to 78.9 or 2.5%, respectively). Also, in the group receiving 0.025% NPG and 10 mg vitamin E, the incidence of normal young was approximately the same as that of the corresponding group fed the basal ration.

On the whole, the supplementation of vitamin E to the rats receiving the ration containing 0.10% NPG gave similar results as those obtained with rats fed the ration with 0.025% NPG. Congenital abnormalities occurred only in the offspring of rats receiving the 2-mg vitamin E supplementation. The incidence of anomalies, however, was approximately one-third of that observed in corresponding rats fed the basal ration. With respect to the incidences of normal young and resorption, the differences between the positive and the negative controls, and the positive controls and the experimental group were statistically significant.

Similarly, the rats fed the ration containing 0.40% NPG and no added vitamin E gave a gestational performance corresponding to that of rats fed the basal ration, except for the minimal production of normal young. The group receiving 0.40% NPG and 10 mg of vitamin E gave the same degree of performance as the corresponding group receiving 0.10% NPG. Also, rats receiving 0.40% NPG responded to the supplementation of 2 mg of vitamin E differently from those fed the ration with 0.10% NPG. Here, besides the occurrence of congenital malformations (4.2%), there was a significant increase in the percentage of normal young (from 20.9 to 49.3%), with a concomitant significant lowering of the incidence of resorption (from 70.9 to 46.5%). Thus the differences in the incidence of normal young and resorption between the positive and negative controls, positive control and experimental group, and the negative control and experimental group fed this ration with 0.40% NPG were all significant statistically.

When the groups receiving the 3 levels of NPG without added vitamin E were compared with the corresponding group fed the basal ration, the beneficial effect of the 0.10% NPG in the ration became evident, since there was a production of 13.6% normal young instead of 0%, with a concurrent decrease in the incidence of resorption (from 100 to 85.2%, table 3). Comparing all the experimental groups fed the rations containing NPG with the corresponding group fed the basal ration, insofar as the normal live young were concerned, NPG at the levels of 0.025 and 0.10% improved the yield more than twofold, whereas at the level of 0.40%, it improved the yield nearly sixfold. Comparing all the positive controls that received NPG, there was a reduction in the incidence of normal young, together with an increase in the incidence of resorption when the level of NPG was increased from 0.025 to either 0.10 or 0.40%.

3. *Experiments with EMQ (ethoxyquin).* When the gestational performance of the 3 groups of rats fed the ration containing 0.00625% EMQ was examined, there was a slight increase in the percentage of the normal young from a value of 64.6% in the negative controls to 68.2% in the experimental group (table 4). A further increase to 91.8% was observed in the positive controls. However, no significant difference was found between any 2 groups fed this ration as far as the incidence of normal young was concerned. Yet, 0.8% abnormal young was born to rats in the negative controls, but no abnormal young were born to rats in the experimental group. The percentage, then, of resorption was non-significantly reduced in the experimental group, but was significantly decreased in the positive controls when compared with the negative controls. The difference in resorption between the positive controls and the experimental group was also statistically significant.

From the standpoint of the increase in the incidence of normal young and the decrease in the incidence of resorption, the gestational performance of the negative controls and the experimental group fed the ration containing 0.0125% EMQ was slightly better than that of rats that received the ration with 0.00625% EMQ. However, the positive controls fed the ration with 0.0125% EMQ did not do as well as those fed the ration with 0.00625% EMQ, due to the higher incidence of resorption. Also, abnormal young were produced by the positive controls. As far as

TABLE 4

Effect of ethoxyquin (EMQ) on gestational performance of vitamin E-deficient rats

Supplement to basal vitamin E-deficient ration		Vitamin E given during gestation	No. rats bred	Live fetuses			Dead fetuses	
				Normal	Abnormal		Dead	Resorbed
	%	mg		% [1]	% [1]	% [2]	% [1]	% [1]
EMQ	0.00625	0	14	64.4	0.8	1.3	1.7	33.1
EMQ	0.00625	2	13	68.2	0	0	1.0	30.8
EMQ	0.00625	10	11	91.8	0	0	2.3	5.9
EMQ	0.0125	0	18	70.3	0	0	0.5	29.2
EMQ	0.0125	2	20	82.6	0.5	0.6	0	16.9
EMQ	0.0125	10	17	88.1	0.7	0.7	0	11.2
EMQ	0.025	0	15	65.0	1.4	2.2	2.1	31.5
EMQ	0.025	2	14	80.2	0.8	1.0	1.7	17.3
EMQ	0.025	10	13	97.0	0	0	1.4	1.6
EMQ	0.050	0	10	78.9	2.2	2.7	4.4	14.5
EMQ	0.050	2	13	75.5	0.9	1.2	0	23.6
EMQ	0.050	10	10	78.8	1.2	2.6	0	20.0
None		0	24	0	0	0	0	100.0
None		2	20	8.5	15.0	63.8	2.0	74.5
None		10	19	100.0	0	0	0	0

[1] In terms of total number of implantation sites.
[2] In terms of total number of viable young.

the normal young were concerned, no significant difference between any 2 groups fed the ration with 0.0125% EMQ was observed. Nevertheless, with respect to resorption, the difference between the positive and the negative controls was significant statistically.

The 3 groups of rats fed the ration containing 0.025% EMQ showed the best gestational performance, in that there was a large increase in the percentage of normal young in the positive controls. The differences, then, in the incidence of normal young between the positive and negative controls and the positive controls and the experimental group were both significant. The only inconsistency was the appearance of abnormal young in the negative controls fed this ration, as was observed in the corresponding group fed the ration with 0.00625% EMQ.

The results from the 3 groups of rats receiving 0.05% EMQ were unique. First, the incidence of normal young was approximately the same, regardless of whether vitamin E was administered during gestation. Second, abnormal young were found in all 3 groups. Third, the incidence of resorption of both the positive controls and the experimental group was non-significantly higher than that of the neg tive controls.

Furthermore, when all the negative co trols that received rations containing EM were compared both with each other a with the group receiving the basal ratio the rats receiving 0.05% EMQ showed t highest incidence of normal young a the lowest incidence of resorption. Wh the absence of abnormal young w used as the criterion of best gestation performance, then the group receivi 0.0125% EMQ and no added vitamin showed the best results. When all t positive controls were examined, the r fed the ration with 0.025% EMQ p sented the best performance by having t highest incidence of normal young a the lowest incidence of resorption. Fr the standpoint of non-occurrence of normal young, then, the positive contr fed the rations containing 0.00625% a 0.025% EMQ exhibited the best resul When all the experimental groups w compared from the standpoint of absen of congenital malformations in the you the group receiving 0.00625% EMQ p duced the best results.

4. *Comparison of the efficacy of DP NPG and EMQ as antioxidants.* A.

TABLE 5

Comparative efficacy of N,N'-diphenyl-p-phenylenediamine (DPPD), N-propyl gallate (NPG) and ethoxyquin (EMQ) as antioxidants

Supplement to basal vitamin E-deficient ration		Vitamin E given during gestation	No. rats bred	Live fetuses			Dead fetuses	
				Normal	Abnormal		Dead	Resorbed
	%	mg		% [1]	% [1]	% [2]	% [1]	% [1]
DPPD	0.025	0	17	93.4	0	0	0	6.6
NPG	0.025	0	10	0	1.3	100.0	0	98.7
EMQ	0.025	0	15	65.0	1.4	2.2	2.1	31.5
DPPD	0.025	2	20	62.9	1.3	2.1	0	35.8
NPG	0.025	2	22	18.7	0	0	2.4	78.9
EMQ	0.025	2	14	80.2	0.8	1.0	1.7	17.3
DPPD	0.025	10	12	84.0	0	0	0	16.0
NPG	0.025	10	10	97.5	0	0	0	2.5
EMQ	0.025	10	13	97.0	0	0	1.4	1.6
DPPD	0.05	0	10	86.8	2.9	3.2	5.9	4.4
EMQ	0.05	0	10	78.9	2.2	2.7	4.4	14.5
DPPD	0.05	2	21	86.3	0	0	1.9	11.8
EMQ	0.05	2	13	75.5	0.9	1.2	0	23.6
DPPD	0.05	10	12	87.1	1.2	1.3	0	11.7
EMQ	0.05	10	10	78.8	1.2	2.6	0	20.0

[1] In terms of total number of implantation sites.
[2] In terms of total number of viable young.

0.025% level. When the gestational performance of the negative controls was examined, rats that received DPPD displayed the best results, giving the highest incidence of normal young and the lowest incidence of resorption (table 5). Those receiving EMQ ranked second, whereas those given NPG were the poorest. For this reason, much higher levels of NPG were tested in succeeding experiments (table 3). When the experimental groups were observed, the group receiving EMQ was found to be the best, that receiving DPPD was second, whereas that given NPG was last. Furthermore, when the positive controls were compared, the groups receiving EMQ and NPG both showed better performance than that given DPPD, in terms of higher incidence of normal young and lower incidence of resorption.

B. At 0.05% level. At this level only DPPD and EMQ could be compared (table 5). The gestational performance of all groups of rats indicated that DPPD was better than EMQ insofar as the increase in the incidence of normal young, the reduction in the incidence of resorption and the non-occurrence of the abnormal young in the experimental groups were concerned. However, the positive and the negative controls fed both rations produced abnormal young.

DISCUSSION

The biochemistry of vitamin E has been reviewed by Vasington et al. (7), and Pitt and Morton (8), and more recent observations have been reported by Wiss et al. (9). It has been known for some time that vitamin E plays a role as an antioxidant (10–13), and the effects of chemical antioxidants on vitamin E deficiency have been reviewed by Dam (14). Experiments by many investigators (15–32) have shown that DPPD can act as a partial substitute for vitamin E. Likewise, Shull et al. (22,23), Mertz and Schwarz (33), Machlin et al. (34), Schwarz (25), Crider et al. (31), Bunyan et al. (32) and Wiss et al. (9) have noted that EMQ can replace vitamin E to a certain extent. Few people, however, have tested NPG as a substitute for vitamin E, except Schwarz and his group (25, 33), and Telford et al. (35). No definite theory regarding the relationship between tocopherol and a large num-

ber of synthetic chemical antioxidants has been established. However, the generally accepted theory has been the sparing action of the antioxidants on vitamin E, which might still be either in the deficient diet or tenaciously held in the body tissues (36), although this theory has been challenged lately (37, 38).

Recently, Wiss et al. (9), in an attempt to learn the differences in biological activity between vitamin E and EMQ, performed experiments to determine the amounts of both substances in the various organs at various time intervals following the administration of C^{14}-labeled *d,l*-α-tocopheryl acetate and EMQ. The results showed that vitamin E was absorbed rather slowly, whereas EMQ was absorbed rapidly. However, high levels of vitamin E remained for longer periods in the tissues, whereas EMQ was eliminated rapidly from the body (39). The bulk of the tocopherol was located in the mitochondria and microsome fractions of the liver, but EMQ was located primarily in the supernatant solution rather than the structural parts of the cells.

Our experiments were started in 1959 and lasted for more than 2 years. The deleterious effect of higher levels of DPPD (21) appears to be borne out by our results which showed a reduction in the percentage of normal young in rats fed the vitamin E-deficient ration containing 0.075% DPPD in the absence of added vitamin E, when compared with the corresponding groups fed rations containing 0.025 and 0.05% DPPD (table 2). The beneficial effect of vitamin E supplementation, as judged by the increase in the incidence of normal young and the decrease in the incidence of resorption, was shown only in the rats receiving this high level of DPPD (0.075%). The increase in the incidence of resorption, in the rats fed diets supplemented with 10 mg of vitamin E, over those of the groups receiving no vitamin E supplement in rations containing 0.025 and 0.05% DPPD, might indicate not only a non-sparing action of DPPD but also an antagonistic action between DPPD and vitamin E under the circumstances. This premise may also explain the "non-consistent" responses, observed by Bunnell et al. (16), of lower values for liver and plasma tocophero live weight, and liver lipid in chicks r ceiving both DPPD and vitamin E th in those in the vitamin E-deficient chic receiving DPPD alone.

The rats in the group receiving 0.025 DPPD in the absence of vitamin E pr duced the most live normal young co pared with all the other groups fed DP (table 2). This indicates that this lev was the best to be incorporated into t basal ration as a replacement for vitam E under the conditions of the experimen

The reason for the occurrence of abn mal young in the offspring of rats fed t rations containing 0.05% DPPD, 0.025 NPG, and 0.00625, 0.025 and 0.05% EM and no added vitamin E was probably th because of their sparing action on vitam E, the incorporation of DPPD, NPG a EMQ into the vitamin E-deficient ratio at the specified levels increased the vi min E content of the body tissues of rats to the marginal level where ter genesis was possible. The appearance some abnormal young in the groups ceiving rations containing 0.05% DP 0.0125, and 0.05% EMQ plus 10 mg vitamin E might also be another mani tation of the antagonism between DP EMQ and vitamin E under these co tions.

The results of our experiments w vitamin E-deficient rations containing ferent levels of DPPD, NPG and EMQ erally indicated a reduction of terato due to maternal vitamin E deficiency. reductions in these cases, however, w due to increases in the number of no young rather than to increases in number of resorptions, as were obse in our previous experiments with est and progesterone (4) and γ-tocophero

Our data from the groups rece DPPD, NPG and EMQ in the absenc vitamin E also substantiated the obs tions of Schwarz (25) concerning the tive potency of these 3 chemical oxidants as substitutes for vitamin E general, DPPD was most potent, EMQ intermediate, and NPG was the potent of all.

[9] Cheng, D. W., and S. Subbammal 1959 of gamma-tocopherol on the incidence of tera due to maternal vitamin E deficiency. J. Anima 18: 1532 (abstract).

Vitamin E and the other antioxidants function by donating hydrogen to a lipid peroxyl radical and breaking the chain reaction of peroxidation (40). They protect the animals from the damage to the cellular and subcellular membranes and widespread metabolic derangements. It would be worthwhile to study the changes in the liver mitochondria, microsomes, and lysosomes of the different groups of rats used in this series of experiments, in order to correlate these results with teratogenesis.

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LITERATURE CITED

1. Thomas, B. H., and D. W. Cheng 1952 Congenital abnormalities associated with vitamin E malnutrition. Proc. Iowa Acad. Sci., *59:* 218.
2. Cheng, D. W., and B. H. Thomas 1953 Relationship of time of therapy to teratogeny in maternal avitaminosis E. Proc. Iowa Acad. Sci., *60:* 290.
3. Cheng, D. W., L. F. Chang and T. A. Bairnson 1957 Gross observations on developing abnormal embryos induced by maternal vitamin E deficiency. Anat. Rec., *129:* 167.
4. Cheng, D. W. 1959 Effect of progesterone and estrone on the incidence of congenital malformations due to maternal vitamin E deficiency. Endocrinology, *64:* 270.
5. Cheng, D. W., K. G. Braun, B. J. Braun and K. H. Udani 1961 Tocopherol content of maternal and fetal rat tissues as related to vitamin E intake during gestation. J. Nutrition, *74:* 111.
6. Cheng, D. W., T. A. Bairnson, A. M. Rao and S. Subhammal 1960 Effect of variations of rations on the incidence of teratogeny in vitamin E deficient rats. J. Nutrition, *71:* 51.
7. Vasington, F. D., S. M. Reichard and A. Nason 1960 Biochemistry of vitamin E. Vitamins and Hormones, *18:* 43.
8. Pitt, G. A. J., and R. A. Morton 1962 Fat-soluble vitamins. Ann. Rev. Biochem., *31:* 491.
9. Wiss, O., R. H. Bunnell and U. Gloor 1962 Absorption and distribution of vitamin E in the tissues. Vitamins and Hormones, *20:* 441.
10. Hove, R. L., and Z. Hove 1944 The effect of temperature on the relative antioxidant activity of alpha-, beta- and gamma-tocopherols and of gossypol. J. Biol. Chem., *156:* 623.
11. Dam, H. 1953 Vitamin E as an in vivo antioxidant. Experientia (Supp.), *1:* 195.
12. Bro-Rasmussen, F., and W. Hjarde 1961 Fat-soluble vitamins. Ann. Rev. Biochem., *30:* 447.
13. Horwitt, M. K. 1961 Vitamin E in human nutrition — an interpretative review. Borden's Rev. Nutrition Res., *22:* 1.
14. Dam, H. 1957 Influences of antioxidants and redox substances on signs of vitamin E deficiency. Pharmacol. Rev., *9:* 1.
15. Bunnell, R. H., L. D. Matterson, E. P. Singsen, L. M. Potter, A. Kozeff and E. L. Jungherr 1955 Studies on encephalomalacia in the chick. 3. The influence of feeding or injecting various tocopherols or other antioxidants on the incidence of encephalomalacia. Poultry Sci., *33:* 1068.
16. Bunnell, R. H., L. D. Matterson, E. P. Singsen and H. D. Eaton 1956 Studies on encephalomalacia in the chick. 5. The effect of fish oil and diphenyl-*p*-phenylenediamine on the vitamin E metabolism of the chick. Poultry Sci., *35:* 436.
17. Scott, M. L., F. W. Hill, L. C. Norris, D. C. Dobson and T. S. Nelson 1955 Studies on vitamin E in poultry nutrition. J. Nutrition, *56:* 387.
18. Singsen, E. P., R. H. Bunnell, L. D. Matterson, A. Kozeff and E. L. Jungherr 1955 Studies on encephalomalacia in the chick. 2. The protective action of diphenyl-*p*-phenylenediamine against encephalomalacia. Poultry Sci., *34:* 262.
19. Ames, S. R., M. I. Ludwig, W. J. Swanson and P. L. Harris 1956 Effect of DPPD, MB, BHT and hydroquinone on reproductive process in the rat. Proc. Soc. Exp. Biol. Med., *93:* 39.
20. Jensen, L. S., J. S. Carver and J. McGinnis 1956 Effects of vitamin E, N, N'-diphenyl-*p*-phenylenediamine and fish liver oil on reproduction in turkeys. Proc. Soc. Exp. Biol. Med., *91:* 386.
21. Draper, H. H., S. Goodyear, K. D. Barbee and B. C. Johnson 1958 A study of the nutritional role of antioxidants in the diet of the rat. Brit. J. Nutrition, *12:* 89.
22. Shull, R., R. B. Alfin-Slater, H. J. Deuel, Jr. and B. H. Ershoff 1957 Comparative effects of alpha-tocopherol, DPPD and other antioxidants on muscular dystrophy in guinea pig. Proc. Soc. Exp. Biol. Med., *95:* 263.
23. Shull, R., B. H. Ershoff and R. B. Alfin-Slater 1958 Effects of antioxidants on muscle and plasma lipids of vitamin E-deficient guinea pigs. Proc. Soc. Exp. Biol. Med., *98:* 351.
24. Tucker, R. G., S. M. Greenberg, A. E. Heming and J. K. Mathues 1957 Iron absorption and metabolism. II. Substitution of N, N'-diphenyl-*p*-phenylenediamine (DPPD) for vitamin E. J. Nutrition, *63:* 33.
25. Schwarz, K. 1956 Effect of antioxidants on dietary necrotic liver degeneration. Proc. Soc. Exp. Biol. Med., *99:* 20.
26. Sharman, I. M., and T. Moore 1959 The partial vitamin E activity of N, N'-diphenyl-*p*-phenylenediamine in the rat. Biochem. J., *69:* 61P.

27. Corwin, L. M., and K. Schwarz 1960 Prevention of decline of alpha-ketoglutarate and succinate oxidation in vitamin E deficient rat liver homogenates. J. Biol. Chem., *235:* 3387.
28. Draper, H. H., and A. S. Csallany 1958 Action of N, N'-diphenyl-p-phenylenediamine in tocopherol deficiency diseases. J. Biol. Chem., *99:* 739.
29. Draper, H. H. 1959 Biopotency of non-tocopheral compounds in vitamin E deficiency diseases. Proc. Soc. Exp. Biol. Med., *102:* 737.
30. Csallany, A. S., and H. H. Draper 1961 On the occurrence of vitamin E in the liver of dystrophic and antioxidant-fed rabbits. Arch. Biochem. Biophys., *92:* 462.
31. Crider, Q., P. Alaupovic and B. C. Johnson 1961 On the function and metabolism of vitamin E. III. Vitamin E and antioxidants in the nutrition of the rats. J. Nutrition, *73:* 64.
32. Bunyan, J., A. T. Diplock, E. E. Edwin and J. Green 1962 Exudative diathesis and lipid peroxidation in the chick. Brit. J. Nutrition, *16:* 519.
33. Mertz, W., and K. Schwarz 1958 Reversal of respiratory decline in necrotic liver degeneration by intraportal antioxidants. Proc. Exp. Biol. Med., *98:* 808.
34. Machlin, L. J., R. S. Gordon and K. H. Meisk 1959 The effect of antioxidants on vitami E deficiency symptoms and production o liver "peroxide" in the chicken. J. Nutrition *67:* 333.
35. Telford, I. R., C. S. Woodruff and R. H Linford 1962 Fetal resorption in the ra as influenced by certain antioxidants. Am J. Anat., *110:* 29.
36. Harris, P. L., and K. E. Mason 1955 Vita min E and metabolic processes in vitamin E Atti del Terzo Congresso Internazionale Venezia, p. 1.
37. Schwarz, K. 1961 A possible site of actio for vitamin E in intermediary metabolism Am. J. Clin. Nutrition, *9:* 71.
38. Diplock, A. T., E. E. Edwin, J. Bunyan an J. Green 1961 The effect of tocopherol vitamin A, selenium and antioxidants o ubiquinone in the rat. Brit. J. Nutrition, 15 425.
39. Wilson, R. H., J. O. Thomas, C. R. Thompson H. F. Launer and G. O. Kohler 1959 Ab sorption, metabolism and excretion of ant oxidant 6-ethoxyl-1,2-dihydro-2, 2,4-trimethyl quinoline. Agr. Food Chem., *7:* 206.
40. Tappel, A. L. 1962 Vitamin E as th biological lipid antioxidant. Vitamins an Hormones, *20:* 493.

Luminescence Analysis of Food Antioxidants

Determination of Propyl Gallate in Lard

H. W. Latz and R. J. Hurtubise

The luminescence properties of the food antioxidants BHA, BHT, and PG are presented for the first time. A simple, rapid, and accurate fluorometric method is used to determine PG directly in lard in the presence of BHA, BHT, or both. Chloroform, used as a solvent, quenches the fluorescence of BHA and BHT, as well as the background fluorescence of lard, but not PG. As little as 0.0005% PG can be determined in a lard sample by this method without interference from BHA or BHT.

Numerous methods have been reported for the determination of the food antioxidants BHA (mixture of isomers, 2-*tert*-butyl-4-methoxyphenol and 3-*tert*-butyl-4-methoxyphenol), BHT (2,6-di-*tert*-butyl-4-methylphenol), and PG (propyl gallate) (Berger *et al.*, 1960; Johnson, 1967; McCaulley *et al.*, 1967; Mahan and Chapman, 1951; Scheidt and Conroy, 1966; Stuckey and Osborne, 1965; Vos *et al.*, 1957). Many methods lack sensitivity and specificity for measuring accurately parts per million concentrations and are lengthy. In most cases it is necessary to isolate the antioxidants and then determine their concentration by some appropriate method of analysis. Stuckey and Osborne (1965) have recently reviewed ultraviolet absorption, colorimetric, and gas chromatographic methods of determining food antioxidants.

EXPERIMENTAL

Apparatus. Fluorescence and phosphorescence spectra were obtained with an Aminco-Bowman spectrophotofluorometer equipped with a phosphoroscope accessory. The source for the excitation monochromator was a xenon lamp (American Instrument Co., Catalog No. 416-992). The detector for the emission monochromator was a 1P21 phototube. Spectra were recorded with an Aminco-Bowman X-Y recorder.

Reagents. ETHANOL. The ethanol was distilled once, using a distilling column similar to the one described by Winefordner and Tin (1964).

CHLOROFORM. Mallinckrodt analytical reagent or Baker analyzed reagent was distilled once with the same column used for purification of ethanol.

BHA, BHT, AND PG. These food grade antioxidants were used without further purification. They were obtained from Eastman Chemical Products, Inc., Kingsport, Tenn., and Universal Oil Products, East Rutherford, N.J.

QUININE SULFATE. A stock solution of 0.8 μg. per ml. of quinine sulfate in 0.1*N* H_2SO_4 was used daily to check the sensitivity of the spectrophotofluorometer. The excitation monochromator was set at 250 mμ, and the emission monochromator was set at 450 mμ. The sensitivity was then adjusted to the value previously obtained.

General Procedure for Standard Addition Method. The cell used to measure the fluorescence intensity of PG must be clean and completely dry before each reading.

A standard solution was prepared containing 100 μg. per ml. of food grade PG in chloroform. PG does not dissolve readily in chloroform; therefore, it was necessary to stir the solution for about 20 minutes. Between 2.400 and 2.600 grams of lard were weighed in a 100-ml. beaker, melted, and dissolved in approximately 50 ml. of chloroform. The sample was transferred to a 100-ml. volumetric flask, the beaker was rinsed, and the rinsings were added to the 100-ml. volumetric flask and diluted to 100 ml. Aliquots of 10 ml. of lard solution were placed in four 25-ml. volumetric flasks. Samples of 0.5, 0.2, and 0.1 ml. of the standard PG solution were added to three of the volumetric flasks. Then the contents of all four flasks were diluted to 25 ml. with chloroform. The spectrophotofluorometer was adjusted with a standard solution of quinine sulfate, using slit arrangement 5. The relative fluorescence intensity of the four lard solutions was measured with the excitation monochromator at 272 mμ and emission monochromator at 352 mμ. The standard addition curve was prepared by plotting relative fluorescence intensity against micrograms per milliliter of PG added to the lard solutions. The line intersecting the relative intensity axis was extrapolated to zero relative intensity to obtain the concentration in the unknown solution of PG in 25 ml. of chloroform.

General Procedure for Obtaining Fluorescence Spectra of Antioxidants. The antioxidant (BHA, BHT, or PG) was dissolved in absolute ethanol and diluted to a 1 to 5 μg. per ml. solution of the antioxidant. The excitation and emission spectra were obtained using slit arrangement 4.

General Procedure for Obtaining Phosphorescence Spectra and Lifetimes of Antioxidants. The antioxidant (BHA, BHT, or PG) was dissolved in absolute ethanol and diluted to a 1 to 5 μg. per ml. solution of the antioxidant. The sample tube containing the antioxidant solution was placed in a Dewar flask which contained liquid nitrogen, and the phosphoroscope was set at

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Table I. Luminescence Properties of Antioxidants

Compound	Solvent	Excitation Max., Mμ	Emission Max., Mμ		Phos. Mean Lifetime, Sec.	Relative Intensity*		Concn., μg./Ml.	
			Fluor.	Phos.		Fluor.	Phos.	Fluor.	Phos.
BHA	Ethanol	293	342	420	2.9	11.7	1.56	5.0	4.0
BHT	Ethanol	287	350	439	0.5	0.069	0.027	5.0	100.0
PG	Ethanol	286	372	454	2.7	4.40	2.70	3.5	4.0
BHA	Chloroform	290	345	...	...	0.009	.....	4.0	...
BHT	Chloroform	290	330	...	...	0.004	.....	4.0	...
PG	Chloroform	272	352	...	...	1.50	.....	4.0	...

* Compared to fluorescence of 0.8 μg. per ml. quinine sulfate solution in 0.1N H_2SO_4, relative intensity 12.0.

maximum speed. The phosphorescence spectrum was then recorded.

The phosphorescence decay times (the time for the relative intensity to drop from a given value to 1/e of that value) were obtained using the recorder, and a blank slit inserted in the slit holder facing the excitation source was manually moved to block the light from the source. The slit program was as follows: 4–4–4–4–4.

RESULTS AND DISCUSSION

Fluorescence and Phosphorescence of Antioxidants in Ethanol. Table I gives the first reported luminescence data on the antioxidants BHA, BHT, and PG. The data and spectra presented have not been corrected for instrumental artifacts. The purpose of this work was to investigate the luminescence properties of the antioxidants and use these properties to develop analytical methods for the quantitative determination of the antioxidants used in foods. The investigation of the luminescence properties indicates that there are many possible approaches utilizing both fluorescence and phosphorescence. For example, Table I indicates that the emission wavelengths of BHA and BHT are close to each other, and an analysis of a mixture of the two might appear to be impossible. However, BHT gives a relative intensity of 0.07 at 350 mμ; while BHA gives a relative intensity of 11.1 at 350 mμ with both antioxidants at the same concentration. Since the fluorescence of BHT is so weak, BHA could be determined quantitatively in a mixture of the two at low concentrations of BHT.

The fluorescence and phosphorescence emission intensities of both BHA and PG are strong compared to BHT and could be used easily for analytical purposes. The fact that their emission maxima are 30 mμ apart suggests further that either fluorescence or phosphorescence could be used for the determination of both antioxidants in a mixture without the need for separation (Figures 1 and 2). A study of phosphorescence decay of the three antioxidants has shown that the mean lifetime of BHT is short compared to BHA and PG (Table I). It should therefore be possible to determine either BHA or PG in the presence of BHT by the use of decay curves (Winefordner, 1966). The above suggestions do not exhaust all possible methods of luminescence analysis for the antioxidants.

Fluorescence of Antioxidants in Chloroform. As can be seen from Figure 3 and Table I, the fluorescence of both BHA and BHT in chloroform is extremely weak. In fact, their fluorescence is almost identical with the background emission of chloroform, and therefore it was not possible to determine accurately a maximum excitation and emission wavelength for BHA and BHT in chloroform. The weak fluorescence of BHA might be due partially to the heavy atom effect (Wehry, 1967), whereby there is an increase in the rate of intersystem crossing from the singlet to the triplet state of the molecule. Since phosphorescence usually occurs from the lowest triplet state of a molecule, the relative phosphorescence intensity of BHA in chloroform should be greater than for fluorescence, if the rate of intersystem crossing is increasing. Even though chloroform forms a snow at liquid nitrogen temperature, which would decrease the measured phosphorescence intensity,

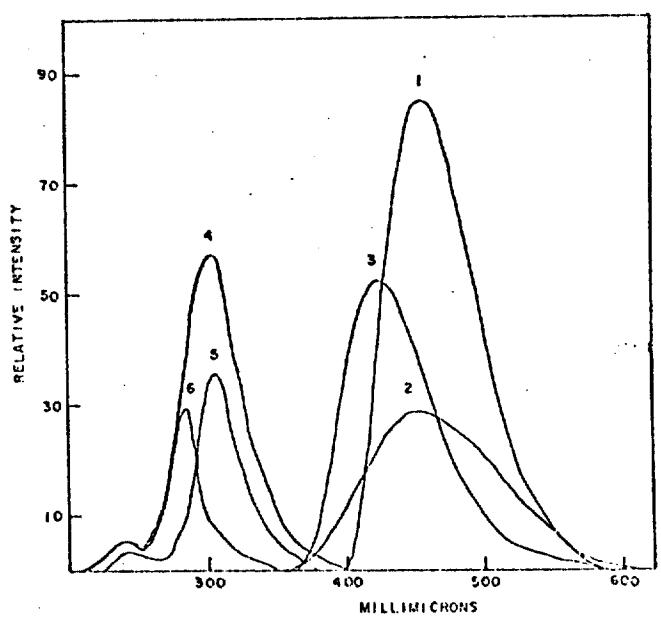

Figure 1. Fluorescence and excitation spectra of antioxidants in ethanol

1. Fluorescence spectrum of PG
2. Fluorescence spectrum of BHA
3. Fluorescence spectrum of BHT
4. Excitation spectrum of PG
5. Excitation spectrum of BHT
6. Excitation spectrum of BHA

No intended correlation from plot to plot of relative intensities

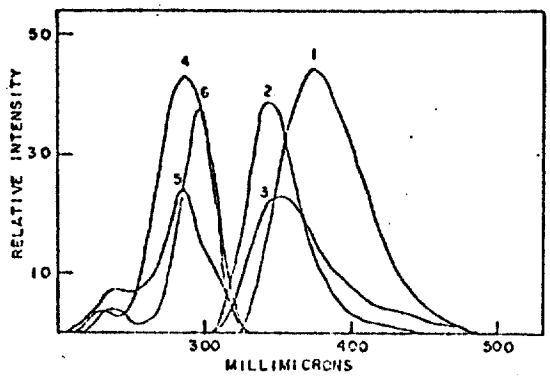

Figure 2. Phosphorescence and excitation spectra of antioxidants in ethanol

1. Phosphorescence spectrum of PG
2. Phosphorescence spectrum of BHT
3. Phosphorescence spectrum of BHA
4. Excitation spectrum of PG
5. Excitation spectrum of BHA
6. Excitation spectrum of BHT

No intended correlation from plot to plot of relative intensities

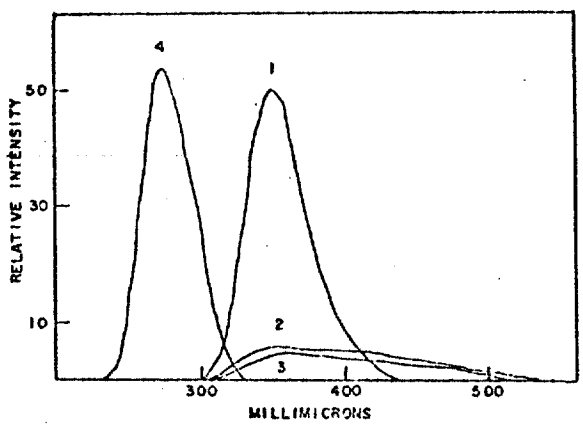

Figure 3. Fluorescence spectra of antioxidants in chloroform

All antioxidants at 4 μg./ml. and excited at 272 mμ

1. Fluorescence spectrum of PG
2. Fluorescence spectrum of BHA (sensitivity × 27 compared to 1)
3. Fluorescence spectrum of BHT (sensitivity × 27 compared to 1)
4. Excitation spectrum of PG

the intensity of BHA was about 10 times greater than the fluorescence intensity of BHA in chloroform at room temperature. The fact that the emission intensity is still low indicates that the absorbed energy is being lost by other processes. No definite conclusions could be drawn for BHT, because the phosphorescence intensity in chloroform was very low.

Determination of PG Directly in Lard in Presence of BHA and BHT. Since BHA and BHT essentially do not fluoresce in chloroform, and the background emission from antioxidant-free lard samples is very low (Table II), it was felt that the fluorescence emission of PG could be used for its qualitative and quantitative determination in lard. Table III gives the results of a number of analyses where the antioxidants were added to uninhibited lard samples. Some of the concentrations that were added were within the range of governmental regulations. The lard samples were checked for the antioxidants by a thin-layer chromatography procedure similar to the one used by Scheidt and Conroy (1966). However, the excitation and emission spectra of PG in a lard sample in chloroform can be used for qualitative identification of PG directly in lard in the presence of BHA, BHT, or both. By comparing the relative intensities of the blank solutions with the solutions containing PG in Table II, it can be seen that as little as 0.12 μg. per ml. and less of PG can be detected. In general Table III indicates that the percentage of PG in a sample will be high if a blank value is not subtracted and can be high or low if a blank value is subtracted. The error involved in both cases, however, is not serious at such low concentrations. Therefore, PG can be determined accurately with or without subtracting a blank value. Samples 7 and 8 contained five times as much BHA and BHT as PG and there was virtually no interference from BHA and BHT in the determination of PG.

Table IV illustrates the determination of PG in inhibited commercial lard samples, all of which contained either BHA or BHT. A blank value was not subtracted in the analysis of these samples. The presence of PG in one supposedly antioxidant-free lard sample was detected by obtaining the fluorescence excitation and emission spectra of PG in lard and then checked by thin-layer chromatography. With another sample it was necessary to spot four times the amount

Table II. Comparison of Relative Fluorescence Intensity of Uninhibited and Inhibited Lard Samples in Chloroform

Sample	Relative Intensity, Uninhibited Lard[a]	Sample	Relative Intensity, Inhibited Lard[b]	PG Concn., μg./Ml.
1	0.06	6	2.37	2.00
2	0.05[c]	7	0.55	0.40
3	0.06	8	0.17	0.12
4	0.06	9	1.04	0.80
5	0.07	10	0.52	0.40

[a] Blank solutions, 0.010 g./ml. of lard.
[b] 0.010 g./ml. of lard, blank value not subtracted.
[c] Contained BHT.

Table III. Analysis of Lard Samples of Known PG Content by Standard Addition Method

Sample	Added, % BHA	Added, % BHT	Added, % PG	PG Found, %, Blank Not Subtracted*	Error, %	PG Found, %, Blank Subtracted	Error, %
1	0.0120	.....	0.0040	0.0040	0.0	0.0037	−7.5
2	0.0120	.....	0.0040	0.0036	−10.0	0.0032	−20.0
3	0.0400	.....	0.0200	0.0215	+7.5	0.0205	+2.5
4	0.0400	.....	0.0200	0.0205	+2.5	0.0190	−5.0
5	0.0080	0.0080	0.0040	0.0042	+5.0	0.0040	0.0
6	0.0080	0.0080	0.0040	0.0050	+25.0	0.0045	+12.5
7	0.0400	0.0400	0.0080	0.0087	+8.8	0.0085	+6.3
8	0.0400	0.0400	0.0080	0.0085	+7.5	0.0080	0.0
9	.....	.....	0.0012	0.0016	+33.4	0.0010	−16.7
10	.....	.....	0.0012	0.0019	+41.6	0.0013	+8.5

* Blank solution 0.010 g./ml. of uninhibited lard in chloroform.

of solution normally specified for TLC analysis (Scheidt and Conroy, 1966). However, by obtaining the excitation and emission spectra of PG in a chloroform lard solution PG was quickly identified.

A standard addition method was used for a number of reasons. First, variations in source intensity and phototube aging can change a calibration curve, even though the instrument sensitivity is adjusted with a standard. By using a standard addition method these problems are practically eliminated, since the relative intensity data for one analysis can be obtained within 30 minutes. More importantly, slight variations in the composition of different lard samples could possibly alter the relative fluorescence intensity of PG from one lard sample to another, but by using a standard addition method this source of error should be eliminated. Also, in the standard addition method the concentration of PG increases with each reading and the background emission from the lard remains constant. Therefore, as the concentration of PG increases the background emission from the lard is less important. For example, one of the antioxidant-free samples in Table IV gives a relative intensity of 0.06. The final relative intensity reading in a typical analysis would be 2.54. Therefore, the background emission does not contribute greatly to the total emission.

The limit of detection by the standard addition method is near 0.0005% PG in a solid lard sample, which would yield a concentration of 0.05 μg. per ml. in the final analysis solution using the method described. At lower concentrations it would be necessary to use larger samples of lard and subtract a blank value from the relative intensity values. By doing this the lower limit of detection can be extended even more. The maximum amount of PG that can be detected in a solution of chloroform is 4 μg. per ml., because concentration quenching begins to occur near this concentration. Therefore, if any of the solutions used in the analysis contained more than 4 μg. per ml. it would be necessary to dilute the solutions to eliminate concentration quenching.

Table IV. Determination of Propyl Gallate in Commercial Lard Samples

Sample	% PG
1	0.0025
	0.0026
2	0.0021
	0.0020
3	0.0042
	0.0039

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LITERATURE CITED

Berger, K. G., Sylvester, N. D., Haines, D. M., *Analyst* 85, 341 (1960).

Johnson, D. P., *J. Assoc. Offic. Anal. Chemists* 50 (6), 1298 (1967).

McCaulley, D. F., Fazio, T., Howard, J. W., DiCiurcio, F. M., Ives, J., *J. Assoc. Offic. Anal. Chemists* 50 (2), 243 (1967).

Mahan, J. H., Chapman, R. A., *Anal. Chem.* 23, 1116 (1951).

Scheidt, S. A., Conroy, H. W., *J. Assoc. Offic. Anal. Chemists* 49 (4), 807 (1966).

Stuckey, B. N., Osborne, C. E., *J. Am. Oil Chemists' Soc.* 42 (3), 228 (1965).

Vos, H. J., Wessels, H., Six, C. W. Th., *Analyst* 82, 362 (1957).

Wehry, E. L., "Fluorescence. Theory, Instrumentation, and Practice," pp. 86–90, Marcel Dekker, New York, 1967.

Winefordner, J. D., "Fluorescence and Phosphorescence Analysis," p. 180, Interscience, New York, 1966.

Winefordner, J. D., Tin, M., *Anal. Chim. Acta* 31, 239 (1964).

Received for review September 3, 1968. Accepted December 5, 1968.

1934. The virucidal action of high fre-
–416.
r, A. 1947. Effects of X-radiation on
), 263–264.
Craig, V. 1949. Effect of electronic
Am. Diet Assoc. 25, 39–45.
sterilization," *Am. Miller* 73 (4), 43.
on of beer by dielectric heat treatment.

The Freezing Preservation of Foods.
Co., New York.
production and characteristics of 3,000
enol. Radium Therapy 49, 532.
948. Irradiation of biological materials
de rays. *J. Applied Phys.* 19, 599–604.
Cloud, R. W. 1940. Cathode rays for
Radium Therapy 43, 728–734.
s of soft X-rays on urease and catalase.
.
ion.
s growth in chicks. *Poultry Supply*

actericidal lamps. *Agr. Eng.* 26, 67–68.
, and Miller, J. I. 1949. The effect of
n the keeping quality of frozen pork.

. ical estimation of nicotinic acid
E. , 221–225.
, A. G. 1946. High-frequency electric
con. Entomol. 39 (4), 487–498.
e bactericidal effects of high frequency
, 485–489.
930. The effect of cathode rays upon
1–932.
E. H. 1947. Electronic pasteurization
.
ce contamination and its control. *Food*

heating pave the way for South Ameri-
.
e on bactericidal effects of short electro-
Shinfield, Eng., Paper No. 1041.
ization of milk by means of radiations.

y voltages on bacteria. *Nature* 166, 434.

The Pharmacological Evaluation of Antioxidants

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I. Introduction

Investigations into the problem of rancidity and methods for stabilizing edible oils and fats have been quite extensive. One of the fundamental problems yet to be solved is the mechanism by which fats become rancid and the role of antioxidants in the prevention or delay of rancidification. Mattill (1947) has reviewed the chemical aspects of the problem. The influence of lipoxidases, yeasts, molds, or bacteria on the decomposition of glycerides of fatty acids has also not been clearly elucidated although it is known that the presence of such entities is not necessary for the production of rancidity.

The importance of preserving the wholesomeness of edible fats is exemplified by the fact that in the United States the average individual derives about one-third of his total caloric requirements from fat. Fat not only functions as a concentrated foodstuff, but also has an important relationship to the availability and utilization of essential dietary substances. Some of these substances are vitamin A, carotene, vitamin E, vitamin D, pantothenic acid, pyridoxine, biotin, ascorbic acid, and linoleic acid (Burr and Barnes, 1943). Considerable experimental evidence is available also which emphasizes that the diet of man and animals is enhanced in some way by the addition of fresh natural edible fats. The presence of rancid fats in the diet, however, has a serious deleterious

effect, which goes beyond the destruction of essential dietary constituents. Fitzhugh *et al.* (1944) have demonstrated that the feeding of rancid lard to rats at a dietary level of 6% resulted in a marked emaciation of the animals by the end of the first year on the diet. A progressive type of paralysis was also noted in these animals at the time of termination of the experiments (2 years). The pathological examination verified the fact that the major symptoms were the result of a vitamin E deficiency, although the byproducts of the oxidized fat appear to have a toxic action of their own (Burr and Barnes, 1943).

From these considerations the indications are that considerable emphasis must be placed on the importance of keeping edible fats palatable and wholesome, especially when storage is necessary. Stabilization of fats has been accomplished by several means, such as choice of raw materials, hydrogenation, proper packaging, deodorization, and exercising every precaution during processing to avoid unnecessary exposure to heat and light, or contamination with prooxidants. All these methods have their limitations in that their applications are not always feasible to all fats and fatty food products; hence reliance for the stabilization of fats has been placed on antioxidants. By the addition of traces of certain chemicals it has been found that oxidative rancidity can be inhibited and the age resistance of fats considerably increased. This phenomenon has been known for more than 100 years and the first utilization for prevention of rancidity was in the pharmaceutical field—employment of gum benzoin to protect lard. This product has been official in the United States Pharmacopoeia since 1880.

Many compounds have been found to possess antioxidant properties, the more common compounds being gum guaiac, gallic acid, catechol, and hydroquinone. Numerous other chemicals of the phenolic and acidic type have also had their proponents. Naturally occurring antioxidants have received considerable attention, the purpose being to develop a nontoxic substance suitable for food use. Lecithin, cephalin, tocopherols, and products obtained from cereals, yeasts, sugars, and other food substances have been proposed. Lundberg (1947) has compiled a list of antioxidants which are in current use or which have been proposed for use in stabilizing edible fats.

The criteria which are considered necessary for establishing the usefulness of a chemical as an antioxidant have been listed by Higgins and Black (1944). According to these authors, the antioxidant should be soluble in fats and impart no foreign color, odor, or flavor to the fat even on long storage and should be an effective inhibitor in the treated product for at least a year after storage at a temperature of 75°–85° F. in unsealed containers. The antioxidant should be unchanged when

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foods prepared from fats
the antioxidant for food
antioxidants which are able
for practical usefulness, bu
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1. Nordihydroguaiaretic
2. Propyl gallate
3. Hydroquinone
4. l-ascorbyl palmitate
5. *d*-isoascorbyl palmi

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heated and should possess the ability of retarding rancidity in baked foods prepared from fats treated with the antioxidant. The safety of the antioxidant for food use must be established. There are many antioxidants which are able to pass the physical and chemical criteria for practical usefulness, but to establish proof of nontoxicity is more difficult.

Antioxidants are chemical preservatives and come under the terms of the Federal Food, Drug and Cosmetic Act when they are present in food shipped in interstate commerce. Any antioxidant which may be legally incorporated into foods must be declared on the label in a way to show it to be a preservative. If the chemical is safe and nonpoisonous, it can be incorporated into unstandardized foods as a preservative if it does not conceal damage or inferiority or make the product appear better or of greater value. It may be used in standardized food only if the standard recognizes the preservative as a legitimate ingredient. If the food is not a standardized item and the antioxidant is nonpoisonous, the only requirement is the declaration of the preservative on the label. If the antioxidant is a poisonous and deleterious substance it must be shown that the preservative is required and cannot be avoided in good manufacturing practice. In this event, the law provides that the Administration shall promulgate regulations limiting the quantity of the substance a food may contain so that it will not constitute a hazard to the public health. It is the manufacturer's and shipper's responsibility to secure adequate evidence of safety for the antioxidant that he intends to use. A general plan for the pharmacological investigation, which permits a thorough appraisal of the toxicology of an antioxidant, has been presented (Lehman, 1948).

Of the many chemicals proposed for antioxidant use and under active investigation, pharmacological data on the compounds listed below have become available.

1. Nordihydroguaiaretic acid
2. Propyl gallate
3. Hydroquinone
4. l-ascorbyl palmitate
5. *d*-isoascorbyl palmitate
6. *d*-isoascorbic acid
7. Thiodipropionic acid
8. Dilauryl thiodipropionate
9. Distearyl thiodipropionate
10. Gum guaiac

The palmitates and *d*-isoascorbic acid are not considered very effective as antioxidants and any discussion of their pharmacology will be incidental only. Phenol and catechol are included for the purpose of comparison, although they are too toxic for food use.

II. Acute Toxicity

A summary of the acute toxicity of the antioxidants is presented in Table I. From a practical standpoint it may be stated that an antioxidant which possesses an LD_{50} (the quantity of the antioxidant which is fatal to 50% of the test animals following oral administration) of 1,000 mg./kg. or above has met at least one important criterion of harmlessness. An inspection of the table reveals that nordihydroguaiaretic acid, propyl gallate, thiodipropionic acid and its derivatives, *d*-isoascorbyl palmitate, gum guaiac, and butylated hydroxy anisole have measured up to this criterion. It is of interest to note that in general hydroquinone and phenol have the same order of toxicity.

Table I

The Acute Toxicity of Antioxidants

Animal	Route of administration	Approximate LD_{50} mg./kg.	Source
		Nordihydroguaiaretic acid	
Mice	Oral	>2000	Bieter (1949)
Mice	Oral	4000	F & D A* (1950)
Rats	Oral	>2000	Bieter (1949)
Rats	Oral	5500	F & D A* (1950)
Guinea pigs	Oral	830	Bieter (1949)
Mice	Intraperitoneal	550	Bieter (1949)
		Propyl gallate	
Mice	Oral	2000	Boehm and Williams (1943)
Mice	Oral	3500	F & D A* (1950)
Rats	Oral	3800	Orten *et al.* (1948)
Rats	Oral	3600	F & D A* (1950)
Rats	Intraperitoneal	380	Orten *et al.* (1948)
		Thiodipropionic acid	
Mice	Oral	>2000	Hazleton (1949)
Mice	Oral	2000	F & D A* (1950)
Mice	Intraperitoneal	250	Hazleton (1949)
Mice	Intravenous	175	Hazleton (1949)
Rats	Oral	>2000	Hazleton (1949)
Rats	Oral	3000	F & D A* (1950)
Rats	Intraperitoneal	500	Hazleton (1949)
Rats	Intravenous	>300	Hazleton (1949)
		Dilauryl thiodipropionate	
Mice	Oral	>2000	Hazleton (1949)
Rats	Oral	>2500	Hazleton (1949)
Mice	Intraperitoneal	>2000	Hazleton (1949)

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Animal	Route of administration	App
		Diste
Mice	Oral	
Rats	Oral	
Mice	Intraperitoneal	
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Mice	Oral	
Rats	Oral	
Mice	Oral	
Rats	Oral	
Rats	Oral	
Guinea pigs	Oral	
Mice	Intraperitoneal	
Mice	Oral	
Rats	Oral	
Guinea pigs	Oral	
Mice	Intraperitoneal	
		Buty
Mi	Oral	
Rats	Oral	
Mice	Oral	
Rats	Oral	
Guinea pigs	Oral	
Pigeons	Oral	
Dogs	Oral	
Cats	Oral	
Rats	Intravenous	
Mice	Oral	
Mice	Oral	
Rats	Oral	
Rats	Oral	
Mice	Intraperitoneal	

* Food and Drug Administration,

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order of toxicity.

Antioxidants

	Source
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	Bieter (1949)
	F & D A* (1950)
	Bieter (1949)
	F & D A* (1950)
	Bieter (1949)
	Bieter (1949)
:ate	
	Boehm and Williams (1943)
	F & D A* (1950)
	Orten *et al.* (1948)
	F & D A* (1950)
	Orten *et al.* (1948)
:c acid	
	Hazleton (1949)
	F & D A* (1950)
	Hazleton (1949)
	Hazleton (1949)
	Hazleton (1949)
	F & D A* (1950)
	Hazleton (1949)
	Hazleton (1949)
--opionate	
	Hazleton (1949)
	Hazleton (1949)
	Hazleton (1949)

TABLE I (*Continued*)

Animal	Route of administration	Approximate LD_{50} mg./kg.	Source
		Distearyl thiodipropionate	
Mice	Oral	>2000	Hazleton (1949)
Rats	Oral	>2500	Hazleton (1949)
Mice	Intraperitoneal	>2000	Hazleton (1949)
		d-Isoascorbyl palmitate	
Mice	Oral	>8000	F & D A* (1950)
Rats	Oral	6000	F & D A* (1950)
		Gum guaiac	
Mice	Oral	>2000	Bieter (1949)
Rats	Oral	>2000	Bieter (1949)
Rats	Oral	>5000	F & D A* (1950)
Guinea pigs	Oral	1120	Bieter (1949)
Mice	Intraperitoneal	>2000	Bieter (1949)
		Catechol	
Mice	Oral	260	Bieter (1949)
Rats	Oral	260	Bieter (1949)
Guinea pigs	Oral	210	Bieter (1949)
Mice	Intraperitoneal	190	Bieter (1949)
		Butylated hydroxy anisole	
Mice	Oral	2000	F & D A* (1950)
Rats	Oral	2200	F & D A* (1950)
		Hydroquinone	
Mice	Oral	400	F & D A* (1950)
Rats	Oral	320	F & D A* (1950)
Guinea pigs	Oral	550	F & D A* (1950)
Pigeons	Oral	300	F & D A* (1950)
Dogs	Oral	200	F & D A* (1950)
Cats	Oral	70	F & D A* (1950)
Rats	Intravenous	115	F & D A* (1950)
		Phenol	
Mice	Oral	520	Bieter (1949)
Mice	Oral	395	F & D A* (1950)
Rats	Oral	440	Bieter (1949)
Rats	Oral	450	F & D A* (1950)
Mice	Intraperitoneal	360	Bieter (1949)

* Food and Drug Administration, unpublished data.

III. Chronic Toxicity

1. *Rats*

Lifetime feeding studies in rats have been carried out with all the compounds listed. One of the criteria for establishing the harmlessness of a substance which may be ingested chronically when added to food is the effect of such a substance on the growth rate of rats. The first six

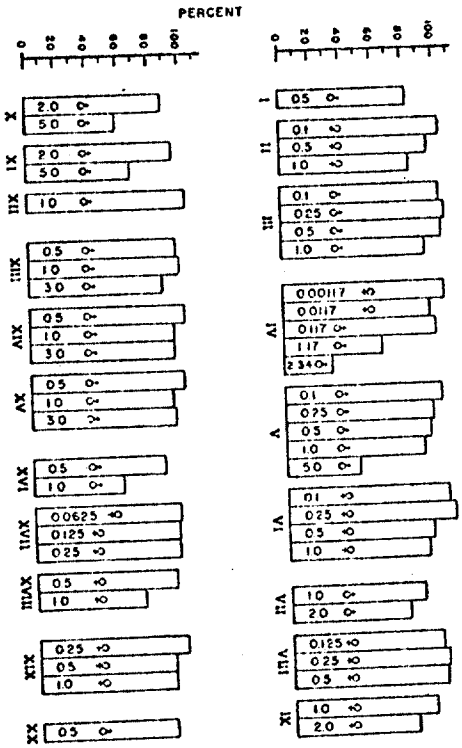

Fig. 1. Mean growth rate of rats fed the antioxidants for 6 months expressed as per cent of the control groups. The growth rate of the control animals was taken as 100 per cent. The figures in each column represent the per cent of the antioxidant in the diet. The symbol ♂ = male; ♀ = female. The Roman numerals identify the antioxidants and source of the data as follows: I, II, Nordihydroguaiaretic acid (Bieter); III, Nordihydroguaiaretic acid (F&DA*); IV, Propyl gallate, (Orten *et al.*); V and VI, Propyl gallate, (F&DA*); VII, VIII, IX, Hydroquinone, (F&DA*); X, *l*-ascorbyl palmitate, (F&DA*); XI, *d*-isoascorbyl palmitate, (F&DA*); XII, *d*-isoascorbic acid, (F&DA*); XIII, Thiodipropionic acid, (Hazleton); XIV, Dilauryl thiodipropionate, (Hazleton); XV, Distearyl thiodipropionate, (Hazleton); XVI, XVII, XVIII, Catechol, (F&DA*); XIX, Phenol, (F&DA*); XX, Gum guaiac, (Bieter).

* Food and Drug Administration, unpublished data.

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Practically all of the antioxi
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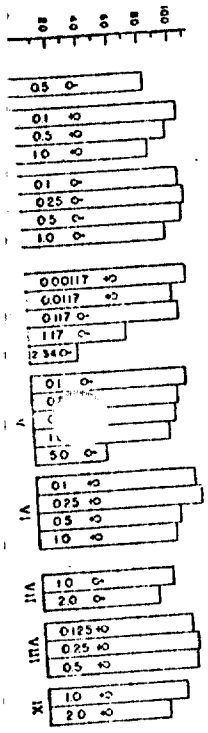

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VII, VIII, IX, Hydroquinone, (F&DA*);
-isoascorbyl palmitate, (F&DA*); XII,
-ropionic acid, (Hazleton); XIV, Dilauryl
ryl thiodipropionate, (Hazleton); XVI,
Phenol, (F&DA*); XX, Gum guaiac,

l data.

months in the life of a rat is usually taken for observing growth changes for the reason that this time period represents the rapidly growing stage. Figure 1 represents a summary of the data of the various compounds. Practically all of the antioxidants are recommended for use in quantities not to exceed 0.01% in animal fats and shortenings. In assessing the harmlessness of the antioxidants, a factor of safety of at least 100 is taken. This requires that when the antioxidant is fed to experimental animals in their total diet at a level 100 times that proposed in fats for human consumption, no significant effect on growth should occur. The results in Fig. 1 are unfavorable to nordihydroguaiaretic acid and hydroquinone. Orten *et al.* (1948) noted some stunting in rats on the 1.17% level of feeding propyl gallate. No significant effect at the 1% level was noted in our rats, but retardation was significant at the 5% level. It may be noted in the figure that we have fed all the antioxidants for a lifetime with the exception of thiodipropionic acid and its esters and gum guaiac. This affords an opportunity to compare the growth effects of the various antioxidants on an equal basis. Since it required 5% of propyl gallate to affect growth in our experiments, it is believed that species difference or some other variable was responsible for the stunting of growth in the 1.17% level of the antioxidant as observed by Orten *et al.*

The dilauryl and distearyl esters of thiodipropionic acid are recommended for use in a concentration of 0.09%. These compounds represent about 0.03% of the acid and since they have been fed at a level of 3.0% without evidence of effect, the 100-times margin of safety appears to have been established.

Gum guaiac is employed in concentrations of 0.1%, and to meet the criterion about a 10% feeding level should have been carried out. Very often technical difficulties do not permit the addition of such high concentrations as would be necessary in the case of gum guaiac to establish a 100-fold margin of safety. This obstacle can be overcome in many instances by supplementary data in other species of animals. This has not been done with gum guaiac, but the long history of its use in medicine without reported injury appears to substantiate the harmlessness of the gum for antioxidant use.

2. *Other Species*

Chronic studies on propyl gallate have been conducted in guinea pigs and dogs by Orten *et al.* (1948). The guinea pigs were fed at a level of 0.011% of the antioxidant and were observed for one year. No detectable effect on the rate of growth was noted. No influence on reproduction was noted.

A group of 7 dogs was fed propyl gallate for 14 months in amounts

similar to that fed the guinea pigs. The animals on the propyl gallate regimen showed no demonstrable effects and were similar in every respect to the 5 animals which served as controls.

Thiodipropionic acid was fed to guinea pigs by Hazleton (1949) at a level of 0.5% in the drinking water and for a period of 120 days. No significant effect was noted on weight or mortality. Dogs were fed a mixture of 10 parts by weight of dilauryl thiodipropionate and one part of thiodipropionic acid and in a concentration of 0.1% and 3.0% of the acid-ester mixture in the diet. The observation period was 100 days. No untoward effects were noted.

Nordihydroguaiaretic acid has not been subjected to additional studies in dogs which is a serious omission in light of the results in rats. Bicter (1949) employed mice in one series of feeding experiments but the results were inconclusive.

Some observations on the blood constitutents were made by Orten *et al.* (1948) on rats receiving propyl gallate in the diet. The lower levels of antioxidant feeding produced no changes, but concentrations above 1.1% produced significantly lower hemoglobin values. Erythrocite and leucocyte counts were not significantly affected. Hazleton (1949) made similar blood studies on the dogs fed the acid-ester mixture of thiodipropionic acid without finding anything of significance.

In the pharmacological appraisal of an antioxidant it is important to determine the effect of heat on the stability of the substance. Reaction products or a modification of the chemical may occur which may have pharmacological effects. Orten *et al.* (1948) included such experiments in their observations. Rats were fed a heated antioxidant mixture (propyl gallate, lecithin, and corn oil) in an amount at least 100 times that which would be consumed by humans if all the dietary fat were treated with the recommended amount of antioxidant mixture effective in preserving edible fat. No detectable toxic effects were noted. Hazleton (1949) also fed thiodipropionic acid in combination with dilauryl thiodipropionate heated with lard in concentrations at least 10 times that recommended for use as an antioxidant. No injury was noted. We have conducted similar experiments with the ascorbyl palmitates without evidence of injury.

3. *Mortality*

The mortality rate of rats fed the several antioxidants is also an index of toxicity. A high mortality in any group of treated animals as compared with the controls indicates that the substance under test is quite toxic. If the mortality rate is about equal to that of the controls, the compound may be considered as not highly toxic. Table II summarizes

the available data on the mort
may be seen that none of the
the rats. None of the treated
the controls in the number of
of the experiment.

Mortality in Rats after 2 Ye

Antioxidant	Dosage per cent
Nordihydroguaiaretic acid	0
	0.1
	0.5
	1.0
Nordihydroguaiaretic acid	0
	0.1
	0.25
	0.5
	1.0
Propyl gallate	0
	0.1
	0.25
	0.5
	1.0
	5.0
Thiodipropionic acid	0
	0.5
	1.0
	3.0
Dilauryl thiodipropionate	0.5
	1.0
	3.0
Distearyl thiodipropionate	0.5
	1.0
	3.0
l-ascorbyl palmitate	0
	0.05
	0.25
d-isoascorbyl palmitate	0.05
	0.25
d-isoascorbic acid	1.0
Gum guaiac	0
	0.5

animals on the propyl gallate
s and were similar in every
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a pigs by Hazleton (1949) at a
for a period of 120 days. No
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in an amount at least 100 times
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toxic effects were noted. Hazle-
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group of treated animals as com-
the substance under test is quite
equal to that of the controls, the
ghly toxic. Table II summarizes

the available data on the mortality rates of the various compounds. It may be seen that none of the compounds affected the mortality rate of the rats. None of the treated groups showed significant differences from the controls in the number of animals living at any time up to the end of the experiment.

TABLE II

Mortality in Rats after 2 Years of Feeding the Several Antioxidant Diets

Antioxidant	Dosage per cent	Number of Animals Per group	Number of Animals Living at 2 years	Source
Nordihydroguaiaretic acid	0	18	13	Bieter (1949)
	0.1	18	12	
	0.5	18	17	
	1.0	18	12	
Nordihydroguaiaretic acid	0	10	3	F & D A* (1950)
	0.1	10	1	
	0.25	10	3	
	0.5	10	3	
	1.0	10	2	
Propyl gallate	0	16	8	F & D A* (1950)
	0.1	16	4	
	0.25	16	6	
	0.5	16	6	
	1.0	16	4	
	5.0	16	3	
Thiodipropionic acid	0	20	17	Hazleton (1949)
	0.5	20	16	
	1.0	20	13	
	3.0	20	15	
Dilauryl thiodipropionate	0.5	20	10	Hazleton (1949)
	1.0	20	13	
	3.0	20	4	
Distearyl thiodipropionate	0.5	20	18	Hazleton (1949)
	1.0	20	14	
	3.0	20	18	
l-ascorbyl palmitate	0	10	4	F & D A * (1950)
	0.05	10	6	
	0.25	10	4	
d-isoascorbyl palmitate	0.05	10	4	F & D A * (1950)
	0.25	10	5	
d-isoascorbic acid	1.0	10	4	F & D A * (1950)
Gum guaiac	0	10	7	Bieter (1949)
	0.5	10	9	

TABLE II (*Continued*)

Antioxidant	Dosage per cent	Number of Animals Per group	Number of Animals Living at 2 years	Source
Catechol	0	12	2	F & D A* (1950)
	0.0625	12	4	
	0.125	12	4	
	0.25	12	1	
Catechol	0	18	5	F & D A* (1950)
	0.5	18	4	
	1.0	18	4	
Hydroquinone	0	12	2	F & D A* (1950)
	0.125	12	5	
	0.25	12	8	
	0.5	12	8	
Hydroquinone	0	18	5	F & D A* (1950)
	1.0	18	7	
	2.0	18	5	
Phenol	0	12	2	F & D A* (1950)
	0.25	12	5	
	0.5	12	6	
	1.0	12	6	

* Food and Drug Administration, unpublished data.

IV. PATHOLOGY

The predominant pathologic lesions produced by the various antioxidants after lifetime feeding to rats are summarized in Table III. If the criterion of a 100-fold margin of safety on a chronic basis is adhered to, then nordihydroguaiaretic acid and hydroquinone would be considered as deleterious substances for addition to food.

V. SUMMARY

Of the antioxidants listed in the discussion above, evidence of safety for use appears to have been established for propyl gallate, thiodipropionic acid and its dilauryl and distearyl esters, and gum guaiac. Nordihydroguaiaretic acid is not included because no experiments have been conducted in nonrodent species. Hydroquinone must be classed as a harmful and deleterious substance, and as such, has no place as an antioxidant in foods. Catechol has been proposed for antioxidant use (Olcott, 1934), but pharmacological evidence clearly classifies this chemical as a poison.

TABLE III

Pathology in Chronically Poisoned Rats (2-Year Feeding)

Substance	Diet concentration Producing injury	Predominant injury	Source
Nordihydroguaiaretic acid	0.5%	Massive cecal hemorrhages with single and multiple cysts in the mesentery in the angle of junction between small and large intestine	Bicter (1949)
Nordihydroguaiaretic acid	0.5%	Inflammatory cecal lesions and slight cystic enlargement of lymph nodes near the cecum	F & D A† (1950)
Propyl gallate	1.17%	Tubular damage and presence of albuminous casts probably as the result of inanition	Orten *et al.* (1948)

...ued)

...er of Animals Living at 2 years	Source
2	F & D A* (1950)
4	
4	
1	
5	F & D A* (1950)
4	
4	
2	F & D A* (1950)
5	
8	
8	
5	F & D A* (1950)
7	
5	
2	F & D A* (1950)
5	
6	
6	

...ts.

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produced by the various anti- are summarized in Table III. If ...ety on a chronic basis is adhered ...hydroquinone would be consid- ...ion to food.

...IARY

...cussion above, evidence of safety ...ed for propyl gallate, thiodipro- ...ryl esters, and gum guaiac. Nor- because no experiments have been ...droquinone must be classed as a ...l as such, has no place as an anti- ...n proposed for antioxidant use ...idence clearly classifies this chem-

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Pathology in Chronically Poisoned Rats (2-Year Feeding)

Substance	Diet concentration Producing injury	Predominant injury	Source
Nordihydroguaiaretic acid	0.5%	Massive cecal hemorrhages with single and multiple cysts in the mesentery in the angle of junction between small and large intestine	Bieter (1949)
Nordihydroguaiaretic acid	0.5%	Inflammatory cecal lesions and slight cystic enlargement of lymph nodes near the cecum	F & D A† (1950)
Propyl gallate	1.17%	Tubular damage and presence of albuminous casts probably as the result of inanition	Orten *et al.* (1948)
Propyl gallate	5.0%	Patchy hyperplasia in the proventriculus	F & D A† (1950)
Hydroquinone	2.0%	Suggestion of increase in incidence of chronic gastrointestinal ulceration and renal tumors over spontaneous incidence	F & D A† (1950)
* *l*-ascorbyl palmitate * *d*-isoascorbyl palmitate * *d*-isoascorbic acid	0.25% 0.25% 1.0%	No lesions present attributable to the compounds fed	F & D A† (1950)
* Thiodipropionic * Dilauryl thiodipropionate * Distearyl thiodipropionate	3.0% 3.0% 3.0%	No pathological changes	Hazleton (1949)
Catechol	0.25%	Beginning hepatic cell hyperplasia	F & D A† (1950)
* Phenol	1.0%	No pathological changes	F & D A† (1950)
* Gum guaiac	0.5%	No damage attributable to the compound	Bieter (1949)

* Highest concentration fed.
† Food and Drug Administration, unpublished data.

References

Bieter, R. N. 1949. Personal communication, University of Minnesota.

Boehm, E., and Williams, R. 1943. A study of the inhibiting actions of propyl gallate (normal propyl trihydroxy benzoate) and certain other trihydric phenols on the autoxidation of animal and vegetable oils. *Quart. J. Pharm. Pharmacol.* 16, 232–243.

Burr, G. O., and Barnes, R. H. 1943. Noncaloric functions of dietary fats. *Physiol. Revs.* 23, 256–278.

Fitzhugh, O. G., Nelson, A. A., and Calvery, H. O., 1944. Rancid fat in experimental diets. *Proc. Soc. Exptl. Biol. Med.*, 56, 129–131.

Hazleton, L. W., 1949. Personal communication, Hazleton Laboratories, Falls Church, Virginia.

Higgins, J. W., and Black, H. C., 1944. Preliminary comparison of the stabilizing effect of several recently proposed antioxidants for edible fats and oils. *Oil & Soap* 21, 277–279.

Lehman, A. J., 1948. Procedures for the appraisal of newer antioxidants. *Official Publication, Association of American Feed Officials, Inc.* 93–94.

Lundberg, W. O., 1947. A survey of present knowledge, researches, and practices in the United States concerning the stabilization of fats. *The Hormel Institute of the University of Minnesota Publication* No. 20.

Mattill, H. A., 1947. Antioxidants. Ann. Rev. Biochem. 16, 177–192.

Olcott, H. S., 1934. Antioxidants and the autoxidation of fats. II. *J. Am. Chem. Soc.* 56, 2492–2493.

Orten, J. M., Kuyper, A. C., and Smith, A. H., 1948. Studies on the toxicity of propyl gallate and of antioxidant mixtures containing propyl gallate. *Food Technol.* 2, 308–316.

Woodard, G., Hagan, E. C., and Radomski, J. L., 1949. The toxicity of hydroquinone for laboratory animals. *Federation Proc.* 8, 348.

Salmonella Infecti

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I. I

Producers, processors, and
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* Based on a review of the lit
the University of California, prev

† Present address: College of V
Illinois.

27

OILS, FATS, AND WAXES

The Multi-Determination of Antioxidants in Lard

By DONALD F. McCAULLEY, THOMAS FAZIO, JOHN W. HOWARD, FRANCES M. DiCIURCIO, and JULIAN IVES (Division of Food Chemistry, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204)

A method has been developed for the multiple determination of antioxidants (BHA, BHT, Ionox-100, PG, THBP, and TDPA) in lard. The procedure consists of vacuum sublimation of the antioxidants and quantitative determination by gas chromatography. UV, IR, and mass spectrometry were used for identification and confirmation. Average recoveries of the antioxidants added to 4 g lard at 100 ppm ranged from 91 to 96%.

Under present food additive regulations, various antioxidants are acceptable for addition, either directly to food products or indirectly through the medium of food packaging materials. These antioxidants are 3-(2-)*tert.*-butyl-4-hydroxyanisole (BHA), 3,5-di-*tert.*-butyl-4-hydroxytoluene (BHT), *n*-propyl gallate (PG), nordihydroguaiaretic acid (NDGA), 2,4,5-trihydroxybutyrophenone (THBP), 3-3′-thiodipropionic acid (TDPA), dilaurylthiodipropionate (DLTDP), and gum guaiac. More recently 4-hydroxymethyl-2,6-di-*tert.*-butylphenol (Ionox-100) has been proposed for use.

In recent years, mixtures of antioxidants have been used extensively, since no single compound is equally effective in all substrates under all conditions, and best results are usually obtained with combinations. It is important, therefore, to have a rapid analytical method which can determine these compounds in food products.

At the present time the most widely used analytical procedures for the determination of antioxidants are colorimetric methods (1–8). Many of these procedures are subject to limitations of nonspecificity, varying degrees of sensitivity and precision, and interference by other antioxidants. In 1956, Anglin, *et al.* (1) described a colorimetric method for the quantitative estimation of BHA, BHT, PG, and NDGA in edible fats. In this method, PG and NDGA are extracted with 72% ethyl alcohol from fat dissolved in petroleum ether or carbon tetrachloride, whereas BHT and BHA are steam distilled. The antioxidants PG and NDGA, however, cannot be estimated in mixtures. Excellent recoveries were obtained of antioxidants added to lard at levels as low as 25 ppm for NDGA and 50 ppm for BHA, BHT, and PG.

In 1964, Sahasrabudhe (9) reported on the quantitative estimation of BHA, BHT, PG, and NDGA in lard. The antioxidants were extracted from *n*-hexane solution of the fat with 80% ethyl alcohol and acetonitrile and separated by two-dimensional thin layer chromatographic techniques. Quantitative measurements were made by colorimetric methods after extraction of the compounds from the adsorbent. Recoveries of antioxidants added to lard at levels of 50 to 200 ppm ranged from 94 to 102%. The advantages of this method, as cited by the author, are that (*1*) all four antioxidants can be estimated in one aliquot of fat, (*2*) PG and NDGA can be estimated when present together in mixtures, and (*3*) the isomers of BHA can be effectively separated.

Gas chromatographic procedures have also been used to determine BHA and BHT in food products. Buttery and Stuckey (10), in 1961, employed a direct extraction of these antioxidants from potato granules fol-

lowed by gas chromatography of the eluate on an Apiezon L column with flame ionization detection. A range of 0.5 to 10 ppm of antioxidant was analyzed with an average error of less than 11%. Schwecke and Nelson (11) and Anderson, *et al.* (12) have also determined BHA and BHT in cereals, potato products, and waxed paper by gas chromatography. These workers used a silicone gum (2% SE-30/1% Tween 80) column and a flame ionization detector. Recoveries of levels in the 5–30 ppm range varied from 95 to 103%.

In a recent publication, Schwien, *et al.* (13) described an acetonitrile extraction and a subsequent cleanup on an alumina acid column for the separation of BHA and BHT from fats and oils prior to determination by gas chromatography. Recoveries from tallow, lard, and corn oil spiked at 16.7 and 66.7 ppm with BHA and BHT ranged from 80 to 95% for BHA and 91 to 115% for BHT.

This report describes a quantitative procedure for the determination of BHA, BHT, PG, Ionox-100, THBP, and TDPA in lard. Gum guaiac was omitted from this study since available information indicates that it is no longer used in the United States. In subsequent investigations, this method will be extended to NDGA and DLTDP and to a wide range of food products.

METHOD

Apparatus

(a) *Vacuum and diffusion pumps.*—(*1*) Welch Duo-Seal (Model 1402B, Welch Scientific Co., Skokie, Ill.), or equivalent, and (*2*) Veeco diffusion pump (Model EP-2-5W, Veeco Instrument Co., Inc., Plainview, N.Y.), or equivalent.

(b) *Recording spectrophotometer and accessories.*—(*1*) Cary 11 (Applied Physics Corp., Monrovia, Calif.), or equivalent; (*2*) fused quartz cells, 1 cm optical path length and 0.35 ml capacity (Optical Cell Co., Inc., Brentwood, Md.), or equivalent.

(c) *Infrared spectrophotometer and accessories.*—Beckman IR-5A, with 5x beam condenser and NaCl ultra-micro liquid cells, 0.1 mm path length, (24496, Beckman Instrument Co., Fullerton, Calif.), or equivalent.

(d) *Sublimation apparatus.* — See Fig. 1. (Custom built by Laboratory Supply and Equipment Co., Chevy Chase, Md.): (*1*) Flat bottom distillation flask: fabricated from a 25 mm O-ring plain joint #6780, Corning Glass Works, Corning, N.Y.). The plain end is sealed off and flask is custom blown to shape. The flask is 52 mm high and has an o.d. of 52 mm at the widest point. (*2*) Column: 102 mm long, fabricated by fusing the plain ends of two 25 mm O-ring joints (#6780, Corning Glass Works) together. Baffles are formed into the lower 45 mm portion of the column in 2 tiers of three, equidistant from each other. Each baffle is produced by pushing the molten glass wall of the column inward with a forming tool. (*3*) Cold finger section: made by fusing the plain end of a 9 mm O-ring joint (#6780, Corning Glass Works) perpendicular to the plain end of a 25 mm O-ring joint (#6780, Corning Glass Works). A 110 mm length of 16 mm o.d. glass tubing is centered inside the 25 mm joint and fused onto the plain end. The finger is formed by sealing off the tube and shaping. Dimensions of unit are as follows: overall length of the cold finger = 110 mm, length from top of unit to joint lower edge = 38 mm, and length of side arm joint = 30 mm. (*4*) Insulated bath: consists of two Pyrex glass beakers placed one inside the other. The inner beaker (800 ml capacity tall form) containing ca 500 g silicone fluid (or other heat transfer liquid) is wrapped in heating tape and is embedded in sand contained by the second beaker (2 L capacity Griffin low form). Fiber glass insulation material (1" thick) is wrapped around the 2 L beaker to prevent heat loss. (*5*) Clamps: custom built, C-shaped, held together by brass screws.

(e) *Stirrer-hot plate.*—Model 11-493 V2 (Fisher Scientific Co., Silver Spring, Md.), or equivalent.

(f) *Volumetric flask.*—1 ml capacity (K-62150, Kontes Glass Co., Vineland, N.J.), or equivalent.

(g) *Concentrator tube.*—4 ml capacity (K-57005, Kontes Glass Co.), or equivalent.

(h) *GLC collection trap.*—10 ml capacity, custom built (Laboratory Supply and Equipment Co.). *See* Fig. 2 for detailed drawing: the inside diameter of the inner tube is just large enough to admit the barrel of a 10 µl Hamilton syringe.

(i) *Syringes.*—10, 25, and 100 µl (Hamilton Co., Inc., Whittier, Calif.), or equivalent.

(j) *Ion gauge.*—Vacronic Laboratory Equipment, Inc., East Northport, N.Y., or equivalent.

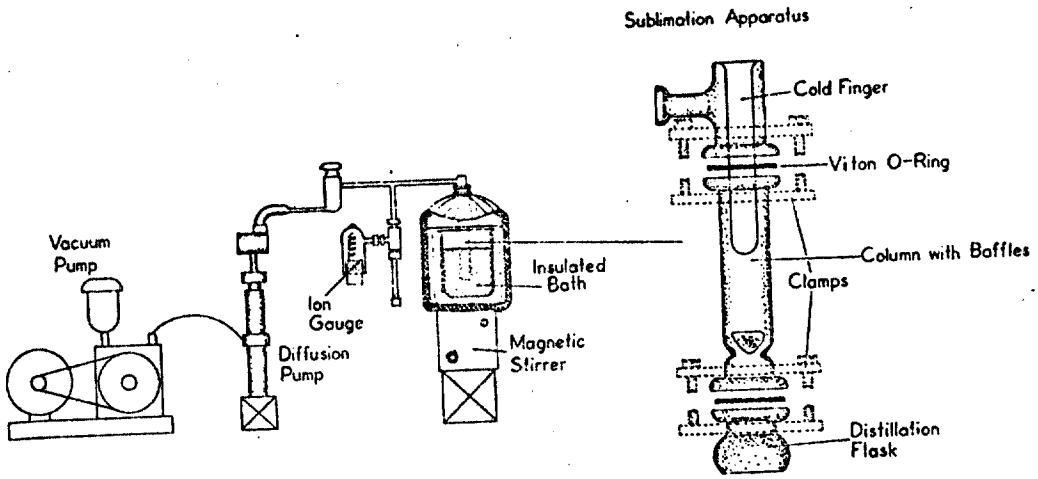

Fig. 1—Sublimation apparatus.

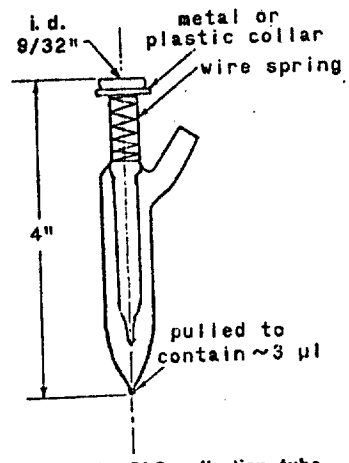

Fig. 2—GLC collection tube.

(k) *Gas chromatograph:* — Barber-Colman Model 5000 with a flame detector, fraction collector, and effluent splitter (20:1 ratio) (Barber-Colman Co., Rockford, Ill.).

Column and conditions: Pack 16′ × 3 mm column with 3% GE-XE-60 liquid phase (nitrile-silicone gum rubber, Analabs, Hamden, Conn.) on 60/80 mesh Gas Chrom-Q (Applied Science Laboratories, Inc., State College, Pa.); precondition by heating the column for 48 hr at 250°C while maintaining a carrier gas (nitrogen) flow of 30 ml/min. Maintain the following parameters throughout the analyses: injector temperature, 130–250°C; detector temperature, 260°C; carrier gas flow rate, 125 ml/min.; hydrogen flow rate, 37 ml/min., air or oxygen flow rate, 500 ml/min.; and attenuation range, X2 at 3×10^{-9} amp. into 1 mv full scale recorder.

The three column temperature programs are *Program I,* from 100 to 150°C at 10°/min. and then to 250°C at 5°/min.; *Program II,* from 100 to 165°C at 10°/min., then operate at 165°C isothermally for 16 min. followed by a manual rise to 250°C and again run isothermally for 10 min.; and *Program III,* from 90 to 130°C at 10°/min., then operate at 130°C isothermally for 10 min.

Reagents

(a) *Ethyl ether.*—Analytical reagent (Mallinckrodt Chemical Works, St. Louis, Mo.), or equivalent.

(b) *Pyridine.* — ACS reagent (Baker and Adamson, New York, N.Y.), or equivalent.

(c) *Methanol.*—Reagent, anhydrous (Merck & Co., Rahway, N.J.), or equivalent.

(d) *Antioxidants.*—Tennox PG (propyl gallate), Tennox BHA (butylated hydroxyanisole), and Tennox BHT (butylated hydroxytoluene), food grade (Eastman Chemical Products, Inc., Kingsport, Tenn.), or equivalent; 2,4,5-trihydroxybutyrophenone (THBP), experimental grade (Eastman Chemical Products, Inc.), or equivalent; Ionox-100 (4-hydroxy methyl-2,6-di-*tert.*-butyl phenol), food grade (Shell Chemical Co., Division of Shell Oil Co., Union, N.J.), or equivalent; thiodipropionic acid (TDPA) (Carlisle Chemical Works, Reading, Ohio), or equivalent.

(e) *Standard solutions of antioxidants.* — Transfer 1 g of each antioxidant (PG, BHA, BHT, THBP, and Ionox-100) to individual 100 ml volumetric flasks and dilute to volume with

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anhydrous ethyl ether. Transfer 2 ml of each stock solution to separate 100 ml volumetric flasks and adjust to volume with anhydrous ethyl ether. (Each solution contains 0.2 mg antioxidant/1 ml.)

Dissolve 1 g TDPA in 25 ml methanol in a 100 ml volumetric flask and dilute to volume with anhydrous ethyl ether. Transfer 2 ml of this stock solution to a 100 ml volumetric flask and adjust to volume with anhydrous ethyl ether. (This solution contains 0.2 mg TDPA/1 ml.)

(f) *Chlorotrimethylsilane.*—Eastman Chemical Products, Inc., Kingsport, Tenn., or equivalent.

(g) *1,1,1,3,3,3-Hexamethyldisilazane.* — Eastman Chemical Products, Inc., Kingsport, Tenn., or equivalent.

(h) *Diazald (N-methyl-N-nitroso-p-toluene sulfonamide).*—Aldrich Chemicals Co., Milwaukee, Wis., or equivalent. Use to prepare diazomethane, according to manufacturer's directions, as follows:

Prepare reagent in a well-ventilated hood. Use cork stoppers (14) for all connections. Fit a 125 ml long-neck distilling flask with a dropping funnel and an efficient condenser. Connect condenser in series to a 500 ml and a 125 ml Erlenmeyer receiving flask, respectively. Place 20 ml ether in the 125 ml receiver and adjust height of inlet tube to below the surface of the ether. Cool both receivers to 0°C.

Dissolve 6 g KOH in 10 ml water in the distilling flask. Add 35 ml 2-(2-ethoxyethoxy)-ethanol (Aldrich) and 10 ml ether. Connect distilling flask to condenser and immerse flask in a water bath maintained at 70°C with a magnetic stirrer-hot plate. Start stirrer and, as distillation begins, add a solution of 21.5 g "Diazald" in 140 ml ether through the dropping funnel over 20-min. period. At end of distillation, add another 20 ml ether and continue to distill until distillate is colorless. Combine contents of the two receivers. Freeze overnight. Decant the diazomethane from the ice crystals into a polyethylene or glass bottle. Stopper with a cork. Store in freezer until ready for use. (*Caution:* Diazomethane is both toxic and explosive.)

(i) *Silicone fluid.*—F-1-0173, 100 cst (Dow-Corning Corp., Midland, Mich., or equivalent).

Procedures

Vacuum separation.—Weigh 4 g lard sample into a 60 ml flat-bottom distillation flask. Attach flask to a glass column containing the built-in baffle system and a cold finger, and connect the sublimation apparatus to the vacuum system as shown in Fig. 1. Fill the cold finger with liquid nitrogen. Maintain liquid N level during sublimation. Start vacuum pump and slowly apply vacuum to the system. (*Note:* The pressure in the system should drop to about 0.5 μ as measured by the gauge attached between the cold finger and the trap.) Lower assembly into insulated bath maintained at 188°C. After 2 hr discontinue vacuum and remove assembly from the silicone oil bath.

Remove retaining clamps and cautiously lift out the cold finger. (*Caution:* Losses occur by accidental scraping of cold finger against the walls of the column.) Pour off liquid nitrogen. Quantitatively transfer the sublimate to the concentrator tube by washing the cold finger with small portions of anhydrous ethyl ether, using a 1 ml hypodermic syringe. Adjust volume to 2 ml by evaporating excess solvent under nitrogen in a warm water bath. Mix and reltain for gas-liquid chromatographic analysis. The entire solution will be required for the quantitative determination of BHA, BHT, Ionox-100, PG, THBP, and TDPA.

Gas liquid chromatography.—Inject 20 μl ether solution of the sublimate to be analyzed into the column, program the temperature according to *Program I*, and collect the effluents corresponding to BHA, BHT, or Ionox-100 in the 10 ml collection trap cooled with liquid nitrogen. Retain for identification by ultraviolet spectral analysis.

For the determination of PG and THBP, pipet 1 ml ether solution of the sublimate in the concentrator tube into a 1 ml volumetric flask. Evaporate solvent almost to dryness in a warm water bath under nitrogen. Adjust volume to 1 ml with the silanizing reagents as follows: 0.5 ml pyridine, 0.3 ml 1,1,1,3,3,3-hexamethyldisilazane, and 0.2 ml chlorotrimethylsilane. Stopper flask, mix well, and let solution stand for 5 min. Inject 20 μl of solution into the column, program according to *Program II*, and collect the PG and THBP effluents in separate 10 ml traps cooled with liquid nitrogen. Retain for confirmation of identity by ultraviolet spectral analysis.

To determine TDPA, use the remaining ether solution in the concentrator tube. Evaporate the solvent to a small volume (ca 0.3 ml) in a warm water bath under nitrogen. Adjust to 1 ml with diazomethane and mix. Inject 30 μl of solution into the chromatograph and program the temperature according to *Program III*. If a peak is obtained (indi-

cating the presence of TDPA), inject a 250 μl aliquot and collect the effluent in the 10 ml liquid nitrogen-cooled fraction collector. Retain for identification by infrared spectrophotometry.

For the quantitation of the antioxidants prepare the standardization curves as follows:

For BHA, BHT, and Ionox-100, inject appropriate aliquots of the standard solutions directly into the gas chromatograph. Use 1 ml aliquots of PG, THBP, and TDPA standard solutions, evaporate each solution under nitrogen, and prepare the silyl and methoxylated derivatives as described above. Collect the effluent corresponding to each antioxidant under study and reserve for confirmatory analysis. Calculate the amounts of the antioxidants present by either the peak height or peak area techniques.

To confirm the identity of BHA, BHT, Ionox-100, PG, and THBP, add 0.4 ml methanol to each of the traps containing the column effluents and swirl. Transfer solutions to 0.35 ml capacity 1 cm path length cells and record the ultraviolet spectra in the wavelength region of 210–400 mμ, using methanol in the reference cell. Compare with spectra obtained for the standards under identical instrumental conditions.

To confirm the identity of TDPA, wash the trap with CS_2 and evaporate the solvent to about 3 μl. Transfer the solution to a Beckman 0.1 ml micro liquid cell and record the infrared spectrum, using the Beckman IR 5A spectrophotometer equipped with a 5x beam condenser. Compare with the spectrum obtained for a TDPA standard under identical instrumental conditions.

Recovery Studies

Most of the recoveries were conducted with lard products obtained from local retail markets. These samples included both stabilized and unstabilized varieties. Preliminary analyses for antioxidants in these products were conducted to establish that they were free of the compounds used in the recovery studies. Two ml aliquots of the standard solutions (0.2 mg/ml) were pipetted into the distillation flask containing 4 g of melted lard. The ether was then removed by evaporation on a steam bath under a stream of nitrogen. In some instances, all six of the above antioxidants were added to the lard in each recovery study. The analysis was then carried out as previously described.

Results

The recoveries of six antioxidants added at a level of 100 ppm to 4 grams of lard are summarized in Table 1. Recoveries ranged from 84 to 104% for these compounds.

Representative ultraviolet absorption spectra obtained for the antioxidants (BHT, BHA, PG, Ionox-100, and THBP) added to and recovered from lard are shown in Figs. 3 and 4. The infrared spectrum of the methoxylated TDPA recovered from lard is shown in Fig. 5. These spectra demonstrate that the antioxidants can be readily recovered and their identification confirmed by the aforementioned techniques.

Studies have been conducted to determine the ultimate sensitivity limits of the method for the antioxidants recovered from lard with respect to their detection by gas chromatography and confirmation by ultraviolet and infrared spectrophotometry. With this method, BHA and BHT can be detected by gas chromatography at about 1 ppm; however, their identities cannot be confirmed. For quantitation and definite confirmation of the antioxidants in the lards used in this investigation it was established that the lower limits of sensitivity range from 10 ppm (BHA and BHT) to 25 ppm (PG, Ionox-100, THBP, and TDPA). At the present time, NGDA and DLTDP can only be determined qualitatively with this method. Studies are now in progress to investigate the parameters necessary for their quantitative determination.

The procedure has been applied to the analyses of various commercial lards for the presence of BHA, BHT, and PG. The results are tabulated in Table 2. Brand A was

Table 1. Recovery of six antioxidants added to 4 g of lard at a level of 100 ppm

Antioxidant	No. of Runs	Recovery Range (%)	Av. %
BHA	10	88–104	95
BHT	11	85–101	91
Ionox-100	5	89–98	93
PG	11	86–102	96
THBP	9	87–101	93
TDPA	3	84–98	92

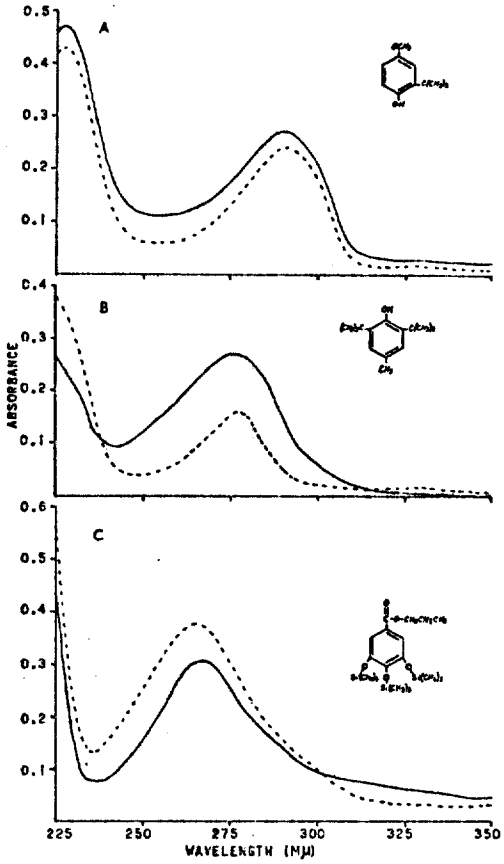

Fig. 3—Ultraviolet absorption spectra of BHA(*A*), BHT(*B*), and silanized PG(*C*), isolated from lard; solid line, unknown; broken line, reference.

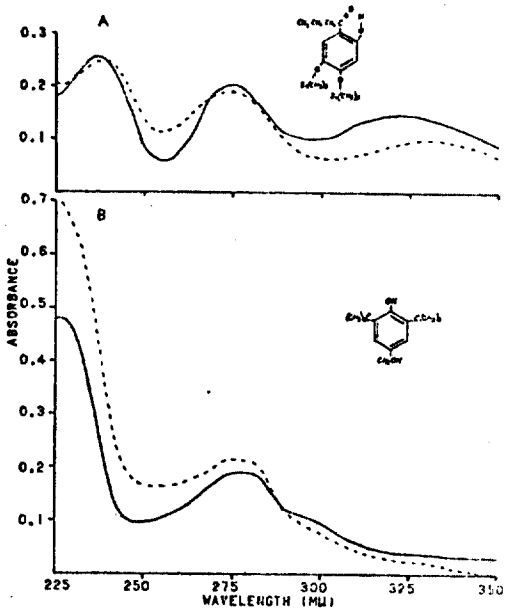

Fig. 4—Ultraviolet absorption spectra of silanized THBP(*A*) and Ionox-100(*B*) recovered from lard; solid line, unknown; broken line, reference.

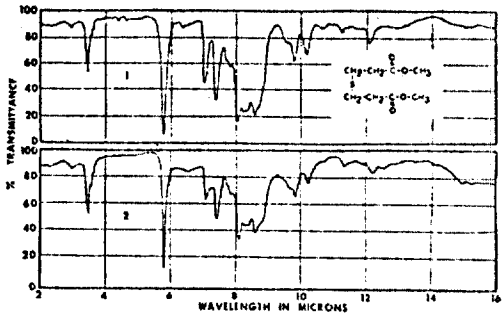

Fig. 5—Infrared spectra of methoxylated TDPA recovered from lard: curve 1, standard; curve 2, unknown.

found to be the most heavily stabilized; however, the antioxidant content of all of the samples analyzed are below the permitted levels.

Discussion

All of the antioxidants employed in this investigation were effectively and quantitatively separated from the lard under the conditions prescribed. Approximately 1% of the starting material (lard) migrates to the cold finger, thus contributing to the background interferences noted in the preliminary gas chromatographic work. These interferences have since been reduced by the use of a baffle system built into the column wall (Fig. 1). Significant losses have been encountered when the sublimate is accidentally scraped from the cold finger as it is removed from the assembly. This was corrected by reducing the overall diameter of the cold finger by 2 mm.

Table 2. Antioxidants (ppm) found in commercial lards

Brand	BHA	BHT	PG
A	80	82	40
B	0	0	25
C	75	77	37
D	35	0	25
E	26	23	0
F	0	0	37
G	86	0	25
H	40	0	0

Preliminary investigations showed that direct chromatography of the ether solutions

for PG and THBP was unsuccessful. Therefore, silyl ether derivatives were prepared; they could be chromatographed because of their increased volatility. Langer, *et al.* (15) first described the use of trimethylsilyl (TMS) compounds to prepare volatile derivatives of phenols for gas chromatographic analysis. However, their method required long reflux times for complete reactions. In our study, the TMS derivatives of PG and THBP were formed by the method of Sweeley, *et al.* (16), using pyridine, hexamethyldisilazane, and trimethyl chlorosilane; this reaction was rapid at room temperature and the analyses can be made within a few minutes.

TDPA also could not be chromatographed directly and attempts to utilize the silanization reaction gave anomalous results. Later studies revealed that the methoxylation reaction with diazomethane could be used to form the dimethyl ester of the acid which can be chromatographed effectively and determined quantitatively.

Mass spectrometry was employed to verify the formation of both the silanized and methoxylated antioxidant derivatives. Mass spectral data showed that silylation was essentially quantitative for BHA, PG, Ionox-100, and THBP (which formed the disilyl ether); BHT remained unsilanized.

In the quest for a quantitative multiple detection method for antioxidants, the following column packing materials were explored: SE-52, SE-30, SE-550, Apiezon L, Carbowax 20M, SAIB, JXR, Epon 1001, DEGS, neopentyl glycol succinate, DC-QF-1, and Versamide 900. BHA, BHT, and Ionox-100 chromatographed well on almost all of these liquid phases. If these compounds are the only antioxidants to be determined, a fast, low-temperature vacuum distillation cleanup (about 20 minutes, 150°C) has certain advantages, particularly with foods of high fat content, since direct extraction techniques remove large amounts of fatty substances along with the antioxidants. These interfering materials not only result in a multiplicity of peaks, but also tend to overload and finally block the column.

Good recovery values were obtained with BHA, BHT, and Ionox-100 when they were vacuum distilled for 45 minutes at 200°C, followed by injection into a 4′ column containing 7.5% GE-XE-60 as the liquid phase. Unfortunately, silanized PG and THBP derivatives were not adequately separated from interfering lard peaks. Recovery ranges were as follows: 86–107% for BHA, 86–104% for BHT, and 87–103% for Ionox-100. The parameters were essentially the same as those previously described for the 16′ column with the exception of the temperature program. In this study the column was heated to 75°C and run isothermally for 2 minutes. Temperature programming was initiated at a rate of 10°C per minute to 150°C, then continued to a maximum of 250°C at a rate of 5°C per minute.

Silanized PG and THBP were separated poorly on most of the liquid phases investigated, and interferences were also encountered from the silanized lard. When prepared as a 16′ column the 3% GE-XE-60 packing material yielded the best overall results by far of any of the phases screened to date.

The response of the flame detector to the antioxidants used in this study was found to be reasonably uniform. Once the operating parameters were determined they were maintained and no attempt was made to readjust to optimum response each day. Therefore, standards were run with each determination. Figure 6 presents the retention time

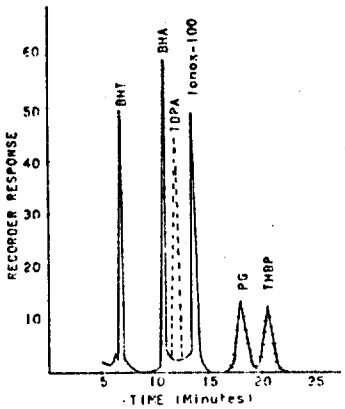

Fig. 6—Gas chromatogram showing typical retention times and responses for six antioxidant standards; ———, *Program I*; —|—|—, *Program II*, and — — — —, *Program III*.

of the compounds and their respective responses when 20 μl are injected into the 16′ column at a concentration of 0.2 mg/ml.

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REFERENCES

(1) Anglin, C., Mahon, J. H., and Chapman, R. A., *J. Agr. Food Chem.*, 4, 1018–1022 (1956).
(2) Filipic, V. J., and Ogg, C. L., *This Journal*, 43, 795–799 (1960).
(3) Gibbs, H. D., *J. Biol. Chem.*, 72, 649 (1927).
(4) Laszlo, H., and Dugan, L. R., Jr., *J. Am. Oil Chemists' Soc.*, 38, 178–180 (1961).
(5) Mahon, J. H., and Chapman, R. A., *Anal. Chem.*, 23, 1116–1123 (1951).
(6) Sloman, K. G., Romagnoli, R. J., and Cavagnol, J. C., *This Journal*, 45, 76–80 (1962).
(7) Szalkowski C. R., and Garber, J. B., *J. Agr. Food Chem.*, 10, 491–496 (1962).
(8) Vos, H. J., Wessels, H., and Six, C. W., *Analyst*, 82, 362–367 (1957).
(9) Sahasrabudhe, M. R., *This Journal*, 47, 888–893 (1964).
(10) Buttery, R. G., and Stuckey, B. N., *J. Agr. Food Chem.*, 9, 283–285 (1961).
(11) Schwecke, W. M., and Nelson, J. H., *ibid.*, 12, 86–89 (1964).
(12) Anderson, R. H., Huntley, T. E., Schwecke, W. M., and Nelson, J. H., *J. Am. Oil Chemists' Soc.*, 40, 349 (1963).
(13) Schwien, W. G., Miller, B. J., and Conroy, H. W., *This Journal*, 49, 809–812 (1966).
(14) Rabjohn, N., *Organic Syntheses, Vol. 4*. John Wiley & Sons, Inc., N.Y., 1963, pp. 250–251.
(15) Langer, S. H., Pantages, P., and Wender, I., *Chem. Ind. (London)*, pp. 1664 (1958).
(16) Sweeley, C. C., Bentley, R., Makiba, M., and Wells, W. W., *J. Am. Chem. Soc.*, 85, 2497–2507 (1963).

MEAT AND MEAT PRODUCTS

Extraction of Lipids from Raw Beef Lean by Using Various Solvent Systems

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Study shows that chloroform-methanol extraction to remove lipids from beef yielded percentages of total fat equal to or greater than those obtained by the AOAC method. The ratio of phospholipid to triglyceride was always higher in the chloroform-methanol extracted samples. Six extraction method-solvent system combinations, three drying procedures, and two sample preparation methods were compared. The extracted lipids were separated by thin layer chromatography. The ratio of phospholipid to triglyceride was calculated after the spectrophotometric determination of the ester groups present. Acid-hydrolysis-Rohrig gave the lowest yield of total lipid and of phospholipid. Sample preparation method or drying method caused few significant differences in the proportions of total solids and of total lipids in the beef cuts analyzed.

blasting action responsible for blasting the concrete blocks can be correlated. The results also establish the superiority of shaped charges over non-shaped charges.

References

1. SHETH, P. G., *Indian J. Technol.*, 2 (1964), 42.

Separation & Identification of Antioxidants in Oils & Fats by Thin Layer Chromatography

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A thin layer chromatographic method employing microscopic slides for the preparation of the thin layer medium, petroleum ether-dioxane (10 : 1) as mobile solvent and 5 per cent alcoholic phosphomolybdic acid as spray reagent is described for the separation and identification of the antioxidants propyl gallate, butylated hydroxyanisole and butylated hydroxytoluene in fats and oils. Satisfactory separation is achieved in 10 min.

THERE are several reports in literature[1-5] on the use of thin layer chromatography for the analysis of synthetic and natural antioxidants. In the present communication a thin layer chromatographic method for the identification and separation of the three common antioxidants for oils and fats, viz. propyl gallate (PG), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) is reported.

The chamber used was a coplar flask (used for cleaning microscope slides), about 4 in. high with a ground glass cover. Microscope slides were used as chromatographic plates. The plates were coated with aluminium oxide (GRE Merck, Brockmann grade, 200 mesh) containing 5 per cent plaster of Paris made into a slurry with twice its weight of water and spread over the slide (preferably on the frosted slide) by passing a glass rod over the slide to obtain uniform coating. Slides were dried in the air for about 1 hr and then kept in an air-oven (150-60°C.) for 4 hr and cooled in a desiccator. It is not advisable to keep the slides for a long time as the aluminium oxide layer undergoes deactivation.

Of the various solvents tried as mobile phase, a mixture of petroleum ether-dioxane (10:1) was found to give best results. In this solvent PG does not move, BHT moves along with the solvent front and BHA goes to the centre. The use of 5 per cent alcoholic (ethyl) solution of phosphomolybdic acid as spray reagent gives well-defined blue spots with the antioxidants.

About 20 ml. of oil or fat containing a mixture of PG, BHA and BHT (0·001 per cent) was shaken vigorously with 10 ml. of methanol and then the mixture centrifuged. The separated alcoholic layer was evaporated under reduced pressure, and directly spotted on the chromatographic slide about 1 cm. from the bottom edge of the slide and in the centre with the aid of a fine capillary or micro-pipette. For comparison, methanolic solutions of PG and BHT were similarly spotted near the edges. The spots were dried, preferably in the air, and carefully kept in the chamber containing the solvent. After the solvent had run up to 1 cm. of the top edge of the slide it was taken out of the chamber and the solvent allowed to evaporate off. The chromatogram was then sprayed with 5 per cent alcoholic solution of phosphomolybdic acid with the aid of a sprayer, when blue spots of antioxidants appeared distinctly against the white background of aluminium oxide layer. The whole operation takes only 10 min. PG does not move in the solvent. BHT moves with the solvent front and BHA goes more or less to the centre.

References

1. SEHER, A., *Fette u. Seif.*, 61 (1959), 345.
2. SEHER, A., *Nahrung*, 4 (1960), 466.
3. SEHER, A., *Microchim. Acta*, (1961), 308.
4. BRENNER, M. & NIEDERWIESER, A., *Experientia*, 17 (1961), 237.
5. PRIVETT, O. S., *Proceedings, Flavor chemistry symposium* (Campbell Soup Co., Camden, NJ), 1961, 147.

АКАДЕМИЯ НАУК СССР

1968 ТРУДЫ ГЕЛЬМИНТОЛОГИЧЕСКОЙ ЛАБОРАТОРИИ ТОМ XIX

* (287) Myuge, S.G. 1968. Effect of propyl gallate on the extraintestinal digestion of phytohelminths. Tr. Gel'mintol. Lab., Akad. Nauk SSSR. 19:137-139.

С. Г. МЮГЕ

ВЛИЯНИЕ ПРОПИЛГАЛЛАТА НА ЭКСТРАКИШЕЧНОЕ ПИЩЕВАРЕНИЕ ФИТОГЕЛЬМИНТОВ

Было показано, что фитогельминты обладают внекишечным пищеварением, выделяя в растительную ткань ряд гидролитических ферментов (Dieter, 1955—1956; Зиновьев, 1956; Мюге, 1956). Протеолиз белка в данном случае осуществляется катепсинами, активность которых зависит от наличия в среде — растительной клетке — их активаторов. Катепсины фитогельминтов активируются донаторами водорода, причем различные виды фитогельминтов требуют определенных активаторов (глютатион, аскорбиновая кислота, цистеин и другие). Наличие в растении-хозяине специфических для паразита активаторов катепсинов в значительной степени обусловливает возможность существования гельминта в растении (Мюге, 1965). Поскольку донаторы водорода играют определенную роль в окислительно-восстановительных процессах растения, то при инвазии фитогельминтами происходит некоторая перестройка этих процессов, так как в клетку вводится новый окислительный фактор — катепсин гельминта. Обнаруживается это явление изменением активности ряда дыхательных ферментов растения, а в некоторых случаях увеличением неорганического фосфора и выделением тепла, что, наряду с повышением активности дыхания, косвенно указывает на разобщение дыхания и фосфорилирования.

Однако в растении, очевидно, включаются «аварийные» окислительные системы и последнее явление через некоторое время после инвазии исчезает.

Влияя на окислительные процессы растения, оказалось возможным ингибировать протеолитическую активность гельминтов, вызывая тем самым уменьшение их патогенного воздействия на хозяина и голодание самих паразитов (Мюге, 1964).

Это влияние может осуществляться или перестройкой окислительных процессов растения таким образом, что участвующие в них донаторы водорода не могут активизировать SH-группы катепсина нематод, так как последний к ним не адаптирован, или нормализацией вызванных гельминтами изменений в цепи окислительных процессов растения.

Для проверки первого предположения мы использовали ингибиторы некоторых ценных окислительных реакций, в частности пропилгаллат (ПГ).

В целях исследования влияния пропилгаллата на питание и ферментативную активность фитогельминтов, паразитирующих в вегетативных органах, мы опрыскивали 0,01%-ным ПГ листья папоротника, зараженного листовой нематодой *Aphelenchoides olisistus*. Внешнее проявление инвазии этой нематоды выражается в некрозе листовых пластинок, причем быстрота распространения некроза (коричневых пятен на листе) пропорциональна количеству особей нематод и их ферментативной активности.

Для опыта были выбраны листья папоротника (*Pteris*) с примерно одинаковыми очагами инвазии. Опытные листья опрыскивались 0,01%-ным раствором ПГ, а контрольные — водопроводной водой. Участки некротизированной ткани замерялись и сравнивались (см. таблицу).

Листья после опыта были пропущены через воронку Бермана. В контрольных листьях № 1 и 2 было обнаружено большое количество живых нематод, в листе № 3 было большее количество мертвых нематод и лишь единицы были слабо подвижны.

В опытных листьях живых нематод обнаружено не было. Это можно объяснить гибелью нематод от голода, так как специальными опытами установлено, что пропилгаллат в данной концентрации для червей не токсичен.

Площадь очага поражения листьев папоротника листовой нематодой (в *см*²)

	№ листа	День опыта								
		1	3	5	10	15	18	25	30	35
Контроль	1	0,25	0,30	0,36	0,64	1,2	2,4	4,0	10	Погибли
	2	0,25	0,35	0,40	0,78	1,4	2,8	4,5	12	
	3	0,30	0,35	0,45	0,66	2,0	2,6	4,3	Весь погиб	
Опыт	4	0,25	0,26	0,30	0,50	0,55	0,55	0,55	0,60	0,6
	5	0,25	0,28	0,30	0,50	0,50	0,55	0,58	0,60	0,6
	6	0,30	0,32	0,36	0,56	0,60	0,65	0,70	0,70	0,7

Наличие мертвых особей в контрольном листе объясняется тем, что к моменту взятия пробы весь лист погиб, а фитогельминты способны питаться только живой растительной тканью.

Одновременно с описанным опытом мы провели и параллельный, с поливом раствора ПГ под корень. Уменьшение инвазии в этом случае замечено не было.

В других сериях опытов показано, что обработка растений ПГ ингибирует протеолитическую активность и ряда седентарных фитогельминтов (Мюге, 1962, 1963, 1964).

Известно, что пропилгаллат наиболее активно реагирует с ферментами, содержащими SH-группы (Агатова, Вартанян, Эмануэль, 1963).

Следовательно, наряду с другими ферментами, находящимися в растительных клетках, связывался и катепсин, выделенный нематодами. Однако ингибирование протеолиза и других ферментов растительных клеток, содержащих SH-группы, приводило к усиленному синтезу последних или к адаптивному синтезу ферментов, катализирующих реакции, аналогичные тем, которые осуществлялись ингибированными ферментами. Это связано с тенденцией клетки стабилизировать биохимическое состояние, нарушенное каким-либо эндогенным фактором (Мойед, 1964; Умбаргер, 1964, Чанс, 1964).

С другой стороны, было замечено, что протеолитическая активность извлеченных из растения фитогельминтов уменьшается со временем.

Аналогичные данные были получены В. Г. Зиновьевым (1957). Поэтому мы поставили перед собой задачу выяснить, не влияет ли ингибирование экзоферментов нематод, выделенных в растительную клетку, на темп выделения этих ферментов нематодой.

Для опыта растения свеклы, зараженные *H. schachtii*, обрабатывались пропилгаллатом. Заражение производилось личинками примерно одного

возраста. Через определенные промежутки времени нематод снимали с корней (по 50 экз. с корня) и переносили в физиологический раствор для экстрагирования ферментов. Одновременно в корешках гистохимически определялся гидролиз белка.

Активность выделенных червями протеолитических ферментов зависела от ингибирования этих ферментов в растении, что указывает на связь между активностью ферментов в среде (а следовательно, и питанием паразита) и темпом их выделения.

Таким образом, ПГ ингибирует как катепсин нематод, так и другие ферменты, содержащие SH-группы, имеющиеся в растении. Однако он различно влияет на их синтез. Стимулируя синтез клеточных ферментов, ПГ не взаимодействует с ферментативными железами паразита, снижая темп образования катепсина.

ЛИТЕРАТУРА

Агатова А. И., Вартанян Л. С., Эмануэль Н. М. 1963. Механизмы взаимодействия свободных радикалов, образующихся на ингибиторов радикальных процессов с SH-группами белков.— Докл. АН СССР, 150, № 3: 547—550.

Зиновьев В. Г. 1956. Ферментативная активность некоторых фитогельминтов и биохимические изменения в поврежденных ими частях растений.— В сб.: «Проблемы паразитологии». Тр. II научн. конф. паразитологов УССР. Киев, стр. 292—293.

Зиновьев В. Г. 1957. Ферментативная активность нематод-паразитов растений.— Зоол. ж., 36, вып. 4, стр. 617—620.

Мойед Г. С. 1964. Некоторые вопросы регуляции активности ферментов конечным продуктом. В кн.: «Регуляторные механизмы клетки». Изд-во «Мир», стр. 417—428.

Мюге С. Г. 1956. Сравнительный анализ фитонематод.— В сб.: «Проблемы паразитологии. Труды II научн. конфер. паразитологов УССР». Киев, стр. 300—301.

Мюге С. Г. 1962—1963. Влияние ингибиторов свободно-радикальных реакций на галлообразование и развитие фитогельминтов.— Helminthologia, 4: 344—349.

Мюге С. Г. 1964. Паразитологические нематоды растений. Изд-во «Колос», 75 стр.

Мюге С. Г. 1965. О физиологической специфичности фитогельминтов.— Труды Гельминтол. лабор. АН СССР, 16, стр. 81—92.

Умбаргер Г. 1964. Подавление активности фермента конечным продуктом (ингибирование конечным продуктом) как механизм обратной связи.— В кн.: «Регуляторные механизмы клетки. Изд-во «Мир», стр. 390—407.

Чанс Б. 1964. Регуляторные механизмы ферментных систем. Регуляторные механизмы клетки. Изд-во «Мир», стр. 371—389.

Dieter A. 1955—1956. Vergleichende experimentelle Untersuchungen an zoophagen und phytophagen Nematode.— Wiss. Z. Martin-Luther-Univ., Halle-Wittenberg. Math.-Naturwiss. Reihe, 5(2) : 157—185.

STUDIES ON THE TOXICITY OF PROPYL GALLATE AND OF ANTIOXIDANT MIXTURES CONTAINING PROPYL GALLATE [a]

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Extensive toxicological studies are a prerequisite to the introduction in foods of new chemical preservatives, synthetic flavoring agents, coloring matter, etc. This paper reports studies undertaken on propyl gallate employing three species of animals, rats, guinea pigs and dogs, and extending over periods running from 14 months to 2 years. No toxicity was observed following the ingestion of amounts of propyl gallate equivalent to at least 100 times that which would be consumed if all dietary fat were treated with effective amounts of this antioxidant.

The use of antioxidants for the preservation of edible fats, certain lipid preparations, and fatty food products is becoming increasingly widespread. Incorporation of these substances in food preparations delays the development of rancidity; this is of importance not only from a commercial but also from a nutritional standpoint. Compounds which have been found to have sufficiently high antioxygenic value for use in foods are of widely different chemical structure and include nor-di-hydro-guiaretic acid, ascorbic acid and its palmityl ester, alpha-tocopherol, and gallic acid and its esters (reviewed by Mattill) *(6)*. As a preliminary condition to the introduction of any of these compounds for general use in foods and medicinal products designed for human consumption, it is necessary that a thorough investigation be made for any possible toxic effects of the antioxidant. This paper reports the results of such an investigation of propyl gallate and of antioxidant mixtures containing propyl gallate.

The literature on the effects of administered propyl gallate and related compounds consists primarily in studies of its acute toxicity. Abderhalden *(1)* reported a very low degree of toxicity for gallic acid itself and Filomeni *(2)* found the minimum lethal dose of gallic acid administered intravenously to rabbits was 3.23 gm. per kilo body weight. Levi *(5)* reported that the isolated frog heart was reversibly stopped by gallic acid in a concentration of 1 part to 2500. Ethyl gallate, even in large doses, produces no symptoms of toxicity when injected subcutaneously into mice, Hilditch *(3)*. Thus the available information suggests that propyl gallate probably has a low toxicity.

The investigation of the toxicity of propyl gallate here reported was divided into two main portions: (1) "acute" studies in rats to determine the LD_{50} of propyl gallate when administered both orally and subcutaneously; (2) "chronic" studies to ascertain the effects of administering smaller amounts of pure propyl gallate and of antioxidant mixtures containing propyl gallate for periods varying from 1 to 2 years to 3 different species—rats, guinea pigs, and dogs. In these studies there was no evidence of toxicity for amounts at least one hundred times that which would be ingested by the human subject if all the fat in his diet were treated with an effective amount of the antioxidant.

EXPERIMENTAL

Acute Toxicity Studies in Rats. The acute toxicity of propyl gallate, of heated propyl gallate, and of antioxidant mixtures containing propyl gallate was determined by administration both intraperitoneally and orally (stomach tube) to adult, hooded

[a] Supported by a grant from The Griffith Laboratories, Inc., Chicago, Illinois.

and albino, male and female rats weighing between 250 and 400 grams. These rats had previously been fed a commercial laboratory ration and were fasted twelve hours previous to the antioxidant administration. No animal was included if the dose was not quantitatively given nor if visible regurgitation or other loss occurred.

The propyl gallate was prepared for injection by adding sufficient water to 20 gm. of solid propyl gallate to bring the total volume to 100 cc., and then homogenizing the mixture in a Waring Blendor. Administered volumes of this solution were corrected for the amount of air entrained during the mixing process. In order to simulate conditions encountered on cooking and baking, the toxicity of a sample of propyl gallate previously heated for one hour at 425-450° F. in an oil bath was also determined. Some decomposition occurred during the period of heating as evidenced by the preparation becoming a light brown color. The dosage at which 50 percent of the animals died (LD_{50}) was the same (3.8 gm. per kilo, Table 1)

TABLE 1

Determination of LD_{50} of Propyl Gallate in Rats

Oral Administration					Intraperitoneal Administration		
Amount (gm. per kilo Body Weight)	Unheated Propyl Gallate		Heated Propyl Gallate*		Amount (gm. per kilo Body Weight)	Unheated Propyl Gallate	
	No. of Animals	No. of Deaths	No. of Animals	No. of Deaths		No. of Animals	No. of Deaths
5.0	6	6	10	8	0.5	6	6
4.0	18	12	10	6	0.4	18	12
3.5	18	5	10	4	0.35	18	5
3.0	12	0	14	4	0.30	12	0
2.5	2	0			0.25	2	0
2.0	9	0			0.20	9	0

*Heated at 425-450° F. for 1 hour.

with heated and unheated samples of propyl gallate; apparently heating does not alter the toxicity of the substance. When administered intraperitoneally, the LD_{50} was one-tenth as high (0.38 gm. per kilo) as when administered orally.

In the fatal cases, following the administration of pure propyl gallate, the time and cause of death appeared to be independent of whether the heated or unheated substance was administered, and of the mode of administration. It usually occurred within 10 to 60 minutes and appeared to be due to asphyxia or cardiorespiratory failure as evidenced by gasping respiration and convulsions before death. Autopsy revealed no abnormalities other than general dilatation and engorgement of both visceral and peripheral blood vessels and sometimes inflation of the lungs. The adrenal glands were particularly hyperemic and the vessels leading to them were markedly engorged with blood.

The acute toxicity of propyl gallate was also studied when it was administered together with other substances in an oily medium (results not shown in the table). A preparation containing 9.16 percent propyl gallate, 6.16 percent citric acid and 30 percent lecithin in corn oil, heated together to 165° C. to effect solution was administered by mouth in an amount (4.2 cc. per 100 gm. body weight) which contained the oral LD_{50} dose of propyl gallate as previously determined. Of thirteen successful administrations, only two animals died within 24 hours; the remaining animals fully recovered within several days. Apparently the oil decreased the rate of absorption of the propyl gallate.

Chronic Toxicity Studies in Rats. Weanling (21-23 day-old) albino male and female rats of the Connecticut Agricultural Experiment Station strain were placed

in individual wire mesh-bottom cages in a room maintained at 78° ± 3° F. and were fed an adequate basal synthetic diet of the following composition:

Casein	20 percent
Sucrose	10 percent
Dextrin (white)	35 percent
Crisco	21 percent
Wesson's Salt Mixture	4 percent
Standardized Cod Liver Oil	5 percent
Dried Brewer's Yeast	5 percent

The rats were divided into ten different groups. The above synthetic diet without supplementation was fed to the control group. The diets fed the other groups of rats were supplemented with various levels of propyl gallate, antioxidant mixture (containing propyl gallate, lecithin, and corn oil) and heated antioxidant mixture (Table 2). The "low" level of propyl gallate (0.00117 percent) was arbitrarily chosen as that amount which would be present in the diet if all the fat of the diet [assuming 23.3 percent fat, the average consumed by the Army during the war—Howe *(4)*] were treated with the recommended amount (0.25 percent) of a commercial antioxidant mixture which contains 2 percent propyl gallate. The "low" level of antioxidant mixture contained this same amount of propyl gallate. The ten-fold successive increases in the "medium," "high," and "sub-acute" levels were also arbitrarily adopted. Even the low level is purposely an exaggeration of the

TABLE 2

Supplements Added to the Various Diets

Group	Number of Rats	Supplement to Basal Diet
Control	15 males, 15 females	None
Propyl Gallate—"Low Level"	10 males, 10 females	0.00117% Propyl Gallate
Propyl Gallate—"Medium Level"	10 males, 10 females	0.0117% Propyl Gallate
Propyl Gallate—"High Level"	10 males, 10 females	0.117% Propyl Gallate
Propyl Gallate—"Sub-acute Level"	10 males, 10 females	1.17% Propyl Gallate
Propyl Gallate—"Sub-acute Level"	10 males, 6 females	2.34% Propyl Gallate
Antioxidant Mixture—"Low Level"	10 males, 10 females	0.06% Antioxidant Mix.*
Antioxidant Mixture—"Medium Level"	10 males, 10 females	0.6% Antioxidant Mix.*
Antioxidant Mixture—"High Level"	3 males, 7 females	6.0% Antioxidant Mix.*
Heated Antioxidant Mixture—"High Level"	5 males, 5 females	0.6% Heated Antioxidant**

*Replacing an equivalent amount of Crisco in the basal diet.
**Antioxidant mixture fortified with 17.4% propyl gallate heated at 425-450° F. for one hour.

amount which might be consumed by human subjects since undoubtedly only a small portion of the total dietary fat would be treated with the antioxidant mixture. The "sub-acute levels" would thus represent more than one thousand and two thousand times, respectively, the level which might be consumed by human subjects.

All rats were placed on their respective diets at weaning, with the exception of the group fed the high level of antioxidant mixture. These rats were placed on this diet at an age of 18 weeks, after receiving 1.17 percent propyl gallate from weaning. With the exception of this group and of several rats sacrificed for histological tissue studies, all animals were continued on their respective diets until death or for a two-year period.

TABLE 3

Growth of Male Rats Fed Diets Containing Various Levels of Propyl Gallate

Age of Rat	Amount of Propyl Gallate Added to Diet																	
	None (Control)		0.00117% Propyl Gallate		0.0117% Propyl Gallate		0.117% Propyl Gallate		1.17% Propyl Gallate		2.34% Propyl Gallate	
	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*
Weeks		gm		gm		gm		gm		gm		gm

TABLE 3

Growth of Male Rats Fed Diets Containing Various Levels of Propyl Gallate

Age of Rat	Amount of Propyl Gallate Added to Diet											
	None (Control)		0.00117% Propyl Gallate		0.0117% Propyl Gallate		0.117% Propyl Gallate		1.17% Propyl Gallate		2.34% Propyl Gallate	
	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*
Weeks		*gm.*		*gm.*		*gm.*		*gm.*		*gm.*		*gm.*
4	15	59 ± 1.5	15	59 ± 2.1	9	59 ± 3.3	7	62 ± 4.1	10	53 ± 3.1	10	52 ± 1.5
6	15	127	15	124	10	121	7	121	8	57	8	45
8	15	211	15	208	10	204	7	198	7	75	6	60
10	15	282	15	264	10	276	7	274	6	105	6	81
12	15	339 ± 12	15	350 ± 11	10	328 ± 10	7	331 ± 17	6	142 ± 13	6	101 ± 6.9
14	15	381	15	391	10	376	7	373	6	177	6	120
16	14	421	15	434	10	405	7	413	6	214	6	140
18	15	444	14	465	10	435	7	441	3**	247	6	158
20	15	468	15	478	10	457	7	462	3	273	6	168
24	15	499 ± 17	15***	522 ± 20	10	487 ± 9.7	7	498 ± 24	3	331 ± 36	6	193 ± 22
28	15	529	10	557	10	495	7	530	3	359	6	206
32	15	540	9	593	9	539	7	555	3	389	6	230
36	14	553	9	608	9	556	7	562	3	408	5	241
39	14	559 ± 18	9	625 ± 30	9	554 ± 10	6	602 ± 43	3	431 ± 8	5	247 ± 17
43	14	572	8	625	9	579	5	636	3	440	5	266
47	13	581	6	620	8	596	5	658	3	445		
51	13	572	6	617	8	602	5	681	3	432		
55	11	550 ± 24	4	657 ± 50	8	583 ± 30	5	689 ± 34	3	422 ± 30		
59	9	547	4	684	7	572	4	692	2	431		
63	8	585	4†	708	6	564	4	695	2	473		
67	6	619			4	612	7†	740	2	445		
71	5	613 ± 50			2	634 ± 21	7	762 ± 54	2††	432		
75	4	624			2	627	7	754				
79	3	613			1	615	4	786				
83					1	570	1	855				
85							1	800				

*Variations give mean deviation of the mean. **3 animals changed to 6% antioxidant mixture on 18th week. ***5 animals changed to heated "antioxidant mixture" diet on 26th week. †animals changed from 0.00117% to 0.117% propyl gallate on 63rd week. ††sacrificed for tissues.

The growth of the rats was followed by weekly weighings during the first year of the experiment and bi-weekly weighings during the second year of the experiment. Because of lack of space, the growth for only one sex is presented for each antioxidant preparation, arbitrarily, for male rats for the groups receiving propyl gallate (Table 3), and for female rats for the groups receiving antioxidant mixture (Table 4). With the exception of the rats given "sub-acute levels" of propyl gallate (1.17 and 2.34 percent) growth of all animals receiving the antioxidant-containing diets was at least equal to that of the control animals.

The somewhat higher values of the mean weights of the rats receiving antioxidant supplements may be due to some protective action of the antioxidant on the fat or perhaps some other dietary constituent. Since food was not allowed to stand in the feeding cups for periods longer than 5 days, the difference would not appear to be due to development of rancidity in the control diet before the food was eaten.

Rats fed diets containing 1.17 percent and 2.34 percent levels of propyl gallate were definitely stunted in their growth (Table 3). At these levels, propyl gallate imparts a distinctly bitter taste to the diet, and the stunted growth may be due to a resultant lowered food consumption. Rats which were stunted in growth by the 1.17 percent level of propyl gallate in the diet resumed a more normal growth rate when they were transferred at 18 weeks of age to a diet containing 6 percent antioxidant mixture (Table 4), Ten animals were transferred at the 26th week from the

TABLE 4

Growth of Female Rats Fed Diets Containing Various Levels of "Antioxidant Mixture"

Age of Rat (Weeks)	Amount of Antioxidant Mixture Added to Diet							
	None (Control)		0.06 Per Cent		0.60 Per Cent		6.0 Per Cent	
	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*	No. of Animals	Mean Weight*
		gm.		*gm.*		*gm.*		*gm.*
4	15	60 ± 1.5	8	51 ± 1.4	7	56 ± 5.0		
6	15	123	10	113	9	110		
8	15	174	10	167	9	166		
10	14	215	10	211	9	204		
12	14	242 ± 5.7	10	237 ± 5.6	9	230 ± 6.3		
14	14	263	10	268	9	255		
16	14	278	10	278	9	272		
20	14	301	10	307	9	302	7**	214
24	14	324 ± 8.6	10	324 ± 6.0	9	337 ± 8.0	7	246 ± 8.0
28	13	331	10	339	9	357	7	273
32	13	345	10	359	9	379	7	294
36	13	360	10	363	9	386	7	314
39	11	361 ± 7.3	10	370 ± 6.8	9	397 ± 16	7	313 ± 18
43	11	366	9	375	8	420	7***	313
47	10	380	8	370	6	430		
51	7	388	8	381	5	464		
55	7	392 ± 21	8	400 ± 21	5	468 ± 34		
59	7	405	7	402	5	460		
63	7	416	6	426	5	469		
67	7	433	6	450	5	476		
71	7	438 ± 23	4	458 ± 30	4	504 ± 60		
75	5	436	4	462	2	505		
79	2	525	3	490	2	500		
83	1	520	2	525	1	650		
87			1	458				
107			1***	480				

*Variations give mean deviation of the mean. **Rats fed from weaning to 18th week a diet containing 1.17% propyl gallate. ***Sacrificed for tissue studies.

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	7**	214
: ± 8.0	7	246 ± 8.0
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	7	294
	7	314
: ± 16	7	313 ± 18
	7***	313
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diet containing 0.00117 percent propyl gallate to a diet containing 0.6 percent "heated antioxidant mixture" fortified with propyl gallate (see Table 2). No change in the rate of growth (data not included in tables) was noticed during the subsequent four weeks, when the animals were sacrificed for the study of tissues.

Except for those animals stunted in growth by the high levels of propyl gallate in the diet, there was no detectable effect of either the antioxidant mixture or of propyl gallate on gross appearance. "Growths" were found only in three groups—one rat receiving the control diet, four rats receiving the diet treated with 0.6 percent antioxidant mixture, and one rat receiving the diet containing 0.00117 percent propyl gallate. The "growth" in the control rat was found histologically to be a hyperplastic lymph node probably related to an extensive pulmonary carcinoma found on autopsy. The "growths" in two of the antioxidant-fed rats were found histologically to be enlarged lymph nodes; the third growth was an actively secreting mammary gland and the fourth consisted of collagenous connective tissue. The remaining growth from the rat fed a diet containing 0.00117 percent propyl gallate was not identified.

It is evident from the data in Table 5 that there is no consistent difference between the hemoglobin values for the animals fed the control diet and the animals

TABLE 5

Hemoglobin Levels of Rats Fed Antioxidant Supplemented Diets

Diet	Mean Hemoglobin Level at Age*				
	6 Weeks	14 Weeks	60-65 Weeks	65-70 Weeks	70-80 Weeks
	gm. %	gm. %	gm. %	gm. %	gm. %
Control	12.3 ± 0.4	15.2 ± 0.5	14.3 ± 0.4	14.6 ± 0.7	14.2 ± 0.4
0.00117% Propyl Gallate			14.6 ± 0.4		
0.0117% Propyl Gallate			13.9 ± 1.0	11.9 ± 0.8	12.1 ± 0.7
0.117% Propyl Gallate			13.3 ± 0.7	14.6 ± 0.8	13.8 ± 0.5
1.17% Propyl Gallate	13.0 ± 0.4	10.9 ± 0.4	10.9 ± 2.0	12.1 ± 1.0	13.7 ± 1.3
2.34% Propyl Gallate	13.4 ± 0.4	11.1 ± 0.3			
0.06% Antioxidant Mixture			13.4 ± 0.4	13.3 ± 0.6	14.2 ± 0.5
0.6% Antioxidant Mixture			14.5 ± 0.4	13.5 ± 0.6	13.8 ± 0.4

*Variations give mean deviation of the mean.

fed the supplemented diet with the lower levels of antioxidant. However, animals fed diets containing "sub-acute levels" of propyl gallate had significantly lower hemoglobin levels during the early part (to 65 weeks of age) of the experiment when there were sufficient animals to yield a reliable mean value. As the animals became older the hemoglobin values appeared to increase toward normal levels. Low hemoglobin levels were encountered in the heavier rats of all groups and appeared to be attributable to chronic blood loss from lesions on the feet caused by standing on the wire screen bottoms of the cages.

Additional hematological data were obtained which are not included in the tables. Erythrocyte counts in the majority of the animals varied directly in all cases with hemoglobin levels. Leucocyte counts on 27 selected rats fed the various diets showed no significant difference with respect to either the total leucocyte count or to the differential count.

Animals which died during the course of the experiment and those which were purposely sacrificed were examined histologically. No consistent pathological changes attributable to antioxidant supplementation were found in the heart, lung, liver, spleen, or gonads of the animals given any of the diets studied. The only consistent abnormality apparent was in the kidneys of the animals fed "sub-acute

levels" (1.17 percent and 2.34 percent) of propyl gallate. Gross examination showed a mottled appearance. Histological examination showed tubular damage and the presence of albuminous casts. It appears that these changes could have been due to severe inanition and a consequent lack of dietary choline and methionine.

The direct cause of death in practically all animals which succumbed during the experiment was respiratory infection. A frequent contributory cause of death especially in the older rats was the presence of large hair balls in the stomach, causing anorexia and a resulting lowered resistance. An examination of the tables shows that, except for the rats fed diets containing "sub-acute levels" of propyl gallate, the mortality was about the same in all groups.

Chronic Toxicity Studies in Guinea Pigs. Young male and female guinea pigs three to four weeks old and weighing between 200 and 300 grams were divided into two groups each comprised of 14 males and 6 females. The animals were housed in large size wire mesh-bottom cages, two or three animals to the cage, and separated as to sex. All animals were given cabbage three times weekly. Animals in the control group were fed Purina Rabbit Chow *ad libitum* and the animals in the experimental group were fed the same ration previously impregnated with propyl gallate. Pellets were impregnated with propyl gallate by spraying them with an alcoholic solution containing sufficient propyl gallate to give a final concentration equal to the "medium level" of the preceding studies on rats (0.0117 percent).

The body weights of the animals were determined weekly for a period of one year. At the end of this time, the female animals in each group were mated with male animals of the same group. As the experiment was completed, the animals were killed, checked at autopsy, and sections were prepared for microscopic examination.

There was no detectable effect of the propyl gallate on the gross appearance of the animals. Neither did it have any influence on their rate of growth (Table 6). Only one litter of three offspring was obtained from the six control female guinea

TABLE 6

Growth of Guinea Pigs Fed Control and Propyl Gallate-Treated Diets

Weeks on Experiment	Control Diet		Propyl Gallate-Treated Diet	
	Mean Weight* (14 males)	Mean Weight* (6 females)	Mean Weight* (14 males)	Mean Weight* (6 females)
	gm.	*gm.*	*gm.*	*gm.*
0	259 ± 13	211 ± 11	251 ± 11	207 ± 15
2	338	275	325	264
4	443	377	434	340
6	554	442	548	420
8	647 ± 18	493 ± 27	639 ± 14	489 ± 28
10	726	560	731	548
12	772	569	759	570
14	851	644	830	647
16	897 ± 25	675 ± 24	865 ± 19	677 ± 41
20	954	718	925	716
24	1023	780	1011	790
28	1085	830	1075	859
32	1066 ± 23	838 ± 42	1095 ± 40	872 ± 54
36	1102	881	1120	845
40	1125	881	1139	864
44	1182	883	1160	896
48	1210 ± 26	921 ± 40	1202 ± 33	947 ± 61
52	1190	932	1202	972

*Variations give mean deviation of the mean.

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allate-Treated Diet	
*	Mean Weight* (6 females)
	gm.
	207 ± 15
	264
	340
	420
	489 ± 28
	548
	570
	647
	677 ± 41
	716
	790
	859
	872 ± 54
	845
	864
	896
	947 ± 61
	972

pigs, while three litters of three pigs each were obtained from the six propyl gallate-fed guinea pigs. The growth of the litter of the control animal and of one of the litters of the propyl-gallate-fed animals was followed for two months, without detecting any significant difference.

No gross abnormalities attributable to propyl gallate feeding were found at autopsy. Histological examination of liver, kidney, spleen, testes and ovary, adrenal, heart and lungs also showed no pathology attributable to propyl gallate.

Chronic Toxicity Studies on Dogs. Twelve young female mongrel dogs weighing from 4.6 to 15.0 kilos were divided into two groups and housed in indoor pens having access to outdoor runways. Animals in the control group (5 dogs) were fed untreated commercial dog chow pellets and those in the experimental group (7 dogs) were fed pellets previously treated with propyl gallate as already described. As a supplement to this diet a mixture of cod liver oil and dried brewer's yeast was given each week to all animals.

Throughout the course of the experiment, which lasted for 14 months, there was no evidence of any toxic effect of the propyl gallate. The groups could not be differentiated on the basis of appearance, activity, or other gross characteristics. Both the control and propyl gallate-fed dogs increased somewhat in weight during the experiment, probably because of improved food and housing conditions. Terminal hemoglobin values determined on the dogs of both groups showed no significant difference. Qualitative urine analyses also performed at the end of the experiment showed no more than a questionable trace of glucose, acetone-bodies, blood, bile, or protein in any animal. The absence of color in Gerhardt's ferric chloride test for aceto-acetic acid not only indicated the absence of aceto-acetate, but also the absence of detectable quantities of propyl gallate in the urine. The specific gravity of urine samples collected after 12-hour periods in which the animals were deprived of water, showed no significant difference. There is thus no suggestion of any abnormality due to propyl gallate feeding, in renal or hepatic function or of any detectable metabolic aberration.

SUMMARY

The LD_{50} of propyl gallate administered to rats orally is approximately 3.8 grams per kilo body weight and administered intraperitoneally is 0.38 grams per kilo. Death from this dose occurs usually within an hour and is due to cardiorespiratory failure. Heating propyl gallate at 425° to 450° F. for one hour has no effect on the LD_{50} or on the toxic manifestations.

The feeding of "sub-acute levels," 1.17 and 2.34 percent, of propyl gallate to rats results in inhibition of growth and a mild anemia probably due to the bitterness of the diet with a resulting low food intake. Death occurred within four weeks in about 40 percent of the animals fed the higher level. The only consistent pathological change found is tubular damage and the presence of albuminous casts in the kidney. Surviving rats have consumed the above amounts of propyl gallate for 10 to 16 months without visible toxic effect other than inhibition of growth.

The prolonged feeding of varying amounts of propyl gallate (0.00117 percent, 0.0117 percent, and 0.117 percent) or of antioxidant mixture (0.06 percent, 0.6 percent, and 6.0 percent) or of heated antioxidant mixture (0.6 percent) for as long as two years to rats or for 14 to 15 months to guinea pigs (0.0117 percent propyl gallate) and dogs (0.0117 percent propyl gallate) had no detectable effect on gross appearance, growth or body weight, reproduction (guinea pigs), hemoglobin, erythrocyte, or leucocyte levels in blood (rats), renal function as reflected by the

qualitative composition of the urine (dogs), or histological appearance of the liver, kidney, spleen, stomach, gonads, lungs, and heart (rats and guinea pigs).

These results thus show that no detectable toxic effects follow the prolonged ingestion of propyl gallate or of antioxidant mixture when these substances are ingested in amounts at least 100 times that which would be consumed by human subjects if all the dietary fat were treated with the effective recommended amounts of propyl gallate or of antioxidant mixture.

ACKNOWLEDGMENT

The authors are indebted to Dr. Arthur L. Amolsch of the Department of Pathology for histological examination of tissues.

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LITERATURE CITED

1. Abderhalden, E. *Biochem. Handlexikon.*, Bd. VII, 15. Berlin (1912).
2. Filomeni, M. The pharmacology of gallic acid. I. Immediate toxicity of gallic acid introduced intravenously and the anatomicopathological lesions of the organs. *Arch. Pharm. sper.*, 63, 183-192; *C. A.*, 31, 6730 (1937).
3. Hilditch, T. P. Some factors affecting the control of oxidative rancidity. *Chemistry and Industry*, 67-71 (1944).
4. Howe, P. E. Nutritional aspects of feeding the army. *J. Am. Med. Assoc.*, 120, 93-96 (1942).
5. Levi, A. Action of gallic acid on frog heart. *Arch. pharm. sper.*, 61, 81-87; *Physiol. Abs.*, 21, 585 (1936).
6. Mattill, H. A. Anti-oxidants and synergists. *Oil and Soap*, 22, 1-3 (1945).

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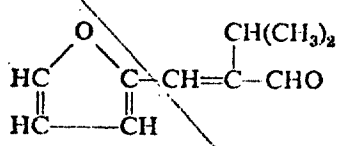

$C_{10}H_{12}O_2 = 164.21$

Yellowish oily liquid. Sp.Gr. 1.03. B.P. 229° C.

Practically insoluble in water, soluble in alcohol and oils.

Pleasant, oily-woody, herbaceous-green odor of moderate to poor tenacity.

This aldehyde has been suggested for use in perfume compositions, but it is rarely offered commercially under its proper chemical n

It was developed in a series of resea periments with Furfural and alifatic hydes, parallel to the more successfu from Benzaldehyde and alifatic aldeh A few of these alkyl-substituted Furfu leins have found use in perfumes, some in flavors.

The title aldehyde gives very pleasan interesting effects with herbaceous-floral rials, including Jasmin and Mimosa, or Amylsalicylate in novel types of Fouge

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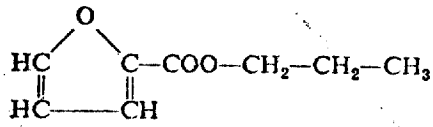
2709: n-PROPYL FUROATE

Propyl-*2*-furoate.
n-Propyl pyromucate.
Furan-*alpha*-carboxylic acid, *n*-Propylester.



$C_8H_{10}O_3 = 154.17$

Colorless liquid. Sp.Gr. 1.07. B.P. 211° C.

Very slightly soluble in water, soluble in alcohol and oils.

Peculiar sweet-oily, herbaceous-earthy odor of moderate to poor tenacity.

Sweet, warm, oily-herbaceous, caramellic-earthy taste in concentrations below 5 ppm. Heavy-earthy, but still sweet at concentrations near 20 ppm.

This ester finds some use in flavor com tions, mainly in Chocolate and Mushr imitation flavor, where the deep-warm, F aceous-nutlike flavor adds power and ch ter. It blends quite well with the more ventional Amylphenylacetate and si esters for above purposes, and it is also patible with *1*-Octen-*3*-ol (see Amyl carbinol).

The concentration of this material in fi ed consumer products will normally be as as 0.05 to 0.30 ppm. It is particularly lo Chocolate flavor, which is a very sens flavor, or rather, a flavor to which the ba clientèle is critically sensitive and alert.

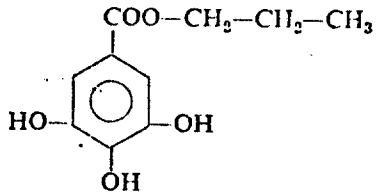
G.R.A.S. F.E.M.A. No.2946.

Prod.: by azeotropic esterification of Furoic acid with *n*-Propanol.

26-664; B-XVIII-275;

2710: n-PROPYL GALLATE

"Tenox P.G." (Eastman Chem. Co.).
Progallin.



$C_{10}H_{12}O_5 = 212$

Almost white, but commonly off-white or greyish microcrystalline powder or small crystals.

M.P. 150° C.

0.3% soluble in water, 50% soluble in alcohol, 1% soluble in most vegetable oils.

Practically odorless when pure, but some commercial products may carry a faintly dry-medicinal odor.

Aqueous solutions of higher than 10 ppm concentration have a perceptibly bitter taste, or bitter-metallic taste and mouthfeel. The material is tasteless in the concentrations recommended for finished products.

This ester is not an active flavor or perfume material, but it serves as an important Antioxydant in many food products and flavor concentrates. It is also used to some extent in essential oils to retard oxidation of Monoterpenes and sensitive aldehydes or ketones, etc. See also monographs: Butyl hydroxy anisole and Butyl hydroxy toluene. The title material is often used in conjunction with the two named materials, and with Citric acid or similar acid as booster/synergist.

The subject ester suffers from the same drawback as other Antioxidants, in the fact that it is a phenol. It discolors in contact with Iron and upon prolonged exposure to air and daylight. The latter type of discoloration may not take place at the low concentration normally used. Only one Antioxidant of importance in food is not a phenol (Ethoxy dihydro trimethyl quinoline).

Propyl gallate is used at the rate of 0.03 to 1 ppm in finished products, mostly in combination as outlined above.

Lemon, Lime, Fruit and Spice flavors are also often treated with this material.

G.R.A.S. F.E.M.A. No.2947.

The American Food and Drug Administration has approved its use and limited the amount to 200 ppm based upon the fat or oil matter present in the consumer product which is treated. The material may be used in freeze dried meats and sausages in the amount of 100 ppm. A further addition of 100 ppm of other Antioxidants is permitted. These figures are based upon the weight of the fat only – in the meat or sausage.

100-864; 162-225; 162-236;

2711: n-PROPYL HEPTOATE

Propyl heptylate.
Propyl heptanoate.
Propyl oenanthate (misleading and abandoned name).

$$CH_3—CH_2—CH_2—OOC(CH_2)_5CH_3$$

$$C_{10}H_{20}O_2 = 172.27$$

Colorless oily liquid. Sp.Gr. 0.87.

B.P. 206° C.

Almost insoluble in water, soluble in alcohol and oils.

Green-fruity, winey, slightly fatty odor of rather poor tenacity. The fruity notes resemble the odor of juice from green Grapes (also called white grapes).

Tart-fruity, winey, mildly oily-green taste in concentrations below 40 ppm. Not very pleasant at higher levels.

This ester, like most other Propylesters, is used only rarely in flavors. Generally speaking, the Propylesters lack natural character and immediately attractive aroma, while there are many such esters of quite pleasant odor.

It finds use in imitation Apple, Coffee, Cognac, Rum, Grape, and in various berry complexes, Tutti-frutti types, etc.

The use level is equivalent to about 5 to 20 ppm in the finished product.

There is only very little use of this ester in perfumery. It may find use as part of imitation Cognac oil and other materials for "winey" topnotes, and in Geranium compositions, Ylang, etc. where the Brandy-winey character can be of interest.

Prod. by azeotropic esterification of *n*-Propanol with *n*-Heptanoic acid.

G.R.A.S. F.E.M.A. N.2948.

33-621; 35-61; 103-104; 163-235;

40. INHIBITION OF BRADYKININ ACTIVITY BY GALLATES AND OTHER ANTIOXIDANTS. L. F. Fossti, K. K. Fox and M. J. Pallansch. Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C. 20250.

In studies of the kinin activity of cow's milk it was observed that antioxidants suppressed the activity of bradykinin used as a control. Gallic acid, its esters and BHA will suppress the contractile response elicited from the smooth muscle of the guinea pig ileum by the action of bradykinin. The initial suppressive effect of members of a homologous series of gallates increases almost directly with chain length. The amyl ester is 150 times as effective as the methyl homolog which requires a concentration of 6.6×10^{-7} gm/ml to start inhibiting the action of 4.0×10^{-8} gm/ml bradykinin. Hexyl gallate is slightly less effective and the higher homologs are too insoluble to produce suppression of bradykinin action in aqueous systems. BHA is the most effective suppressing agent of the antioxidants tested. A plot of the reciprocal of gut response against the reciprocal of bradykinin concentration for different antioxidant concentration indicates that the antioxidants might act as competitive inhibitors. The suppressive effect of long chain gallates, when used in sufficient quantity to markedly depress the gut response to bradykinin, is only partially reversible.

41. SYNTHESIS OF THE FOURTH COMPONENT OF THE BOLL WEEVIL SEX ATTRACTANT, 2-ISOPROPENYL-1-METHYLCYCLOBUTANEETHANOL. J. H. Tumlinson, R. C. Gueldner, D. D. Hardee, A. C. Thompson, P. A. Hedin, Boll Weevil Research Laboratory, Box 5367, State College, Miss., 39762; and J. P. Minyard, Miss. State Chemical Lab., Box CR, State College, Miss. 39762.

The structures of the four compounds (I, II, III, and IV) which comprise the boll weevil sex attractant have been previously reported.

I II III IV

Additionally, the identity of three of these compounds was proved by synthesis. Subsequently, synthesis of the fourth component has been completed, and a synthetic mixture which is equivalent to the natural pheromone in attractancy has been formulated. Synthesis of 2-isopropenyl-1-methylcyclobutaneethanol, was achieved by a four-step synthesis which includes photolysis, Grignard addition, hydroboration, and dehydration. The results of bioassays and field tests will be reported.

42. AN IMPROVED SYNTHESIS OF THE BOLL WEEVIL SEX ATTRACTANT COMPONENTS. R. C. Gueldner, J. H. Tumlinson, D. D. Hardee, A. C. Thompson, P. A. Hedin - Boll Weevil Research Laboratory, Box 5367, State College, Miss. 39762 - and J. P. Minyard, Miss. State Chemical Lab., Box CR, State College, Miss. 39762.

An improved synthesis of the previously reported boll weevil sex attractant components, cis-3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol, cis-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde and trans-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde, has been accomplished. The synthesis provides high yields of the two desired olefinic isomeric precursors and allows their separation by distillation. The elucidation of the stereochemistry of the isomeric precursors by NMR spectra will be discussed. The synthesis of analogs and structure-activity relationships will also be presented.

43. THE APPLICATION OF THE TRICHLOROACETYL ISOCYANATE REACTION WITH TERPENE ALCOHOLS FOR THE QUANTITATIVE FRACTIONATION OF ESSENTIAL OILS. P. A. Hedin, R. C. Gueldner, and A. C. Thompson, USDA Boll Weevil Research Laboratory, Box 5367, State College, Miss. 39762.

The tertiary alcohols of essential oils are often difficult to separate from other oxygenated classes by existing techniques. Previously, Goodlett reported the employment of trichloroacetyl isocyanate (TCAIC) to esterify hydroxy compounds for characterization of alcohols by nuclear magnetic resonance. As applied to gas-liquid chromatographic (GLC) profile analysis of oils, injection of the TCAIC reaction mixture gave a reduction in peak numbers; however removal of the TCAIC esters from the oil with subsequent regeneration to the original alcohol would provide additional valuable information. This report describes the development of a quantitative procedure for the solvent extraction of terpene alcohol esters from the essential oil with their concomitant hydrolysis to carbamates, and the conditions for quantitative regeneration (saponification) of the alcohols.

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Inhibition of Bradykinin by Gallates

Linda P. Posati, Kenneth K. Fox, and Michael J. Pallansch

Gallic acid and its esters will suppress the contractile response elicited from the smooth muscle of the guinea pig ileum by the action of bradykinin. The initial suppressive effect of members of a homologous series of gallates increases almost directly with chain length. Plots of reciprocal of gut response against the reciprocal of bradykinin concentration for different concentrations of these food grade antioxidants indicate that they might act as competitive inhibitors. The mechanism of their inhibitory action is apparently complex and, in the case of the longer chained esters, is only partially reversible.

Bradykinin is a vasoactive peptide whose physiological role in living systems has undergone considerable investigation since its presence was first reported in human blood plasma (Roche e Silva *et al.*, 1949). Some organic compounds related to tranquilizers antagonize the bradykinin induced smooth muscle contractions of isolated guinea pig ileum (Roche e Silva and Garcia Leme, 1964). The type of drug inhibition exhibited by some of these chemicals has been characterized (Garcia Leme and Roche e Silva, 1965).

During the course of our research we noted that some common food grade antioxidants could likewise suppress smooth muscle response to bradykinin. Because antioxidants of this type are employed for food preservation, we report here results obtained during an investigation into the nature of the inhibition of guinea pig ileum response effected by gallic acid and a homologous series of its esters.

MATERIALS AND METHODS

Assays were conducted using a 2- to 3-cm section of the terminal segment of the guinea pig ileum which was suspended in a 5-ml capacity perfusion bath held at 37° C. A steady stream of air was bubbled through the reaction chamber for stirring and oxygenation of the ileum preparation. The ileum was attached to a frontal lever pen which recorded change in length on a kymograph. Before being used for analytical work, the gut was equilibrated for 1 hr in a carrier solution consisting of Tyrode's buffer containing 2 mg per liter of atropine and 40 μg per liter of pyribenzamine. Stock solutions of synthetic bradykinin (BRS 640, Sandoz Pharmaceutical Co.) were prepared in Tyrode buffer. Control responses to the kinin were determined by injecting 0.2 ml of the stock solution into the reaction chamber after buffer flow had been interrupted. After a 30-sec exposure of the smooth muscle to the drug, the cell was flushed by buffer at a rate of 15 ml per min. The height of the peak produced on the kymograph was taken as a direct measurement of the amount of smooth muscle contraction produced by bradykinin. Control responses to histamine dihydrochloride (Nutritional Biochemicals Corp.) were established.

Assay in the Presence of Inhibitors. The same conditions as in the previous assay were adhered to. Stock solutions of gallic acid (Fisher Chemical Co.), ascorbic acid (Fisher Chemical Co.), and the gallate esters, methyl, ethyl, *N*-propyl, isopropyl, *N*-butyl, *N*-amyl, and *N*-hexyl, prepared according to the procedure of Russell and Tebbens (1942), were made up in distilled water. Suitable concentrations of these antioxidants were mixed with varying concentrations of bradykinin immediately before injection into the bath. Decreases in peak heights effected by the presence of the antioxidants in the test mixtures were used to evaluate their inhibitory action.

Computation of Data. All concentrations are presented as concentrations in the reaction bath.

The nature of the inhibition of the smooth muscle response to bradykinin, which these antioxidants produce, was determined according to the general method of Roche e Silva (1959). Results were interpreted by plotting reciprocals of effects ($1/Y$) *vs.* reciprocals of doses of bradykinin ($1/X$). Y is the height of the peaks produced on the kymograph chart as measured in mm and X is the concentration of bradykinin in μg/ml.

The inhibitory potency of the gallate esters was evaluated from these plots and expressed as the pK_i value. Roche e Silva has employed these double reciprocal plots to substantiate linearity between the number of receptors occupied by a drug and the effect as measured on a kymograph. The action of the drug is applicable to Clark's equation:

$$K_c X = \frac{Y}{1 - Y} \tag{1}$$

where X is the concentration of drug in g/ml, Y the response obtained on the chart, and K_c is Clark's constant. If the straight line obtained when such a plot is normalized, that is, the Y intercept or maximum drug response is taken as 1, and the responses taken as percentages of the maximum response, the slope of this line K_n is the reciprocal of Clark's constant. Inversion of Clark's equation gives:

$$\frac{K_n}{X} = 1/Y - 1 \tag{2}$$

and pK_n is a measure of the affinity of the drug for the receptors.

Introduction of an inhibitor into the system follows Gaddum's equation:

$$K_c X = (1 + I/K_i)\frac{Y}{1 - Y} \tag{3}$$

where I is the concentration of inhibitor, K_c Clark's constant, and K_i a constant dependent on the nature of the inhibitor. Inversion of this equation gives:

$$\frac{\beta K_n}{X} = 1/Y - 1 \tag{4}$$

where β is $1 + I/K_i$.

The effect of the inhibitor is to change the slope of the

Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. 20250

normalized line by the multiple β without, in the case of competitive inhibition, changing the intercept. For competitive inhibition the pK_i value can be calculated from β according to the equation:

$$-pK_i = \log (I) - \log (\beta - 1) \quad (5)$$

pK_i is a measure of the affinity of the inhibitor for ileum receptor sites. Experimentally, β is the ratio of the slopes of the lines with inhibitor to the slope of the control line. In the case where β equals 2, the pK_i value is equivalent to a pA_2 value; that is, the concentration of the inhibitor reduces the effect of a double dose of drug to that produced by a single dose. The pK_i values presented here are calculated from the slopes of lines where β was as close to 2 as was possible to attain experimentally.

RESULTS AND DISCUSSION

The threshold levels at which gallic acid and its six homologous esters begin to produce an inhibition of ileum response to 0.04 μg/ml bradykinin is related to the number of carbons in the ester side chain (Figure 1). As the chain length of the acid substitution group increases, the kinin inhibitory power of these antioxidants also increases. The only deviation from this pattern is with hexyl gallate. No difference is noted in the repressive strength of the isomeric forms of propyl gallate.

The observed suppressive effect of the gallates cannot be ascribed to some impurity in the preparations, since all the esters, except hexyl, were made from chromatographically pure alcohols, and all melted at values reported in the literature. Furthermore, the esters migrated as a single spot when analyzed by thin-layer chromatography.

The effect of increasing levels of the phenolic antioxidants on the muscular response to 0.04 μg/ml bradykinin is related to the chain length for gallic acid and the methyl, ethyl, and propyl derivatives (Figure 2). As the chain length is increased to 3 carbons, the change in concentration of antioxidant required to significantly depress gut response becomes greater. When high concentrations of these esters were employed in the reaction mixture, the ileum did not return quickly to normal kinin sensitivity. Flushing of the cell for a period of 30 min was required to produce a normal response to the control after application of propyl gallate in an amount sufficient to completely suppress gut response to bradykinin.

The effect of chain length on the muscle response becomes less obvious as it is increased beyond 3 carbons (Figure 3). The results presented in this figure are complicated by the fact that the ileum never returned to normal kinin sensitivity after exposure to high levels of these compounds and after repeated doses of low levels. The raw data were corrected for loss in kinin sensitivity and are presented as percent suppression of responses to control doses run within 5 to 10 min after flushing the cell of the previous reaction mixture.

To establish the nature of the bradykinin inhibition of the gallate esters, a drug dose study was conducted. A typical double reciprocal plot (Figure 4) shows inhibition of gut contractions in systems containing fixed levels of antioxidant as the kinin dose is decreased. The relatively straight lines passing through a common point on the Y axis are typical of those obtained when a competitive inhibitor is influencing a biological system. Similar curves were obtained for methyl and ethyl gallate and for the higher members of the homologous series at low concentrations. In those instances where repeated small doses of the butyl, amyl, and hexyl gallates decreased the ileum's normal kinin sensitivity, the data were

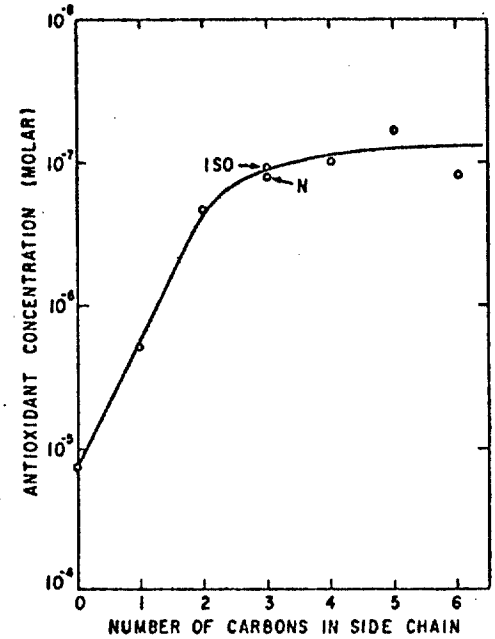

Figure 1. Minimum antioxidant concentrations required to detectably inhibit ileum response to 0.04 μg/ml bradykinin

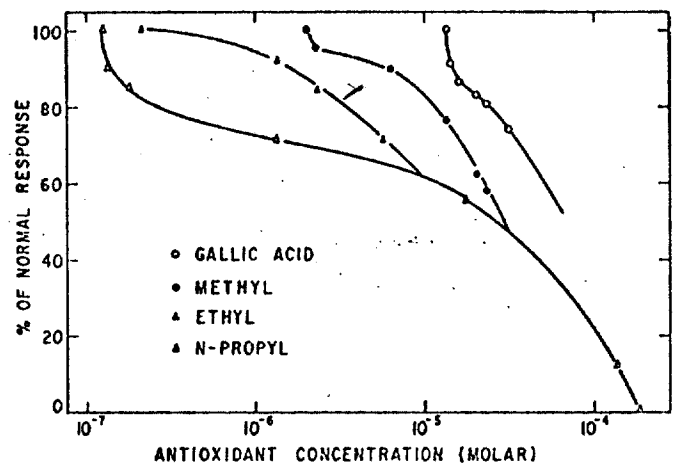

Figure 2. Suppression of ileum response to test solutions of bradykinin containing increasing levels of gallic acid, methyl, ethyl, and *N*-propyl gallate

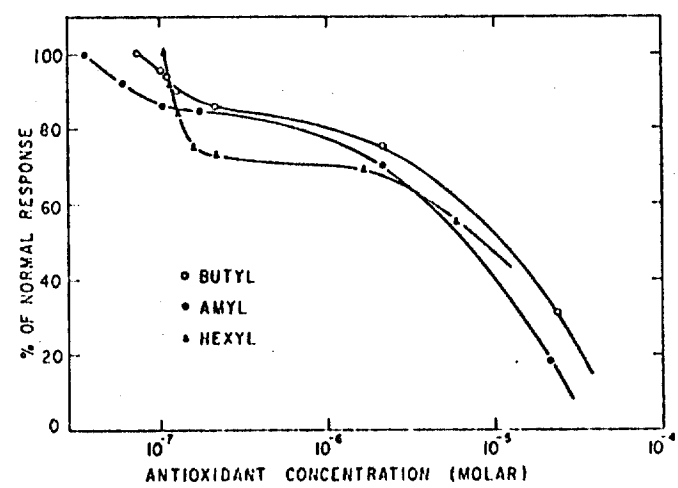

Figure 3. Suppression of ileum response to test solutions of bradykinin containing increasing levels of butyl, amyl, and hexyl gallate

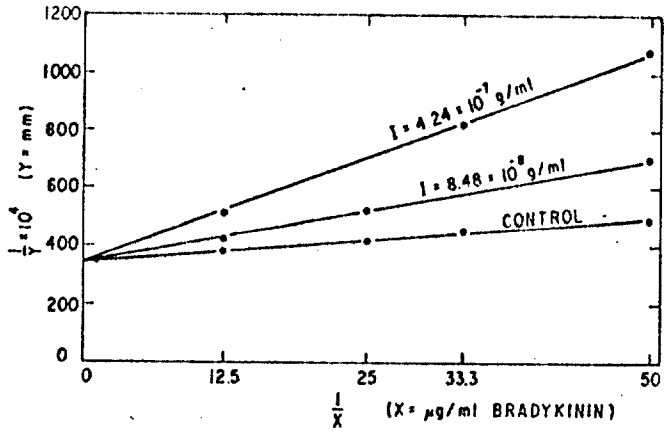

Figure 4. Drug dose (X)-response (Y) plot for N-propyl gallate

I = gallate concentration

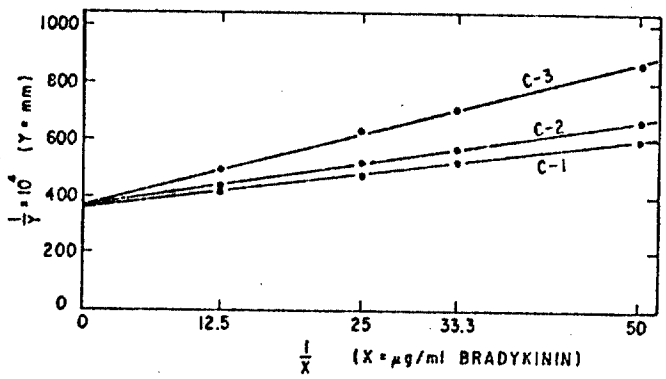

Figure 5. Drug dose (X)-response (Y) plot of control values for bradykinin

Points on line C-1 are original responses
Points on line C-2 are obtained after first series of tests with butyl gallate
Points on line C-3 are obtained after 2nd series of tests with butyl gallate

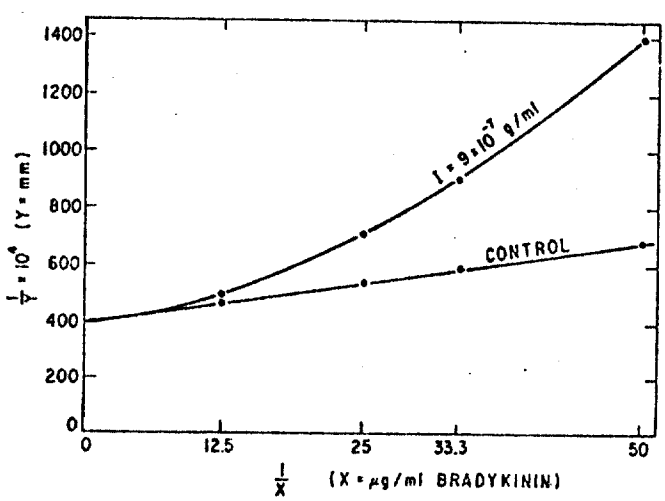

Figure 6. Drug dose (X)-response (Y) plot for butyl gallate

I = gallate concentration

corrected for change in gut response to the kinin standard, and the straight line plots were always obtained.

pK_i values (Table I), calculated from the slopes of the experimental lines, show methyl and ethyl gallate to be relatively poor inhibitors of bradykinin. The other antioxidants are relatively strong inhibitors.

Table I. pK_i Values (w/v) of Gallate Esters

Gallate Ester	pK_i(w/v)
Methyl	4.12
Ethyl	6.00
N-Propyl	7.10
iso-Propyl	7.06
Butyl	7.07
Amyl	7.02
Hexyl	6.80

The decrease in smooth muscle sensitivity effected by butyl, amyl, and hexyl gallate is only partially reversible (Figure 5). Control values for all bradykinin doses are decreased by repetition of doses of these esters. The time for recovery to a stable response, after addition of samples, lengthens with their number and concentration. Once this irreversible effect sets in, control responses do not return to normal.

When high concentrations of all the antioxidants are employed, the straight line relationships between the reciprocals of doses and responses are not obtained (Figure 6). Deviation from linearity is most conspicuous with low kinin doses, and occurs with concentrations of gallate sufficient to more than double the slope of the control line. This effect is most noticeable beyond the propyl derivative.

The inhibitory action of the antioxidants studied is complex. From the graphical data, the esters appear to exhibit competitive drug antagonism. However, it is not a classical competitive antagonism whereby small changes in antioxidant concentration produce marked changes in the slopes of the reciprocal dose effect curves. Large changes in antioxidant concentration are required to turn the angle of the line with inhibitor to produce any significant changes in β.

The inhibitory effects of the methyl, ethyl, and propyl derivatives of gallic acid are largely reversible. Irreversible changes occur in the gut tissue when exposed to the higher members of this series. These higher gallates may decrease gut sensitivity by binding strongly or irreversibly to sites on or near those utilized by the kinin. It is doubtful that the higher homologs change bradykinin sensitivity by destroying the general cell function, because full response of gut tissue to histamine occurs in the presence of all the antioxidants tested up to 5×10^{-4} M concentration.

We are aware of the fact that these antioxidants are slowly oxidized in distilled water and more rapidly oxidized in basic solutions producing detectable spectral changes. Fresh stock solutions of these compounds were therefore prepared every several days and kept refrigerated to insure their stability.

To determine whether there was any chemical interaction between the antioxidants and bradykinin prior to addition of the mixture into the cell, the effect of the sequence of the addition was examined. No differences in inhibitory action were noted between injection of the antioxidants followed by injection of bradykinin and injection of the mixture of the two materials. In one experiment a low concentration of methyl gallate was introduced into the Tyrode buffer supply and doses of bradykinin were injected into the bath. The results were the same as when the mixture of the two compounds were simultaneously injected into the bath containing only Tyrode buffer.

The observed inhibition may result from the presence of phenolic groups in the molecule which give rise to directed binding. The reducing capacity of the molecules is probably not involved, as ascorbic acid, another antioxidant, does not

inhibit smooth muscle contraction produced by bradykinin. The contractile action of this physiologically active peptide is definitely inhibited by gallic acid and six of its homologous esters, one of which is an important food grade antioxidant. The significance of these studies in terms of the usage of gallates for food preservation remains unknown.

ACKNOWLEDGMENT

The authors thank D. G. Cornell for the samples of gallate esters along with data regarding their purity, and W. J. Stein for the supply of guinea pigs.

LITERATURE CITED

Garcia Leme, J., Roche e Silva, M., *Brit. J. Pharmacol. Chemother.* **25**, 50 (1965).

Roche e Silva, M., *Arch. Int. Pharmacodyn. Ther.* **118**, 74 (1959).

Roche e Silva, M., Beraldo, W. T., Rosenfeld, G., *Amer. J. Physiol.* **156**, 261 (1949).

Roche e Silva, M., Garcia Leme, J., Proc. Intern. Pharmacol. Meeting, 2nd, Prague, 1963, **9**, 33 (1964).

Russell, A., Tebbens, W. G., Jr., *J. Amer. Chem. Soc.* **64**, 2274 (1942).

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FOOD ADDITIVES

Application of Thin-Layer Chromatography to the Quantitative Estimation of Antioxidants: BHA, BHT, PG, and NDGA

By MADHU R. SAHASRABUDHE (Food and Drug Directorate, Tunney's Pasture, Ottawa, Ontario, Canada)

A procedure is described for the quantitative estimation of BHA, BHT, PG, and NDGA in lard. The antioxidants are extracted from *n*-hexane solutions of the fat with 80% ethyl alcohol and acetonitrile, separated by two-dimensional thin-layer chromatography using benzene and acetonitrile, and estimated quantitatively by colorimetric methods after extraction of the spots. A special flask for concentration and a suction tube assembly for spot pickup are described. Recoveries of the antioxidants from lard were in the following ranges: BHA, 98–101%; BHT, 96–101%; PG, 90–101%; NDGA, 82–98%. The advantages of the method over others in use are that (1) all four antioxidants can be estimated in one aliquot of fat, (2) PG and NDGA can be estimated when present together in mixtures, and (3) the isomers of BHA can be effectively separated.

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and nordihydroguaiaretic acid (NDGA) are the four antioxidants most commonly used in foods. Anglin, Mahon, and Chapman (1) described methods which have been widely used for quantitative estimation of the above antioxidants in mixtures. In their method, PG and NDGA were extracted with 72% ethyl alcohol from fat dissolved in petroleum ether or carbon tetrachloride, while BHA and BHT were steam distilled. The only limitation of the method was that PG and NDGA could not be estimated in mixtures.

Lundberg and Halvorson (2) reported that a number of antioxidants including BHA, PG, and NDGA could be quantitatively recovered from fat by extraction with 80% ethyl alcohol. Mahon and Chapman (3) found 72% alcohol more suitable for the extraction of BHA, PG, and NDGA since it excludes tocopherol. Under these conditions BHT is only partially extracted. Our preliminary experiments indicated that acetonitrile effectively removed BHT from a glyceride oil in *n*-hexane solutions.

Qualitative separation of the antioxidants by thin-layer chromatography (TLC) has been used successfully for pure antioxidant mixtures (4, 5). However, lipid materials associated with antioxidants interfere in the determination. Quantitative estimation by TLC depends upon (1) effective extraction of the antioxidants from the fat, (2) separation of the antioxidants by TLC, and (3) quantitative recovery from TLC plates.

The present paper describes a method for determination of BHA, BHT, propyl gallate, and NDGA in all combinations.

METHODS

Apparatus

As TLC requires concentrated solutions for effective application, it is desirable to evaporate the solvent. A special evaporation flask was devised for the purpose (Fig. 1 A).

For the recovery of the antioxidants from TLC plates a suction tube assembly was devised (Fig. 1 B).

Reagents

(a) *Silica Gel-G.*—(According to Stahl.)

(b) *Benzene.*—Reagent grade.

(c) *Acetonitrile.*—Freshly distilled.

(d) *2,6-Dichloroquinonechloroimide* (CQC) —0.1% in absolute ethyl alcohol spray reagent.

(e) *Ethyl alcohol.*—Distill commercial absolute alcohol in an all-glass apparatus with 0.1% KOH and 0.1% $KMnO_4$. Take the distillate as 100% and dilute to 80%, 50%, and 25% (v/v).

(f) *Ferrous sulfate.*—0.04% $FeSO_4.7H_2O$ in distilled water. (Prepare daily.)

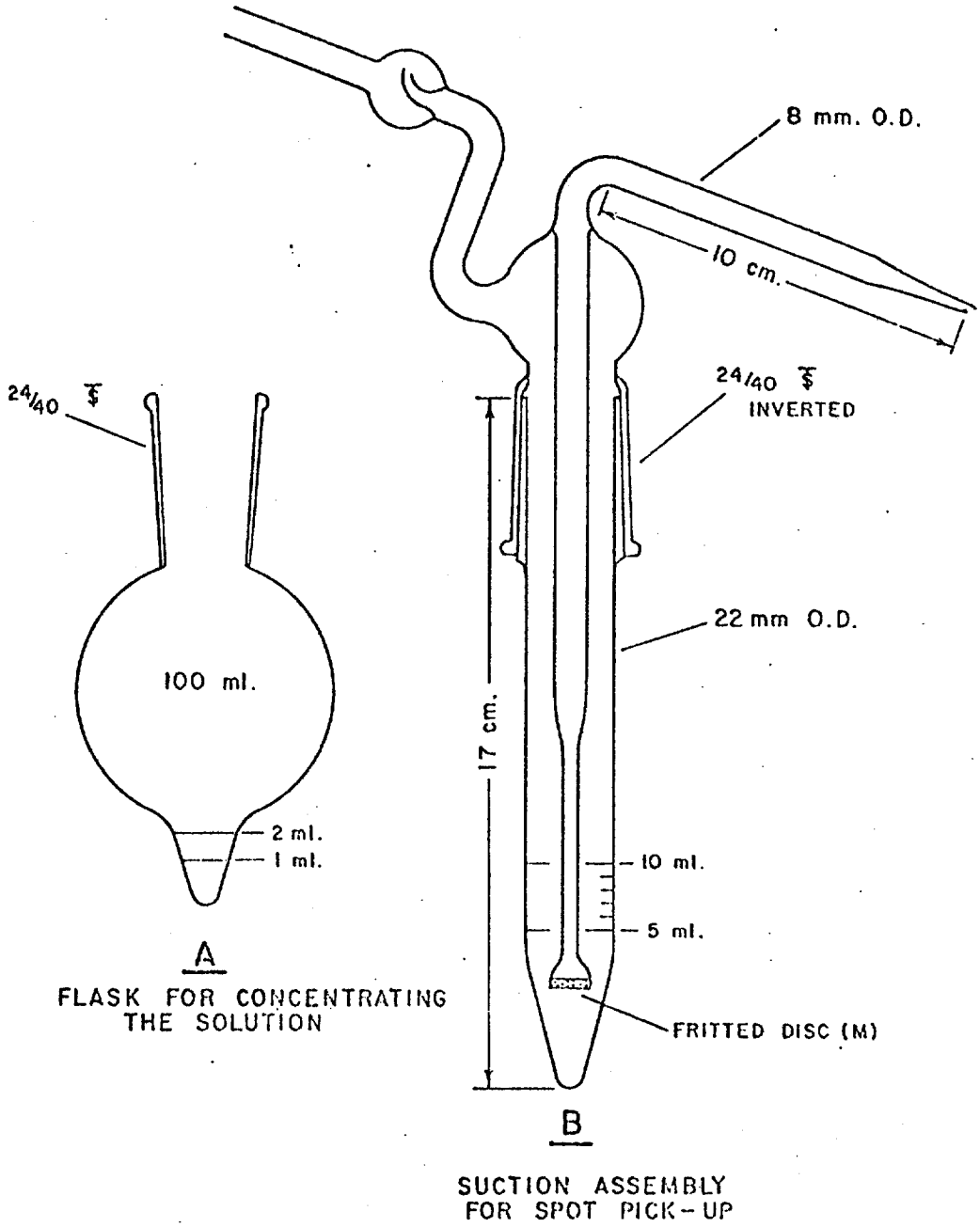

Fig. 1—A, evaporation flask for concentration of the antioxidant solutions. B, suction assembly for spot pickup.

(g) *Ferric chloride.*—0.2% $FeCl_3.6H_2O$ in distilled water.

(h) *Sodium carbonate-bicarbonate buffer.*—5.3% anhydrous sodium carbonate and 4.2% sodium bicarbonate in distilled water.

(i) *2,2'-Bipyridine reagent (BiP).*—Dissolve 200 mg 2,2'-bipyridine in 1 ml ethyl alcohol and dilute to 100 ml with distilled water. If 2,2'-bipyridine is discolored and sticky, it should be purified as described by Mahon and Chapman (3).

(j) *2,6-Dichloroquinonechloroimide (CQC) reagent.*—Dissolve 10 mg CQC in 100 ml ethyl alcohol. Prepare fresh daily.

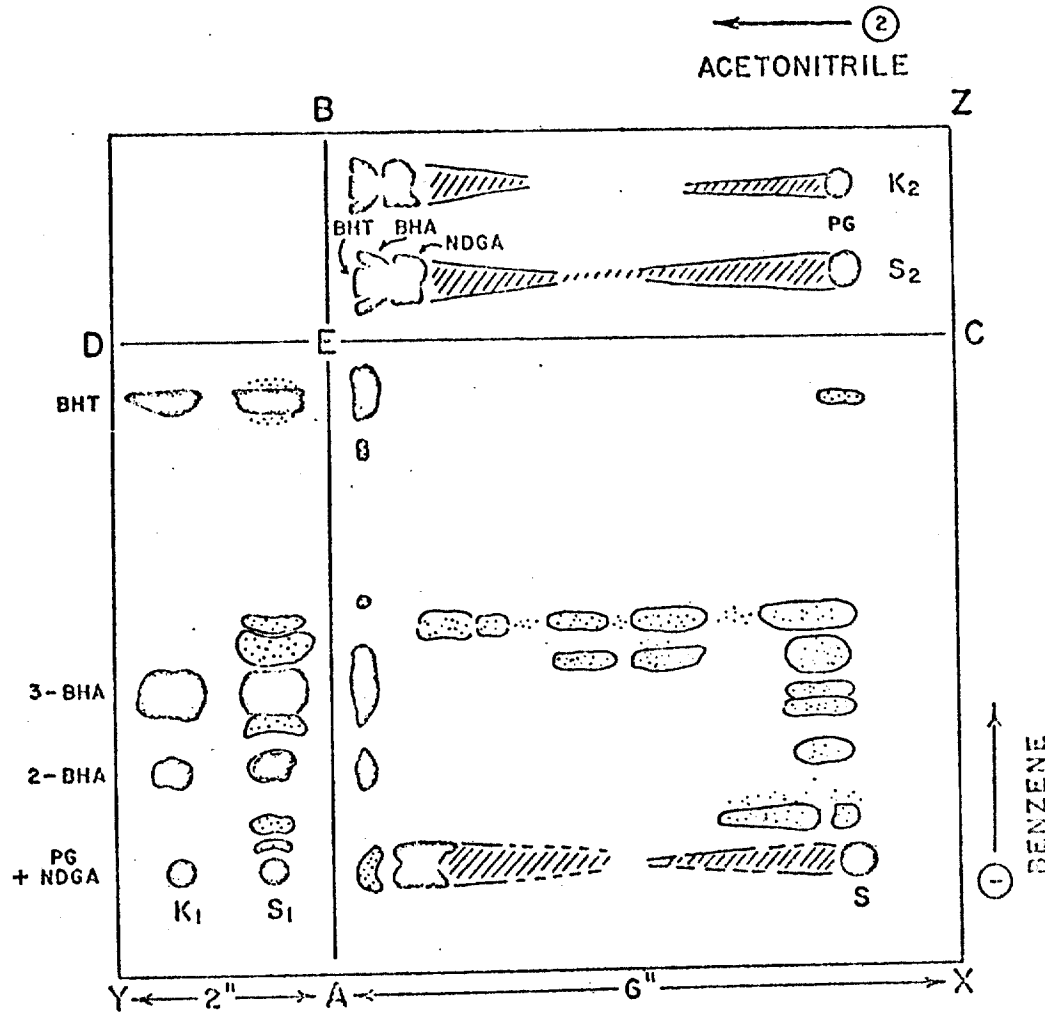

Fig. 2—Diagram of a TLC plate drawn from an actual tracing. Solid black spots are the antioxidants. Streaks obtained with PG and NDGA are shown as shaded areas. Dotted spots and areas represent lipid material. These spots become visible in iodine vapor. In the method, BHA and BHT spots in the area XAE are not visible. On spraying with CQC the antioxidants are seen as different colored spots, viz., BHA, blue; BHT, yellow; NDGA, purple; and PG, greyish purple.

(k) *Borax buffer.*—2% $Na_2B_4O_7.10\ H_2O$ in distilled water.

(l) *Ammonium acetate.*—2.5% in distilled water.

Determination

Extraction of antioxidants.—Dissolve 10 g lard in 100 ml *n*-hexane in a separatory funnel; extract with four 25 ml portions of 80% ethyl alcohol by inverting the funnel 5 times for each extraction. Then, extract with eight 25 ml portions of acetonitrile in the same manner. After each extraction, transfer the clear lower layer to the evaporation flask (Fig. 1 A) and remove the solvent on a flash evaporator. During evaporation the flask is partially immersed in a water bath maintained at 40 ± 2°C.

When all the solvent is removed, dissolve the residue carefully in a few drops of absolute ethyl alcohol. Care should be taken to wash the sides of the flask. Bring final volume to 2 ml in the graduated projection of the flask.

Thin-layer chromatography.—Any equipment for the preparation of 8 × 8" TLC plates coated with Silica Gel-G (250 μ) is suitable.

Mark off the glass plates which are coated with Silica Gel-G as shown in Fig. 2. Apply 20, 40, and 60 μl of the sample on separate plates at S and S_1. A 50 μl syringe is suitable. Spot a standard mixture of the antioxidants on each of these plates at K_1 and develop

Table 1. Outline of the procedure for colorimetric determinations of antioxidants

Procedure	3-BHA	2-BHA	BHT	PG	NDGA
1. Place 5 ml solvent in suction tube	50% alcohol	50% alcohol	100% alcohol	50% alcohol	25% alcohol
2. Wash tube with 2 ml solvent	50% alcohol	50% alcohol	25% alcohol	2.5% NH_4 acetate	25% alcohol
3. Make up volume to 10 ml	50% alcohol	50% alcohol	water	2.5% NH_4 acetate	25% alcohol
4. Add reagents to develop color	2 ml Borax 2 ml CQC	2 ml BiP 2 ml $FeCl_3$	2 ml BiP 2 ml $FeCl_3$	1 ml $FeSO_4$	1 ml $FeSO_4$ 1 ml carb.-bicarb. buffer
5. Reaction time	15 min.	30 min.	30 min. IN DARK	10 min.	10 min.
6. Add *n*-butanol	5 ml	5 ml	5 ml	—	—
7. Read absorbance on a suitable spectrophotometer or colorimeter	620 mμ	515 mμ	522 mμ	515 mμ	515 mμ

benzene until the solvent front is about 2 cm from the line CD. After air drying, apply 50 μl sample and standard mixture at S_2 and K_2, respectively. Develop the plates in acetonitrile and again air dry. Cover the area XAEC with paper and spray the exposed surfaces, AEDY and CEBZ, with CQC reagent. Locate the antioxidant spots in the area XAEC with reference to the standards.

Recovery of antioxidants from the TLC plate.—Place 5 ml appropriate solvent (Table 1) in the tube (Fig. 1 B) and apply suction. By properly positioning the tip end, suck the silicic acid layer into the inner tube. To obtain a consistent area of the silicic acid layer, cut a plastic sheet into rings with an inner diameter of 1". Place the ring on the plate to enclose the area of the spot and pick up all silicic acid inside the area. Immerse the tip in 2 ml of the appropriate solvent to wash the silicic acid onto the fritted disc. Dissolve the antioxidant by applying mild suction alternately on the tip and suction ends. (Five alternate applications of suction were found to be sufficient.) Remove the inner tube and treat the antioxidant solution with appropriate reagents for *Colorimetric analysis*. Rinse the inner tube carefully with up to 1 ml of appropriate solvent.

Colorimetric analysis.—The methods were essentially the same as described by Anglin, Mahon, and Chapman (1). A Beckman DU spectrophotometer was used in this study. The analytical procedure is outlined in Table 1.

Calculations.—Standard *K* values (absorbance per microgram of the antioxidant): *K* values for each antioxidant were calculated by spotting 20, 40, and 60 μg of the individual antioxidant on a Silica Gel-G plate. The spots were extracted and developed in the manner described earlier. This was considered necessary, as some of the reactions involved are influenced by the pH of the medium (6). However, recoveries of the antioxidants from the plate as compared to direct estimation were in the range of 98–100%. The respective concentrations of the antioxidant in the aliquot of fat used are obtained by dividing the observed absorbance by the *K* value.

Example (BHA):

Sample (fat): 10 g
Final volume in evaporation flask: 2 ml
Spot application (TLC): 0.05 ml

Absorbance (BHA) 0.108
K BHA 620: 0.0013

$$\% \text{ BHA} = \frac{0.108 \times 2 \times 100}{0.0013 \times 0.05 \times 10 \times 10^6} = 0.01001$$

Table 2. Recovery of antioxidants

Antioxidant Added to Lard, %		Antioxidant Found, %	Recovery, %
Individual			
BHA[a]	0.005	0.0044 (3-BHA) + 0.0006 (2-BHA)	100
	0.010	0.0091 (3-BHA) + 0.0010 (2-BHA)	101
	0.020	0.0180 (3-BHA) + 0.0017 (2-BHA)	98
3-BHA	0.010	0.0102	102
	0.020	0.0196	98
2-BHA	0.010	0.0098	98
	0.020	0.0200	100
BHT	0.005	0.0048	96
	0.010	0.0101	101
	0.020	0.0197	98
PG[b]	0.005	0.0051	102
	0.010	0.0090	90
NDGA[b]	0.005	0.0040 (+0.0010)[c]	100
	0.010	0.0082 (+0.0013)[c]	95
Mixtures			
1. PG	0.010	0.0092 (+0.0006)[c]	98
NDGA	0.010	0.0086 (+0.0011)[c]	97
2. 3-BHA	0.010	0.0101	101
2-BHA	0.010	0.0098	98
3. BHA[a]	0.007	0.0063 (3-BHA) + 0.006 (2-BHA)	98
BHT	0.007	0.0068	97
PG[b]	0.005	0.0047	94
NDGA[b]	0.005	0.0048	96
4. BHA[a]	0.010	0.0089 (3-BHA) + 0.0011 (2-BHA)	101
		0.0088 (3-BHA) + 0.0010 (2-BHA)	99
		0.0090 (3-BHA) + 0.0010 (2-BHA)	100
BHT	0.010	0.0098	98
		0.0097	97
		0.0099	99
PG[b]	0.007	0.0069	98
		0.0068	97
		0.0069	98
NDGA[b]	0.007	0.0068	97
		0.0067	96
		0.0068	97

[a] Commercial BHA is a mixture of 3-BHA and 2-BHA. Each isomer is determined separately.
[b] PG and NDGA often streak on TLC. These spots and streaks are visible on the plate without spraying and can be effectively picked up.
[c] Figures in parentheses account for the streaks obtained with PG and NDGA.

Results and Discussion

The four antioxidants were added to 100 g samples of antioxidant-free lard, individually as well as in mixtures. The amounts used, shown in Table 2, were in the range permitted in fats and oils. Results of analysis show recoveries of BHA and BHT in the ranges 98–101% and 96–102%, respectively. PG and NDGA gave streaks, obviously because of the impurities associated with the antioxidants. The spots and streaks of these antioxidants are visible without spraying and the entire area can be picked up effectively to give recoveries up to 98%. Reproducibility of the method was tested and found satisfactory by applying a mixture of the four antioxidants (Mixture 1, Table 2) on three separate plates.

Commercial BHA is a mixture of 3-*tert*-butyl-4-hydroxyanisole (3-BHA) and 2-*tert*-butyl-4-hydroxyanisole (2-BHA) and may contain from 5 to 37% of 2-BHA. 3-BHA is more effective as an antioxidant than 2-BHA (7, 8). Chapman and Mahon reported a method for the estimation of isomers in commercial BHA (9) based on relative absorbances of the mixture obtained with 2,6-dichloroquinonechloroimide-borax reagent and ferric chloride-bipyridine reagent. The proportions of the two were calculated by difference. In the present investigation it was found that the isomers can be quantitatively separated by thin-layer chromatography. BHA used in this study contained 10.5% 2-BHA (Table 2).

The advantages of the above method are that (*1*) all four antioxidants can be estimated in one aliquot of the fat, (*2*) PG and NDGA can be estimated when present together in mixtures, and (*3*) the isomers of BHA can be effectively separated. The data demonstrate that this is a decided improvement over accepted methods of analysis of antioxidants.

Acknowledgments

The author wishes to thank Robert Chambers for his technical assistance in the earlier stages of the study.

The evaporation flask and the suction assembly were fabricated in the Food and Drug Laboratory by R. G. Campbell.

BHA, BHT, and PG used in the study were obtained from Eastman Chemical Products, Inc.; NDGA was a commercial product of Wm. Stange & Co.

References

(1) Anglin, C., Mahon, J. H., and Chapman, R. A., *J. Agr. Food Chem.*, 4, 1018 (1956).
(2) Lundberg, W. O., and Halvorson, H. O., *Proc. Inst. Food Technologists*, p. 115 (1945).
(3) Mahon, J. H., and Chapman, R. A., *Anal. Chem.*, 23, 1116 (1951).
(4) Scher, A., *Fette, Seifen, Anstrichmittel*, 61, 345 (1959); *Nahrung*, 4, 466 (1960); *Michrochim. Acta*, 303 (1961).
(5) Conroy, H. W., and Barnes, R., Private Communication, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, 1961.
(6) Mahon, J. H., and Chapman, R. A., *Anal. Chem.*, 23, 1120 (1951).
(7) Dugan, L. R., Jr., Hoffert, E., Blumer, G. P., Dabkiewicz, I., and Kraybill, H. R., *J. Am. Oil Chemists' Soc.*, 28, 493 (1951).
(8) Rosenwald, R. H., and Chenicek, J. A., *ibid.*, 28, 185 (1951).
(9) Mahon, J. H., and Chapman, R. A., *Anal. Chem.*, 241, 531 (1952).

OILS, FATS, AND WAXES

Detection of PG, NDGA, BHT, and BHA in Fats and Oils by Thin Layer Chromatography

By SHIRLEY A. SCHEIDT and HARRY W. CONROY (Food and Drug Administration, 1009 Cherry St., Kansas City, Mo. 64106)

PG, NDGA, BHT, and BHA were extracted with acetonitrile from fat or oil, followed by thin layer chromatographic separation and identification. The four antioxidants were distinctly separated from one another by one-dimensional solvent development of the silica gel adsorbent layer. The chromatography required < 1 hour from spotting to visualization with 2,6-dichloroquinone chlorimide. As little as 1 μg of BHT and 0.5 μg of PG, NDGA, and BHA were detected by this method.

Since mixtures of antioxidants are widely used as preservatives in fats and oils, a rapid method is needed for their extraction, separation, and identification. This study was limited to four common antioxidants; propyl gallate (PG), nordihydroguaiaretic acid (NDGA), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA).

A simple extraction of fat or oil to prepare the antioxidants for thin layer chromatography was sought. Lundberg and Halvorson (1) extracted several antioxidants from fat with 80% ethyl alcohol, but BHT was not removed. Schwien and Conroy (2) and Sahasrabadhe (3) extracted BHT from fat with acetonitrile. We found that acetonitrile extracted all four antioxidants from fat or oil dissolved in petroleum ether and that the extract could be used for thin layer chromatography without further purification.

Thin layer chromatography was used for simultaneous separation and identification. Several thin layer methods have been described but none has completely separated PG, NDGA, BHT, and BHA. Seher (4) used two-dimensional thin layer chromatography to separate 12 antioxidants but could not separate PG and NDGA. The two-dimensional method of Sahasrabadhe (3) separated BHT, BHA, PG, and NDGA. However, our laboratory was repeatedly unable to separate PG and NDGA by this method. Conroy and Barnes (5) could not separate PG and NDGA by one-dimensional thin layer chromatography because the antioxidants streaked together on the chromatogram. Salo, Mackinen, and Salminen (6) suggested that PG and NDGA formed a "kritisches Paar" which could not be separated by thin layer chromatography.

Copius-Peereboom (7) used polyamide as an adsorbent, rather than the usual silica gel, and cleanly separated PG and NDGA; BHA, BHT, and NDGA were not separated. Meyer (8) mixed silica gel and kieselguhr together to separate seven antioxidants. Even though the thin layer chromatogram was developed twice in the same solvent, PG and NDGA were only slightly separated.

This paper describes complete separation and identification of PG, NDGA, BHA, and BHT by one-dimensional chromatography on silica gel. Moreover, the persistent problem of streaking has been eliminated.

Samples for this study were prepared by adding 0.003% BHT, BHA, PG, and NDGA to antioxidant-free lard, tallow, and oil.

METHODS

Reagents and Apparatus

(*Note:* All solvents were reagent grade.)

(a) *Mobile solvent.*—Chloroform + methanol + acetic acid, 90:10:2.

(b) *Chromogenic agent, 2.0%.* — Dissolve 2,6-dichloroquinone chlorimide in absolute ethyl alcohol.

(c) *Adsorbent.* — MN-Silica gel G-HR. (Brinkmann Instruments, Inc., Westbury, N.Y.).

(d) *Antioxidants.*—BHT and BHA (Eastman Chemical Products). *n*-Propyl gallate, practical grade (Matheson, Coleman and

Bell). NDGA (nordihydroguaiaretic acid) (Nordigard Corp.). Dissolve 10 mg of each in 10 ml acetone.

(e) *Thin layer applicator.*—To coat plates 0.25 mm thick (Desaga-Brinkmann, Model 3-11, or equivalent).

(f) *Solvent trough.*—About 10" long and deep enough to hold 0.5 cm solvent.

(g) *Developing tank.*—Glass or metal with tight closure for holding 8" plate. Line all sides with blotting paper.

Determination

Extraction.—Add 100 ml petroleum ether (30–60°) to 15 g melted fat or oil and transfer to 250 ml separator. Extract with four 25 ml portions of acetonitrile saturated with petroleum ether. Combine acetonitrile extracts in 250 ml beaker and evaporate to 2–4 ml on steam bath with aid of air current. Transfer extract to 5 ml volumetric flask and dilute to volume with acetone. Use this solution for spotting thin layer plates. (Appropriate dilutions can be made for antioxidants present in concentrations higher than 0.003%.)

Preparation of chromatographic plates.—Mix 30 g MN-silica gel G-HR (c) with 60 ml H_2O to form slurry for five 8×8" plates. Apply layer 0.25 mm thick. Let plates set at room temperature for at least 15 min. Activate plates at 100°C for 30 min. in forced draft oven, cool to room temperature, and store in desiccator.

Preparation of developing tank.—About 30 min. before use, pour mobile solvent (a) into solvent trough to a depth of 0.5 cm. Also, pour mobile solvent (a) on floor of developing tank and place blotting paper in contact with solvent pool.

Spotting and development of thin layer plate.—Spot (microliter pipet) ca 2 μg of each antioxidant (d) and sample 1 cm apart and 1" from bottom of plate. Develop plate in tank trough, letting solvent front move 10 cm from spotting line. Remove plate and let solvent evaporate at room temperature. (If greater separation between PG and NDGA is desired, return plate to tank and let solvent front move in same direction to 15 cm from spotting line. Remove plate and let solvent evaporate.) Spray with chromogenic agent (b), using a fine mist atomizer. Color development takes ca 5 minutes and may be hastened to ca 30 seconds by carefully exposing the sprayed plate to ammonia vapor. PG appeared as a greenish-gray spot, NDGA gave a red spot, BHT appeared as a yellow spot, and BHA was an orange spot which changed to purple on exposure to ammonia.

Table 1. R_f values of four antioxidants

Antioxidant	Single Solvent Development	Two Solvent Development
NDGA	0.26	0.37
PG	0.32	0.45
BHA	0.74	0.84
BHT	0.90	0.94

Results and Recommendation

Typical R_f values of PG, NDGA, BHT and BHA are given in Table 1. The thin layer chromatogram was developed either to 10 cm, or to 10 cm and then redeveloped to 15 cm.

When silica gel G (Merck) was used, PG formed a brown spot as it was applied to the adsorbent layer and the spot was streaked upon development. However, PG neither formed a brown spot when it was applied to the adsorbent layer nor streaked when it was developed, if the iron in the silica gel was complexed either by prewashing the layer with 2% citric acid or by adding various amounts of citric acid to the slurry. Neither method of treating the adsorbent layer with citric acid aided the separation of PG from NDGA.

When MN-silica gel G-HR (Fe content less than 0.001%) was used, PG did not form a brown spot, did not streak, and was completely separated from NDGA. A slight tail on the PG and NDGA spots was eliminated by adding 2% acetic acid to the mobile solvent, as suggested by Tabbitt (9). Less than one hour was required from spotting to visualization. As little as 1 μg of BHT and 0.5 μg of PG, NDGA and BHA were detected.

This method has made possible a quick extraction, separation, and identification of PG, NDGA, BHT, and BHA with minimal manipulations.

It is recommended that the thin layer

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chromatographic method described be studied collaboratively for the quantitative determination of antioxidants.

Acknowledgments

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REFERENCES

(1) Lundberg, W. O., and Halvorson, H. O., *Proc. Inst. Food Technologists*, 1945, pp. 115–125.
(2) Schwien, W. G., and Conroy, H. W., *This Journal*, 48, 489–492 (1965).
(3) Sahasrabudhe, M. R., *ibid.*, 47, 888–893 (1964).
(4) Scher, A., *Fette Seifen Anstrichmittel*, 61, 345–351 (1959).
(5) Conroy, H. W., and Barnes, R., Food and Drug Administration, private communication, 1961.
(6) Salo, T., Mackinen, R., and Salminen, K., *Z. Lebensm. Untersuch.-Forsch.*, 125, 450–453 (1965).
(7) Copius-Peereboom, J. W., *Nature*, 204, 748–750 (1964).
(8) Meyer, H., *Deut. Lebensm. Rundschaw.*, 57, 170–175 (1961).
(9) Bobbitt, J. M., *Thin-Layer Chromatography*, Reinhold Publishing Corp., New York, 1963, p. 65.

The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee C, and was accepted by the Association. See *This Journal*, 49, 167–172 (1966).

Extraction, Cleanup, and Gas Chromatographic Determination of BHA and BHT in Fats and Oils

By WILLIAM G. SCHWIEN, BOB J. MILLER, and HARRY W. CONROY (Food and Drug Administration, 1009 Cherry St., Kansas City, Mo. 64106)

In preparing samples for analysis of antioxidants in fats and oils, melted fat and/or oil is mixed with Celite, blended with acetonitrile, and the acetonitrile is extracted with mixed ethers. The mixed ethers are evaporated, and the oily residue is incorporated with alumina acid. The alumina mixture is placed in a chromatographic column and eluted with aqueous methanol, and the methanolic eluate is extracted into mixed ethers. The ether extract is clean enough for TLC as well as GLC determinations. Recoveries from tallow, lard, and corn oil spiked at 16.7 and 66.7 ppm with BHA and BHT ranged from 80 to 95% for BHA and 91 to 115% for BHT.

Current methods for butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in fatty foods include colorimetric determinations from their steam distillates (1, 2) or gas chromatographic procedures by a direct extraction technique (3–5). Colorimetry produces an extract free from fats and oils but requires specialized distilling equipment. Gas chromatography is quite rapid but removes large amounts of fats and oils, along with the antioxidants, which may cause multiple peak interference and column contamination and make any TLC confirmatory testing unworkable. If direct extractions are made on pure fats, residual oil contamination is even more troublesome.

The proposed method involves an acetonitrile extraction and a subsequent cleanup on an alumina acid column. Resulting extracts are clean enough for thin layer chromatography (TLC) as well as gas-liquid chromatography (GLC). The method is rapid and relatively simple, and requires no special equipment for extracting BHA and BHT from fats and oils.

METHOD

Apparatus

(a) *Gas chromatograph.*—Barber-Coleman Model 5000 with an argon ionization detector using a 1 mv strip chart recorder, or equivalent apparatus. A chromatographic column

A Review of Antioxidant Analysis in Food Products

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Abstract

To permit proper usage of antioxidants by the food processor and permit control under applicable governmental regulations, it is necessary to have adequate analytical methods. Such methods should both positively identify and provide a quantitative assay for the additive. Due to the complex nature of foods, analysis of an antioxidant in the very small quantities used presents a very real problem. Solvent extraction and/or steam distillation techniques are employed to separate the antioxidants from the food. Ultraviolet spectra, gas chromatography and colorimetric techniques are used to identify and determine the quantity of each antioxidant present. Slight variations are necessary for each type of food.

Introduction

THE FOOD PROCESSOR is faced with the problem of using sufficient antioxidant to provide adequate shelf life. He is limited on the amount of antioxidant permitted by various government regulations, by economics, and by the fact that extreme quantities of some antioxidants act as prooxidants. Some antioxidant is often lost or utilized during processing; thus the finished product must be analyzed for antioxidant content rather than depending on the quantity of antioxidant added as a quality control check.

One point should be emphasized. The actual antioxidant content of a food will vary inversely with time lapsed since addition of the antioxidant. Figure 1, taken from Anderson et al. (1), shows this loss of antioxidant during storage of wheat flake cereals.

The complex nature of foods causes a problem in analysis of an antioxidant in the small quantities used. Two general routes are used—one for high-fat foods such as nuts, prepared meat foods, and some baked goods; and another for low fat foods such as potato flakes, rice, and some cereals.

High-Fat Foods

The initial step in the isolation of antioxidants from high-fat foods is Soxhlet extraction with petroleum ether or similar solvents. The extract contains the fats, oils, antioxidants and probably some other products. The high-mol-wt proteins, phospholipids, etc., remain in the Soxhlet cup. Of course, with pure fats such as lard or shortening, extraction is not necessary. Incidentally, extraction is a field which often causes trouble and deserves further investigation.

Anderson et al. (1) have shown a direct correlation between extractable antioxidant and peroxide number of stored samples of wheat flakes, corn flakes and puffed oats. However, experience in the Eastman laboratories shows that antioxidant extraction from high-fat foods often does not correlate with shelf life and particularly peroxide value. The question arises as to whether the extraction removes antioxidant which has migrated to the center of a food particle. In addition, phenolic antioxidants may complex or react with the naturally occurring fats, such as the phospholipids, and are not extracted; thus there the question as to whether or not these "bound" an oxidants still function as such or whether they longer provide protection from oxygen attack.

The extract from a high-fat food can be treated two ways. One procedure is to analyze directly, a this technique will be treated subsequently. By a other method, the solvent is evaporated from t extract and the 3-t-butyl-4-hydroxyanisole (BHA and 3,5-di-t-butyl-4-hydroxytoluene (BHT) remov from the fat by the method of Anglin, Mahon a Chapman (3) which involves steam distillation about 160C. BHA and BHT steam distill, but prop gallate (PG) and nordihydroguaiaretic acid (NDGA either do not steam distill or are destroyed by t high temp. Superheated steam is necessary becaus under normal steam distillation conditions, BHA a BHT distill from this fat system too slowly for pra tical purposes.

Low-Fat Foods

For determination of BHA and BHT in low-fa foods, an initial extraction is not required. The fo is steam-distilled directly by the method of Filip and Ogg (8) which involves a rapid steam distillatio using atmospheric steam. As sodium sulfite is us in the processing of potato flakes, sulfur dioxide mu be removed by means of a magnesium oxide scrubb in analyzing this product because it interferes i subsequent analysis.

The next step is to analyze the extract and/or stea distillate for antioxidant. The following methods a used.

Ultraviolet Spectra

Each phenolic antioxidant has a characteristic UV spectra, but naturally occurring materials absorbi in the UV range interfere with the measurement. A UV method for BHT in lard has been reported (13 The lard is dissolved in cyclohexane and passed ov a silicic acid column. The eluate is analyzed for BH at 240 and 320 mμ. BHA, PG, tocopherols and nord hydroguaiaretic acid do not interfere.

BHT can also be determined in lard by extracti the fat with isooctane and passing the isooctane sol tion over alumina (14). Absorbance measurement a 276 and 283 mμ will determine BHT concentrations a low as 0.001%.

Berger, Sylvester and Haines (4) have develop a method for separating BHA, BHT and gallate este from other fat constituents by column chromato raphy over Silastic 181, a synthetic rubber. BHT determined in the UV region by measuring the dr in optical density at 306 mμ when the eluate is ma basic. BHA and the gallates do not interfere wi this change in absorbance, and are determined b colorimetric methods discussed below.

2,4,5-Trihydroxybutyrophenone (THBP) can be d termined in fats and oils by dissolving the samp in aqueous isopropyl alcohol and passing the soluti over alumina (7). The THBP is retained on t column while interfering substances are eluted. T THBP is removed from the alumina by treating wi

hydrochloric acid and is then dissolved in isoamyl alcohol. The amount of THBP is determined by measuring the absorption at 350 mμ.

The methods described above all depend on separation of the antioxidant by column chromatography which involves a fairly tedious elution and the handling of large volumes of solvent. Since other antioxidants are strongly adsorbed on the columns, the method is in general limited to the much less strongly adsorbed BHT. THBP is so firmly adsorbed by the column packing that interfering materials can be eluted without moving the THBP so that separation is achieved. Any other alkyl substituted phenol which is eluted with BHT would give a positive interference with the BHT analysis. Because of the tedious elution and the possibility of interferences, BHT in general is not analyzed in foods by this method in the Eastman laboratories.

However, since the method is extremely sensitive, it can be very valuable for routine analysis of BHT in a given food when it can be shown that no interferences exist by carrying out the analysis on the food with no antioxidant present and where the elution apparatus can be set up on a permanent basis.

Colorimetric Methods

Currently, the most widely used analytical procedures involve colorimetric methods. Ideally, a food extract, steam-distillate or fat solution should be treated directly with reagents to give noninterfering colors specific for each antioxidant. In practice, of course, such is not the case. Generally used methods for the various antioxidants are as follows:

BHA

2,6-Dichloroquinonechloromide Method (3, 8). The steam distillate, from either the Anglin, Mahon and Chapman or the Filipic and Ogg methods, as a 50% ethanol solution is treated with 2,6-Dichloroquinonechloromide [Gibbs reagent (9)] and with borax buffer to give a pH of 9.4. A blue compound is formed, and the amount is analyzed by measurement of absorption at 620 mμ. This reaction is specific for BHA in the presence of BHT because the reactive sites ortho and para to the phenolic hydroxy group in BHT are blocked. In a fat containing only BHA, the BHA can be extracted directly with 70% ethanol and the extract analyzed colorimetrically with ferric chloride-2,2′-bipyridine (11) or Gibbs reagent (12). It has been found in the Eastman laboratories, however, that small amts of naturally occurring material, presumably phenols, present in commercial lards and other pure fats give positive results with these reagents. In our laboratories therefore, the Anglin, Mahon and Chapman steam distillation method is always used for these products.

Sulfanilic Acid Method. Lazlo and Dugan (10) have described the use of the color produced by reaction of BHA and diazotized sulfanilic acid in basic solution as a quantitative analysis method. BHT also reacts with the reagent but so slowly that little interference is encountered if measurements are made within 5–10 minutes after adding the reagent. PG inhibits the color development, so must be removed prior to analysis by extraction with ammonium acetate solution.

BHT

Ferric Chloride—2,2′-Bipyridine Method (3, 8). The steam distillate, from either the Anglin, Mahon and Chapman or Filipic and Ogg methods, as 50% alcohol solutions is treated with ferric chloride—2,2′-bipyridine reagent, and the absorbance determined at 515 mμ. The result is a total of the BHA and the BHT concns. Subtraction of the BHA contribution as determined by Gibbs reagent gives the amount of BHT. The reaction of BHT with the ferric chloride is not complete within the normal analysis time; thus controlling the time of color development is very important. As a further complication, BHA accelerates the BHT reaction rate. However, addition of *n*-butyl alcohol to the reaction mixtures just prior to spectrophotometric measurement inhibits the color reaction and results in reproducible results.

3,3-Dimethoxybenzidine (Dianisidine) Method. Szalkowski and Garber (17) developed a specific method for determining BHT based on its reaction with dianisidine and nitrous acid. Of antioxidants, only ethoxyquin interfered enough to give an error of greater than 3%. The procedure involves a modified Anglin, Mahon and Chapman steam distillation followed by adjustment of the distillate to give a 50% methanol solution. The dianisidine-nitrous acid is added, the colored product is extracted with chloroform, and the absorbance is measured at 520 mμ.

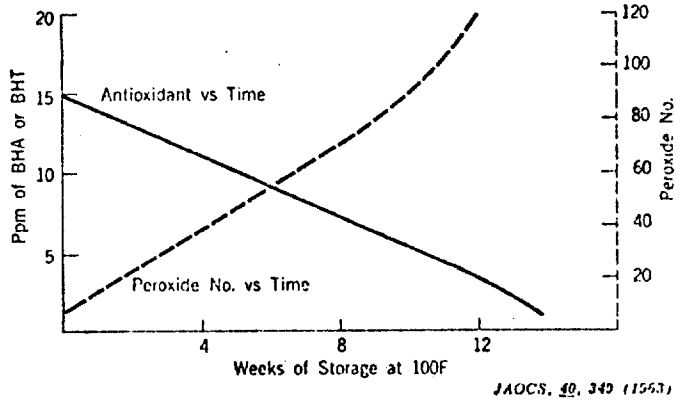
Sloman, Romagnoli and Cavagnol (16) have combined the Gibbs' reagent method for BHA and the dianisidine method for BHT in an over-all scheme for analysis of these two antioxidants in food products.

Propyl Gallate (PG)

PG does not steam distill; thus its analysis must be conducted on an extract.

Ferrous Ion Method (8). The fat is dissolved in carbon tetrachloride and extracted with 50% aqueous ethyl alcohol. The extract is diluted with water to give 25% ethyl alcohol; the solution is buffered at pH 10 with sodium carbonate-bicarbonate and ferrous sulfate is added. The absorbance of the purple solution is measured at 515 mμ. NDGA interferes with this analysis, but NDGA and PG have similar antioxidant properties and are not normally used together.

In the United Kingdom, propyl, octyl and dodecyl gallates are all used. Vos, Wessels and Six (18) developed a scheme for their analysis using the ferric ion reagent. By this method, PG is selectively extracted with water from a petroleum ether solution of the fat. The amt of PG is determined by adding ferrous tartrate at a pH of 7.0–7.6 and measuring the optical density at 530 mμ. The higher gallates are extracted with methanol. After addition of ferrous



JAOCS, 40, 349 (1963)

Fig. 1. Wheat flake cereals to which 15 ppm of each BHA and BHT were added. Stored at 100F in packages with double glassine paper liners.

TABLE I

Chromatographic Columns for BHA/BHT Separation[a]

Column[c]	Approximate Retention Times, Min[b]			Column Temp
	BHA	BHT	Di-BHA	
2 ft 33% Carbowax 20M	10.5	4.5	7	200—280C[d] @ 5C/min.
4 ft 15% Oxiron 2000	9.5	5	7.5	200—260C[d] @ 10C/min.
4 ft 15% Tween 61	14	6.5	11	200—225C[d] @ 5C/min.
4 ft 5% Tween 61, 25% Silicone SE-30[e]	10.5	8	14	215C
4 ft 20% Silicone-Fluoro-FS-1265-QF-1	7.5	6	9.5	125—180C[d] @ 10C/min.

[a] Instrumental conditions: Sample size 100 μl; Helium flow 60 ml/min; Injection port temp 325C; Detector temp 290C; Detector current 200 ma; Sensitivity XI

[b] The exact retention time will vary somewhat with different preparations of the same column packing.

[c] Support in all cases was untreated Chromosorb W (product of Johns-Manville).

[d] The temp was held at the higher point until all three products eluted.

[e] This column was excellent, but subsequent attempts to duplicate the packing met with only partial success.

tartrate, the methanol solution is diluted with water, extracted with a mixture of isoamyl alcohol and petroleum ether, and the absorbance of the aqueous layer then determined at 550 mμ. Dodecyl and octyl gallates are distinguished by a qualitative color reaction in which ferrous tartrate is added to the methanol solution. This solution is then shaken with a mixture of isoamyl alcohol and petroleum ether. Formation of a violet-blue color in the upper layer indicates the presence of dodecyl gallate since octyl gallate gives no color with this reagent. A similar method for total gallate content based only on extraction with 95% aqueous alcohol has also been developed (6).

Colorimetric reactions in general are excellent for BHA, BHT and PG analyses in food. However, they are not completely specific. 2,6-Dichloroquinonechloromide and diazotized sulfanilic acid will react with any phenol with a vacant ortho or para position, not just with BHA; ferric chloride — 2,2′-bipyridine will give color with any phenol; PG analysis depends on formation of a colored, iron complex and, as already pointed out, NDGA will interfere. In the Eastman laboratories, experience has shown that these objections are not too serious since interfering color has not been encountered in PG analyses, and steam distillation procedures apparently remove most interferences in the BHA and BHT analyses. As with the UV analyses, one is on much firmer ground when the analysis is a routine one where the food containing no antioxidant can be run through the analysis to determine if interferences exist.

Gas Chromatography

Since its development in the 1950's, gas chromatography has become one of the more powerful analytical tools. The fact that excellent commercial instruments can be purchased in the range of only $2000-$4000 has led to its widespread use throughout the chemical

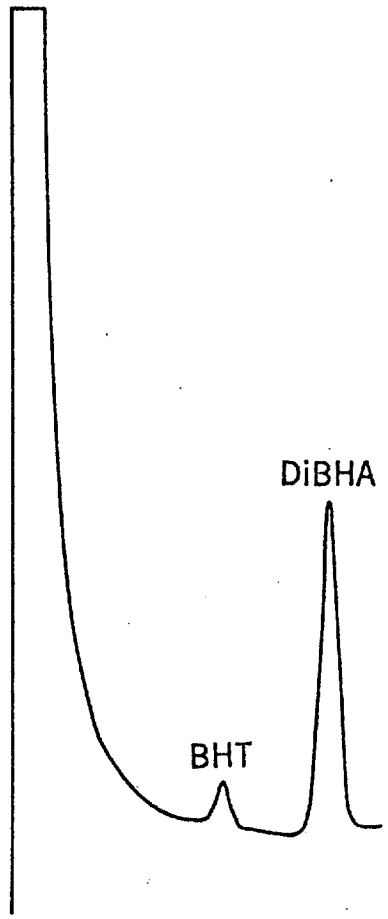


FIG. 2. BHT separation with Carbowax 20M Column.

and allied industries. One major advantage is that detection of very small quantities of material is possible. Analysis of antioxidants in foods involves quantities in the ppm range, thus it is natural that this tool should be applied in this area.

Buttery and Stuckey (5) described direct extraction of BHA and BHT from potato granules followed by gas chromatography of the eluate on an Apiezon L Column using flame ionization detection. A range of 0.5 ppm to 10 ppm of antioxidant was analyzed with an average error of less than 11%.

Anderson, Schwecke, Nelson and Huntley (1) extended the gas chromatographic work using the direct extraction technique. Schwecke and Nelson (15) introduced the use of 3,5-di-t-butyl-4-hydroxyanisole (DiBHA) as an internal standard. In the internal standard method, the unknown amounts of BHA and BHT are determined by comparison with a known concentration of Di-BHA in the same sample. This procedure obviates having to measure carefully the amt of a standard solution. These workers analyzed in the range of 5–30 ppm antioxidant using a Silicone Gum SE-30-Tween 80 column and flame ionization detection. Anderson et al. (1) used this method in studying the decrease in antioxidant content in cereals with storage time.

TABLE II

Sensitivity of a 33% Carbowax 20M Column

Sample	Determinations	BHA, ppm				BHT, ppm			
		Actual	Anal. Av.	Range	Std. Dev.	Actual	Anal. Av.	Range	Std. Dev.
40B	8	10	10.2	9.5–11.0	0.55	10	9.95	9.0–11.0	0.80
40D	8	5	5.70	5.5– 6.0	0.28	5	5.45	5.0– 6.0	0.42
40C	9	2.5	2.52	2.3– 2.7	0.16	2.5	3.02	2.8– 3.6	0.27
40D	8	1	1.00	0.8– 1.2	0.13	1	0.91	0.85– 1.0	0.05

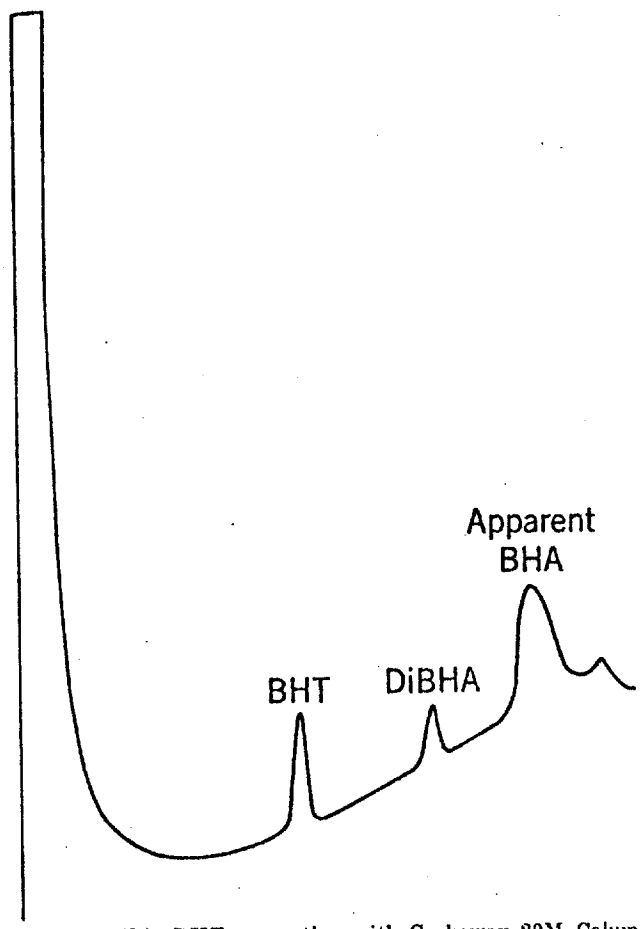


FIG. 3. BHA, BHT separation with Carbowax 20M Column.

These reports show that gas chromatography is an excellent tool for BHA and BHT analyses. In the Eastman laboratories, however, some problems have occurred in use of the flame ionization detection system, the major problem being column "bleed." In the published reports, the techniques followed used less than 1-μl samples and a very high detector sensitivity. This procedure results in detection of any small amount of column packing which bleeds off and confuses the analysis. Under these conditions, the column must be conditioned for an extended period of time to drive off column packing which is volatile at the column temp employed. In addition, flame ionization detection does not give an equal response for equal amts of different compounds, so calibration curves must be prepared. Anderson and Nelson (2) described the use of the much less sensitive thermal conductivity detection system using 23% Silicone Gum SE-30 column packing. A 100-μl sample must be used to obtain sufficient detector response. The response is essentially the same for BHA, BHT and the internal standard, Di-BHA. Similar work in the Eastman laboratories confirms the utility of this method using an F and M Scientific Company Model 720 Gas Chromatograph. A discussion of some of this work follows:

A 10- to 15-g sample of food was packed in a 13 × 20-mm glass column with a 55 × 30-mm solvent reservoir at the top. The sample was washed with one 10-ml portion and four 5-ml portions of ethyl ether. About 1 lb nitrogen pressure was used to obtain reasonable flow. Concurrently, a gentle nitrogen stream was used to evaporate the emerging eluate. Toward the end of the elution, 500 μg of Di-BHA in ether solution was added to the conc. The final volume was about 1 ml.

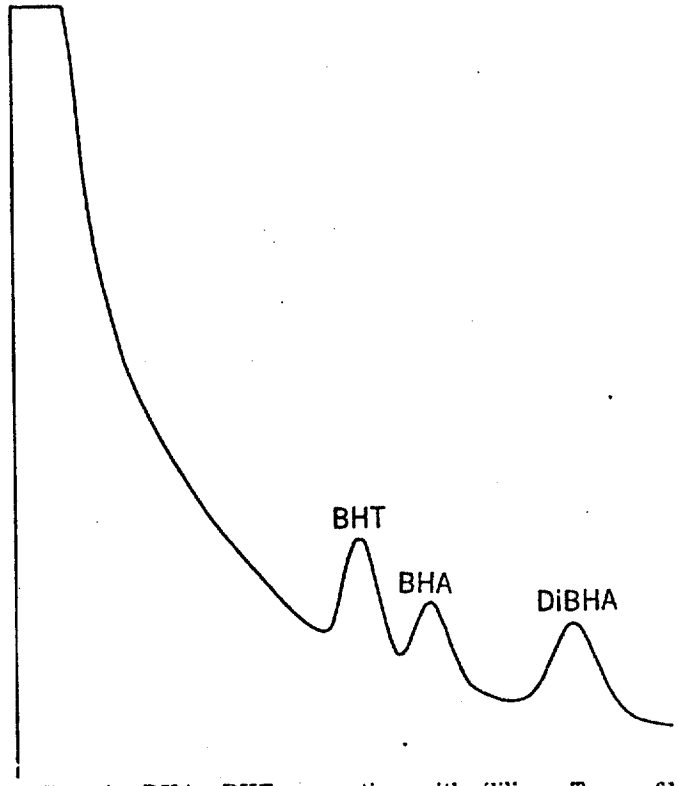


FIG. 4. BHA, BHT separation with Silicone-Tween 61 Column.

Instrument conditions for the F and M Scientific Company Model 720 are found in Table I. A number of columns have been devised which adequately separate BHA, BHT and Di-BHA. In our laboratories, the separation of BHA and BHT using the 23% Silicone Gum SE-30 of Anderson and Nelson (2) was not as good as is desired. We have developed the columns listed in Table I which give adequate separation and reasonable retention times.

In no case was column bleeding a problem and only about 30 min was required to obtain a steady baseline. However, it should be pointed out that the Model 720 is a dual column instrument where a reference column is in parallel with the analysis column which results in compensation of bleeding. We have also found that the response of the BHA, BHT and Di-BHA is equal for equal amts, within experimental error, so that no calibration need be made and direct peak area comparisons are valid. Table II shows the sensitivity of the 33% Carbowax 20M Column. These data were carried out with solutions prepared to correspond to the indicated concns of antioxidant.

This column was used to analyze rice samples for BHT in the 3–30 ppm range; the chromatogram contained no peaks other than those of the BHT and Di-BHA. Figure 2 illustrates the chromatogram obtained. A problem involving BHA and BHT in the 3–30 ppm range in potato flakes was also investigated using this column. However, it was found that a constituent from the potatoes came off the column in the BHA region, completely obscuring the BHA peak. Figure 3 shows this interference. Changing to a 4 ft 5% Tween 61, 25% Silicone SE-30 column eliminated this problem; clean, sharp peaks with no interference were obtained. These are shown in Figure 4. In the potato flake analysis, the concentrated extract contained a good deal of insoluble material. However, allowing it to settle as much as possible minimized the amount of solid taken with the sample, and even

when a relatively large amount of solid was occluded in the sample shot on the column, there was no interference in the BHA, BHT, Di-BHA peaks. With the variety of columns developed here, it should be possible to eliminate such interference in other problems by changing columns. Another positive advantage in having various columns available is that BHA and BHT can be identified positively since not only can peak times be measured, but order of elution can be varied from BHT, BHA, Di-BHA to BHT, Di-BHA, BHA.

The problem encountered in this potato flake analysis illustrates quite clearly a general point which is indicative of our experience in antioxidant analyses. Each type of food presents a slightly different problem and while general methods can be discussed, variations may be necessary for individual cases.

The gas chromatographic technique is valuable in eliminating interferences since, as in the case of the potatoes, interference is immediately obvious and methods can be varied to eliminate it. Interference in colorimetric work would be far harder to detect in individual cases, especially where a single wavelength reading is used with no check of the rest of the spectrum for other peaks.

Gas chromatographic analysis needs further investigation, particularly in high fat food where the direct extraction technique removes large amounts of various fats and oils along with the antioxidants. These interfering materials not only result in a multiplicity of peaks, but also completely plug up the column on occasion.

The use of the gas chromatographic technique to analyze BHA and/or BHT in a single food product is relatively simple and can be handled by a technician with little supervision. However when substrates are varied or when instrumentation difficulties arise, an operator with a thorough knowledge of gas chromatographic techniques and instrumentation experience is a must.

REFERENCES

1. Anderson, R. H., T. E. Huntley, W. M. Schwecke and J. H. Nelson, JAOCS *40*, 349 (1963).
2. Anderson, R. H. and J. P. Nelson, Food Tech. *17*, No. 7, 95 (1963).
3. Anglin, C., J. H. Mahon and R. A. Chapman, J. Agr. Food Chem. *4*, 1018 (1956).
4. Berger, K. G., N. D. Sylvester and D. M. Haines, Analyst 85, 341 (1960).
5. Buttery, R. G., and B. N. Stuckey, J. Agr. Food Chem. *9*, 288 (1961).
6. Cassidy, W., and A. J. Fisher, Analyst *85*, 295 (1960).
7. ECPI Food Laboratory Standard Procedure No. 25.
8. Filipic, V. J., and C. L. Ogg, J. Assoc. Offic. Agr. Chem. *43* 795 (1960).
9. Gibbs, H. D., J. Biol. Chem. *72*, 649 (1927).
10. Lazlo, H., and L. R. Dugan, Jr., JAOCS *38*, No. 4, 178 (1961)
11. Mahon, J. H., and R. A. Chapman, Anal. Chem. *23*, 1116 (1951)
12. *Ibid.* *23*, 1120 (1951).
13. Phillips, M. A., and R. D. Hinkel, J. Agr. Food Chem. *5*, 379 (1957).
14. Roos, J. B., Fleischwistschaft *11*, 667 (1959).
15. Schwecke, W. M., and J. H. Nelson, J. Agr. Food Chem. *12*, 86 (1964).
16. Sloman, K. G., R. J. Romagnoli and J. C. Cavagnol, J. Assoc. Offic. Agr. Chem. *45*, 76 (1962).
17. Szalkowski, C. R., and J. B. Garber, J. Agr. Food Chem. *10*, 491 (1962).
18. Vos, H. J., H. Wessels and C. W. T. Six, Analyst 82, 362 (1957).

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Arch. Path., 1956, v. 62, p. 218. — 34. Hughes P. E., Cancer Res., 1958, v. 18, p. 426.— 35. Koller P. C., Progr. Biophys., 1954, v. 4, p. 195. — 36. Miller E. C., Miller J. A., Kline B. E. et al., J. exp. Med., 1948, v. 88, p. 89. — 37. Neish W. E., Rylett A., Biochem. Pharmacol., 1963, v. 12, p. 893. — 38. Neukomm S., Oncologia (Basel) 1957, v. 10, p. 107. — 39. Potter V. K., Price J. M., Miller E. C. et al., Cancer Res., 1950, v. 10, p. 28. — 40. Rabbi A. et al., Boll. Soc. ital. Biol. sper., 1962, v. 38, p. 1909. — 41. Reid E., Cancer Res., 1962, v. 22, p. 398. — 42. Terayama H., Otsuka M., Gann, 1959, v. 50, p. 57.—43. Truhaut R., Druckrey H., Presse méd., 1958, v. 66, p. 1180.—44. Smith W. E., Bull. N. Y. Acad. Med., 1956, v. 32, p. 77.— 45. Striebich M. J., Shelton E., Schneider W. C., Cancer Res., 1953, v. 13, p. 279. — 46. Suntzeff V., Croninger A. B., Wynder E. L. et al., Cancer, 1957, v. 10, p. 250.

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UTILIZATION OF BIOCHEMICAL INDICES FOR EVALUATION OF POSSIBLE CARCINOGENIC PROPERTIES OF FOODSTUFF ADMIXTURES

(Literature survey)

B. L. Rubenchik (Kiev)

УДК 613.29 (439.1)

ИССЛЕДОВАНИЕ ВЛИЯНИЯ НА ОРГАНИЗМ ТЕПЛОКРОВНЫХ ПРИМЕСЕЙ И ДОБАВОК, СОДЕРЖАЩИХСЯ В ОТЕЧЕСТВЕННЫХ ПИЩЕВЫХ ПРОДУКТАХ ВЕНГРИИ

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Оценка влияния на организм примесей и добавок (чужеродных веществ), содержащихся в пищевых продуктах, с точки зрения гигиены питания является все более насущной задачей.

В Венгрии материал по этому вопросу объединен в работе Tarján [1]. Частично эти вопросы освещены также в сообщениях Cieleszky [2, 3, 4], Sándi [5, 6], Dénes [7], Truhaut [8, 9]. Truhaut и Druckrey даже предполагают наличие взаимосвязи между увеличением использования чужеродных веществ и злокачественными заболеваниями [10].

Имеющиеся в литературе данные освещают вопрос влияния одного какого-либо чужеродного вещества или только одной группы чужеродных веществ. Так, Hecht [11] занимался токсикологией красителей пищевых продуктов, а Souci и Mergenthaler [12] — консервирующими агентами. Однако литературных данных, которые бы освещали совместное влияние ряда чужеродных веществ, попадающих в организм вместе с продуктами питания, мы не встречали. По нашему мнению, этот вопрос является одним из неразрешенных и очень важных, поскольку известно, что в повседневной жизни множество чужеродных веществ всевозможных химических структур попадает в организм и влияет на него. Поэтому целью нашей работы было, с одной стороны, изучить, какие чужеродные вещества чаще всего присутствуют в пищевых продуктах, точнее говоря установить, в каком количестве попадают эти вещества в организм человека, а с другой стороны, мы попытались исследовать совместное влияние на организм ряда чужеродных веществ при скармливании их нескольким генерациям животных.

Чужеродные вещества, находящиеся в продуктах питания, разделятся на 2 группы. К одной группе относятся преднамеренно добавляемые к пищевым продуктам вещества, так называемые дополнительные

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вещества — добавки (например, ароматизаторы, красители, консервирующие агенты и др.), а к другой группе — загрязняющие пищевые продукты примеси, попадающие в них, например, в ходе технологических процессов. Гигиеническое законодательство ограничивает выбор пищевых добавок, их применяемые количества и максимально допустимое содержание примесей в продуктах питания.

С целью изучения потребления чужеродных веществ человеком мы использовали статистические данные, которые ориентировали нас в отношении количества употребляемых пищевых продуктов, и литературные данные [13—20] о количестве примесей в отдельных пищевых продуктах. Однако из официальных статистических данных не всегда можно установить качество и количество потребленных пищевых продуктов. Так, например, известно, что в Венгрии на каждого человека приходится по 100 *кг* муки в год. Но только часть потребленной муки содержит такие вещества, как карбонат аммония, бикарбонат натрия и виннокаменную кислоту, т. е. те вещества, которые употребляются в хлебопекарной промышленности.

В таких и подобных случаях можно только приблизительно предполагать количество чужеродных веществ. В отношении муки мы установили, что загрязнение мучных изделий чужеродными веществами равно 10%.

Для мяса характерен фосфат, используемый в вареных колбасных изделиях, нитрит, встречающийся в маринованных мясных изделиях, а также примеси олова и свинца (в минимальном количестве), содержащиеся в мясных консервах. По нашему мнению, 30% всего количества потребленного мяса перерабатывается и, таким образом, к покупателю попадает, содержа чужеродные вещества.

Что касается жира, то предположительно 80% его потребленного количества содержит пропиловый эфир галловой кислоты в качестве антиоксиданта, а в качестве примеси в свином жире встречается ДДТ; около 20% жира попадает к потребителям в виде маргарина, окрашенного красителем «Аннатто» и содержащего в качестве примеси некоторое количество никеля.

Относительно сахара мы предположили, что 20% его используется в виде конфет, джемов, а также фруктовых консервов или в виде фруктовых напитков. Эти продукты содержат различные красители, ароматические вещества и бензойную кислоту как консервирующее вещество, а также виннокаменную кислоту.

Мы допускаем, что все молочные продукты загрязнены ДДТ и, кроме того, 5% из них потребляются в виде плавленого сыра, а это значит, что 5% употребляемых молочных продуктов содержат фосфаты и натриевые соли лимонной и виннокаменной кислот, которые добавляются при плавлении сыров.

Овощи и фрукты могут содержать остатки инсектофунгицидов, а консервированные овощные и фруктовые продукты содержат также и консервирующие вещества.

Табл. 1 была составлена на основании упомянутых выше соображений, а также с учетом данных потребления пищевых продуктов в Венгрии за 1961 г.

На основании расчетов, приведенных в табл. 1, мы приблизительно определили количество чужеродных веществ, приходящееся на 1000 *кг* продуктов питания.

Токсикологические опыты проводили на крысах, добавляя к обыкновенному корму крыс чужеродные вещества в удесятеренном количестве. Равномерность смешения чужеродных веществ в корме устанавливали количественным определением меди.

Состав корма для крыс, а также количества чужеродных веществ, добавленных к 100 *кг* корма, приведены в табл. 2.

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Таблица 1

Количество чужеродных веществ, попадающих в организм с пищевыми продуктами

Пищевые продукты	Количество продукта на 1 человека в год (в *кг*)	Количество данного продукта в 1000 *кг* всех потребленных продуктов (в *кг*)	Количество данного продукта, содержащее чужеродные вещества		Чужеродные вещества	Количество чужеродных веществ в 1 *кг* данного продукта (в *мг*)	Количество чужеродных веществ в 1000 *кг* всех потребленных пищевых продуктов (в *г*)
			в *кг*	в %			
Мука	100	140	14	10	$(NH_4)_2CO_3$	1 000	14,0
					$NaHCO_3$	1 000	14,0
					Виннокаменная кислота	1 000	14,0
Мясо	70	98	29,5	30	Фосфаты	2 000	60,0
					Нитрит	150	4,4
					Олово	100	
					Свинец	5	0,15
Жир	20	28	22,4	80	Пропилгаллат	100	2,2
					ДДТ	1,5	0,035
			5,6	20	Краситель «Аннатто»	100	0,56
					Никель	1,0	0,006
Сахар	30	43	8,6	20	Красители	100	0,86
					Ароматические вещества	10	0,09
					Бензойная кислота	1 000	8,6
					Виннокаменная кислота	15 000	130,0
Молоко	200	280	280	100	ДДТ	0,045	0,013
Масло и др.			14	5	Фосфаты	3 000	42,0
Овощи	100	140	70	50	ДДТ	0,2	0,014
					ТМТД	1,0	0,07
					Цинеб	1,0	0,07
			14	10	Медь	10,0	0,14
			2,8	2	Салициловая кислота	1 000	2,8
Фрукты	100	140	70	50	ДДТ	1,0	0,07
					ТМДТ	1,0	0,07
					Цинеб	1,0	0,07
					Медь	10,0	0,7
			14	10	Бензойная кислота	500	7,0
					Сорбиновая кислота	500	7,0
					Красители	100	1,4
Картофель	80	112					
Яйца	12 (240 шт.)	17					
Итого . . .	712	998					

Предполагалось, что если крыса с живым весом 1 *кг* ежедневно потребляла 100 *г* корма с составом, указанным в табл. 2, тогда она получала 14 *мг* карбоната аммония, 14 *мг* бикарбоната натрия, 144 *мг* виннокаменной кислоты и т. д. в день, в расчете на 1 *кг* живого веса. В отличие от крыс человек употребляет ежедневно только 28,5 *г* пищи в среднем в расчете на 1 *кг* веса тела. Значит крысы потребляют в 3½ раза больше пищи (в расчете на 1 *кг* веса тела), чем люди. Следовательно, подопытные крысы получили чужеродных веществ в среднем в 35 раз больше, чем их попадает в пищу человека, так как человек ежедневно потребляет на 1 *кг* веса тела в среднем 0,4 *мг* карбоната аммония, 0,4 *мг* бикарбоната натрия, 4,1 *мг* виннокаменной кислоты и т. д.

Опыты были проведены на белых крысах-самках, которые были разведены в нашем институте селективным, ротационным методом. В опытные (т. е. получающие чужеродные вещества) и контрольные группы мы выбирали животных из одного рода. В каждую клетку (площадью 3000 *см*²) помещали по 8 самок и к ним подсаживали

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Таблица 2

Состав рациона для подопытных животных (из расчета на 100 *кг* корма)

Смешанный корм (в *кг*)		Смесь солей (в *г* на 1 *кг* смеси солей)		Смесь витаминов		Смесь чужеродных веществ (в *г* на 1 *кг* смеси чужеродных веществ)	
Пшеница	18	$MnSO_4$	35	Витамин А	600 000 МЕ на 1 *кг* смеси витаминов	Карбонат аммония	14
Ячмень	18	$MgSO_4$	50	Витамин B_1 (продукт завода Кинонн)	300 *мг*	Бикарбонат натрия	14
Рожь	14	$FeSO_4$	5	Витамин B_2 (продукт завода Рош)	600 *мг*	Виннокаменная кислота	144
Овес	14	$CuSO_4$	2,5	Ниацин	3 000 *мг*	Фосфаты	102
Кукуруза	4	$ZnSO_4$	0,4	Кальциевая соль пантотеновой кислоты (продукт завода Пфицер)	1 200 *мг*	Нитриты	4,4
Очищенные семечки подсолнуха	8	KJ	0,2	Витамин D_2	100 000 МЕ	Олово	3
Мука люцерны	2	NaCl	500	Витамин Е	6 000 *мг*	Свинец	0,15
Рыбья мука	5	Отруби	До 1 000	Холинхлорид	200 *г*	Пропилгаллат	2,2
Казеин	5			Метионин	400 *г*	ДДТ	0,13
Обезжиренный молочный порошок	4			Этоксиметилквинолин	12,5 *г*	Краситель «Аннатто»	0,56
Сухие дрожжи	4			Отруби добавляли	До 1 000 *г*	Никель	0,006
Фоскал (продукт завода «Филаксия»)[1]	2					Красители[2]	2,26
Смесь солей	1					Бензойная кислота	15,6
Смесь витаминов	1					ТМТД	0,14
						Медь	0,84
						Салициловая кислота	2,8
						Сорбиновая кислота	7
						Отруби добавляли до	1 000

[1] Вторичный ортофосфат кальция.
[2] Смесь красителей (в *г*): неукокцин 0,50, амарант 0,50, тартразин 0,50, бриллиантовый черный 0,50, индигокармин 0,13, кислотный желтый 0,13.

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по одному самцу с высокой способностью к оплодотворению, который был старше самок на 1—2 месяца. В клетках одновременно находилось не более 9 животных, что было необходимо с точки зрения биологии размножения плотности популяции по Lane-Petter [21].

Исследования начали проводить на 46 подопытных и на 46 контрольных крысах-самках. Для случки было использовано 12 самцов, которые через 5 месяцев были заменены. Самцов меняли между 6 опытными и 6 контрольными группами через каждые 2 недели, по методу циклической меновой случки.

Крысы-самки питались упомянутым выше кормом 10 месяцев, и на протяжении этого периода мы наблюдали особей F поколения из шестикратного выводка. Для дальнейших исследований из 1573 подопытных животных F поколения мы оставили всего 43 животных (24 самок и 19 самцов), а из 1374 контрольных животных — всего 24 (11 самок и 13 самцов).

Ежедневно животные однократно получали размешанный в воде корм. Воду и корм они потребляли ad libitum. Беременных самок помещали в отдельные клетки. Животные из опытной группы во время стельности, родов и кормления продолжали получать корм, дополненный чужеродными веществами.

Кроме клинического наблюдения над животными по влиянию хронического кормления их смесью чужеродных веществ, проводили также наблюдения за размножением животных, жизнеспособностью и развитием их потомства.

Известно, что организм приспособляется к факторам окружающей среды, в относительно незначительной степени его повреждающим. Повреждению организма препятствуют механизмы адаптации. Предполагается, однако, что в таких случаях организм может защищаться менее успешно против новых воздействий окружающей среды вследствие перегруженности адаптационного механизма. В связи с этими предпосылками для выяснения разницы между подопытными и контрольными животными обе группы были подвергнуты физической нагрузке (плавание), а также токсическому воздействию (отравление тетрахлорметаном).

Крысы плавали в бассейне с поверхностью 0,6 $м^2$ и температурой воды 10°. Одновременно в бассейн помещали не более 10 животных с приблизительно одинаковым весом тела (с разницей ±10 *г*). В каждом случае плавало одинаковое число крыс из опытной и контрольной групп. Продолжительность плавания (от начала до первого погружения животного в воду) измеряли секундомером. Извлеченных из бассейна крыс вытирали сухой тканью и помещали под лампу с инфракрасными лучами. Через 3 дня процедуру с плаванием повторяли, затем животных обезглавливали, их органы взвешивали и помещали в закрепители, необходимые для гистохимических исследований. Подобные же исследования проводили на других 2 группах подопытных и контрольных крыс, но перед плаванием животных отравляли ежедневно в течение 5 дней DL_{25} тетрахлорметана, который вводили при помощи желудочного зонда (0,25—0,50 *мл* на 100 *г* веса тела).

Органы 30 подопытных и 27 контрольных крыс были исследованы гистологически. Свежие органы были помещены в фиксирующий раствор по Carnoy, т. е. в безводный ацетон с температурой 4°. После заливания парафином мы окрашивали гематоксилин-эозином печень, почки, тонкие кишки, поджелудочную железу, легкие, а также проводили реакцию на щелочную фосфатазу в почках и реакцию на эстеразу в поджелудочной железе и кишечнике. После фиксации надпочечников в 4% растворе формалина в течение 48 часов мы приготовляли из них замороженные срезы размером 15—20 *мк*, затем наносили на них глицерин-желатину и исследовали с помощью поляризационного микроскопа количество двоякопреломляющих липоидов в отдельных слоях надпочечников.

Биометрическая обработка данных была осуществлена по методам, изложенным в книге Weber [22]. Статистическая оценка количества животных, погибших от отравления тетрахлорметаном, составлена по методу Heath и Irwin [23] и затем вычислена по методу Van der Waerden и Nievergelt [24].

В начале опыта подопытных самок взвешивали еженедельно, но вследствие вскоре наступающей беременности (в течение 3—4 недель) взвешивание животных прекращали.

Из 92 самок 90 принесли приплод по нескольку раз, и только 2 из контрольных самок были беременны по 1 разу. Животные в группе, получающей чужеродные вещества, беременели 207 раз (т. е. каждая самка по 4,7 раза), а контрольные — 198 раз (т. е. каждая самка по 4,3 раза). У 4 самок из группы крыс, получавших чужеродные вещества, и 11 самок из группы контрольных животных детеныши погибали. У большинства животных в опытной группе детеныши двух приплодов вырастали во взрослые особи, а в контрольной группе только один. Число самок, у которых детеныши погибали, было практически одинаковым в обеих группах. Число всех выкидышей в опытной группе равнялось 30, а в контрольной — 50.

* Смесь красителей (в г): нуклокцин 0,50, амарант 0,50, тартразин 0,50, бриллиантовый черный 0,50, индигокармин 0,13, кислотный желтый 0,13

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В течение 10 месяцев опыта от самок, получавших чужеродные вещества, родилось 1573 живых детеныша, т. е. в среднем по 35,8 животного. В контрольной группе родилось 1374 детеныша, т. е. по 29,9 животного. Таким образом, самки, потреблявшие чужеродные вещества, принесли в среднем 8,9 детеныша в выводке, а самки контрольной группы — в среднем 9,3.

Данные, приведенные в табл. 3, иллюстрируют развитие потомства.

В живом весе животных, отлученных от матерей, и взрослых животных значительной разницы между опытной и контрольной группой не наблюдалось. Развитие потомства в обеих группах было удовлетворительным.

Таблица 3

Живой вес (в г) животных, отлученных от матерей, и взрослых животных F поколения, потреблявших чужеродные вещества, и контрольных

	Подопытные животные	Контрольные животные
Живой вес отлученных от матерей животных:		
самцы	64,3	65,1
самки	63,9	63,4
Живой вес взрослых животных перед плаванием:		
самцы	322,4	371,9
самки	222,6	232,2

В табл. 4 представлены данные, полученные при нагрузке плаванием подопытных и контрольных животных.

Полученные данные свидетельствуют о том, что продолжительность плавания подопытных и контрольных животных была практически одинаковой. Не наблюдалось заметной разницы и при введении тетрахлорметана. Под воздействием тетрахлорметана из числа животных, получавших смесь чужеродных веществ, погибло 48%, а из контрольных — 30%. Эта разница является незначительной. Если мы перечислим погибших животных по методу Heath и Irwin [23] в порядке их падежа и отметим подопытных животных буквой А, а контрольных животных буквой Б, то получим следующий ряд:

Таблица 4

Результаты нагрузки плаванием подопытных и контрольных животных

	Подопытные крысы		Контрольные крысы	
	количество животных	продолжительность плавания (мин., сек.)	количество животных	продолжительность плавания (мин., сек.)
1-е плавание матерей	33	11,19	32	11,19
2-е плавание матерей	16	11,48	20	11,42
Плавание матерей после отравления тетрахлорметаном	17	13,55	14	13,07
1-е плавание потомства:				
самцы	19	16,55	13	16,44
самки	24	10,00	11	10,00
2-е плавание потомства:				
самцы	8	18,48	6	19,12
самки	9	12,49	5	12,42
Плавание потомства после отравления тетрахлорметаном	4	13,30	4	14,36
Итого	130	12,53	105	12,46

у матерей — Б Б Б А А Б; у потомства — А Б А Б Б А Б А А А А А Б Б А А.

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В отношении веса органов различия между животными опытной и контрольной групп также не наблюдалось.

При сравнении органов подопытных и контрольных животных значительного различия между ними с точки зрения гистологии и гистохимии установить не удалось. В печени и почках как подопытных, так и контрольных животных, отравленных тетрахлорметаном, наблюдались в одинаковой степени выраженные ожирение, кровоизлияния, некробиоз и микронекрозы.

Таким образом, на основании проведенных наблюдений можно сделать следующий вывод: ежедневное введение с рационом в хроническом опыте 2 поколениям белых крыс смеси всех чужеродных веществ, содержащихся в продуктах питания, потребляемых населением Венгрии, при аггравации входящих в смесь ингредиентов в 35 раз против их обычного содержания в продуктах (при пересчете на 1 *кг* веса тела), существенно не отразилось на состоянии подопытных животных.

Никаких значительных различий между животными, получавшими чужеродные вещества, и контрольными не было обнаружено ни в прибавлении в весе, ни в данных о размножении, ни в физической нагрузке животных (плаванием), ни под влиянием отравления тетрахлорметаном. Вес органов, гистологические и гистохимические исследования также не выявили отличий между опытной и контрольной группой.

ЛИТЕРАТУРА

1. Tarján R., Egészségtudomány, 1960, т. 4, стр. 77. — 2. Cieleszky V., Pintér M., Élelmiszervizsgálati Közlemények, 1955, т. 1, стр. 56. — 3. Cieleszky V., Növénytermelés, 1957, т. 6, стр. 41.—4. Idem, Nahrung, 1958, Bd. 2, S. 801.—5. Sándi E., Elelm. Ipar, 1962, т. 16, стр. 51.—6. Idem. Élelmiszervizsgálati Közlemenyek 1957, т.3, стр. 199. — 7. Dénes A., Nahrung, 1962, Bd. 6, S. 48. — 8. Truhaut R., Bull. Soc. sci. Hyg. alim., 1955, v. 43, p. 79.—9. Idem., Ann. Falsific. Fraudes, 1957, c. 15, p. 412.—10. Truhaut R., Druckrey H., Presse méd., 1958, v. 66, p. 1180.—11. Hecht G., Toxikologische Daten von Farbstoffen und ihre Zulassung für Lebensmittel in verschieden Ländern. Mitteilung 6. der "Deutschen Forschungsgemeinschaft". Wiesbaden, 1957. — 12. Souci S. W., Mergenthaler E., Fremdstoffe in Lebensmitteln mit besonderer Berücksichtigung der Konservierung. München, 1958.—13. Druckrey H., Kopfmüller K., Z. Naturforsch., 1948, Bd. 36, S. 254. — 14. Day P., Am. J. clin. Nutr., 1961, v. 9, p. 283. — 15. Goodman R. N., Ibid., p. 269. — 16. Hazleton L. W., J. Agric. Food Chem., 1957, v. 5, p. 336. — 17. Tarjan R., Lindner K., Élelmezésegészségügyi zsebkönyv (Tápanyagtáblázat). Budapest, 1962. — 18. Tarján R., Nahrung, 1963, Bd. 7, S. 21.—19. Rudas I., Népegészségügy, 1955, т. 10, стр. 62.—20. Tarján R., Lamm G., Szalay E., Egészségtudomány, 1958, т. 2, стр 1. — 21. Lane-Petter W., Brit. J. animal Behaviour, 1953, v. 1, p. 124. — 22. Weber E., Grundriss der biologischen Statistik. Jena, 1956. — 23. Heath D. F., Irwin J. O., Nature, 1962, v. 194, p. 1192. — 24. Van der Waerden B. L., Nievergelt E., Tafeln zum Vergleich zweier Stichproben mittels X-test und Zeichentest. Berlin, 1956.

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EFFECTS PRODUCED ON THE ORGANISM OF WORMBLOODED ANIMALS BY ADMIXTURES AND ADJUVANTS CONTAINED IN FOOD-STUFFS OF HUNGARIAN ORIGIN

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Fetal Resorption in the Rat as Influenced by Certain Antioxidants[1]

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Some prenatal mortality is perhaps a normal phenomenon accompanying gestation in all mammals. Yet there is a paucity of definitive data on total embryonic or fetal wastage as compared with the wealth of information on the pathologic, genetic, physiologic and nutritional causes of prenatal death. Of the literature which deals with this problem in man or domesticated animals, most of it deals with fetal mortality, either as stillbirths or abortions.

In the rat there are many factors that may increase fetal death and subsequent resorption as, insufficient protein (Barry, '20), elevated environmental temperature (Sundstroem, '27; Hsu, '48; MacFarlane, Pennycuik and Thrift, '57), vitamin deficiencies, e.g., biotin (Kennedy and Palmer, '45), pantothenic acid (Nelson and Evans, '46), pyridoxine (Nelson and Evans, '48), pteroylglutamic acid (Nelson, Asling and Evans, '52), tocopherol (Cheng and Thomas, '53) and riboflavin (Nelson et al., '56). Other causes of resorptions are ovariectomy (Haterius, '36), trypan blue injections (Wilson, '54), Lathyrus odoratus feeding (Walker and Wirtschafter, '56) and others. There is also evidence that loss due to resorption is not confined to any particular period of pregnancy but occurs throughout gestation (wild rat, Perry, '46; wild rabbit, Brambell, '48; mouse, Hollander and Strong, '50).

Because the rat resorbs rather than aborts an imperfect conceptus, a record of all implantations is retained in the uterine mucosa at the implantation sites. This record is represented by remnants of implantation ranging from resorbed fetuses to inconspicuous cicatricial plaques. A careful macroscopic examination of the incised term uterus will therefore supply data on total implantations as well as normal young.

Resorption data secured from the examination of nearly 500 pregnant rats involving approximately 5,000 fetuses and over 500 resorptions is herewith presented. In addition, we investigated the influence of certain maternally administered compounds, mostly antioxidants, on the incidence of fetal loss as compared to normal animals held on normal diets. These data are the outgrowth of a larger problem concerned with the possible value of these antioxidants and related compounds in the prevention or amelioration of lesions arising from neonatal anoxia.

MATERIALS AND METHODS

Walter Reed-Carworth Farms strain rats in their first gestation were used exclusively and weighed approximately 200 gm at time of breeding. After positive mating the females were randomly distributed into experimental and control groups and the former were given *per os* or directly into diet one of the various compounds listed in table 1. Twenty-two days after positive mating the pregnant rat was killed in 100% nitrogen and after 21 minutes the young delivered by caesarian section. Both horns of the uterus were completely incised and a careful macroscopic survey made for resorption sites. The resorptions were charted as to location in the respective region of the horns and classified as to degree of resorption. Seasonal variation in the number of resorptions were recorded.

RESULTS

In 126 normal untreated gravid animals 40.8% had one or more resorptions and 10.6% of all recognizable implantations had terminated in resorptions. Of the 29 dietary regimens used, animals receiving

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TABLE 1

Effect of antioxidants and other compounds on fetal resorption[1]

	Total dose	Per os or in diet	Litters	Implantations	Normal fetuses	Litters with resorptions	Resorptions
	gm					%	%
Butylated hydroxytolulene (BHT)	0.5	D	11	124	121	18.2	2.4
d-l,alpha tocopherol acetate	0.2	O	12	169	162	41.7	4.1
2,5-di-tert-amylhydroquinone	0.25	D	10	134	128	60.0	4.5
d gamma tocopherol	0.2	O	11	127	121	45.5	4.7
Butylated hydroxyanisole (BHA)	0.5	D	12	145	137	51.7	5.5
d gamma tocopherol	0.07	O	12	157	148	50.0	5.7
2,6-di-tert-butyl-4-methyl-phenol (DBMP)	0.5	D	11	140	131	54.5	6.4
Thiodipropionic acid	0.5	D	10	128	119	60.0	7.0
1,2-di-hydro-2,2,4 trimethyl-6-ethoxyquinoline (Santoquin)	0.25	D	12	147	136	33.3	7.5
1,2-di-hydro-2,2,4 trimethyl-6-phenylquinoline (Santoflex B)	0.25	D	11	139	128	54.5	7.9
Nordihydroguaiaretic acid (NDGA)	0.5	D	8	86	79	75.0	8.1
Pangamic acid sodium (vit. B_{15})	0.25	D	11	138	126	27.2	8.7
Thiamine hydrochloride	2.0	O	13	160	145	69.2	9.4
Ascorbic acid	2.0	O	14	146	132	71.4	9.6
Glucose	30% of diet	D	25	280	251	68.0	10.3
Normal			126	1430	1278	40.8	10.6
Thiamine hydrochloride	0.5	D	8	75	67	62.5	10.7
2,2-methylene bis(4-methyl-6-tertiary butyl phenol)	0.25	D	8	101	90	37.5	10.9
Polymerized 1,2-dihydro-2,2,4-trimethyl quinoline (Flectol H)	0.25	D	17	148	136	52.9	12.1
Cytochrome C	0.05	D	8	84	73	50.0	13.1
Glutathione	0.25	D	6	65	56	66.6	13.9
d-l,alpha tocopherol acetate	0.1	O	14	150	129	71.4	14.0
Ascorbic acid	0.5	D	20	235	199	70.0	15.3
N,N'-diphenyl-p-phenylene-diamine (DPPD)	0.5	D	23	275	233	60.8	15.3
Phenothiazine	0.25	D	10	89	75	70.0	15.7
2,2-methylene bis(4-ethyl-6-tertiary butyl phenol)	0.25	D	9	111	92	66.6	17.1
n propyl gallate	0.5	D	9	93	76	77.7	18.3
Paraminobenzoic acid (PABA)	0.5	D	9	105	80	77.7	23.8
Hydroquinone	0.5	D	10	105	77	100.0	26.8
Methylene blue	0.5	D	10	100	62	90.0	38.0

[1] For samples of antioxidants we are indebted to the American Cyanamid Company for 2,2-methylene bis (4-methyl-6-tertiary butyl phenol) and 2,2-methylene bis(4-ethyl-6-tertiary butyl phenol), Distillation Products Industries for dl-alpha tocopherol acetate and gamma tocopherol, the B. F. Goodrich Company for DPPD, the Monsanto Chemical Company for Santoflex B, Santoquin and Flectol H and Hale Laboratories for vitamin B_{15}.

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one of 15 showed less resorptions (range 2.4–10.1%) than the controls, while rats receiving one of the other 14 diets showed an increased percentage of resorptions (10.7–38.0%) (table 1). In the dosage given none of these compounds appeared to be toxic to the mother with the possible exception of methylene blue, where poor pregnancy weight gain was noted. This compound yielded, incidentally, the highest percentage of resorptions (38.0%). Of particular interest is the observation that two of the widely used commercial antioxidants, butylated hydroxytolulene (BHT) and butylated hydroxyanisole (BHA), as well as the antioxidant vitamins alpha and gamma tocopherol, at 200 mg level, and 2,5-di-tert-amylhydroquinone gave the lowest percentage of resorptions.

Data on the distribution of resorption sites in all groups revealed that 47.6% of all resorptions were located in the cranial third of the horns, 20.6% in middle third and 31.8% in the caudal third. Most of the sites seen in this cranial region were small inconspicuous cicatrixic plaques suggestive of an early embryonic death.

Using the classification of Walker and Wirtschafter ('56) most of the resorptions, regardless of dietary supplement, belonged either to grade 3, representing complete resorption of the embryo but with small placenta still present, or grade 4, which are cicatrized uterine plaques marking previous placental sites. Grade 2, consisting of small macerated recognizable fetuses, were seldom encountered. The number of fetuses falling into grade 1, e.g., dead, but fully formed, could not be determined since many of our fetuses were killed by anoxic treatment of the mother.

DISCUSSION

Since our observations dealt with the identification of placental sites *in situ*, the question might be raised if identifiable plaques form at all implantation sites. That such scars do form where embryos have been resorbed is well documented by the work on rats of Davis and Emlen ('48). They reported more scars present than young born, the excess being ascribed to resorbed embryos. They also noted that scars do not occur spontaneously in nonpregnant rats. Moreover, these authors and Conaway ('55) reported that the resorption sites may remain identifiable for at least a year in the rat, and Deno ('41) made the same observation on the mouse. Therefore our use of first gestation animals avoided the possible error of counting sites of earlier pregnancies and ascribing them to the gestation under study.

The range of reported resorptions in normal rats by other investigators (11.8–6.5%) (table 2) may be due to seasonal variations in the fertility of the animals. It is generally accepted by rat breeders that fertility is lowest in winter and highest in summer (Main, '39). Since our data was collected throughout the year under controlled environmental conditions, we were able to separate our findings on a seasonal basis. We noted a reduction in the percentage of resorptions during the spring and summer (8.1%) and a marked increase (20.7%) during the winter and fall. Frazer ('55) using Wistar albino rats reported that 31% of all litters had some prenatal mortality on a yearly average, but that it was lowest in July-September (17%) and highest from October to December (86%). Other workers failed to present their prenatal mortality data on a seasonal basis, therefore an accurate comparison of animal groups is difficult, if not impossible.

Another variable influencing the percentage of resorptions in the rat may be the strain difference. Data on 6 different strains, including the wild rat, are summarized in table 2 and, with the exception of our WR-CF animals (10.6%) and Mason's O.M.–Mc. rats (11.8%) the 4 remaining groups agree rather closely on resorption percentages (6.6–8.7%). Yet the strain of the animal may be important, for the findings of Fekete ('47) in the mouse that fertilized ova transplanted to different strains of mice have a significantly better chance of survival in one strain than another, implies that one strain provides a better environment for implantation and development of the ovum. Hollander and Strong ('50) also noted significant differences in fetal mortality among various strains of mice. It is certainly possible that in the rat also, strain differences may exist which could

methylene bis(4-ethyl-6-tertiary butyl phenol), Distillation Products Industries for dl-alpha tocopherol acetate and gamma tocopherol, the B. F. Goodrich Company for DPPD, the Monsanto Chemical Company for Santoflex P, Antioquin and Flectol H and Hale Laboratories for vitamin B12.

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TABLE 2

Prenatal mortality in mammals

Animal	Litters	Implantations	Normal fetuses	Resorptions	Corpora lutea	Total prenatal mortality	Reference
				%			
Striped hamster	29	193	119	38.3			Fortuyn, '29
Stoat	12	108	103	4.6	135	23.7	Deanesley, '35
Grey squirrel	9		27		30	10.0	Deanesley and Parkes, '33
Sheep	80	109	101	7.2	116	12.9	Hammond, '21
Pig	22	316	267	15.5	396	32.5	Hammond, '21
Pig[1]	495	3528	3485	1.2	4480	22.2	Corner, '23
Pig	102	1117	1028	8.0	1396	26.1	Crew, '25
Rabbit (wild)	68	350	340	2.9	390	12.8	Hammond, '21
Rabbit (wild)	1819		8877		10574	16.0	Brambell, '48
Rabbit (wild)	2179	11363			12551	9.4	Brambell and Mills, '47
Rabbit (wild)	1834	9658	8927	7.6			Brambell and Mills, '48
Rabbit (tame)	56	441	391	11.3	578	32.3	Hammond, '21
Mouse	1080	7967	6672	16.3			Hollander and Strong, '50
Mouse[2]	500	3175	2930	7.7			Danforth and deAberle, '28
Rat (wild brown)	131	1238	1135	8.3	1420	20.0	Perry, '45
Rat (OM-Mc)[3]	50	492	434	11.8			Mason
Rat (Wistar)	25	226	211	6.6			MacFarlane et al., '57
Rat (Wistar)[4]	6	57	52	8.7			Wilson, '54
Rat (Long-Evans)	156		998		1498 (ova count)	33.4	Long and Evans, '22
Rat (Long-Evans)[5]	42	447	410	8.3			Nelson et al., '52
Rat (Holtzman)	35	371	347	6.5			Cheng and Thomas, '53
Rat (WR-CF)[6]	126	1430	1278	10.6			Telford, Woodruff, Linford

[1] Omitted from this data were 13 litters with all implanted embryos resorbing.
[2] From data on 500 pregnancies during last third of gestation as compared to 500 litters 1–3 days old.
[3] OM-Mc designates a mixed strain derived from Osborn-Mendel and McCollum piebald strains (data from personal communication).
[4] Reports present resorptions about 4–5% (personal communication).
[5] Pteroylglutamic acid control animals. Current resorptions in Long-Evans rats about 5% (personal communication).
[6] WR-CF indicates original Carworth Farms stock but inbred at Walter Reed Army Research Center.

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account for the fairly wide range of prenatal death rates.

Our analysis of the location of resorption sites in the various regions of the uterine horn revealed that 47.6% of all resorptions were found in the cranial third of the horn, with 20.7% in middle third and 31.8% in caudal third, confirming our impression that the cephalic tapered extremity of the horn was, perhaps, an unfavorable location for the developing embryo. This concept is supported by the observation of Fawcett, Wislocki and Waldo ('47) that the overcrowding by the developing mouse ovum in the anterior chamber of the eye inhibited the growth of adjacent implanted ova. However, Frazer's ('55) data on the rat does not favor this idea, for he demonstrated that the percentage resorption was the same in both horns even though different number of ova were implanted in the different horns. Furthermore, the overcrowding of a mouse uterine horn with an unusually larger number of implanted ova did not influence adversely the resorption rate, as shown by Hollander and Strong ('50).

Another variable influencing fetal loss is the maternal age or weight. Frazer ('55) noted in two strains of rats that the heavier the mother, the higher resorption rate. Similar findings had been reported earlier by Hollander and Strong ('50). All of our animals were standardized at approximately 200 gm giving us uniform test animals thus eliminating the possible discrepancies due to age or weight.

Combining the data in table 2 for all groups of the same species, reveals that the tame rat values fall roughly in the middle of the other mammalian data. For example, the tame rat data show that 9.2% of all implantations end in resorptions. Those mammals with a lower percentage of resorptions are, pig (3.7%), stoat (4.6%), sheep (7.2%), wild rabbit (7.3%) and wild rat (8.3%). Those animals suffering on the average greater fetal loss than the rat are, tame rabbit (11.3%), mouse (15.9%) and wild hamster (38.3%). While fetal wastage is probably common to all mammals considerable species variation does exist.

Basic to our study of the value of antioxidants in the reduction of fetal mortality is the question of their mode of action and whether the site of action of the compound is on the fetal or maternal side of the placenta or both. Most of the agents reported here have been previously used in animal experimentation (Bunnell et al., '55; Schwarz, '58; Mertz and Schwarz, '58; Dam et al., '48, '51). From the results of these workers we assumed that the compound could be assimilated into the blood stream and may exert some influence on the developing placenta and might affect fetal growth. Evidence of placental transfer, however, is available only on a limited number of the tested agents, such as glucose (Huggett, '54), α-tocopherol (Neuweiler, '49), thiamine chloride (Slobody, Willner and Mestern, '49), ascorbic acid (Hamil et al., '47) and phenothiazine (Behn, Frahm and Fretwurst, '56). The rate of placental transfer of fat and water soluble vitamins is quite different, with the lipid soluble vitamins being much slower. In fact in the rat the transfer may be so slow that the cord blood content of vitamin A (Lund and Kimble, '43) or E (Straumfjord and Quaife, '46) may, under certain circumstances, be less than in maternal blood. In the rat Mason and Bryan ('40) reported only limited placental transfer of vitamin E derived from a wheat germ oil concentrate. Yet if 250 mg tocopherol is administered to women 4–8 hours before term, the vitamin E level in the cord is raised significantly (Varangot, Chailley and Rieux, '44). By contrast the water soluble vitamins, thiamine chloride (Slobody, Benson and Mestern, '46) and ascorbic acid (Manahan and Eastman, '38; Chow and Okuda, '60) showed consistently higher concentrations in cord than maternal blood, suggesting even perhaps a concentration of the vitamins by the placenta.

There is a great deal of evidence that in the rat vitamin E is essential for normal fetal development, for example, Cheng, Chang and Bairnson ('57) showed that in vitamin E deficiency 100% resorption of embryos occurred, while no resorption occurred in pregnant rats receiving daily 2 mg d,l-α-tocopherol acetate. Our data showed only 4.1% resorption in rats receiving 10 mg *per os* for 20 days during gestation as compared to 10.6% for all

[5] Pteroylglutamic acid control animals. Current resorptions in Long-Evans rats about 5% (personal communication).
[6] WR-CF indicates original Carworth Farms stock but inbred at Walter Reed Army Research Center.

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normal untreated controls. Daily 5 mg dosages of α-tocopherol administered to another group of pregnant animals had no beneficial effect on resorption rate, in fact some negative response was noted (14% resorptions).

In addition to high dosages of α- and γ-tocopherol our data suggest some beneficial effects on resorption rate were obtained from 2,5-di-tert-amylhydroquinone, butylated hydroxytolulene (BHT), butylated hydroxyanisole (BHA), 2,6-di-tert-butyl-4-methylphenol (DBMP), thiodipropionic acid, 1,2-di-hydro-2,2,4-trimethyl-6-ethoxyquinoline (Santoquin), 6-phenyl-1, 2-dihydro-2,2,4-trimethylquinoline (Santoflex B), nordihydroguaiaretic acid (NDGA); while methylene blue, hydroquinone, paraminobenzoic acid (PABA), n propyl gallate, 2,2-methylene bis (4-ethyl-6-tertiary butyl phenol), phenothiazine and N, N-diphenyl-p-phenylenediamine (DPPD) all substantially increase resorption rates.

One is impressed from the data summarized in table 2 that the total prenatal mortality in mammals is much higher than might be suspected from the resorption data alone. Brambell ('48) contends in the rabbit, "that the loss between ovulation and full term is not less than 43% of ova ovulated and may be, and indeed probably is, substantially heavier." Data on total wastage in the rat is scanty, limited to the early work of Long and Evans ('22) and later Perry ('45) on the wild rat. These workers reported respectively 33.4% and 20.0% total prenatal mortality while resorption rate was 8.3% for the two strains of rats.

Other mammals follow a similar trend, ranging from an elevenfold increase in total mortality over resorptions in pig (Corner, '23) to less than a twofold increase in sheep (Hammond, '21). Such observations certainly provide a challenge to animal research workers to seek out the causative factors for such an enormous wastage. These factors are legion, but include such environmental disturbances as abnormalities of blood supply of placenta, an unfavorable implantation site, faulty development of the decidua, abnormal environmental temperature and others. Maternal influences may adversely affect normal implantation and development, such as, inanition, avitaminoses, blood dyscrasias, endocrine disorders, infections and the presence of unphysiologic compounds in the blood such as some of the antioxidants herein reported.

SUMMARY AND CONCLUSIONS

1. In 126 untreated normal pregnant rats 40.8% had one or more resorptions and 10.6% of all implantations terminated in resorptions.

2. The cranial third of the uterine horn contained 47.6% of all resorptions, while the middle third held only 20.6% of the total resorptions.

3. The percentage resorptions in the tame rat falls intermediate among the other mammalian groups.

4. Of the maternally administered compounds, the tocopherols (α and γ), BHT, BHA, amylhydroquinone and DBMP appear to have definite prophylactic value in reducing the frequency of resorptions in the rat, while methylene blue, hydroquinone and paraminobenzoic acid markedly increase resorption rates.

5. Possible extrinsic and intrinsic factors that may influence prenatal mortality in the rat are discussed.

LITERATURE CITED

Barry, L. W. 1920 The effects of inanition in the pregnant albino rat, with special reference to the change in the relative weights of various parts, systems and organs of the offspring. Contr. Embryol. Carnegie Inst., *11:* 91–136.

Behn, W., M. Frahm and E. Fretwurst 1956 Ueber den diaplacentaren Uebergang von Phenothiazin Derivaten. Klin. Wchnschr. *34:* 872.

Brambell, F. W. R., and I. H. Mills 1947 Studies on sterility and prenatal mortality in wild rabbits. III. Loss of ova before implantation. J. Exp. Biol., *24:* 192–210.

——— 1948 Studies on sterility and prenatal mortality in wild rabbits. IV. The loss of embryos after implantation. Ibid., *25:* 241–269.

Brambell, F. W. R. 1948 Prenatal mortality in mammals. Biol. Rev., *23:* 370–407.

Bunnell, R. H., L. D. Matterson, E. P. Singsen, L. M. Potter and A. Kozeff 1955 Studies on encephalomalacia in the chick. 3. The influence of feeding or injecting various tocopherols and other antioxidants on the incidence of encephalomalacia. Poultry Sci., *34:* 1068–1075.

Cheng, D. W., and B. H. Thomas 1953 Relationship of time of therapy to teratogeny in maternal avitaminosis E. Proc. Iowa Acad. Sci., *60:* 290–299.

Cheng, D. W., L. F. Chang and T. A. Bairnson 1957 Gross observations on developing abnor-

Attention is drawn to the possibility that this text may be copyrighted. The reader is urged to investigate its copyright status.

mal embryos induced by maternal vitamin E deficiency. Anat. Rec., *129:* 167–186.

Chow, B. F., and K. Okuda 1960 Transfer of vitamins from mother to fetus. J.A.M.A., *172:* 422–426.

Conaway, C. H. 1955 Embryo resorption and placental scar formation in the rat. J. Mammal., *36:* 516–532.

Corner, G. W. 1923 The problem of embryonic pathology in mammals, with observations upon intra-uterine mortality in the pig. Am. J. Anat., *31:* 523–545.

Crew, F. A. E. 1925 Prenatal death in the pig and its effect upon sex ratio. Proc. Royal Soc. Edin., *46:* 9–14.

Dam, H., I. Kruse, I. Prange and E. Sondergaard 1948 Influence of dietary ascorbic acid, nordihydroguaiaretic acid, and cystine on vitamin E deficiency symptoms in chicks. Biochemica et Biophysica, *2:* 501–513.

——— 1951 Substances affording a partial protection against vitamin E deficiency symptoms. Acta Physiol. scand., *22:* 299–310.

Danforth, C. H., and S. B. de Aberle 1928 The functional interrelation of the ovaries as indicated by the distribution of foetuses in mouse uteri. Am. J. Anat., *41:* 65–74.

Davis, D. E., and J. T. Emlen 1948 The placential scar as a measure of fertility in rats. J. Wildlife Mgt., *12:* 162–166.

Deanesley, R., and A. S. Parkes 1933 The oestrous cycle of the grey squirrel (Sciurus carolinensis). Phil. Trans. Royal Soc. b., *222:* 47–96.

Deanesley, R. 1935 Growth and reproduction in the stoat (Mustela erminea). Ibid., *225:* 459–492.

Deno, R. A. 1941 A criterion for distinguishing between virgin and parous animals. Pharm. Arch., *12:* 12–16.

Fawcett, D. W., G. B. Wislocki and C. M. Waldo 1947 The development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. Am. J. Anat., *81:* 413–443.

Fekete, E. 1947 Differences in the effect of uterine environment upon development in DBA and C57 strains of mice. Anat. Rec., *98:* 409–415.

Fortuyn, A. B. D. 1929 Prenatal death in the striped hamster (Cricetulus griseus). Arch. de Biol., *39:* 583–606.

Frazer, J. F. D. 1955 Foetal death in the rat. J. Embryol. and Exp. Morph., *3:* 13–29.

Hamil, B. M., B. Munks, E. Z. Moyer, M. Kaucher and H. H. Williams 1947 Vitamin C in the blood and urine of the newborn and in the cord and maternal blood. Am. J. Dis. Child., *74:* 417–433.

Hammond, J. 1921 Further observations on the factors controlling fertility and foetal atrophy. J. Agric. Sci., *11:* 337–366.

Haterius, H. O. 1936 Reduction in litter size and maintenance of pregnancy in the oophorectomized rat: evidence concerning the endocrine role of the placenta. Am. J. Physiol., *114:* 399–406.

Hollander, W. F., and L. C. Strong 1950 Intra-uterine mortality and placental fusion in the mouse. J. Exp. Zool., *115:* 131–147.

Hsu, Chin-Yun 1948 Influence of temperature on development of rat embryos. Anat. Rec., *100:* 79–90.

Huggett, A. St. G. 1954 The transport of lipids and carbohydrates across the placenta. Cold Spring Harbor Symposia Quant. Biol., *19:* 82–92.

Kennedy, C., and L. S. Palmer 1945 Biotin deficiency in relation to reproduction and lactation. Arch. Biochem., *7:* 9–13.

Long, J. A., and H. M. Evans 1922 The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif., *6:* 1–148.

Lund, C. J., and M. S. Kimble 1943 Plasma vitamin A and carotene of newborn infant, with consideration of fetal-maternal relationships. Am. J. Obst. and Gynec., *46:* 207–221.

MacFarlane, W. V., P. R. Pennycuik and E. Thrift 1957 Resorption and loss of fetuses in rats living at 35°C. J. Physiol., *135:* 451–459.

Main, R. J. 1939 The care of a small rat colony. C. V. Mosby Company, St. Louis.

Manahan, C. P., and N. J. Eastman 1938 Cevitamic acid content of fetal blood. Bull. Johns Hopkins Hosp., *62:* 478–481.

Mason, K. E., and W. L. Bryan 1940 Placental and mammary transfer of vitamin E in the rat. J. Nutrition, *20:* 501–517.

Mertz, W., and K. Schwarz 1958 Reversal of respiratory decline in necrotic liver degeneration by intraportal antioxidants. Proc. Soc. Exp. Biol. and Med., *98:* 808–812.

Nelson, M. M., and H. M. Evans 1946 Pantothenic acid deficiency and reproduction in rat. J. Nutrition, *31:* 497–507.

——— 1948 Effect of desoxypyridoxine on reproduction in the rat. Proc. Soc. Exp. Biol. and Med., *68:* 274–276.

Nelson, M. M., C. W. Asling and H. M. Evans 1952 Production of multiple congenital abnormalities in young by maternal pteroylglutamic acid deficiency during gestation. J. Nutrition, *48:* 61–80.

Nelson, M. M., C. D. E. Baird, H. V. Wright and H. M. Evans 1956 Multiple congenital abnormalities in the rat resulting from riboflavin deficiency induced by antimetabolite galactoflavin. Ibid., *58:* 125–134.

Neuweiler, W. 1949 Vitamin E — Uebertritt von Mutter auf Kind. Internat. Ztschr. Vitaminforsch., *21:* 83–87.

Perry, J. S. 1945 The reproduction of the wild brown rat (Rattus norvegicus Erxleben). Proc. Zool. Soc. London, *115:* 19–46.

Schwarz, K. 1958 Effects of antioxidants on dietary necrotic liver degeneration. Proc. Soc. Exp. Biol. and Med., *99:* 20–24.

Slobody, L. B., R. A. Benson and J. Mestern 1946 A comparison of the vitamin C in mothers and their newborn infants. J. Pediatrics, *29:* 41–44.

Slobody, L. B., M. M. Willner and J. Mestern 1949 Comparison of vitamin B_1 levels in mothers and their newborn infants. Am. J. Dis. Child., *77:* 736–739.

Straumfjord, J. V., and M. L. Quaife 1946 Vitamin E levels in maternal and fetal blood plasma. Proc. Soc. Exp. Biol. and Med., *61:* 369–371.

... is drawn to the possibility that this text may be copyrighted. The read... ...aged to investigate its copyright status.

Sundstroem, E. G. 1927 Physiological aspects of tropical climates. Physiol. Rev., *7:* 320–362.

Varangot, J., H. Chailley and N. Rieux 1944 Sur la teneur du sang du cordon en vitamine E et sur le passage du tocophérol de la mère au fetus. Compt. rend. Soc. de biol., *138:* 24–25.

Walker, D. G., and Z. T. Wirtschafter 1956 Resorption of embryos in rats on Lathyrus odoratus diet. J. Nutrition, *58:* 147–159.

Wilson, J. G. 1954 Withdrawal of claim that azo blue causes congenital malformations. Proc. Soc. Exp. Biol. and Med., *87:* 1.

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Fd Cosmet. Toxicol. Vol. 6, pp. 25–31. Pergamon Press 1968. Printed in Great Britain

The Influence of Propyl Gallate and α-Tocopherol on the Survival Time of Rats during Fasting*

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Abstract—A toxicological investigation was carried out in order to repeat and extend the findings of Bukhman (*Vop. Pitan.* 1962, 21, 68) concerning the toxicity of propyl gallate in fasting rats. Rats were given a diet containing 0·002 or 0·004% propyl gallate or α-tocopherol. After 13 wk some of the animals were sacrificed, while the remaining rats were placed on a partial starvation diet until death. The survival time was recorded. Chemical determinations of water, protein and fat content were made on the sacrificed animals as well as on those that died. No difference in survival time or in protein and fat utilization was found between the control groups and the groups which received propyl gallate or α-tocopherol. The results of Bukhman could not be confirmed.

INTRODUCTION

Propyl, octyl and dodecyl gallates are extensively used as antioxidants in fats. On the basis of detailed toxicity studies, an acceptable daily intake for man of 0–0·5 mg gallates/kg body weight was estimated by the Joint FAO/WHO Expert Committee on Food Additives (1965).

Our attention has been directed to an unconventional approach to toxicity testing. In a study by Bukhman (1962) rats were fed for 13 wk on a diet containing 15% fat to which was added 0·02% propyl gallate, a concentration equivalent to 0·003% of the total diet. After this period, the rats were placed on a partial starvation diet until death. At the end of the first period, several animals were sacrificed and the fat and protein content of their bodies determined. Animals which died during the fasting period were treated in a similar manner. From Bukhman's results it appeared that the average survival time of the animals was considerably reduced by the propyl gallate, from 26·8 days in the control group to 14·7 days in the experimental group. According to the author, the control animals utilized their body fat and proteins better than the animals which had been fed propyl gallate. It was postulated that the oxidation processes in the organism were inhibited by the antioxidant and that this resulted in an incomplete utilization of the body fat and protein. To study the value of this type of toxicological investigation, a comparative investigation has now been carried out, in which propyl gallate and α-tocopherol were tested.

*Publication of this paper has been agreed upon because the work upon which it is based arose from the following comment by the Joint FAO/WHO Expert Committee on Food Additives (1965, p. 25): "... The results of the experiments involving partial starvation are of great interest and indicate a higher toxicity of propyl gallate than the other studies. Evaluation of these experiments is difficult, however, in the absence of comparable studies with other food additives. ..." By demonstrating that the claims made by Bukhman could not be substantiated, the authors have shown that cruel and unphysiological experiments which involve starving animals to death have little scientific merit and absolutely no justification as a method of safety-evaluation—Editor.

EXPERIMENTAL

Materials. Propyl gallate was obtained from Chemische Fabriek "Naarden", Naarden, The Netherlands, and α-tocopherol from Hoffmann-La Roche, Basel, Switzerland. Fresh lard was obtained weekly from the slaughterhouse and melted down.

Animals and diets. Six groups of Wistar rats (initial body weight 40–60 g) were used in the experiment. The control lard group consisted of 30 male and 30 female rats which received 15% lard in a diet containing 59% whole-wheat flour, 25% skim-milk powder and 1% salt mixture. Four other groups, each consisting of 15 male and 15 female rats received the same diet to which 0·002 or 0·004% propyl gallate or α-tocopherol was added. In Bukhman's experiment only one dietary level of propyl gallate (0·003%) was used. In the present study, an additional control group containing 15 male and 15 female rats was included for purposes of comparison. These animals (control WMS group) received a standard diet consisting of 66% whole-wheat flour, 33% whole-milk powder and 1% salt.

Methods. For histopathological studies, organs and tissues were fixed in 4% formalin. Paraffin sections were stained with haematoxylin and eosin; frozen sections were used for Oil Red O staining. The test of Wilcoxon (1945) was used for statistical calculations, unless otherwise stated.

Feeding study and analyses. During the first 13 wk, tapwater and diets were given *ad lib.* and growth and food intake were recorded. At the end of this period, 10 males and 10 females from the control lard group and 5 males and 5 females from each of the other groups were sacrificed. The liver, kidneys, heart, spleen, thymus, adrenals, thyroid, pituitary, pancreas, brain, uterus, ovaries, testes and prostate were weighed. Histopathological studies of the organs of the rats of both control groups and of the groups on 0·004% propyl gallate and 0·004% tocopherol were carried out. Blood was taken for haemoglobin estimation and erythrocyte and leucocyte counts. The protein content of the liver was determined. Organs, head, tail, feet and skin were removed and the rest of the carcass was packed in a sheet of plastic and frozen at −20°C. The frozen carcass was ground in a meat mincer and weighed. A small part was taken out for a protein determination and the rest was dried to constant weight at a temperature of about 90°C, in order to determine the water content. A part of the ground dried carcass was used for fat determination by petroleum ether extraction in a Soxhlet apparatus. The protein content of the liver was estimated after homogenization of about 60 mg in water and that of the ground carcass after homogenization of 60 mg in N-NaOH solution. The homogenates were subjected to protein determination by the method of Lowry, Rosebrough, Farr & Randall (1951).

Starvation and analyses. After 13 wk, the remaining animals were placed in individual cages on a fat-free diet having 20% of the calorific value of the normal mean daily food intake for male rats. Each animal received 3·5 g food/day, and the animals were maintained on this diet until they died. The survival time of each rat was recorded and the same chemical determinations were carried out as on the sacrificed rats.

Recovery period. After 9 wk on the partial starvation diet (22 wk from the beginning of the experiment), the surviving animals were fed the control WMS diet *ad lib.* to detect any eventual difference in recovery between the groups. These animals were sacrificed 2 or 4 wk later and haematological and chemical analyses were carried out.

RESULTS

Growth, food intake, haematology and organ weights

During the first 13 wk, growth and food intake of the animals receiving antioxidants did

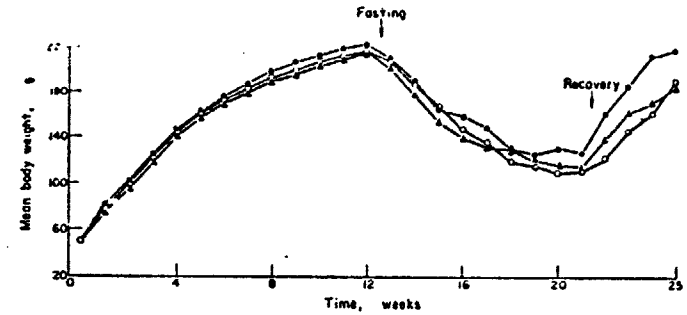

FIG. 1. Growth curves of female rats during a feeding period of 13 wk, a subsequent partial starvation period of 9 wk and a recovery period. Numbers of animals: wk 1–13, at least 30; wk 13–22, 20–3; wk 22–26, 3 or 4. ○, control lard group; ●, 0·002+0·004% propyl gallate groups; ▲, 0·002+0·004% α-tocopherol groups.

not differ significantly from those of the control groups. In Fig. 1 the growth curves of the female control rats and female rats on propyl gallate and on α-tocopherol are given. It is clear that these animals did not show any difference in growth. The same was the case for the male animals. The partial starvation diet, starting after 13 wk caused a rapid weight loss in all groups. After a 9-wk fasting period, the 1 male and 13 females still alive were placed on the control WMS diet. The body weight of these rats recovered rapidly. No significant differences in the blood composition or in organ weights relative to body weight or heart weight were found that could be ascribed to the administration of the antioxidants.

Survival time after fasting

A striking difference in survival time was found between male and female rats. Since there was no difference between the two dose levels of antioxidants, data from the groups receiving 0·002 and 0·004% propyl gallate were combined for statistical calculations. The same was done for the groups receiving 0·002 and 0·004% α-tocopherol. The difference in survival time between males and females was statistically significant for the control and the tocopherol groups ($P<0·05$) and for the gallate groups ($P<0·01$). In Table 1 the cumulative percentage mortality after varying periods on the partial starvation diet is given for male and female animals.

Table 1. *Effect of prior administration of antioxidants on cumulative percentage mortality of rats during a period of partial starvation*

Diet	Cumulative mortality (%) at day*						
	15	20	25	30	40	50	65
			Males				
Control lard	5	25	55	80	95	100	100
Gallate (both levels)	14	57	67	81	86	90	95
Tocopherol (both levels)	15	40	70	95	95	100	100
Control WMS	0	50	70	80	100	100	100
			Females				
Control lard	5	35	45	65	65	85	85
Gallate (both levels)	0	26	42	42	42	74	84
Tocopherol (both levels)	5	30	35	60	75	85	85
Control WMS	10	30	50	50	60	60	60

*From beginning of starvation period.

The data given in Table 1 do not show a clear difference between the rats of the control groups and the rats that had received propyl gallate or α-tocopherol in their diet. Most of the animals died in the first 15–30 days after the beginning of the fasting period, but there was a large variation. After a 9-wk fasting period, 14 animals were still alive and it was therefore difficult to calculate a mean survival time. An attempt was made to do this by giving the surviving animals an arbitrary survival time of 65 days, since from that time on they were placed on a normal diet. The results of this calculation are presented in Table 2 and show no statistically significant difference between the groups for either sex.

Table 2. *Effect of antioxidants on "mean survival time" of rats during fasting*

	Males		Females	
Diet	Death rate	Mean survival time (days)	Death rate	Mean survival time (days)
Control lard	20/20	26·0	17/20	32·6
Gallate, 0·002%	11/11	21·2	8/9	40·8
Gallate, 0·004%	9/10	28·1	8/10	38·0
Tocopherol, 0·002%	10/10	24·3	8/10	37·3
Tocopherol, 0·004%	10/10	20·9	9/10	27·2
Control WMS	10/10	22·6	6/10	40·0

Chemical analyses

Animals sacrificed after 13 wk. The water, fat and protein content of the carcasses as well as the protein content of the liver are summarized in Table 3. The results, except in the case of the liver, are expressed as percentages of the minced carcass.

Animals dying during fasting. In all rats that died during the fasting period the same chemical analyses were carried out as in the rats that were sacrificed after the 13-wk feeding period. The results are given in Table 3. When these results are compared with those of the rats that were sacrificed before the fasting period, a statistically significant difference ($P<0·001$ in all cases) in body weight and water and fat content of the animals is apparent. The decrease in body weight was considerable, averaging 43·9% for the males and 42·5% for the females, and became more pronounced with longer survival. With Spearman's rank correlation test (Jonge, 1963) a significant negative correlation ($P<0·01$) was found between the survival time and the body weight for both male and female rats when data for all groups were combined.

Animals sacrificed after recovery. One male and 13 female rats still alive after the 9-wk fasting period were placed on the control WMS diet. From Fig. 1 it is clear that the body weight recovered rapidly. Half of the animals were sacrificed after 2 wk and the rest after 4 wk. The same chemical analyses were carried out as in the other groups of rats. Since there appeared to be no difference (except in body weight) between the animals sacrificed after 2 or 4 wk, the results have been combined in Table 3.

These results show that a normal food intake for 2 wk is sufficient to restore the water and fat content of the rats to the same level as before the fasting period. Only the animals of the control WMS group had a tendency to a higher fat and a lower water content than before. The weight gain after 2 wk was 69% for the control WMS group and averaged 42% for the other groups. After 4 wk these figures had increased to 83% and 74% respectively. The organ weights and haematological findings did not show any deviations.

Table 3. *Water, fat and protein content of the rats at various stages of the test*

			Carcass			Liver
Diet	No. of animals	Body weight (g)	Water (%)	Fat (%)	Protein (%)	Protein (%)
Males		Animals sacrificed after 13 wk				
Control lard	10	280	67·3	6·21	19·7	22·8
Gallate, 0·002%	5	284	67·4	4·94	21·0	23·9
Gallate, 0·004%	4	301	67·8	5·15	20·0	21·4
Tocopherol, 0·002%	4	289	68·0	5·75	19·5	20·5
Tocopherol, 0·004%	5	285	67·4	6·05	19·7	20·7
Control WMS	5	302	67·4	6·55	19·3	20·9
Females						
Control lard	10	194	67·2	7·62	19·9	22·2
Gallate, 0·002%	5	205	65·1	7·63	19·7	22·1
Gallate, 0·004%	5	206	64·7	9·09	21·0	22·5
Tocopherol, 0·002%	5	206	64·5	8·79	18·7	20·6
Tocopherol, 0·004%	5	196	66·0	7·62	19·6	21·0
Control WMS	5	199	65·5	7·94	18·4	21·4
Males		Animals dying during fasting				
Control lard	19	161	70·8	0·34	17·6	20·8
Gallate, 0·002%	11	175	71·1	0·29	18·0	19·7
Gallate, 0·004%	9	150	71·1	0·33	18·1	19·8
Tocopherol, 0·002%	10	152	70·4	0·34	19·4	19·9
Tocopherol, 0·004%	10	159	70·4	0·33	18·7	21·1
Control WMS	10	171	70·3	0·32	19·1	21·6
Females						
Control lard	17	122	69·9	0·38	18·3	20·8
Gallate, 0·002%	8	107	70·2	0·48	17·2	21·5
Gallate, 0·004%	8	116	69·6	0·41	18·6	21·0
Tocopherol, 0·002%	8	111	70·0	0·47	17·3	21·7
Tocopherol, 0·004%	9	117	70·4	0·44	18·3	21·6
Control WMS	6	116	69·4	0·38	19·6	21·4
		Animals sacrificed after recovery				
Control lard	3	—	65·9	7·48	18·0	21·1
Gallate (both levels)	4	—	65·3	9·51	19·0	21·0
Tocopherol (both levels)	3	—	66·1	7·38	19·6	20·8
Control WMS	4	—	63·0	12·79	20·4	20·0

In any given period, no statistically significant difference was found between the groups for any of the determinations made.

Histopathology

Extensive histopathological examinations were carried out on all the organs and tissues which were weighed, except the brain and the thymus. The digestive tract and the bladder were also studied. Because many of the fasting animals died during the night, a marked autolysis was found in the tissues of these animals, especially in the digestive tract. All animals which died during the starvation period were extremely cachectic and most of them showed oedema.

Neither the sacrificed animals, nor those which died after fasting showed any pathological changes which could be attributed to the treatment with antioxidants.

DISCUSSION

This experiment was carried out in order to repeat and extend a toxicity study with propyl gallate carried out by Bukhman (1962). In his experiments, animals given propyl gallate in their diet showed a reduction in weight gain and delayed development. During partial fasting the rats receiving propyl gallate failed to utilize their body fat and protein adequately and succumbed at an earlier date. Bukhman found a mean survival time of 14·7 days for these groups and of 26·8 days for the groups not receiving propyl gallate. He used only male animals.

In our experiment, rats of both sexes were used. It appeared that the survival time for female animals was longer than that for male animals. In fact there were 13 females and 1 male still alive 9 wk after the beginning of the starvation period. When these animals were given an arbitrary survival time of 65 days, an average of 23·9 days was found for the male animals of all groups and 36·1 days for the females. No difference in survival time was observed between the control and test groups. The longer survival of the female animals can probably be explained by the fact that the starvation diet (20% of the calorific value of the normal daily food intake) was based on the food consumption of the male animals. The females were given the same quantity of food, although they normally need less food than the males.

The determination of the water, fat and protein content of the carcasses did not show any differences between control and treated animals. Animals that died during the partial starvation period had a significantly higher water content than the animals sacrificed after the 13-wk feeding period. This was possibly due to starvation oedema. The fat content of the animals that died was significantly lower than that of the sacrificed animals. There was no difference in fat content between the animals that died 10–15 days after the beginning of the starvation period and those dying after more than 40 days starvation. Obviously the fat was utilized quite rapidly at the beginning of this period in spite of the administered antioxidant, but a very small amount, less than 0·5%, always remained.

The protein content of the rats showed a tendency to be lower in the animals dying during the starvation period than in the sacrificed animals, especially when they survived more than 40 days, but the difference was not significant. The protein content of the liver was not affected by fasting.

In contrast to Bukhman's postulation, our biochemical analyses did not show that rats fed diets containing propyl gallate use their fat and protein resources less effectively than the controls. In no case was there a significant difference in fat or protein content between the groups. Possibly this can be explained by the fact that Bukhman expressed his results in terms of absolute amounts of protein and fat and not as percentages of the body weight.

We have found a strongly negative correlation between survival time and body weight. In Bukhman's paper no body weights were given, but since our results indicated a relatively constant protein content of 20% for all rats, we can calculate from his figures the body weight before fasting and when the animals died. In this way we have also calculated the weight loss of his animals when they died, and found for the groups without propyl gallate a mean value of 33·5% and for the groups receiving fats containing 0·02% propyl gallate a mean value of 21·9%.

In our investigation a loss in body weight of 43·9% for the males and 42·5% for the females was found. It seems that in our test, animals consuming antioxidants utilized their protein more effectively than the animals on a gallate-free diet in Bukhman's study. Since no

pathological findings were reported by Bukhman, it is not known why the animals with propyl gallate in their fat died when they had lost only 21·9% of their body weight.

Our conclusions on propyl gallate also apply to α-tocopherol, since, as the tables and figure show, the results for the two compounds are closely comparable.

Since we could not confirm Bukhman's results, it has been concluded that this type of study makes no useful contribution to the toxicological investigation of antioxidants or other substances.

REFERENCES

Bukhman, N. D. (1962). Der Einfluss des Propylgallat-antioxydants auf den Nährwert des Fettes. *Vop. Pitan.* 21, 68.

Joint FAO/WHO Expert Committee on Food Additives (1965). Specifications for Identity and Purity and Toxicological Evaluation of some Antimicrobials and Antioxidants. *WHO/Food Add./24.65; FAO Nutr. Meet. Rep. Ser. No. 38A.*

Jonge, H. de (1963). *Inleiding tot de Medische Statistiek*. 2nd ed. Part I, p. 305. Nederlands Instituut voor Praeventieve Geneeskunde, Leiden.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurements with the Folin phenol reagent. *J. biol. Chem.* 193, 265.

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometr. Bull.* 1, 80.

Influence du gallate de propyle et de l'α-tocophérol sur la durée de survie des rats pendant le jeûne

Résumé—Une étude toxicologique a été conduite afin de reproduire et de compléter les données de Bukhman (*Vop. Pitan.*, 1962, 21, 68) en ce qui concerne la toxicité du gallate de propyle chez les rats soumis au jeûne. Des rats ont reçu un régime contenant 0,002 à 0,004% de gallate de propyle ou d'α-tocophérol. Au bout de 13 semaines, certains de ces animaux ont été sacrifiés, les autres étant mis sous un régime de famine partielle jusqu'à leur mort. On a relevé la durée de leur survie. On a procédé à des dosages de l'eau, des protéines et des lipides aussi bien chez les animaux sacrifiés que chez ceux qui sont morts. On n'a trouvé aucune différence quant à la durée de survie ou à l'utilisation des protéines et des lipides entre le groupe témoin et celui auquel était administré le gallate de propyle ou l'α-tocophérol. On n'a pas pu confirmer les résultats qu'avait obtenus Bukhman.

Der Einfluss von Propylgallat und α-Tocopherol auf die Überlebenszeit von Ratten während des Fastens

Zusammenfassung—Es wurde eine toxikologische Untersuchung durchgeführt, um die Ergebnisse von Bukhman (*Vop. Pitan.* 1962, 21, 68) hinsichtlich der Toxizität von Propylgallat in fastenden Ratten zu wiederholen und zu ergänzen. Ratten erhielten Futter mit einem Gehalt von 0,002 oder 0,004% Propylgallat oder α-Tocopherol. Nach 13 Wochen wurden einige Tiere getötet, während die verbleibenden Ratten ein zum Tode führendes unzureichendes Futter erhielten. Die Überlebenszeit wurde bestimmt. Chemische Bestimmungen des Wasser-, Protein- und Fettgehalts wurden an den getöteten und an den verendeten Tieren durchgeführt. Hinsichtlich der Überlebenszeit oder der Protein- und Fettverwertung wurde kein Unterschied zwischen den Kontrollgruppen und den Gruppen festgestellt, die Propylgallat oder α-Tocopherol erhielten. Die Ergebnisse von Bukhman konnten nicht bestätigt werden.

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METABOLIC PATHWAYS OF THE PHENOLIC ANTIOXIDANTS

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INTRODUCTION

The addition of phenolic antioxidants to fats and foods containing fats intended for human consumption is practised in many countries throughout the world today. Their purpose is to prevent edible fats and oils from becoming rancid by autoxidation. The compounds that are at present being used, their permitted levels in the fat, and the number of countries using them are set out in Table 1. Over 1,000 tons of the antioxidants BHA, BHT and PG were added to food and animal fodders in the U.S.A. during 1956; over 400 tons of this amount were incorporated in foods for human consumption. Of these phenolic compounds, the most commonly used are BHA (55%), BHT (25%) and PG (20%) while THBP is the most recently developed of them and at the time of writing has been approved for use only in the U.S.A. Legislative approval for the use of any of the antioxidants listed in Table 1 has not yet been given in New Zealand.

Table 1: The phenolic antioxidants, their permitted maximum allowable concentrations and the number of countries that have approved their use in foods (as at Dec., 1959).

Antioxidant, accepted abbreviation	*Chemical name*	*Maximum allowable concentration in fats (%)*	*Number of countries permitting their use*
BHA[1]	2-*t*-butyl-4-methoxyphenol (85%) and 3-*t*-butyl-4-methoxyphenol (15%)	0.02	16
BHT[2]	2:6-di-*t*-butyl-4-methylphenol	0.02	8
PG	*n*-propyl gallate	0.01	19
OG	octyl gallate	0.015	16
DG	dodecyl gallate	0.02	
NDGA[3]	4:4′-(2:3-dimethyltetramethylene)-dipyrocatechol	0.01	12
THBP	2:4:5-trihydroxybutyrophenone	0.01	1

[1] Also known as 3-*t*-butyl-4-hydroxyanisole (major isomer) and 2-*t*-butyl-4-hydroxyanisole (minor isomer).
[2] Also known as 3:5-di-*t*-butyl-4-hydroxytoluene.
[3] Commonly called nordihydroguaiaretic acid.

Extensive toxicity studies have been carried out on all these compounds using experimental animals. These include acute toxicity tests, particularly for the determination of the LD_{50},* and both short- and long-term toxicity tests which include usually a histopathological examination of the test species. The recommended species for this testing are the rabbit, rat and dog.

An antioxidant is at present regarded as safe for human use if it satisfies two conditions. First, the LD_{50} must be not less than 1,000 mg/kg animal body weight; second, it must not have any significant effect on growth when fed to experimental animals in their total diet for long periods (up to two years) at a level one hundred times that proposed for fats for human consumption. All the approved antioxidants have, of course, passed these tests. Quinol (hydroquinone), one of the first phenolic compounds to be used as an antioxidant, has a LD_{50} well below this safe level and is not now used. The approximate LD_{50}'s of the phenolic antioxidants compared with phenol, quinol and *p*-cresol are set out in Table 2.

Is the acute and chronic toxicity study a sufficient criterion for assessing the safety of these compounds? The joint FAO/WHO expert committee on food additives recently considered this question (World Health Organization, 1958). In addition to the routine toxicity tests, the report recommended that additional knowledge of the metabolic and biochemical effects of these additives should be provided for their evaluation. It is necessary to have as much information as possible about an additive before it appears in our food.

The Toxicology Research Unit of the Medical Research Council of New Zealand was formed in 1954 to study the possible dangers to health arising from the increasing use of chemicals as food additives. Because of the growing uneasiness among those people responsible for establishing the safety for use of food additives, a research programme was planned for an investigation of the toxicity and metabolic fate of some of the phenolic antioxidants. Until we commenced this work, virtually nothing was known on how the body copes with these compounds. From the point of view of public health protection, information was required to supply the answers to the following:

* LD_{50} (Lethal Dose 50%) is a measure of toxicity—*i.e.*, the quantity of the compound, preferably expressed in mg/kg of animal body weight, which when administered to experimental animals will be 50% fatal. Toxicity may also be expressed as LD_{80}, LD_{90}, etc.

Table 2: The approximate LD_{50} (mg/kg) by oral administration to rats, mice and rabbits of the phenolic antioxidants, compared with phenol, quinol and *p*-cresol.

	Approximate LD_{50} (mg/kg)		
Antioxidant	*Rats*	*Mice*	*Rabbits*
BHA	2,200	1,250	
	2,900	2,000	
	4,130 (non-fasted)		
	>5,000 (fasted)		
BHT	1,700 (female)	2,000	2,100-3,200
	1,970 (male)		
	2,450		
PG	2,600	1,700	
	3,600	2,000	
	3,800	3,500	
OG	4,500		
DG	6,500		
NDGA	>2,000	>2,000	
	5,500	4,000	
THBP	3,200-6,400	800-1,600	
Phenol	440	395	420
	450	520	
	1,500		
Quinol	320	400	
p-Cresol	1,800		620

(i) What effects on animals have very high doses of antioxidants? It is not sufficient to find the dose level at which some adverse effect is produced; it is necessary to know precisely what the effect is and how it is produced.

(ii) How does the animal's body deal with the strange chemical? This involves a study of the chemical changes that the antioxidant undergoes in the animal's body.

(iii) If the dose is repeated daily, will the material accumulate in the animal body and in one way or another produce cumulative effects?

This paper summarizes the results of investigations that have been carried out in the last six years in an attempt to answer the above questions. It is hoped that metabolic studies of this kind will provide the essential additional evidence required for assessing the safety for use of these antioxidants in our foods.

DETOXICATION PATHWAYS OF PHENOLIC ANTIOXIDANTS

Phenolic compounds are detoxified in the body by conjugation with various compounds involving both the hydroxyl group and the benzene ring system. In certain cases other groups attached to the phenolic ring may undergo some metabolic reactions also. These conjugation reactions involve glucuronic acid in glucuronide formation, sulphuric acid in ethereal sulphate formation, glycine in hippuric acid formation, and rarely *O*-methylation or hydroxylation of the aromatic ring.

The first section of this account considers the origin of the principal compounds in the body that are available to conjugate with the phenolic antioxidants to form the metabolites that are ultimately excreted in the urine. The second section outlines the forms in which the phenolic antioxidants take on conjugation—*i.e.*, the structural patterns of the metabolites of which the antioxidant constitutes the aglycone portion.

A. Enzymic pathways for the conjugation of the phenolic antioxidants with glucuronic acid, sulphuric acid and glycine.

When phenols are administered to animals, the main portion of a dose can be accounted for by the excretion in the urine of the corresponding aryl-sulphuric acid and β:D-glucosiduronic acid. Aromatic and phenolic acids are also excreted by conjugation with sulphuric and glucuronic acids, and a portion of the dose may be combined with glycine as the hippuric acid.

1. Glucuronide synthesis.

Within the last few years, the steps in the enzymic synthesis of glucuronides have been fairly well established. The steps in the formation of a phenolic glucuronide, which takes place in the liver, are shown in the following scheme:

glycogen
↓
α:D-glucose-1-phosphate + uridine-5-triphosphate
↓ *uridyl transferase*
uridine diphosphate-α:D-glucose + pyrophosphate
↓ DPN+ *UDPG-dehydrogenase*
uridine diphosphate-α:D-glucosiduronic acid + DPNH
↓ Ar-OH *glucuronyl transferase*
Ar-β:D-glucosiduronic acid + uridine diphosphate
(Ar-OH = phenolic antioxidant)

The important point to note is that at no time does glucuronic acid occur in the free state; it is produced from the glucose of uridine diphosphate-α:D-glucose by oxidation. The conjugated glucuronic acids of the phenolic antioxidants are all β:D-glucuronides and in the last step in their synthesis, which is catalysed by glucuronyl transferase, a Walden inversion reaction probably takes place. The same enzyme systems are involved in the formation of the ester-linked type of glucuronide (conjugation through a carboxy acid group) as distinct from the ether-linked type (conjugation through the phenolic hydroxyl group).

2. Ethereal sulphate synthesis.

It is now quite clear that the enzymic synthesis of the aryl sulphates takes place in the liver also. A process of sulphate activation is brought about which requires both ATP and magnesium ions. Sulphate then is transferred enzymically from the active sulphate (PAPS) to the phenolic antioxidant to form the ethereal sulphate as shown in the following tentative scheme:

Adenosine-5′-triphosphate (ATP) + SO_4^{--}
↓ *ATP-sulphurylase*
Adenosine-5′-phosphosulphate (APS) + pyrophosphate
↓ ATP *APS-kinase*
Adenosine-3′-phospho-5′-phosphosulphate (PAPS) + ADP
↓ Ar-OH *phenol sulphokinase*
$Ar\text{-}SO_4^-$ + 3′:5′-diphosphoadenosine (PAP)
(Ar-OH = phenolic antioxidant)

3. Hippuric acid synthesis.

The synthesis of hippuric acids occurs in the liver and the kidney in some animals. The process requires an activated form of the benzoic acid derivative which appears to be a benzoyl-coenzyme A. The pathway can be represented as follows:

Benzoic acid derivative + ATP ⟶ Adenylbenzoate + pyrophosphate
Adenylbenzoate + CoA ⟶ Benzoyl-CoA + adenylic acid
Benzoyl-CoA + glycine ⟶ Hippuric acid derivative

This enzymic synthesis involves a phenolic carboxy group only.

B. Structural patterns of the metabolites of the phenolic antioxidants.

The study of the metabolism of these compounds is essentially an investigation of the urinary excretion following administration of

the phenolic antioxidant to the animal. An initial analysis of the urine for the different conjugates has been carried out for the majority of them. The percentage excretion of the urinary metabolites of the phenolic antioxidants (including quinol) is summarized in Table 3.

Table 3: Urinary metabolites of the phenolic antioxidants
(Results expressed as a percentage of the dose)

Antioxidant:	*BHA*		*BHT*	*PG*	*THBP*		*Quinol*
Species used:	Rabbit	Rat	Rabbit	Rabbit	Dog	Rat	Rabbit
Dose level:	1g	0.08-0.1g	0.8-1.6 g/kg	1g	0.3g/kg	0.4g/kg	0.1-0.2 g/kg
Metabolites:							
Glucuronide conjugate	46.4	71.5	35.9	72.0	30	23	42.9
Ethereal sulphate	9.2	13.5	8.0	—	45	52	30.0
Unconjugated phenol	5.8	5	8.4	6.7	2.3	6.3	+
Glycine conjugate	0	—	1.8	—	—	—	—
Total recovery:	61.4	90.0	54.1	78.7	77.3	81.3	>72.9

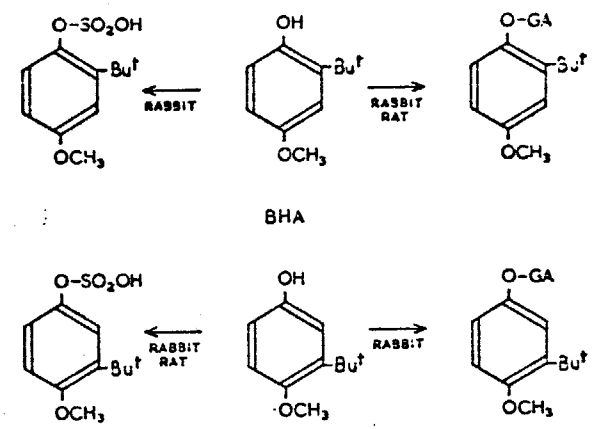
The next section outlines the structures of the urinary metabolites of the individual antioxidants as determined by analysis, by degradation and in some cases by their synthesis, which naturally follows after their isolation from urine.

1. Metabolites of BHA.

BHA was the first phenolic antioxidant to be studied in any detail. The metabolism of BHA and of its isomers (2-*t*-butyl- and 3-*t*-butyl-4-methoxyphenol) in the rabbit was investigated by Dacre *et al.* (1956). They found BHA to be conjugated with both glucuronic acid and sulphuric acid. A small part of the BHA was excreted unchanged in the urine. The glucuronide conjugates of the two isomers were isolated by these workers as the crystalline barium salts; the free glucuronides were found to have the structures 2-*t*-butyl- and 3-*t*-butyl-4-methoxyphenyl-β:D-glucosiduronic acid. From the urine of rats after the administration of BHA, the glucuronide of the 2-*t*-butyl isomer was isolated as the crystalline triacetyl methyl ester (Dacre, 1958). Astill *et al.* (1960) found that rats metabolized the 2-*t*-butyl isomer chiefly to the glucuronide and the 3-*t*-butyl isomer to the corresponding

ethereal sulphate. The ethereal sulphates of the two isomers of BHA were synthesized and shown to be constituted as 2-*t*-butyl- and 3-*t*-butyl-4-methoxyphenyl hydrogen sulphate. Both the sulphates were isolated from rat urine and shown to be identical with the synthetic compounds. These workers also presented evidence for the probable formation by demethylation of 2- (or 3-*t*-butyl-4-hydroxyphenyl hydrogen sulphate.

The metabolic pathways of BHA are set out in Scheme 1.



Scheme 1.

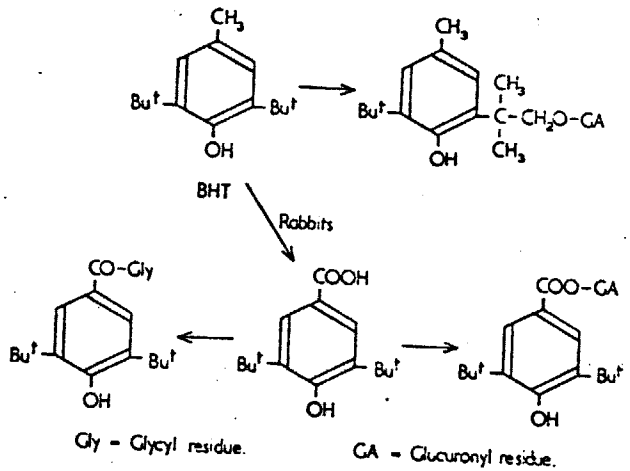
2. Metabolites of BHT.

The metabolism of BHT in the rabbit was investigated by Dacre (1960a, 1960b). This antioxidant presents an unusual metabolic problem to the animal because the hydroxyl group is sterically blocked by the two large *t*-butyl groups in the 3 and 5 positions. Conjugation of this hindered group with glucuronic acid and sulphuric acid in the conventional manner cannot, of course, take place. How then does the animal manage to cope with this antioxidant? The compound was found to be metabolized by oxidation along two separate pathways: firstly, one of the *t*-butyl groups is converted by ω-oxidation to the 2:2-dimethyl-2-ethyl structure; and, secondly, the methyl group in position 1 is oxidized to the carboxy acid. The conjugates are then formed through one of these two new groups.

Four metabolites of BHT were isolated and characterized by Dacre (1960a) from the urine after feeding BHT to rabbits. The major portion of the dose of BHT was conjugated with glucuronic acid as both ester-linked and ether-linked glucuronides; some was

excreted as a glycine conjugate; and some was present in an uncombined but oxidized form. The two glucuronides were isolated as the crystalline triacetyl methyl ester derivatives. Acid and enzyme hydrolysis experiments showed that these conjugates have the structures 3:5-di-*t*-butyl-4-hydroxybenzoyl-β:D-glucosiduronic acid and β:β'-dimethyl-β''-(2-hydroxy-3-*t*-butyl-5-methylphenyl) -ethyl-1-β:D-glucosiduronic acid. Similar hydrolysis experiments on the glycine conjugate show that it is formulated as 3:5-di-*t*-butyl-4-hydroxyhippuric acid. The tree phenol was identified as 3:5-di-*t*-butyl-4-hydroxybenzoic acid. Analysis of the urine indicated some excretion of BHT as an ethereal sulphate but nothing is known at present about the structure of this metabolite. The same benzoyl-β:D-glucosiduronic acid and hippuric acid conjugates were found in rabbit urine following the oral administration of 3:5-di-*t*-butyl-4-hydroxybenzoic acid in the amounts 84.0% and 8.8% respectively of the dose (Dacre, 1906b).

The metabolic pathways of BHT are set out in Scheme 2.



Scheme 2.

The nature of the cause of death when relatively large, repeated doses of BHA and BHT were given orally to experimental animals was studied by Denz and Llaurado (1957). The fatal effects of large doses in rabbits are due to a gross disturbance of water and salt balance. There is an increased urinary excretion of sodium, potassium and aldosterone, associated with a fall in serum and muscle potassium, a rise in muscle sodium, and a fall in extracellular fluid volume. Large doses of BHA do not produce in the rat the

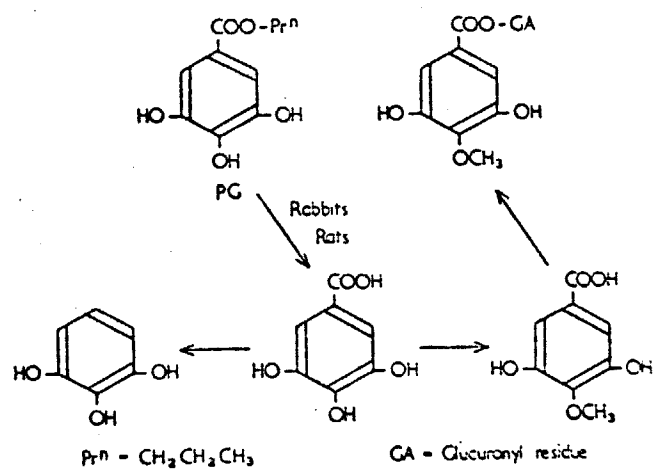
large increase in potassium excretion that is found in the rabbit. It is well known that phenolic compounds are toxic in large doses but this is the first evidence of the way in which they produce their toxic effects.

3. *Metabolites of PG.*

It is only recently that the metabolic fate of gallic acid and its related compounds, propyl gallate and lauryl gallate, was investigated (Booth *et al.* 1959). When PG was given to rats by stomach tube, the metabolites identified chromatographically in the urine were mainly 4-*O*-methyl gallic acid and smaller amounts of gallic acid. Some evidence for a conjugate of 4-*O*-methyl gallic acid was found, when this acid was given to rats.

The metabolism of PG was studied by Dacre (unpublished results). The initial analysis of rabbit urine for conjugates following the administration of PG is summarized in Table 3. A paper chromatographic analysis for metabolites in the urine showed the presence of a glucuronide as the major urinary metabolite. Other metabolites identified were 4-*O*-methyl gallic acid, gallic acid and pyrogallol. From its chemical reactions, the glucuronide conjugate almost certainly has the structure 4-*O*-methyl galloyl-β:D-glucosiduronic acid. It seems clear then that the main pathway is by cleavage of the ester linkage followed by methylation of the hydroxyl group in the 4 position and/or conjugation with glucuronic acid.

The metabolic pathways of PG are set out in Scheme 3. There are no studies reported on the metabolism of OG and DG in animals.

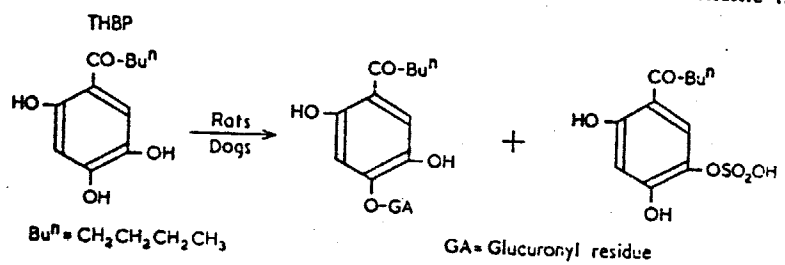


Scheme 3.

4. Metabolites of THBP.

The metabolism of the antioxidant THBP in the rat and dog was recently investigated by Astill *et al.* (1959). They showed that this compound was metabolized largely by ethereal sulphate formation, with a lesser amount of conjugation with glucuronic acid. The butyryl side-chain appeared to survive intact and no evidence of oxidation to more complex phenols was obtained. The ethereal sulphate conjugate was isolated as its potassium salt and shown to have the structure 5-butyryl-2:4-dihydroxyphenyl hydrogen sulphate. The glucuronide of THBP was not isolated but experiments showed that its structure was 4-butyryl-2:5-dihydroxyphenyl-β:D-glucosiduronic acid. There was also some evidence for a third conjugate (an ethereal sulphate) of THBP in both rat and dog urine. Small quantities of THBP were also excreted unchanged in the urine.

The metabolic pathways outlined above are set out in Scheme 4.



Scheme 4.

5. Metabolites of Quinol.

This was the first phenol to be used as an antioxidant. Garton and Williams (1949) studied its metabolic fate in the rabbit as part of their extensive investigations of the detoxication of benzene. Small doses were excreted, chiefly as the glucuronide and to a lesser extent as the ethereal sulphate conjugate. The glucuronide was isolated as the crystalline triacetyl methyl ester and shown to have the structure *p*-hydroxyphenyl-β:D-glucosiduronic acid. The ethereal sulphate could not be isolated from rabbit urine but their experiments showed that it is almost certainly the monosulphate of the compound—*i.e.*, it is *p*-hydroxyphenyl hydrogen sulphate. Some of the dose of quinol is excreted uncombined in the urine.

6. Metabolites of NDGA.

Nothing is known of the metabolism of this antioxidant.

There is very little known about other methods of excretion of the antioxidants in experimental animals. There is some excretion in the faeces of animals but the evidence so far obtained shows little or no storage of them in organs and tissues. The further fact that recovery of these compounds in the urine is by no means completely accounted for (see Table 3) indicates the need for further studies. Other metabolic pathways may possibly be found.

SUMMARY

1. The metabolic pathways of the phenolic antioxidants in experimental animals have been outlined.

2. The studies have shown that these compounds are metabolized along well-known and recognized routes—*i.e.*, by conjugation with glucuronic acid, with sulphuric acid or with glycine.

3. In the case of BHT conjugation takes place only after the compound has undergone oxidation along two different pathways; in the case of PG only after it has undergone ester cleavage and *O*-methylation.

4. No evidence has so far been found which suggests that these antioxidants produce adverse biochemical or metabolic effects in the animal body. The studies provide additional evidence for establishing the safety for use of these food additives.

REFERENCES

Astill, B. D., Fassett, D. W., and Roudabush, R. L. (1959): *Biochem. J.*, *72*: 451-9.

Astill, B. D., Fassett, D. W., and Roudabush, R. L. (1960): *Biochem. J.*, *75*: 543-51.

Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., and DeEds, F. (1959): *J. Biol. Chem.*, *234*: 3,014-6.

Dacre, J. C. (1958): In *Ann. Rep. Med. Res. Council of N.Z.*, p.32.

Dacre, J. C. (1960a): *Proc. Univ. Otago Med. Sch.*, *38*: 1-2.

Dacre, J. C. (1960b): *Biochem. J.*, *77* (in the Press).

Dacre, J. C., Denz, F. A., and Kennedy, T. H. (1956): *Biochem. J.*, *64*: 777-82.

Denz, F. A., and Llaurado, J. G. (1957): *Brit. J. Exp. Path.*, *38*: 515-28.

Garton, G. A., and Williams, R. T. (1949): *Biochem. J.*, *44*: 234-8.

World Health Organization (1958): In *Procedures for the Testing of Intentional Food Additives to Establish Their Safety For Use* (Second report of the joint FAO/WHO expert committee on food additives), pp. 1-19. *Tech. Rep. Ser. No. 144.*

The following updates were Recd in DPP 11-9-72 #'s 53, 54, 84, 85, 151, 208, 209, 210, 287, 315, 407

(53)

The Effect of Phenolic Antioxidants on the Assimilability of Rendered Lard

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In recent years, both in the domestic (Soviet) and foreign literature, there have been published a number of papers covering the study of various antioxidants that prevent the spoilage of fats. In our country, in this direction, there are a number of papers (studies) of N. M. Emanuel and others showing the considerable effect of phenolic antioxidants: of butyloxyanisol, butyloxytoluol and of gallic acid esters. It turned out that, in adding them in small amounts (0.01 - 0.02%) to fat, there was a retardation of the oxidizing processes and there was a lengthening of the storage time for the latter. The problem concerned with the possibility of utilizing butyloxyanisol, butyloxytoluol and propylgallate in the capacity of antioxidants of alimentary fats, from the hygienic point of view, has been given special study by I. A. Karplyuk (1959, 1960). She investigated both the organoleptic properties of fat containing the abovementioned antioxidants, as well as the effect on the organism of animals (dogs and white rats) in the case of prolonged feeding. On the basis of the investigations that had been carried out, I. A. Karplyuk came to the conclusion that butyloxyanisol and butyloxytoluol are completely harmless, but not so in the case of propylgallate.

Proceeding from the fat that the abovementioned compounds inhibit the oxidizing processes in fats, we considered it interesting to study the possibility of their effect on the assimilability of fat. We did not find any data in the literature on this problem.

In the capacity of an investigatory subject use was made of fresh rendered lard to which had been added butyloxytoluol, butyloxyanisol and propylgallate, calculating 0.2 gram for 100 grams of fat.

The experiments were conducted on 10 mature white male rats of one generation,

altogether 5 experiments were carried out with a duration of 7 days. The rats were fed a diet consisting of a dry concentrated feed with the addition of 2 gr of lard -- in 2 control experiments, without any antioxidants, and in the 3 rem ing ones, fat was added with the abovementioned antioxidants. Thus, the rats, over the course of a 7-day experimental period, received 14 grams of lard and 2 milligrams of butyloxytoluol, butyloxyanisol and propylgallate. For a uniform distribution of the rendered lard added to the diet, and in order to eliminate the possibility of the scattering of the food by the rats, a dry concentrate fe was ground, carefully mixed with fat, wetted with water and rolled in the form little balls, which were fed to the animals.

In order to assure a follow-up (calculation) of the uneaten feed and of the excreted feces, all the rats were kept in individual cages of the following construction. The floor of the cage, in which the rats were found, consisted of little glass tubes that loosely adjoined one another. Underneath the little tu at a distance of 5 centimers, there was located a metallic screen onto which the fell the feces and large feed residues; smaller particles of food were retained on the underlying screen that was sheathed with a Kapron fiber (the distance between the second and third screen was 2.5 centimeters). For the purpose of accustoming the animals to the conditions of the experiment, preliminarily, over the course of 2 weeks, they were kept in cages that had been prepared for the experiment, and they were given the above-indicated feed with lard, but without any antioxidants. In order to separate one experimental period from the other, carmine (0.03 gram) was added to the feed at the end of the experiment. The feces colored with carmine was discarded and not considered in the experiments.

Between the experiments, when fat with antioxidants was fed, there was an interval of 4 - 5 days. The gathering of the feces and of the feed residues was not carried out during these days. In the course of the 7-day experimental peri the feces excreted by each rat and the residues of the uneaten food were gathere daily and dried.

The feed residues, which had been gathered together and dried for 7 days, wer brought to an absolutely dry state, and after that, a calculation was made of

the actually eaten feed and of the fat contained in it for the dry substance. Th feces that had been gathered together for 7 days was ground and from its average weighted portion of 5 grams fat was extracted by the Soxhlet method. Following this, a calculation was made of the fat excreted with the feces during the entire experimental period for an absolutely dry substance. In the dry concentrated feed a determination was made of the fat content, by the Soxhlet method, and of amount of moisture.

The experiments showed that the consumption of the feed by the rats was a varying one. A poor consumption on the part of the rats was observed in experiments in which the rats were fed a diet with the addition, to the fat, of butyloxytoluol and particularly, propylgallate. The rats consumed the feed reluctantly, even in the final control experiment.

The poor consumption of the feed we are inclined to explain, in the first place, by the possible influence of the high temperature of the air. In June an in the first half of July, when the experiments were carried out, the average daily temperature of the air fluctuated within the range of 21 - 22 deg. In the vivarium, where the rats were kept, and where the ventilation was insufficient, the temperature of the air, during the day, reached 28 deg. It is also possible that by the end of the experiments, the rats may have developed a dislike for the feed, since there was added, to the diet, a relatively large amount of fat. To the extent that the consumption of the feed by the rats was a varying one, in order to compare the data that we had obtained, a calculation was made of the relative amount of unassimilated fat:

$$X = (b \cdot 100).(a),$$

where "a" represents the amount of fat, in grams, that had been actually consumed by the rats; b represents the amount of fat excreted with the feces, in grams; X represents the amount of unassimilated fat in percents. It turned out that in both control experiments (in the first one -- at the beginning of the study, and in the second one -- at the end), in a daily feeding to the rats of 2 grams of fat, without any antioxidants, the average amount of unassimilated fat comprised 5.3%. On adding to 2 grams of fat 4 milligrams of antioxidants, the

average amount of unassimilated fat comprised, respectively, for butyloxytoluol 6.1%, for butyloxyanisol -- 5.8%, and for propylgallate -- 6.2%. Thus, in a comparison with the control experiments, the assimilability of fat with antioxidants proved to be somewhat reduced. The drawing illustrates the effect of antioxidants on the assimilability of fats.

As may be seen from the drawing, the amount of unassimilated fat, in the last control experiment, was within the same range (limits) as in the first control experiment. In essence, the assimilability of fat in the first and last control experiments was almost the same in all 10 rats in spite of the fact that in the last control experiment the rats consumed the feed reluctantly. On adding propylgallate to fat, along with a poor consumption of the feed, there was noted, in 8 rats, also a higher percent of unassimilated fat. On adding butyloxytoluol and butyloxyanisol to fat, there was observed an increase in the amount of unassimilated fat; in the first case -- in 6 rats, and in the second one -- in 5 (in comparison with the control experiments). Evidently, the poor consumption of the feed and the reduced assimilability of the fat with antioxidants are associated not so much with the external conditions of the surrounding environment and with the possible growing dislike of the food as with the action of antioxidants on the assimilation process.

((Illustration on pg. 49 of text))((to the left of this illustration)): Amount of unassimilated fat, in %; ((immediately underneath the same illustration))((reading from left to right)): Control; Butyloxytoluol; Butyloxyanisol; Propylgallate; Control; ((below that)): Effect of antioxidants on the assimilability of alimentary fats.

1 and 5 -- control; 2 -- butyloxytoluol; 3 -- butyloxyanisol; 4 -- propylgallate

((text continued)):

It should be noted that in the course of the entire period (2½ months), the external appearance of the rats and their behavior were normal; the animals became accustomed rapidly to the new cages and to the experimental conditions. By the end of the experiments, all the rats added weight ranging from 30 to 100 gram

We consider it necessary to note that fat, which had been extracted from the feces of the animals, obtained during the control periods and during the period with antioxidants, showed a distinction, namely: fat which did not contain anti oxidants had a yellowish color and an odor that was customary and inherent to rat feces. The fat readily washed off from the walls of the flask upon rinsing it with a soap solution. Fat, which contained butyloxytoluol, butyloxyanisol and propylgallate had a dark reddish color and a weak aromatic odor, and washed off with difficulty from the walls of the flask even when rinsing it with an alkaline solution. The change in color and odor is explained, evidently, by th fact that all the antioxidants employed in our study represent derivatives of t phenol series. It is generally known that ordinary phenol, under the influence o oxygen in the air, converts to phenoquinone, which has a red color. Upon findi its way into the gastroinestinal tract, both rendered lard, as well as, possibl antioxidants, are subjected to hydrolysis. As a result of hydrolysis, there are separated, fromthe fat, free fatty acids -- oleic and others, and from the anti-oxidants, alcohols -- butyl, methyl and propyl. This makes it possible to form esters, which have a fruity odor, as it were.

Conclusions

1. With a daily feeding to rats of 2 grams of fat with the addition of 4 milligrams of butyloxytoluol, butyloxyanisol and propylgallate, the assimilability of fat is somewhat reduced. The most definite data were obtained in feeding fat with propylgallate.

2. The problem involving the reason for a somewhat reduced assimilability of alimentary fats with antioxidants is in need of further study.

Literature (Bibliography)

Karplyuk, I. A., Vopr. pitaniya (Nutritional Problems), 1959, No. 4, p. 24.

Karplyuk, I. A., Effect of the Antioxidants of Alimentary Fats on the Animal Organism and Their Hygienic Evaluation, Avtoref. diss. kand. (Authors' Abstracts of Candidate Dissertations), M(oscow), 1960.

Emanuel', N. M. and others, Myasnaya industriya (Meat Industry), 1958, No. 2, p. 52.

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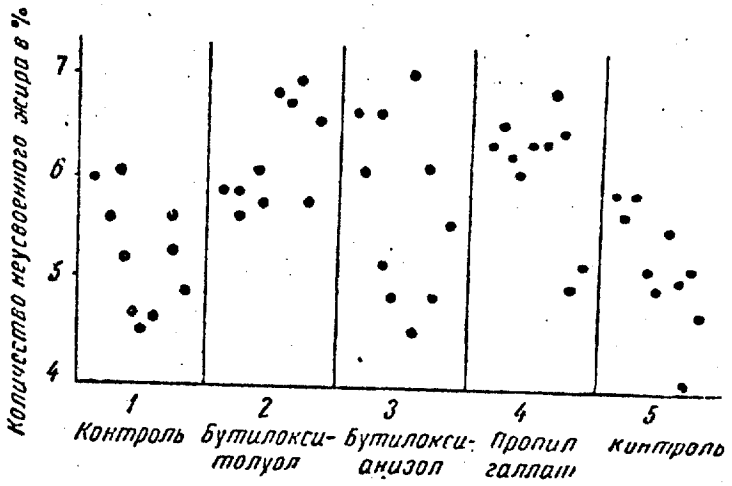
контрольных опытах была почти одинаковой у всех 10 крыс несмотря на то, что в последнем контрольном опыте крысы неохотно поедали корм.

При добавлении в жир пропилгаллата наряду с плохой поедаемостью корма у 8 крыс отмечается и более высокий процент неусвоенного жира. При добавлении в жир бутилокситолуола и бутилоксианизола также наблюдается повышенное количество неусвоенного жира: в первом случае — у 6 крыс, во втором — у 5 (по сравнению с контрольными опытами). Очевидно, плохая поедаемость корма и пониженная усвояе-



Влияние антиокислителей на усвояемость пищевых жиров.
1 и *5* — контроль; *2* — бутилокситолуол; *3* — бутилоксианизол; *4* — пропилгаллат.

мость жира с антиокислителями связываются не столько с внешними условиями окружающей среды и возможной приедаемостью, сколько с действием антиокислителей на процесс усвоения.

Следует отметить, что в течение всего опытного периода ($2^1/_2$ месяца) внешний вид крыс и их поведение были нормальными; животные очень быстро привыкли к новым клеткам и опытному режиму. К концу опытов все крысы прибавили в весе от 30 до 100 г.

Считаем необходимым отметить, что жир, экстрагированный из кала животных, полученного за контрольные периоды и периоды с антиокислителями, отличался между собой, а именно: жир, не содержащий антиокислителей, имел желтоватый цвет и обычный, свойственный крысиному калу, запах. Жир легко смывался со стенок колбы при споласкивании ее мыльным раствором. Жир, содержащий бутилокситолуол, бутилоксианизол и пропилгаллат, имел темный красноватый цвет и слабый ароматический запах, трудно смывался со стенок колбы даже при споласкивании ее щелочным раствором. Изменение цвета и запаха кала объясняется, очевидно, тем, что все использованные в нашей работе антиокислители являются производными фенольного ряда. Общеизвестно, что обыкновенный фенол под влиянием кислорода воздуха превращается в фенохинон, имеющий красный цвет. При попадании в желудочно-кишечный тракт как топленый свиной жир, так и, возможно, антиокислители подвергаются гидролизу. В результате гидролиза от жира отщепляются свободные жирные кислоты — олеиновая и др., а от антиокислителей спирты — бутиловый, метиловый и пропиловый. Это дает возможность образования сложных эфиров, которые имеют как бы фруктовый запах.

Effect of Propylgallate Antioxidants on the Alimentary (Nutritive) Value of Fat

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Fats, being products of poor stability, upon being stored, are rapidly subject to spoilage. Oxidized fats, as is known, lose their nutritive value (N. D. Bukhman, K. M. Levkovich, 1953; N. A. Zapol'skaya, E. F. Solov'eva, 1953; B. I. Kadykov, 1959), and in a number of cases, can even prove to be harmful (Creach, 1955; Johnson and Sakuragi, 1956; Kaunitz, Slanetz, Johnson, 1956).

With the purpose of keeping fats from oxidizing, recourse is had to a chemical method of inhibiting the fat spoilage, consisting in the utilization of antioxidants.

The effectiveness of various antioxidants, from the technological point of view, is dissimilar and depends, on the one hand, on the nature of the antioxidant, and on the other -- on the form of fat. As has been indicated by Tollenaar and Vos (1956), in the food industry of a number of foreign countries, wide use is being made of synthetic antioxidants (butyloxyanisol, butyloxytoluol and propylgallate). In the U.S.S.R. antioxidants are being employed with great care and after a painstaking and comprehensive approval of them. In recent years, at the All-Union Scientific-Research Institute of Fats, there has been a preoccupation with the technological verification of a number of synthetic antioxidants. According to the data of this institute, the most effective antioxidant, which significantly increases the stability of various fats, on storage (upon adding it an amount of 0.01 - 0.02%), is propylgallate. In order to incorporate into the fat industry the abovementioned antioxidant, along with its technological characteristics, it is necessary, in the first place, to resolve the problem dealing with its toxicity, and after that, with its biological value. The toxicological characteristics of propylgallate have been comprehensively studied at the Department of Nutritional Hygiene of the Central Institute of

Advanced Training for Doctors (I. A. Karplyuk, 1959). At the same time, at the Institute of Radiational Hygiene, an approach was made to studying the effect of propylgallate on the nutritive value of fat. The latter, according to the data of B. I. Kadykov (1959), N. D. Bukhman (1959), N. A. Zapol'skaya (1959), E. F. Romanovskaya (1959), M. A. Pogorelko (1959), L. I. Yazeva (1959) and others, varies considerably through an addition, to the fat, of small amounts of various substances (vitamins, emulsifiers, and so forth). In connection with this, it was possible to assume that propylgallate, too, would exert an influence on the characteristics of fat, changing the entire course of digestion, absorption and subsequent use of fat in the organism.

There was submitted for study refined sunflower oil, beef fat and mixed fat. To each fat there was added 0.02% propylgallate (a concentration that, according to foreign authors, is considered to be harmless).

For the solution of the problem that was posed, use was made of 6 groups of white male rats, with 35 rats in each group. All the animals were kept for a prolonged time (3½ months) on so-called synthetic feeding. In the composition of the diet was included potato starch (52% caloricity), casein (18 caloricity) and one or another of the fats under study (30% caloricity). In addition to this, all the animals received a salt mixture, yeast and fish oil, and water in an unlimited amount. Individual groups of the animals differed in that they received fat without any antioxidants and others with antioxidants. Thus, rats in the first group received, in their diet, beef fat, and those in the second group -- the same fat with 0.02% propylgallate, and the third and fourth groups -- respectively, mixed fat and mixed fat with 0.02% propylgallate; the fifth and sixth groups -- respectively, refined sunflower oil, and the same oil with an addition of 0.02% propylgallate. In the course of the experiment, observation was carried out of the weight and development of the young rats, of the survival capacity of the animals and of their utilization of their reserve substances (resources) during moments that were unfavorable for the organism, in the given case -- partial starvation. Upon the expiration of 3½ months of keeping the

animals on the abovementioned diet, 5 rats in each group were killed. In their organs and tissues a determination was made of the albumin (protein) and fat content (on the average, for one rat), in accordance with the procedure of Bukhman and Levkovich (1953). The remaining animals in all the groups were transferred to partial starvation (a fatless diet and a reduction in the caloricity of the diet by 4 - 5 times in comparison withthe initial one); they were kept on this up to the moment of succumbing. In the tissues and organs of the rats that had succumbed, a determination was also made of the albumin (protein) content.

((Graph on pg. 69 of text)) ((to the left of this graph)): Weight, in grams; ((within the same graph, reading down)): 1st group; 2nd group; ((immediately underneath this graph)): Initial; Weeks; ((below that)): Fig. 1. Change in the weight of rats in the first and second groups.

((Two graphs on pg. 70 of text))((to the left of the graph at the top)): Weight, in grams; ((within this graph, reading down)): 3rd group; 4th group; ((immediately underneath this graph)): Initial; Weeks; ((below that)): Fig. 2. Change in the weight of rats in the third and fourth groups. ((to the left of the graph at the bottom of the page)): Weight, in grams; ((within the same graph)): 5th group; 6th group; ((immediately underneath the same graph)): Initial; Weeks; ((below that)): Fig. 3. Change in the weight of rats in the fifth and sixth groups. ((text continued)):

Biological observation has shown that, in young animals of all groups, which had received fat with propylgallate, there was some reduction in the growth, and there was a reduction in the addition of weight. Thus, from Fig. 1 it may be seen that the addition in weight of rats that had received beef fat, constituted, during the observation period, 143 grams, while the increase in weight in animals that had received the same fat along with an antioxidant, -- 126 grams.

Rats, which had been kept on mixed fat, added 159 grams in weight, and rats, which had received the same fat along with an antioxidant, -- 142 grams (Fig. 2). A lesser distinction in the addition in weight was observed in feeding animals with refined sunflower oil along with an antioxidant and without it (Fig. 3).

Analogous data on the addition in weight in rats were obtained by I. A. Karplyul (1959) on feeding the animals lard with an addition of propylgallate (in it).

Observations, during the period of partial starvation of the animals, showed that all the rats that had received propylgallate in their diet were noticeably weaker, and the average duration of their life was considerably shorter than was the case for those animals that had received pure fats (Table 1).

As has been indicated above, in resolving the problem with respect to the biological value of fat with an antioxidant, we were interested in learning to what extent the reserve substances (resources) in the organism of the animals in the individual groups, during the period of partial starvation, are fully utilized.

As may be seen from Table 2, in all the animals that had received, in their diet, fat along with the addition of propylgallate, the percent of utilization of the reserve substances (resources) in the organism, and of albumin (protein), particularly, was noticeably reduced.

From the data set forth it follows that the animals, which had received customary fats, withstood partial starvation over a longer period of time. At the same time, they used up their tissue albumins (proteins) more slowly, and consequently, more economically. However, as a result of greater duration of li up to the moment of succumging, more albumin (protein) was used up in them and the degree of its utilization was greater.

((Two tables on pg. 71 of text))

Table 1

Average Life Duration of Animals in Different Groups

During the Period of Partial Starvation

Group of Animals	Average Duration of Life (in days)
First -- diet with beef fat	27.0
Second -- diet with the same fat and 0.02% antioxidant	13.6
Third -- diet with mixed fat	28.5
Fourth -- diet with the same fat and 0.02% antioxidant	16.0

((table continued from the preceding page))

Fifth -- diet with refined sunflower oil	25.0
Sixth -- diet with the same oil and 0.02% antioxidant	14.5

Table 2

Utilization of the Reserve Substances (Resources) of the Organism in Various Groups of Animals During the Period of Partial Starvation

Group of Rats	Fat Content			Albumin (Protein Content		
	at the end of normal feeding	at the moment of succumbing	Utilization of fat (in %)	at the end of normal feeding	at the moment of succumbing	Utilization of albumin (protein (in %)
	(for 1 rat, on average)			(for 1 rat, on an average)		
First -- diet with beef fat . . .	((See Table 2 on pg. 71 of text for all figures pertaining thereto))					
Second -- diet with the same fat and 0.02% antioxidant . . .						
Third -- diet with mixed fat . . .						
Fourth -- diet with the same fat and 0.02% antioxidant . . .						
Fifth -- diet with refined sunflower oil . . .						
Sixth -- diet with the same oil and 0.02% antioxidant . . .						

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Таблица 1

Средняя продолжительность жизни животных различных групп в период частичного голодания

Группа животных	Средняя продолжительность жизни (в днях)
Первая — рацион с говяжьим жиром	27,0
Вторая — рацион с тем же жиром и 0,02% антиокислителя	13,6
Третья — рацион с комбижиром	28,5
Четвертая — рацион с тем же жиром и 0,02% антиокислителя	16,0
Пятая — рацион с рафинированным подсолнечным маслом	25,0
Шестая — рацион с тем же маслом и 0,02% антиокислителя	14,5

Таблица 2

Использование резервных веществ организма у различных групп животных в период частичного голодания

Группа крыс	Содержание жира		Использование жира (в %)	Содержание белка		Использование белка (в %)
	к концу нормального питания	к моменту гибели		к концу нормального питания	к моменту гибели	
	(в среднем на 1 крысу)			(в среднем на 1 крысу)		
Первая—рацион с говяжьим жиром	25,9	1,0	95,9	36,5	22,3	38,3
Вторая—рацион с тем же жиром и 0,02% антиокислителя	18,7	1,1	93,3	32,1	24,6	23,4
Третья—рацион с комбижиром	34,4	1,0	97,0	37,9	26,2	30,8
Четвертая — рацион с тем же жиром и 0,02% антиокислителя	33,6	1,3	96,0	35,7	29,3	18,4
Пятая — рацион с рафинированным подсолнечным маслом	25,9	1,3	95,0	37,2	25,8	33,6
Шестая—рацион с тем же маслом и 0,02% антиокислителя	18,1	1,3	92,8	37,6	28,4	24,5

межутка времени. При этом они медленнее, следовательно экономнее, расходовали свои тканевые белки. Однако благодаря большей длительности жизни к моменту гибели у них расходовалось больше белка и степень использования его была выше.

Животные же, получавшие жиры с добавлением антиокислителя, оказались менее устойчивыми к частичному голоданию. Гибель их наступала при меньшей степени использования тканевых жиров и белка, чем у контрольных животных.

Из предыдущих наших работ известно, что менее совершенное использование белка всегда сопровождается повышением окислительного коэффициента мочи, указывающего на накопление в организме большого количества токсических, недоокисленных продуктов межуточного обмена (Н. Д. Бухман, 1959). На основании данных настоящей работы можно высказать предположение, что наличие в диете крыс антиокислителя вызывает снижение окислительных процессов в их организме. И. А. Карплюк (1959) в своих выводах так же указывает, что фенольные антиокислители, к которым относится и пропилгаллат, пони-

((text continued)): The animals, however, that had received fats with an addition of an antioxidant, proved to be less resistant to partial starvation. Thei succumbing had its onset at a lesser degree of utilization of tissue fats and albumin (protein) than in the case of the control animals.

From our preceding studies it is known that a less complete utilization of albumin (protein) is always accompanied by an increase in the oxidizing coefficient of the urine, which points to the accumulation, in the organism, of a larg amount of toxic, incompletely oxidized products of interstitial metabolism (N.D. Bukhman, 1959). On the basis of the data in the present paper (study), the assumption may be expressed that the presence, in the diet of rats, of an antioxidant, brought about a reduction in the oxidizing processes in their organism. I. A. Karplyuk (1959), in her conclusions, has also shown that phenolic antioxidants, among which belongs propylgallate, lower the level of the oxidizing processes in the organism and are far from neutral (indifferent) for the organis of animals.

The study that has been carried out once again shows that the presence, in th fats under study (beef fat, mixed fat and refined sunflower oil), of 0.02% propylgallate, considerably reduces their biological value.

We believe that the utilization of propylgallate as an antioxidant in the fat industry is not expedient (practical). It is very desirable, in the capacity of antioxidants, to make use of biologically fully-valuable substances. At the present time, indications have appeared in the literature, that good antioxidant are represented by: pyridoxine (Sakuragi, Kummerow, 1958), Phosphatides, ascorbi acid and vitamin P (N. V. Novotel'nov, M. T. Golovkina, and E. R. Stavrova, 1959

Literature (Bibliography)

Bukhman, N. D., Levkovich, K. M., Vorp. pitaniya (Nutritional Problems), 1953, No. 3, p. 49.

Bukhman, N. D., Proceedings of the 1st Scientific Session with Respect to the Problem: "The Problem of Fat in Nutrition", L(eningrad), 1959, p. 124.

Zapol'skaya, N. A., Solov'eva, E. F., Vopr. pitaniya (Nutritional Problems), 1953, No. 4, p. 47.

Zapol'skaya, N. A., Proceedings of the 1st Scientific Session on the Problem: "Problem of Fat in Nutrition", L(eningrad), 1959, p. 179.

Kadykov, B. I., Proceedings of the 1st Scientific Session on the Problem: "The Problem of Fat in Nutrition", L(eningrad), 1959, p. 3.

Karplyuk, I. A., Vopr. pitaniya (Nutritional Problems), 1959, No. 14, p. 24.

Novotel'nov, N. V., Golovkina, M. T., Stavrova, E. R., Nauchn. dokl. Vyschei shkoly, biol. nauki (Scientific Proceedings of the Higher Institutions of Biological Sciences), 1959, No. 1, p. 137.

Pogorelko, M. A., Proceedings of the 1st Scientific Session on the Problem: "Problem of Fat in Nutrition", L(eningrad), 1959, p. 214.

Romanovskaya, E. F., Ibid., p. 67.

Yazeva, L. I., Proceedings of the 1st Scientific Session on the Problem: "Problem of Fat in Nutrition", L(eningrad), 1959, p. 210.

Creach, M. P., Oleagineux, 1955, v. 3, p. 161.

Johnson, O. C., Sakuragi, T., J. Am. Oil Chem. Soc., 1956, v. 33, p. 433.

Kaunitz, H. Slanetz, C., Johnson, R. E., Ibid., p. 630.

Sakuragi, T., Kummerow, F., Ibid., 1958, v. 35, p. 401.

Tollenaar, E. D., Vos, H. J., Fette, Seifen, Austrichmittel, 1956, v. 58, p. 112.

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Эффективность различных антиокислителей с технологической точки зрения неодинакова и зависит, с одной стороны, от природы антиокислителя, а с другой — от вида жира. Как указывает Tollenaar и Vos (1956), в пищевой промышленности ряда зарубежных стран широко используются синтетические антиоксиданты (бутилоксианизол, бутилокситолуол, пропилгаллат). В СССР антиокислители применяют с большой осторожностью после тщательной и всесторонней апробации их. В последние годы во Всесоюзном научно-исследовательском институте жиров занимаются технологической проверкой ряда синтетических антиокислителей. По данным этого института, наиболее эффективным антиоксидантом, значительно повышающим стойкость различных жиров при хранении (при добавлении его в количестве 0,01—0,02%), является пропилгаллат. Для внедрения в жировую промышленность указанного антиокислителя наряду с технологической характеристикой его необходимо в первую очередь решить вопрос о токсичности, а затем и биологической ценности его. Токсикологическую характеристику пропилгаллата всесторонне изучали на кафедре гигиены питания Центрального института усовершенствования врачей (Н. А. Карплюк, 1959). Одновременно в Институте радиационной гигиены приступили к изучению влияния пропилгаллата на пищевую ценность жира. Последняя, по данным Б. И. Кадыкова (1959), Н. Д. Бухман (1959), Н. А. Запольской (1959), Э. Ф. Романовской (1959), М. А. Погорелко (1959), Л. И. Язевой (1959) и др., значительно изменяется от добавления к жиру малых количеств различных веществ (витаминов, эмульгаторов и пр.). В связи с этим можно было предположить, что и пропилгаллат окажет влияние на характеристику жира, меняя весь ход переваривания, всасывания и дальнейшего использования жира в организме.

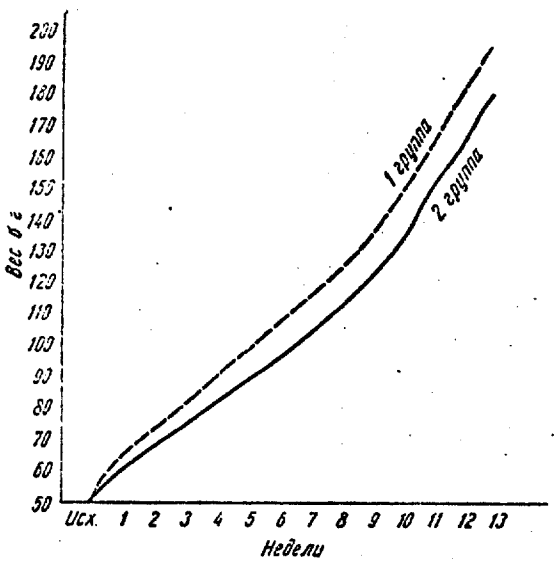

Рис. 1. Изменение веса крыс первой и второй групп.

Изучению подвергалось рафинированное подсолнечное масло, говяжий жир и комбижир. К каждому жиру было добавлено по 0,02% пропилгаллата (концентрация, которая, по данным зарубежных авторов, считается безвредной).

Для решения поставленной задачи было использовано 6 групп белых крыс-самцов, по 35 крыс в каждой группе. Всех животных содержали длительное время ($3^1/_2$ месяца) на так называемом синтетическом питании. В состав диеты входили картофельный крахмал (52% калорийности), казеин (18% калорийности) и тот или иной изучаемый жир (30% по калорийности). Кроме того, все животные получали солевую смесь, дрожжи и рыбий жир и в неограниченном количестве воду. Отдельные группы животных отличались тем, что получали жир без антиокислителя и с антиокислителем. Так, крысы первой группы получали в диете говяжий жир, второй группы — тот же жир с 0,02% пропилгаллата, третьей и четвертой группы — соответственно комбижир и комбижир с 0,02% пропилгаллата, пятой и шестой группы — соответственно рафинированное подсолнечное масло и то же масло с добавлением 0,02% пропилгаллата.

В процессах опыта наблюдали за весом и развитием молодых крыс, за выживаемостью животных и использованием ими своих резервных веществ в неблагоприятные для организма моменты, в данном случае — при частичном голодании. По истечении $^1/_2$ месяцев содержания животных на указанной диете забивали по 5 крыс каждой группы. В их органах и тканях определяли содержание белка и жира (в среднем на

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одну крысу) по методике Бухман и Левкович (1953). Остальных животных всех групп переводили на частичное голодание (безжировая диета и уменьшение калорийности рациона в 4—5 раз по сравнению с исходной), на котором и содержали их до момента гибели. В тканях и органах погибших крыс также определяли содержание белка и жира.

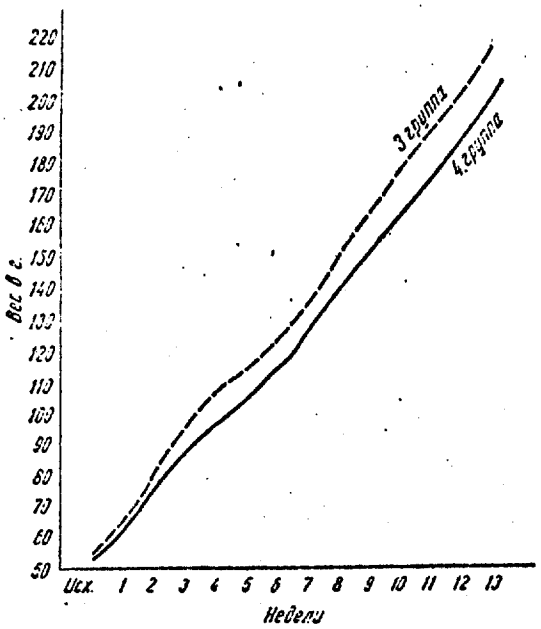

Рис. 2. Изменение веса крыс третьей и четвертой групп.

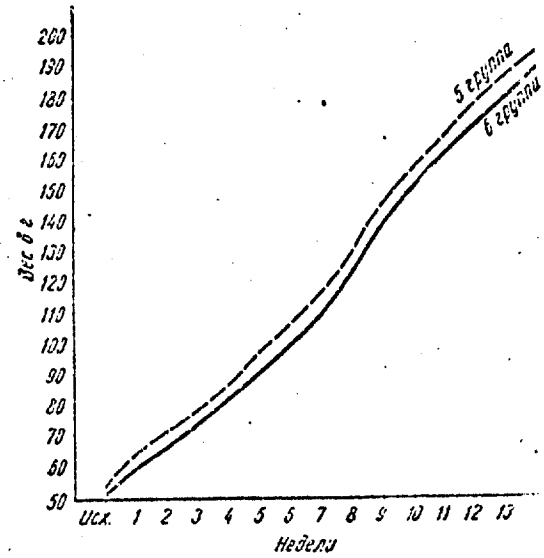

Рис. 3. Изменение веса крыс пятой и шестой групп.

Биологическое наблюдение показало, что у молодых животных всех групп, получавших жир с пропилгаллатом, несколько задерживался рост, снижалась прибавка веса. Так, из рис. 1 видно, что привес крыс, получавших говяжий жир, составил за период наблюдения 143 г, а привес животных, получавших тот же жир с антиокислителем, — 126 г.

Крысы, содержавшиеся на комбижире, прибавили в весе 159 г, а крысы, получавшие тот же жир с антиокислителем, — 142 г (рис. 2) Меньшее различие в приросте веса наблюдалось при питании животных рафинированным подсолнечным маслом с антиокислителем и без него (рис. 3).

Аналогичные данные о привесе крыс были получены И. А. Карплюк (1959) при скармливании животным свиного жира с добавлением к нему пропилгаллата.

Наблюдения в период частичного голодания животных показали, что все крысы, получавшие в рационе пропилгаллат, были заметно слабее и средняя продолжительность их жизни была значительно меньшей, чем тех животных, которые получали чистые жиры (табл. 1).

Как указано выше, при решении вопроса о биологической ценности жира с антиокислителем нас интересовало, в какой степени полно используются резервные вещества в организме животных отдельных групп в период частичного голодания.

Как видно из табл. 2, у всех животных, получавших в рационе жир с добавлением пропилгаллата, процент использования резервных веществ в организме, особенно белка, заметно снижался.

Из приведенных данных следует, что животные, получавшие обычные жиры, выдерживали частичное голодание в течение большего про-

#84

Effect of the Antioxidants of Alimentary Fats -- Propylgallate and Butyloxyanisol -- on the Animal Organism.

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UDK (Universal Decimal Classification):

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Fats, and the products that they contain, are subject to rapid spoilage principally because of the oxidation of fats even upon storing them at a temperature below 0 deg. Highly unsaturated fatty acids and fat- soluble vitamins, with a high physiological activity, are oxidized in the first place.

Papers have made their appearance that attest to the toxic action of a number of oxidation products, such as peroxides, aldehides, ketones and polymers. This explains the interest that is manifested in processes that take place in fats and in the methods of protecting them from spoilage.

The chemical process is one of the most effective methods of protecting fats from oxidation. For this purpose, recently, there have been proposed a great number of compounds and their combination; they have been given the designation of antioxidants ((Anglicized version of the Russian word -- Translator)) or antioxidants ((Russian version of the same word -- Translator)). However, many of them do not satisfy the strict requirements of physicians and of healths supervision agencies. An illustration of the foregoing may be found in the figures (statistics) set forth by Kraybill and Dugan (1): in the U.S.A. alone, in 10 years (from 1944 to 1954), there have been granted 600 patents for proposals of substances and their combination in the capacity of antioxidants of alimentary fats. Antioxidants and their various combinations are employed for addition to nutritive products in more than 20 countries. In many of them, such as in the U.S.A., the South African Republic, Finland, Sweden, Yugoslavia and Canada, there is utilized a considerable amount of various antioxidants (up to 25 designations). In the Soviet Union, there has been permitted the use of two antioxi-

dants in food products: butyloxyanisol and butyloxytoluol.

Many antioxidants may intensify their effect in conjunction with other substances. This synergism is also manifested in mixtures of various antioxidants. In studying various mixtures of antioxidants, Gearhart and Stuckey (2) came to the conclusion that the best is represented by a combination of butyloxyanisol (BOA) and propylgallate (PG). N. Emanuel', D. Knorre, Yu. Lyaskovskaya and V.Piul'skaya (3) have shown that the most synergistic effect is manifested by PG in a concentration of 0.005% , and BOA -- in 0.01%. Kraybill and Dugan (1) discovered that if, to 0.01% BOA, there is added 0.003% PG, and 0.002% citric acid, then the stability of the fat under study will increase by two times in comparison with fat containing only BOA, and by 13 times in comparison with control fat without any antioxidants.

The possibility of adding a small amount of antioxidants (as a result of their synergism) is very tempting. I. Smorodintsev believes that it is more feasible (practical) to add the least possible amount of phenolic anti-oxidants, t to intensify their action through the introduction of one or several synergists.

Graham and his co-authors (1) studied a mixture of TENOKS-2, consisting of 20% BOA, 6% PG, 4% citric acid and 70% propylene glycol (as a solvent). Other groups of rats were fed doses of TENOKS-2 that were increased, in comparison with possible penetration into the human organism, by 6 and 300 times. It was disclosed that a combination of BOA and PG did not manifest any toxic action on the animals; the latter had been observed for a period of a year. A mixture of TENOKS-2 has received permission to be used in the U.S.A. for the purpose of stabilizing fish oil, and also, fat in pork and in the meat of turkeys(turkey hens).

The data published in the foreign literature with regard to the toxicity of a mixture of BOA and PG antioxidants are insufficient, in view of the fact that, in conformity with the materials set forth, the action of the mixture was observed only for some general biological indices.

In connection with the foregoing, and also, for the reason that in the U.S.S.R., the toxitity of the mixture was not studied, for the complete toxicological characteristics of the mixture of antioxidants, we carried out acute, subacute and chronic experiments.

The purpose of the acute experiments was to determine the LD_{50} of the mixture.

Inoculation was carried out with a solution of antioxidants (the ratio of BOA to PG was 2 : 1). The inculation mixture consisted of 20% BOA, 10% PG and 70% sunflower oil in the capacity of a solvent. The inoculation was accomplished on 7 groups of white male rats (with 10 animals in each group; the average weight of the animals was 100 - 120 grams) by means of administering the mixture of antioxidants directly into the stomach through a metallic probe. The LD_{50} was calculated by the method of Miller and Teitner[1]. The results are set forth in Fig. 1. ((Graph on pg. 77 of text)) ((underneath this graph)): Fig. 1. Determination of the LD_{50} by the method of probit (probability unit)-analysis. On the axis of ordinates, there is a linear expression of the the probits (probability units), and on the axis of abscissas, above -- the doses being tested (in grams/kilogram), and below -- the result obtained (number of animals that has succumbed). CIRCLE -- graphic expression for the LD_{50} of the mixture of anitoxidants. ((text continued)):

The LD_{50} obtained for the mixture of antioxidants is equal to 2633 plus or minus 140 milligrams/kilogram; it proved to be between the LD_{50} for BOA (2950 milligrams/kilogram) and that for PG (2500 milligrams/kilogram). These data have shown that a mixture of BOA and PG, in acute experiments, does not manifest any synergistic action and that a mixture of BOA and PG may be figured among the weakly toxic substances.

Subacute experiments were carried out on young white male rats and on females having a weight of 100 - 120 grams. In the experiment were 40 animals (20 males and 2 0 females.) The purpose of the subacute experiments was a study and selection of the most sensitive tests for carrying out chronic experiments.

The feeding was carried out over a period of $2\frac{1}{2}$ months. A mixture of antioxidants was given in rendered lard with a No.1 ground feed briquet. The amount of antioxidants was aggravated by 500 times (500 - fold) in comparison with the amount that could find its way into the human organism: BOA - 100 milligrams/kilogram; PG -- 50 milligrams/kilogram.

In the course of the experiment, observations were carried out daily of the interrelationship between the consumption of the feed and the external appearance of the animals in the experimental and control groups; the weight was determined every 10 days.

Proceeding from the fact that antioxidants, in the live organism, manifest, possibly, an effect on the oxidation processes, we carried out an investigation of the exit-oxygen of the blood, and also, of the blood catalase and kcholinesterase activity.

In breaking off the oxidizing processes in its or in inhibiting them, the antioxidants could exert an influence on the fat metabolism in the organism of the experimental (test) animals; for this reason, we made a study of the amount of ketone bodies in the blood. In addition to this, the diastase of the urine and the sugar of the blood were subjected to investigation.

The animals remained in the experiment from 60 to 70 days. Investigations were carried out on the 20th, 40th and 60th day of the inoculation. In the treatment of the material, those results in which P is equal to or less than 0.05 were taken as being statistically reliable.

Over the extent of the entire experiment, no changes were observed in the animals with respect to the behavior, external appearance and the consumption of their feed. Experimental (test) and control animals added weight equally well. On the 20th day of the inoculation, there was some reduction in the activity of the cholinesterase (P is less than 0.02). Subsequently, evidently, an adaptation took place on the part of the organism to the introduction of antioxi-

dants, and the difference between the animals in the experimental and control groups leveled off (Table 1) . Such an effect was disclosed only in females. In the males, a difference between the control and experiment was not noted.

Investigations of the catalase activity indicated that the antioxidants, in the beginning, inhibit the action of this enzyme (on the 20th day), and after that, its activity increases (on the 40th day). These shifts were also noted in the females (Fig. 2).

((Table on pg. 78 of text))

Table 1

Blood Cholinesterase Activity

Animals	Days of investigation	Control			Experiment						
		n	M	m_M	n_o	M_o	m_M	M_o-M	m_{M_o}-M	t	P
Males	Prior to the experiment										
	20										
	40										
	60										
Females	Prior to the experiment										
	20										
	40										
	60										

((See Table 1 on pg. 78 of text for all figures pertaining thereto))

((Two illustrations on pg. 78 of text)) ((to the left of the upper illustration)):
Catalase activity; ((immediately underneath this illustration)):

((from left to right)) Prior to the experiment; 20th day of the experiment; 40th day of the experiment; 60th day of the experiment; ((below that)): Fig.2. Catalase activity expressed in the catalase index (females).

Dark circles -- experiment; light circles - control; continuous line -- average (mean) magnitude. ((to the left of the lower illustration)): Blood sugar (in milligram %); ((immediately underneath this illustration)): ((from left to right)): Prior to the experiment; 20th day of the experiment; 40th day of the experiment; 60th day of the experiment; ((below that)): Fig. 3.

Blood sugar (males). The legend (conventional) designations) is the same as it is in Fig. 2. ((text continued)):

An analogous action dynamics was disclosed (by us) relative to the blood sugar: in the beginning there takes place a diminution in the amount of suga and after that, an increase in it (Fig. 3). These changes were observed only in the males. In the females , there was no difference between the experiment and the control.

On the 40th day of the inoculation, we disclosed a statistically reliable increase in the ketone/bodies in the males (P is less than 0.02), and a statistically reliable reduction in the ketone bodies in the females (P is less than 0.02). These data are set forth in Table 2. Changes in the exit - oxygen of the blood were not noted.

((Table on pg. 79 of text))

Table 2

Ketone Bodies in the Blood

Animals	Days of Investigation	Control			Experiment			M_o-M	m_{M_o}-M	t	P
		n	M	m_M	n_o	M_o	m_M				
Males	Prior to the experiment										
	20										
	40										
	60										
Females	Prior to the experiment										
	20										
	40										
	60										

((See Table 2 on pg. 79 of text for all figures pertaining thereto))

((text continued)): The antioxidants likewise showed no effect on the pancreas: investigations of the diastase activity of the urine of the lipase activity, directly in the pancreas, showed no difference between the animals in the control and experimental groups.

Following the experimental period, all the animals were destroyed by decapitation. Investigations carried out on the liver glycogen did not disclose any differences between the control and the experiment. The ratio of the weight of the liver to the weight of the body showed a tendency to an increase in this ratio in the experimental animals (P is less than 0.1).

Conclusions

1. BOA (Butyloxyanisol and PG (propylgallate), individually, and also a mixture of these antioxudants, do not pertain to strongly toxic substances.

2. In the action of the mixture of BOA and PG antioxidants on the organism, in acute experiments, there is no disclosure of synergism, nor of antagonism.

3. Large concentrations of the mixture of antioxidants (BOA -- 100 milligrams/kilogram and PG -- 50 milligrams/kilogram) are not neutral (indifferent)for the blood organism. They exert an influence on the blood catalase and cholinesterase activity, and on the amount of ketone bodies and blood sugar ; this attests to their negative action on the organism.

4. The white rat females proved to be more sensitive to the administration of a mixture of antioxidants than did the males.

5. In order to permit the application of BOA and PG antioxidants (in mixture) in alimentary fats and in fat-containing products, it is necessary to carry out investigations under conditions of chronic inoculation of the animals; and this is what represents the subject of investigation at the present time.

Literature (Bibliography)

1. Kraybill, H.R., Dugan, L.R, J. Agr. Food Chem., 1954, v.2,p.81
2. Gearhart, W.M., Stuckey, B.N.,J.AmOil Chem. Soc., 1955, v. 32,p.386
3. Emanuel' , N., Knorre, D. Lyashovskaya, Yu. and others, Myasn.industr.(Meat Industry) of the U.S.S.R., 1958, No. 2, p. 52.
4. Graham, W.RD, Feed , H., J. Pharm.Pharmacol., 1954, v.6,p.543.

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Footnote

(a) Quoted from L.M. Bien'kii, Elements in the Quantitative Evaluation of the Pharmacological Effect, L(eningrad), 1963, p. 63.

(Translated by Carl Demrick Associates Inc. LB/fs)

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антиокислителей, и разница между животными опытной и контрольной групп нивелировалась (табл. 1). Такой эффект был выявлен только у самок. У самцов разница между контролем и опытом не была отмечена.

Исследования каталазной активности показали, что антиокислители вначале ингибируют действие этого фермента (на 20-й день), а затем

Таблица 1

Активность холинэстеразы крови

Животные	Дни исследования	Контроль			Опыт			M_0-M	m_{M_0-M}	t	p
		n	M	m_M	n_0	M_0	m_M				
Самцы	До опыта	10	1,22	0,024	10	1,23	0,02	0,01	0,032	0,31	>0,5
	20	10	1,26	0,02	10	1,26	0,015	0	0,022	0	—
	40	10	1,27	0,02	10	1,27	0,012	0	0,016	0	—
	60	10	1,32	0,02	10	1,34	0,014	0,02	0,026	0,79	>0,25
Самки	До опыта	10	1,30	0,03	10	1,31	0,007	0,01	0,03	0,3	>0,5
	20	10	1,33	0,007	10	1,30	0,003	0,03	0,011	2,5	<0,02
	40	10	1,29	0,01	10	1,29	0,007	0	0,012	0	—
	60	10	1,34	0,03	10	1,35	0,033	0,01	0,043	0,25	>0,5

его активность возрастает (на 40-й день). Эти сдвиги также отмечены только у самок (рис. 2).

Аналогичную динамику действия мы обнаружили в отношении сахара крови: вначале происходит уменьшение количества сахара, а затем

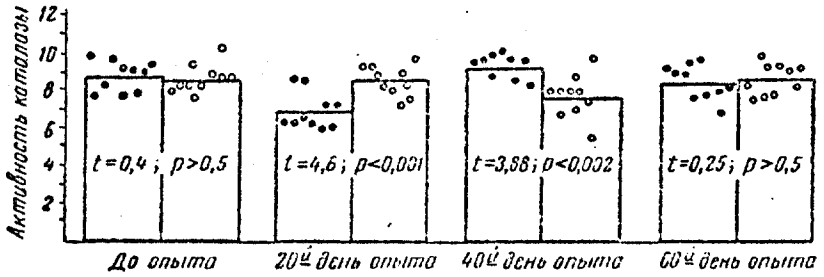

Рис. 2. Активность каталазы, выраженная в показателе каталазы (самки).

Темные кружки — опыт; светлые кружки — контроль; сплошная линия — средняя величина.

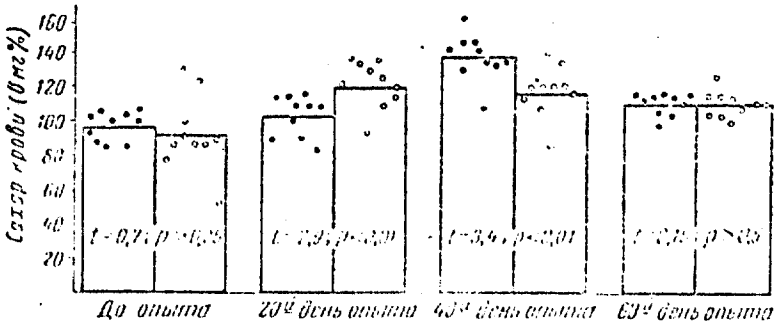

Рис. 3. Сахар крови (самцы). Условные обозначения те же, что на рис. 2.

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is urged to investigate its copyright status.

Опубликованные в зарубежной литературе данные о токсичности смеси антиокислителей БОА и ПГ недостаточны, так как, согласно представленным материалам, наблюдалось действие смеси лишь на некоторые общебиологические показатели.

В связи с изложенным выше, а также потому, что в СССР токсичность смеси не изучалась, для полной токсикологической характеристики смеси антиокислителей нами проведены острые, подострые и хронические опыты.

Целью острых опытов было определение LD_{50} смеси.

Затравку проводили раствором антиокислителей (соотношение БОА к ПГ — 2 : 1). Затравочная смесь состояла из 20% БОА, 10% ПГ и 70% подсолнечного масла в качестве растворителя. Затравку осуществляли на 7 группах белых крысцамцов (по 10 животных в каждой группе, средний вес животных 100—120 г) путем введения смеси антиокислителей непосредственно в желудок через металлический зонд. LD_{50} была рассчитана по методу Миллера и Тейтнера[1]. Результаты представлены на рис. 1.

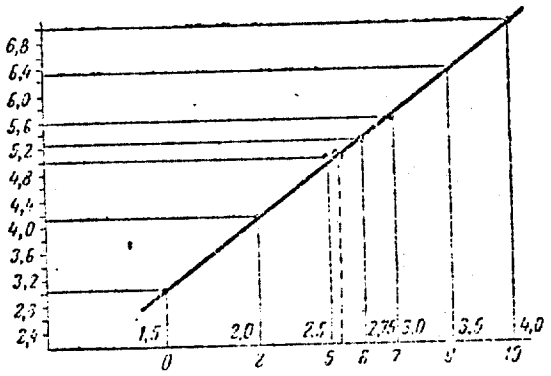

Рис. 1. Определение LD_{50} методом пробит-анализа. На оси ординат — линейные выражения пробитов, на оси абсцисс сверху — испытываемые дозы (в г/кг), снизу — полученный результат (число погибших животных).

О — графическое выражение LD_{50} смеси антиокислителей.

Полученная LD_{50} для смеси антиокислителей равна 2633±140 мг/кг; она оказалась между LD_{50} БОА (2950 мг/кг) и ПГ (2500 мг/кг). Эти данные показали, что смесь БОА и ПГ в острых опытах не проявляет синергетического действия и что смесь БОА и ПГ может быть причислена к слаботоксичным веществам.

Подострые опыты были проведены на молодых белых крысах-самцах и самках весом 100—120 г. В опыте было 40 животных (20 самцов и 20 самок). Цель подострых опытов — изучение и выбор наиболее чувствительных тестов для проведения хронических опытов.

Скармливание проводили в течение 2½ месяцев. Смесь антиокислителей давали в свином топленом жире с молотым кормовым брикетом № 1. Количество антиокислителей аггравировали в 500 раз по сравнению с количеством, которое может попасть в организм человека: БОА — 100 мг/кг; ПГ — 50 мг/кг.

В течение опыта ежедневно проводились наблюдения за взаимосвязью между поеданием корма и внешним видом животных опытной и контрольной групп, вес определялся через каждые 10 дней.

Исходя из того, что антиокислители в живом организме, возможно, оказывают влияние на процессы окисления, мы исследовали вахат-кислород крови, а также активность каталазы и холинэстеразы крови.

Прерывая окислительные процессы в жирах или тормозя их, антиокислители могли повлиять на жировой обмен в организме подопытных животных, поэтому мы изучали количество кетоновых тел в крови. Кроме того, исследованию были подвергнуты диастаза мочи и сахар крови.

Животные находились в опыте от 60 до 70 дней. Исследования проводились на 20-й, 40-й, 60-й день затравки. При обработке материала за статистически достоверные результаты принимались те, в которых P 0,05.

На протяжении всего опыта у животных не было отмечено изменений в поведении, внешнем виде и поедании корма. Подопытные и контрольные животные одинаково хорошо прибавляли в весе. На 20-й день затравки несколько снижалась активность холинэстеразы ($P<0,02$). В дальнейшем, видимо, происходила адаптация организма к введению

[1] Цит. Л. М. Беленький. Элементы количественной оценки фармакологического эффекта. Л., 1963, стр. 63.

Hygienic Evaluation of a Mixture of Butyloxyanisol and Propylgallate Antioxidants of Alimentary Fats

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UDK (Universal Decimal Classification): 613.291 :

(613.268 + 613.288)

The problem of keeping fats from spoiling has great significance for the national economy. In connection with the fact that even at relatively low temp atures there takes place a fat spoilage (for the main part, as a result of oxid tion), the periods for their storage are limited to several months. During oxi ation, there is a change not only in the organoleptic indices (taste, color, an odor of the fats), but they also lose valuable constituent parts -- fat-soluble vitamins and highly unsaturated fatty acids, which are subjected to oxidation i the first place.

In addition to this, the experimental papers of a number of authors (1, 2, 3 4) attest to the fact that the toxic action of the oxidation products of fats - peroxides,aldehydes, ketones, and polymers. Some papers point out the fact tha heated fats, as well as those that have been oxidized, may bring about a develo ment of tumors in the organism (5, 6, 7).

In recent years, in order to prevent fats from being oxidized, there have be proposed chemical compounds that have received the designation of antioxidants antioxidants ((Russian version of the same word -- Translator)). These are che ical substances that are capable of breaking off the chain reaction of oxidatio in rats; they are added in a small amount (0.01 - 0.02%). In investigating the various mixturesof antioxidants, Gearhart and Stuckey (8) came to the conclusio that the most effective one is a mixture of propylgallate and butyloxyanisol; this mixture is 2 times more effective than each component individually. N. Emanuel' and his co-authors (9) have established that the most synergistic effe that these substances yield is in the following concentrations -- 0.01% butyl-

oxyanisol and 0.005% propylgallate.

In the U.S.S.R., for the stabilization of alimentary fats there is permitted application of butyloxytoluol and butyloxyanisol. Propylgallate has been acknowledged as not being harmless for the organism, and it is not recommended to be applied to nutritive products (10). In our investigations, we made use of this antioxidant in doses 4 times smaller than the doses that manifested a harmful action.

In order to study the toxicity in the mixture of butyloxyanisol and propylgallate antioxidants, we carried out acute, subacute and chronic experiments. The purpose of the acute experiments was the determination of the LD_{50} in the mixtur of butyloxyanisol and propylgallate antioxidants, in the ratio of 2 : 1. The purpose of the subacute experiments was the disclosure of the most sensitive tes for utilizing them in chronic experiments.

In selecting the tests, we proceeded from the results of the papers (studies) carried out in our department, assuming that antioxidants, even in the live organism, act on the oxidizing-reduction processes and on the fat metabolism. I acute experiments, which had been carried out on white male rats, having a weigh of 100 - 130 grams, it was disclosed that a mixture of butyloxyanisol and propyl gallate antioxidants did not manifest any synergistic nor antagonistic action. The LD_{50} (lethal dose) of the mixture of antioxidants, which had been determine by the method of probit (probability unit)-analysis (11), proved to be equal to 2033 plus or minus 140 milligrams/kilogram. It was found to be between the LD_{5} for butyloxyanisol (2950 milligrams/kilogram) and that for propylgallate (2300 milligrams/kilogram).

In subacute experiments, a mixture of butyloxyanisol and propylgallate antioxidants (butyloxyanisol, 100 milligrams/kilogram, and propylgallate, 50 milligrams/kilogram) was administered with a 500-fold aggravation in comparison with the amount that may find its way into the human organism; this led to a change in the content, in the blood, of ketone bodies, catalase and cholinesterase, an also, of sugar.

In the chronic experiments, which had been conducted on white male rats and on female ones, a clarification was made of the possibility of utilizing a mixture of antioxidants for the stabilization of the alimentary fats.

In the process of investigation, we made a determination of the weight of the animals (in the beginning, after every 10 days, and after that, when the additio (increase) became negligible, after each month), of the activity of the catalase in the blood, in accordance with the procedure of Bakh and Zubkova (12), of the activity of the cholinesterase in the blood, in accordance with the procedure of Khestrin, in the modification of the Kiev Institute of Labor and Trade-Union Diseases, and of the quantitative sugar content in the blood, in accordance with the procedure of Khagedorn (Hagedorn) and Iensen (Jensen)(13). In addition to this, a clarification was made of the content of ketone bodies in the blood and of the albumin (protein) fractions in the blood serum. Daily observations were carried out of the external appearance, behavior of the animals, and of the feed consumption. The experiment was conducted on young, growing animals with a weig of 120 plus or minus 20 grams -- 3 groups of males (1st, 2nd, 3rd) and on groups of females (4th, 5th, 6th), with 10 animals in each one. The animals in the 1st and 4th groups were administered antioxidants (butyloxyanisol and propylgallate) in a 100-fold aggravation (butyloxyanisol, 20 milligrams/kilogram, and propylgallate, 10 milligrams/kilogram) in comparison with the amount that might find way into the human organism; animals of the 2nd and 5th group were administered mixture of antioxidants in a 10-fold aggravation (butyloxyanisol, 2 milligrams/kilogram; propylgallate, 1 milligram/kilogram); the 3rd and 6th group served as control. The antioxidants were given in rendered lard with a feed briquet. Investigations were carried out prior to the beginning of the inoculation and on the 3rd, 6th and 9th months of the inoculation.

In biochemical investigations on the 3rd and 6th month of the experiment, no difference was disclosed between the control and experimental (test) animals. the 6th month, the animals of both groups were joined for the purpose of obtaining a 2nd generation. It turned out that the rats which had received antioxid-

ants with their feed were not capable of reproducing progeny: out of 5 animals o the 4th group, which had been subjected to the action of antioxidants, in a 100-fold aggravation, not a single one had any progeny; out of 5 females in the 5th group, which had received antioxidants in a 10-fold aggravation, progeny made their appearance in only one, whereas among the control females, offspring was found in three (see table).

((Table on pg. 49 of text))

Results of Experiment on Obtaining Progeny

First Mating					
Antioxidants				Control	
100-fold aggravation		10-fold aggravation			
No. of animal	Amount of offspring	No. of animal	Amount of offspring	No. of animal	Amount of offspring
31	None	41	4	5	Remains of baby rats disclosed
33	"	43	None	53	Same
35	"	45	"	55	None
37	"	47	"	57	"
39	"	49	"	59	7
Second Mating					
32	None	42	2	52	8
34	"	44	None	54	None
36	"	46	"	56	8
38	"	48	"	58	2
40	"	50	"	60	1

((text continued)): For the purpose of verifying the results, the experiment w carried out a second time on other animals. In a secondary mating, out of 5 rats, which had received a mixture of antioxidants in a 100-fold aggravation, r

a single one had any progeny; out of 5 animals to whom antioxidants had been administered in a 10-fold aggravation, only one had any offspring; among the control animals, progeny made its appearance in 4 (see table). A secondary mating confirmed that the animals, which had received antioxidants with their feed, were not capable of reproducing progeny.

On the 9th month of the inoculation, we carried out a sequential series of biochemical investigations. There was disclosed a statistically reliable reduction in the amount of ketone bodies in the blood: P is less than 0.001 in the males, and 0.01 (in the females). Those results were considered as being statis tically reliable in which the error probability, P, was less or equal to 0.05 (see illustration). We did not disclose any changes in other biochemical tests. The percent of mortality among the rats that received antioxidants was greater than it was in the control; thus, out of 20 animals to whom had been administere with the feed, antioxidants in a 100-fold aggravation, by the 9th month of the inoculation, 14 remained alive; out of 20 animals that had received antioxidants in a 10-fold aggravation -- 18, in the meantime, in the control groups, out of 2 rats 19 remained alive.

Thus, a mixture of antioxidants consisting of butyloxyanisol and propylgallat proved to be toxic for rats, and in connection with this, should not be permitte for the stabilization of alimentary fats.

Conclusions

1. A mixture of antioxidants, consisting of butyloxyanisol and propylgallate in a 100-fold aggravation, in the case of its prolonged feeding to rats, increases the mortality of the experimental (test) animals in comparison with the control (ones).

2. The administration, to the experimental (test) animals, of the indicated mixture of antioxidants, brings about sterility in the animals.

3. Butyloxyanisol and propylgallate, in their action on the organism, lead to a reduction in the amount of ketone bodies in the blood. Changes in the sug content, and also, in the activity of enzymes (catalase and cholinesterase) in

the blood, were not observed.

4. A mixture of butyloxyanisol and propylgallate antioxidants appears to be toxic for the organism and cannot be employed for the stabilization of alimentar fats.

((Double illustration on pg. 50 of text))((to the left of both parts of the illu tration)): Amount of ketone bodies in the blood (in % milligrams); ((within the same illustration, at the top of each part)): Males; Females: ((immediately und neath the lower part of this illustration)): Prior to the experiment; 3rd month 6th month; 9th month; Time of observation; ((below that)): Change in the conten of ketone bodies in the blood of experimental (test) and control rats.

Designations: a -- animals that had received antioxidants in a 100-fold aggravation; ((Russian)) b -- animals that had received antioxidants in a 10-fold aggra vation; ((Russian)) v -- control (having received no antioxidants). The numbers inside the columns designate the average (mean) magnitudes of the results obtained. P represents the probability of error.

((within the upper part of the abovementioned illustration, at the lower left)): a; ((Russian)) b; ((Russian)) v.

Literature (Bibliography)

1. Kadykov, B. I., Theses of Proceedings of the 5th Session of the Ukrainian Scientific-Research Nutritional Institute, Kiev, 1958, part 1, p. 19.

2. Kudryashov, B. A., Agatov, P. A., Gin. i akush. (Gynecology and Obstetric 1935, No. 6, p. 1.

3. Dangouman, A., Berlureau, F., Debruyne, H., Rev. franc. corps gras., 1957, v. 4, p. 541.

4. Holman, R. T., Greenberg, S. L., J. Am. Oil Chem. Soc., 1958, v. 35, p. 707.

5. Beck, S., Brit. J. exp. Path., 1941, v. 22, p. 299.

6. Beck, S., Kirby, A. H. M., Peacock, P. R., Cancer Res., 1945, v. 5, p. 135.

7. Kirby, A. H. M., Ibid., p. 129.

8. Gearhart, W. M., Stuckey, B.N., J. Am. Oil Chem. Soc., 1955, v. 32, p. 386.

9. Emanuel', N., Knorre, D., Lyakovskaya, Yu. and others, Myasnaya industriy (Meat Industry) of the U.S.S.R., 1958, No. 2, p. 52.

10. Karplyuk, I. A., Effect of antioxidants of alimentary fats (butyloxyanisol, butyloxytoluol and propylgallate) on the animal organism and their hygienic evaluation, Candidates' Dissertations, M(oscow), 1960.

11. Belen'kii, M. L., Elements of a quantitative evaluation of the pharmacological effect, L(eningrad), 1963, p. 63.

12. Balakhovskii, S. D., Balakhovskii, I. S., Methods of chemical analysis of the boold, M(oscow), 1953, p. 592.

13. Travina, O. V., Textbook (Manual) of Biochemical Investigations, M(oscow) 1955, p. 171.

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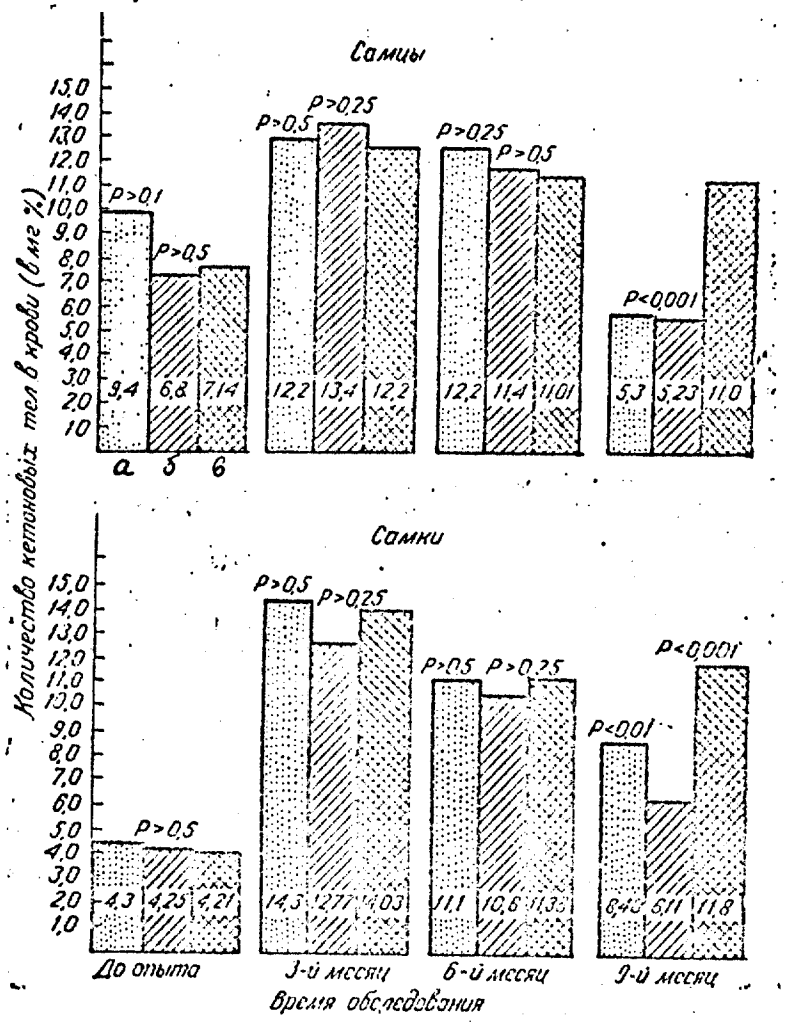

Изменение содержания кетоновых тел в крови подопытных и контрольных крыс.

Обозначения: *а* — животные, получавшие антиокислители в 100-кратной аггравации; *б* — животные, получавшие антиокислители в 10-кратной аггравации; *в* — контроль (не получавшие антиокислителей). Цифры внутри столбцов обозначают средние величины полученных результатов. *P* — вероятность ошибки.

ний в содержании сахара, а также активности ферментов (каталаза и холинэстераза) в крови не наблюдалось.

4. Смесь антиокислителей бутилоксианизола и пропилгаллата является токсичной для организма и не может применяться для стабилизации пищевых жиров.

ЛИТЕРАТУРА

1. Кадыков Б. И. Тезисы докл. 5-й Научной сессии Украинск. научно-исслед. ин-та питания. Киев, 1958, ч. 1, с. 19.—2. Кудряшов Б. А., Агатов П. А. Гиг. и акуш., 1935, № 6, с. 1.—3. Dangouman A., Berlureau F., Debruyne H., Rev. franç. corps gras., 1957, v. 4, p. 511.—4. Holman R. T., Greenberg S. L., J. Am. Oil Chem. Soc., 1958, v. 35, p. 707.—5. Beck S., Brit. J. exp. Path., 1941, v. 22, p. 299.—6. Beck S., Kirby A. H. M., Peacock P. R., Cancer Res., 1945, v. 5, p. 135.—7. Kirby A. H. M., Ibid., p. 129.—8. Gearhart W. M., Stuckey B. N., J. Am. Oil Chem. Soc., 1955, v. 32, p. 386.—9. Эмануэль Н., Кнорре Д., Лясковская Ю. и др. Мясная индустрия СССР, 1958, № 2, с. 52.—10. Карплюк

Retardation of Tumor Growth and RNA Biosynthesis by Administration of Phenolic Compounds to Tumor-Bearing Animals

L. B. Gorbacheva, G. V. Kukushkina and O. E. Petrov (Institute of Chemical Physi of the AN SSSR (Academy of Sciences of the U.S.S.R.), Moscow)

Investigations carried out at the Institute of Chemical Physics of the AN SSSR (Academy of Sciences of the U.S.S.R.) have shown the promise (outlook) of using phenolic compounds as chemotherapeutic agents (1, 2). It appeared that these compounds possess a high biological activity which is manifested in suppre ing the activity of oxidation-reduction enzymes (3, 4), in anhibiting glycolysis and respiration (5, 6), in suppressing the mitotic activity and in disturbing th normal passage (flow) of the life cycle of the cell (7). In addition to this, i has been shown, recently, that these compounds represent good radiosensitizers (8) and possess radioprotective properties (9/11).

It is known that the development of the tumoral process is accompanied by a vigorous (rapid) unregulated reproduction of cells; at the basis of this lie th intensively flowing processes of biosynthesis of albumins (proteins) and nucleic acids. The possibility of impairing and braking these processes represents one of the essential indices of the antitumoral activity of the preparations. The present paper is devoted to an investigation of the changes that develop in the process of biosynthesis of albumins (proteins) and neuclic acids under the influ ence of 4-methyl-2,6-di-tertiary butylphenol (ionol) and of propylgallate (PG).

Investigations were carried out on mice with an ascitic Ehrlich carcinoma and a substantial hepatoma, 22a (transplatable tumors). The operational procedure with these tumors has been described previously (12).

In mice with an ascitic Ehrlich carcinoma, where ionol has been administere intraperitoneally, there was observed a retardation, depending on the concentra tion, of the inclusion of L-C^{14} amino acids into the cellular albumins (prot in this, with a dose of 75 milligrams/kilogram, there is observed a 30% retardatio with a dose of 115 milligrams/kilogram -- a 55% one; and the administration of ionol in a concentration of 200 milligrams/kilogram brings about a complete sup-

pression of the inclusion. In addition to this, ionol and PG (propylgellate) inhibit the biosynthesis of RNA; at the same time, the biosynthesis of RNA is mo sensitive to the action of these compounds than is the biosynthesis of albumins (proteins); this was also confirmed on a cell culture from a laryngeal cancer of the individual Ner-2 (13). For a more intensified study of the character of act of ionol on the biosynthesis of RNA, we carried out a chromatographic separation of the preparations of total RNA secreted from the ascitic cells of animals on columns with methylated albumin adsorbed on kieselguhr (MAK) in a NaCl gradient (14).

The secretion (separation) of RNA from the ascitic cells was carried out in accordance with the procedure of Sherrer and Darnell with a five-fold deproteinization without polyvinylsulfate (15). The specimens of RNA thus obtained contained no more than 0.5% albumin (protein) and were practically free of DNA. In the capacity of a tagged (labelled) precursor use was made of $NaHP^{32}O_4$ (100 microcuries to a single mouse).

The optical density of the eluate was determined spectrophotometrically at 230 millimicrons. In order to obtain the profile of the elution that reflects the distribution of the isotopic tag (label), following the measurement of the optical density, the RNA was precipitated from the solution and the precipitates thus obtained were gathered on ultrafilters. A calculation of the precipitates was carried out by the customary method (16).

((Four graphs on pg. 343 of text)) ((in the upper left-hand corners of each gra Pulses/minute; ((immediately underneath each graph)): Test number; ((below the upper two graphs)): Fig. 1 Chromatographic separation of the preparations of total RNA on columns with methylated albumin adsorbed on kieselguhr in a NaCl gradient. The preparations were isolated from animals that were kept for 1 hour on $NaHP^{32}O_4$.

a - - RNS from control animals; b - - RNA from animals that were administered ionol; 1 -- ultraviolet absorption; 2 - - radioactivity.

((below the two lower graphs)): Fig.2 Chromatographic separation of the

preparation of total RNA on columns with methylated albumin adsorbed on kieselg[illegible] in a NaCl gradient. The preparations were isolated from animals that were kept [illegible] for 30 minutes on $NaPH^{32}O_4$.

The legend (symbols) are the same as in Fig. 1.

((text continued)): In analyzing the profiles of ultraviolet absorption and of the distribution of the isotopic tag (label), it was indicated that in a prolong[illegible] maintenance of the animals on a tagged (labelled) phosphate (2.5 and 4 hours), [illegible] observable retardation in the biosynthesis of RNA, under the influence of ionol, is not accompanied by changes in the profile of ultraviolet absorption and in t[illegible] distribution of the isotopic tag (label) Fig. A, B). Where there has been a sh[illegible] exposure (30 minutes), there is noted a disappearance of the peak of radioactiv[illegible] in the "rapidly (ex)changing" (metabolizing) RNA fraction, which, apparently, i[illegible] more sensitive to the action of ionol (Fig. 2, A, B).

At the present time, it has been shown that this fraction is inhomogeneous and consists of self-information RNA, the precursors of ribosomal RNA and a [illegible] fraction that fulfills a function that is not associated with the matrix synthe[illegible] of albumin (protein) (17 - 20). It may be assumed that the retardation, which [illegible] have observed, in the biosynthesis of rNA and of the albumins (proteins) in the cells of the ascitic Ehrlich carcinoma, to a considerable extent, is the result of inhibition in the synthesis of RNA and of the precursors of ribosomal RNA.

It is interesting to note that the observable effect of the inhibition of albumin (protein) and RNA biosynthesis correlates with the noticeable retardation in the development of the transplantable tumor. This may be seen especially [illegible] phically as illustrated by a diminution in the weight of the solid (substantial) tumor that has been grafted onto the mice. If, on the fourth day following the grafting of the tumor, the mice should have an everyday administration of ionol a dose of 100 and 150 milligrams/kilogram, then, on the 11th day (the terminal phase of the tumoral development), there takes place a retardation in the growth of the tumor and of the spleen. An increase in the ionol dose being administered brings about a greater retardation in the growth of the tumor. The develop[illegible]

of this tumor is accompanied by an increase in the weight of the spleen by 2.5 - 3 times. The administration of ionol also leads to a considerable retardation in the growth of the spleen and does not exert an influence on the development of the liver.

Even a single administration of PG (propylgallate) in a dose of 100 milligrams/kilogram, which brings about a 60% retardation in the inclusion of L-C^{14} amino acids into the albumins (proteins) of the hepatoma, is accompanied by a retardation in the growth of the hepatoma and of the spleen.

From the data set forth it follows that the administration of ionol and PG in animals with transplantable tumors brings about a noticeable retardation in t biosynthesis of albumins (proteins) and RNA, which is accompanied by an inhibiti of the tumoral growth.

Literature (Bibliography)

1. N. M. Emanuel', L. N. Lipchina, Dokl. AN SSSR (Proceedings of the Academy of Sciences of the U.S.S.R.), 1960, 131, 141.

2. N. M. Emanuel', V (Fifth) International Biochemical Congress, Symposium M(oscow), 1961.

3. N. M. Emanuel', L. N. Lipchina, I. I. Pelevina, T. E. Linatova, Dokl. AN SSSR (Proceedings of the Academy of Sciences of the U.S.S.R.), 1960, [illegible]

4. N. M. Emanuel', L. S. Vartanyan, I. I. Pelevina, Theses of Proceedings o the I (First) All-Union Biochemical Congress, L(eningrad), 1[illegible].

5. N. M. Emanuel', E. A. Neifakh, Dokl, AN SSSR (Proceedings of the Acad of Sciences of the U.S.S.R.), 1960, 1[illegible], [illegible].

6. L. M. Smanova, T. A. Nikol'skaya, N. M. Emanuel', Dokl, AN SSSR ((see above)), 1[illegible], 1[illegible],[illegible].

7. G. S. Frankfort, L. N. Lipchina, N. M. Emanuel', Dokl, AN SSSR ((see above)),1[illegible], 1[illegible],[illegible].

8. V. N. Andreev, I. I. Pelevina, G. S. Afanas'ev, L. N. Lipchina, N.M. Emanuel', Dokl. AN SSSR ((see above)), 1[illegible], [illegible] 1[illegible],[illegible].

9. N. M. Emanuel', Proceedings of the Moscow Society of Naturalists),1[illegible],

7, 73.

10. E. B. Burlakova, V. D. Ganitseva, L. V. Slepukhina, N. G. Khrapova, N. M. Emanuel', Dokl. AN SSSR (((see above)), 1964, 155,1308.

11. E. B. Burlakova, V. D. Ganitseva, L. V. Slepukhina, N. G. Khrapov, N. M. Emanuel', Dokl. AN SSSR ((see above)), 1965, 164,934.

12. G. V. Kukushkina, L. M. Gorbacheva, N. M. Emanuel', Vopr. med. khimii (Problems in Medical Chemistry), 1966, No. 5, 452.

13. G. V. Kukushkina, L. M. Gorbacheva, N. M. Emanuel', Vppr. onkologii (Problems in Oncology), 1966, 12, 54.

14. I. D. Handel, A. D. Hershey, Analyt. Biochem. 1960, 1, 66.

15. K. Scherrer, I. E. Darnell, Biochem. and Biophys. Res. Communs., 1962, 7, 486.

16. A. Reid, 1948, in the Collection: "Derivation and Determination of Tagged (Labelled) Atoms."

17. D. D. Brown, E. Littna, J. Mol. Biol., 1964, 4, 669.

18. M. I. Lerman, E. A. Vladimirtseva, Biokhimiya (Biochemistry), 1965, 30, 875.

19. V. A. Gvosdev, V. Kh. Tikhonov, Biokhimiya (Biochemistry), 1964, 29, [illegible] 1033.

20. N. V. Belitsyna, M. A. Mitkhozhin, Biokhimiya (Biochemistry) 1964, 29, 388.

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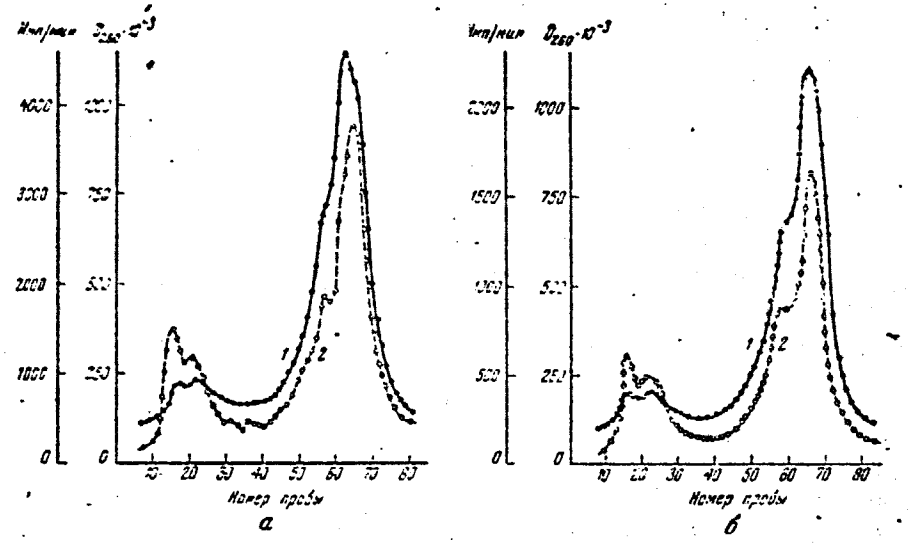

Рис. 1. Хроматографическое разделение препаратов тотальной РНК на колонках с метилированным альбумином, адсорбированным на кизельгуре в градиенте NaCl. Препараты выделены из животных, которые 4 часа выдерживались с $NaHP^{32}O_4$

а — РНК из контрольных животных; б — РНК из животных, которым вводили понол; 1 — УФ-поглощение; 2 — радиоактивность

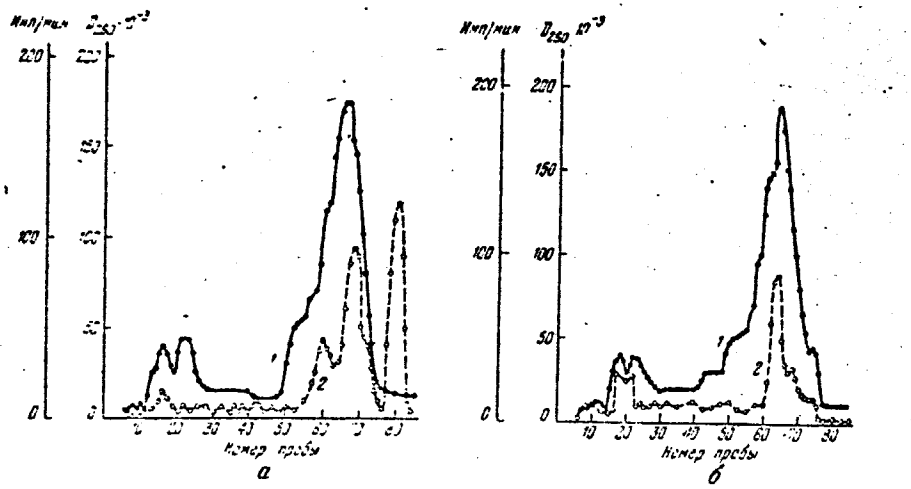

Рис. 2. Хроматографическое разделение препаратов тотальной РНК на колонках с метилированным альбумином, адсорбированным на кизельгуре в градиенте NaCl. Препараты выделены из животных, которые 30 *мин* выдерживались с $NaHP^{32}O_4$

Обозначения те же, что на рис. 1

винилсульфата [15]. Полученные образцы РНК содержали не более 0,5% белка и были практически свободны от ДНК. В качестве меченого предшественника использовали $NaHP^{32}O_4$ (100 *мккюри* на одну мышь).

Оптическую плотность элюата определяли спектрофотометрически при 260 *ммк*. Для получения профиля элюции, отражающего распределение изотопной метки, после измерения оптической плотности РНК из раствора осаждалась и полученные осадки собирались на ультрафильтрах. Счет осадков проводился обычным методом [16].

При анализе профилей УФ-поглощения и распределения изотопной метки оказалось, что при длительном выдерживании животных с меченым фосфатом (2,5 и 4 часа) наблюдаемое торможение биосинтеза РНК под влиянием понола не сопровождается изменениями в профиле УФ-поглощения и распределения изотопной метки (рис. 1, *А*, *Б*). При короткой экспозиции (30 мин.) отмечается исчезновение пика радиоактивности «быстрообменивающейся» фракции РНК, которая, по-видимому, является более чувствительной к действию понола (рис. 2, *А*, *Б*).

В настоящее время показано, что эта фракция неоднородна и состоит из собственно информационной РНК, предшественников рибосомальной РНК и фракции РНК, выполняющей функцию, не связанную с матричным синтезом белка [17—20]. Можно предположить, что наблюдаемое нами торможение биосинтеза РНК и белков в клетках асцитной карциномы Эрлиха в значительной степени обусловлено ингибированием синтеза мРНК и предшественников рибосомальной РНК.

Интересно отметить, что наблюдаемый эффект ингибирования биосинтеза белков и РНК коррелирует с заметным торможением развития перевиваемой опухоли. Особенно наглядно это можно видеть на примере уменьшения веса солидной опухоли, привитой мышам. Если на четвертый день после перевивки опухоли мышам ежесуточно вводить понол в дозе 100 и 150 *мг/кг*, то на 11-е сутки (терминальная фаза развития опухоли) происходит торможение роста опухоли и селезенки. Увеличение вводимой дозы понола приводит к большему торможению роста опухоли. Развитие этой опухоли сопровождается увеличением веса селезенки в 2,5—3 раза. Введение понола приводит также к значительному торможению роста селезенки и не влияет на развитие печени.

Даже однократное введение ПГ в дозе 180 *мг/кг*, которая вызывает 60%-ное торможение включения L-C^{14}-аминокислот в белки гепатомы, сопровождается задержкой роста гепатомы и селезенки.

Из приведенных данных следует, что введение понола и ПГ животным с перевиваемыми опухолями приводит к заметному торможению биосинтеза белков и РНК, которое сопровождается ингибированием роста опухоли.

Toxicologic Characteristics of Phenol Antioxidants of Alimentary Fats (Acute and Subacute Experiments)

I. A. Karplyuk

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Fats, which represent a valuable nutritional product, are subject to rapid spoilage, which, basically, results from oxidizing processes. Oxidation, at low temperatures, slows down somewhat, but it is not discontinued; for this reason, even in the refrigerator, fats may not be preserved for no longer than half a year. In the oxidation process, fats lose their taste (flavor) and nutritive properties and may produce a harmful action (I. Smorodintsev, 1947; Burr and Barues, 1943; Johnson, Perkins, Sugal and Kummerow, 1957, and others).

It has not yet been clarified, definitively, whether the very products of fat oxidation (peroxides, polymeric products) produce a direct harmful effect, or whether, during the oxidation of fats (during rancidification), there is an oxidation and inactivation of such important constituent parts of the diet as vitamins A, D, E, K, pantothenic acid, pyridoxine, biotin, ascorbic acid and vitally necessary unsaturated fatty acids (linoleic, linolenic, and arachidonic). Pathological phenomena, which developed during the feeding of rats with a rancidified experimental diet, are explained by a shortage of these nutritive substances in the diet (Quackenbusch, 1945; Greenberg and Frazer, 1953, Witting, Hishida, Johnson and Kummerow, 1957).

Data also exist with reference to the fact that thoroughly (deeply) oxidized fats possess a carcinogenic activity (Lavik and Baumann, 1941, 1943; Roffo, 1938). The spectrum of thermally oxidized fat has a resemblance to the spectrum of carcinogenic derivatives of phenanthrene (Roffo, 1938).

One of the effective methods of keeping fats from oxidation is the introduction of antioxidants in the fats. The greatest interest is being shown in phenolic

antioxidants as being the most effective: butyloxyanisol, butyloxytoluol and gallic acid esters. In a number of countries, these antioxidants have found wi usage in the food industry (Tollenaar and Vos, 1956).

At the present time, in the Soviet Union, the All-Union Scientific-Research Institute of the Meat Industry and the All-Union Scientific-Research Institute of Fats have proposed the introduction of butyloxyanisol (BNA), butyloxytoluol (BNT) and propylgallate (PG) into rendered fats and vegetable oils. It is recommended that the antioxidants be introduced in an amount of 0.01 - 0.02%. With such an amount of butyloxyanisol or butyloxytoluol, the fats increase their stability by 3 - 5 times, and in the case of adding gallates -- up to 10 times (N. Emanuel', D. Knorre, Yu. Lyaskovskaya and V. Piul'skaya, 1955, 1958). These antioxidants, in the indicated amounts, do not bring about any organoleptic changes in the fat.

When these preparations were proposed for their application in the food industry, a question with regard to their toxicity arose. A study of the toxicity of butyloxyanisol was taken up by Lehmann, Fitzugh, Helson and Woodard (1951), Wilde Ostby and Gregory (1956) and others. Takata, Nagata (1952), Deichmann, Clemmer, Rakoczy and Bianchine (1955) made a study of butyloxytoluol. The toxicity of the gallates was determined by Boehm and Williams (1943), Orten, Knyper and Smith (1948), Allen and De Eds (1951), Roy (1953), Sporn and Peretianu (1956). By the papers (studies) of the above-enumerated authors there were established the LD_{50} of these preparations for mice, rats, rabbits, guinea pigs, and cats; at the same time, the method of establishing this dose is different in the case of each author: some established it by subcutaneous administration, and others -- by an intraperitoneal one, and still others -- by an enteric one (enterally); solutions of various concentrations were administered. It is understandable that the results obtained differed from one other. In the case of a prolonged administration (1 - 3 months) of large doses of phenolic antioxidants (close to 1% of the weight of the feed), the authors noticed an impairment in the growth and development of the animals. Propylgallate, inthe case of such concentrations, led, in addition to this, to a diminution in the amount of blood hemoglobin and to patho-

logical phenomena with respect to the kidneys (the appearance of albumen in the urine). Butyloxyanisol, with a concentration of 0.25 gram for 1 kilogram of weight, in dogs, brought about degenerative changes in the parenchymatous tissue of the liver. The feeding of higher gallates (octyl, dodecyl) to rat females, a concentration of 0.5% of the weight of the feed, brought about an impairment lactation and manifested a harmful effect on the young generation. It should b noted that all the authors, in subacute experiments, made use of a very limited number of tests: the weight of the animals, a study of the blood, and pathomorp logical studies of the organs. Only Deichmann and others (1955), in studying t toxicity of butyloxytoluol, determined the prothrombin time and measured the blood pressure. It seems to us that the amount of tests employed by the author is insufficient in order to judge with respect to the fuctional state of the organism. In our experiments we made an attempt, to the broadest extent possibl to study the various sides of the organism's active life (life activity).

For the comparative toxicologic characteristics of the preparations under study (butyloxyanisol, butyloxytoluol, and propylgallate), acute experiments we set up on rats and mice. For the experiment were taken growing, healthy animal (males and females). Antioxidants were administered in the morning, on an empty stomach, in a solution of sunflower oil, directly into the esophagus from a syringe through a metallic probe, represented by an ordinary thick injection needle with an "olive"soldered on at the end. The animals that remained alive after the administration of the preparation stayed under observation over a per of 20 days. Various amounts of the preparatinns under study were administered the animals (7 - 8 doses). Each dose was received by 8 rats and 10 mice.

By carrying out inoculations on the animals, in various doses, of the prepar ations under study, we established the toxicity of these substances from a minimal toxic dose to an absolutely fatal one. After that, having carried out integratinn, according to Berens (N. S. Pravdin, 1947; V. S. Shadurskaya, 1953) which has been accepted in toxicology, we plotted a mortality curve, and accord ing to it, determined the LD_{50} of all three antioxidants under study for rats and mice. The results of the experiments are set forth in Table 1.

((Table on pg. 25 of text))

Table 1

Results of Determining the Toxic and Fatal Doses of the Antioxidants Under Study

Antioxidants		Rats		Mice	
	minimal toxic dose in milligrams for 1 kilogram of weight	LD_{50} in milligrams for 1 kilogram of weight	LD_{100} in milligrams for 1 kilogram of weight	LD_{50} in milligrams for 1 kilogram of weight	LD_{100} in milli-grams fo: 1 kilogra of weight
Butyloxyanisol (BNA)	1000	2900	4500	1250	3000
Butyloxytoluol (BNT)	500	2450	3500	2000	2500
Propylgallate (PG)	500	2600	3500	1700	2500

((text continued)): From the data obtained it may be seen that the toxicity of these preparations is very close with respect to one another and that phenolic antioxidants are comparatively low in toxicity.

Subacute experiments were conducted with the purpose of disclosing functional impairments under the influence of the preparations under study, in deliberately (admittedly) large, aggravated doses (in comparison with those that may find their way into a person's organism). In the event of the discovery of such deviations, attention was fixed precisely onthose tests in chronic experiments i the definitive solution of the problem concerned with the possibility of introducing phenol antioxidants into food fats. In subacute experiments, there were utilized four groups of rats: the first group received, in its diet, fat with B in a dose of 1/5 of LD_{50}, i.e., 600 milligrams for 1 kilogram of body weight; th second group received BNT, initially 1/5 of LD_{50}, i.e., 500 milligrams for 1 kilogram of weight; in the future, this dose was reduced to 250milligrams for 1 kilogram of body weight because of a poor consumption of feed in the case of a higher content of BNT; the third group received 1/5 of LD_{50} with respect to

— 500 milligrams for 1 kilogram of body weight; the fourth group was a control group.

Antioxidants were injected into rendered lard which was mixed in with the ba feed. The control group received pure lard. The rendered lard and the antioxi ant preparations, which are necessary for the setting up of the experiments, we furnished to us by the All-Union Scientific-Research Institute of the Meat Indu try.

Each rat was kept in an individual cage; this made it possible to keep track of the amount of the feed and fat, with antioxidants, which were consumed.

For the purpose of observing the functional state of the experimental (test) animals, we dwelt on (emphasized) the following indices.

1. Daily observation of the animals.

2. Weighing of the animals once very 10 days.

3. Carrying out a morphological examination of the blood, considering that it is known that phenol and its derivatives frequently produce a haematopoiesis (blood formation) pathology. In the morphological investigation, a count of th regular (prescribed) elements was made: of the erythrocytes, reticulocytes, thrombocyes, and leucocytes; a determination was made of the hemoglobin content of the leucocytic formula.

4. Proceeding from the fact that the oxidizing processes lie at the basis o the majority of biochemical reactions in the organism, and that antioxidants, possibly, even in the live organism, manifest an effect on the oxidation proces an investigation was made of the activity of oxidizing enzymes, and to be precise, catalases and peroxidases. These enzymes were selected also for the reas that Tappel and Marr (1954), while studying in vitro the action of propylgallate and nordihydroguaiaretic acid (also that of a phenol antioxidant) on 14 enzymat systems, discovered that they, in a concentration of $1 \times 10^{\text{minus } 4}$ M, inhibit the activity of a catalase and that of a peroxidase. The inhibiting action of phenolic compounds on a catalase was also shown by Goldacre and Galston (1953). The catalase was determined according to the method of Bakh and Zybkova and was expressed in the form of a catalase index. The peroxidase was determined from

the oxidation of indigocarmine and was expressed in the time (seconds) that was required for its oxidation.

5. An investigation was made of the cholinesterase activity of blood serum, which, in the opinion of physiologists (K. M. Bykov, 1949; Kh. S. Koshtoyants, 1946), is closely associated with the functional state of the nervous system.

6. An investigation was made of the prothrombin time (it depends on the amou of prothrombin that is synthesized in the liver). Clinical physicians acknowle the value of this determination for the purpose of disclosing the functional im- pairments of the liver.

All the selected indices were verified in the animals prior to the experiment and following the administration of the antioxidants -- each 2 weeks. The anim were kept on the experiment from 68 to 82 days. At the end of the experimental period, all the animals were destroyed by decapitation. There were carried out pathological-anatomical dissection and a histological investigation of the inte al organs. In addition to a pathohistological examination, the liver was sub- jected to a chemical study; a determination was made of the content of dry sub- stances, of the amount of glycogen, fat and lipoid phosphorus for the purpose of revailing any possible impairment of some aspects of the carbohydrate and fa metabolism in this organ.

During the entire experimental period, each experimental (test) animal recei the following amount of preparations: BNA -- from 5 to 7 grams; BNT -- from 2 t 4 grams; PG -- from 4 to 6 grams.

No changes were observed in the behavior and outward appearance of the exper- imental (test) animals. Weighing of the animals indicated that prior to the adm istration of antioxidants, the animals in all groups added weight equally well. From the beginning, however, of introducing antioxidants into the diet, the wei of the experimental (test) animals started to lag behind the weight of the cont (ones) considerably (Fig. 1). The lag in weight of the animal experimental (te groups was especially pronounced in the first month of the experiment, and less in the second month.

In animals that received BNA and BNT in their diet there was disclosed a reduction in the activity of the blood catalase. In the control rats, at the end of the experimental period, the activity of the catalase comprised 9.5 - 13.5 milligrams of hydrogen peroxide; in the group with BNA -- 6.7 - 8.8 milligrams; in the group with BNT, by the 43rd day of the experiment -- 6.1 - 8.8 milligrams by the 60th day, the activity of several increased but did not attain the catala level of the control animals. Propylgallate did not bring about any changes in the catalase activity.

The blood of all three experimental (test) groups of animals indicated a redu tion in the peroxidase activity (the amount of seconds devoted tothe oxidation o the substrate increased). The peroxidase activity of the blood in the control animals, during the period of the experiment, fluctuated within the range of 56 - 86 seconds, whereas in the group that received BNA, it comprised, by the 42nd day, 105 - 147 seconds, and by the end of the experiment -- 91 - 135 second The group of animals that received BNT indicated a time of 83 - 116 seconds by the end of the experimental period, and the group, which had received propylgallate -- 84 - 110 seconds.

((Graph at the top left of pg. 27 of text)) ((to the left of this graph)): Weight (in grams); ((immediately underneath the same graph)): Weighing days; ((below that)): Fig. 1. Change in weight of rats in the course of the experime tal period.

I -- weight of the control animals; II -- Weight of the animals that received propylgallate; III -- butyloxyanisol; IV -- butyloxytoluol.

((text continued)): The cholinesterase activity was reduced in animals in whose diet there was mixed in fat with BNT and PG.

By the end of the experiment, the activity was as follows: in control animals -- there was 19.7 - 25.4% disintegrated acetylcholinchloride; inthe group that received BNT, -- 10.4 - 16.3%; in the group that received PG, -- 13.2 - 16.3%; in the group that received BNA, there was no distinction from the control group. No deviations were noted in the morphological composition of the blood and in examining the urine of the experimental (test) animals. The prothrombin time o

all the animals in the experimental (test) groups did not differ from that of th control group.

((Two graphs on pg. 27 of text, in the right-hand column)) ((to the left of the upper graph)): % fat; ((underneath the same graph)): Fig. 2. Fat content in t liver (for a dry substance).

I -- control group; II -- animals that had received butyloxyanisol; III -- those that had received butyloxytoluol; IV -- those that had received propylgallate. ((to the left of the lower graph)): M2% lipoid phosphorus; ((underneath this graph)): Fig. 3. Content of lipoid phosphorus in the liver (for a dry substanc Legend (conventional symbols) the same as in Fig. 2. ((text continued)):

In dissecting the animals groups no pathological-anatomical changes were observed. The weight of the liver in rats that had received BNA and BNT was greater than that in the control ones and than that of the group that had receiv PG. In the histological examination of the internal organs (the cerebrum (brain heart, lungs, stomach, duodenum, small intestine, caecum (blind gut), colon, liver, spleen, kidneys, urinary bladder, and the sex organs), all three experimental (test) groups did not indicate any significant changes in comparison with the control animals. Only in one rate (No. 15) from the propylgallate group was there an edema of the stroma in a testicle, with a discontinuance of spermatogenesis. In another rat (No. 17) of the same group, the villi of the small intestine were edematic with markedly dilated lymphatic capillaries. Pathomorph logical investigations were carried out in the Nutritional Hygiene Section of th Nutrition Institute of the AMN SSSR Yu. P. Kukelum (Yu. P. Kukelem Academy of Medical Sciences of the U.S.S.R.).

The fat content in the liver was identical with that in the control group and with that in the groups that had received BNA and PG (from 14.6 to 20.6%). The liver of the rats, however, that had received BNT, contained an increased amount of fat (20.8 - 27.4%) (Fig. 2). At the same time, in the liver of these animals there was little lipoid phosphorus (Fig. 3). This points to the fact that, in animals that had received BNT, there was an impairment in the synthesis of phos-

pholipids and in the processes of breaking up and secretion of neutral fat from the liver (true, still without any histologically disclosed fatty infiltration of the liver). There was a somewhat reduced content of lipoid phosphorus also those animals that had received BNA.

The percentage content of dry substances and glycogen was approximately the same in all four groups.

The acute and subacute experiments that had been carried out yield a basis for drawing the following conclusions.

1. Phenol antioxidants (butyloxyanisol, butyloxytoluol, and propylgallate) not pertain to highly toxic substances(LD_{50} is greater than 1000 milligrams for 1 kilogramof body weight), and their toxicity is close with reference to one another.

2. Subacute experiments have shown that phenol antioxidants, even though th did not bring about any histological changes in the organs with the administrat of large doses, represent far from neutral substances for the organism of anima

a) experimental (test) rats lagged in weight in comparison with the control ones; this points to the fact that the doses of preparations that had been fed brought about an impairment in the processes of assimilation in the organism of the animals;

b) the antioxidants under study lowered the level of the oxidizing processes in the organism (a reduction in the activity of the oxidizing enzymes);

c) butyloxytoluol and propylgallate reduced the activity of cholinesterase i the blood serum;

d) Butyloxytoluol showed the greatest toxicity. Chemical examinations of th liver disclosed that butyloxytoluol manifests a harmful action on the liver, in pairing the process of phospholipoid synthesis, and also, that entailed in the breaking up and secretion of neutral fat from the liver.

3. For a definitive conclusion with regard to the possibility of introducin phenol antioxidants into industrial practice, chronic experiments are being con ducted, at the present time, with small doses of the preparations. This is nec essary in order to eliminate completely the possible toxic action (effect) on a

individual of fat containing butyloxyanisol, butyloxytoluol and propylgallate.

LITERATURE (BIBLIOGRAPHY)

Bykov, K. M., in the book;"Problems of Soviet Physiology, Biochemistry, Pharmacology", M(oscow), 1949, Book 1, p. 17.

Koshtoyants, Kh. S., Turpaev, T. M., Dokl. AN SSSR (Proceedings of the Acaden of Sciences of the U.S.S.R.), 1946, vol.54, No. 2, p. 181.

Pravdin, N. S., Low Toxicology Procedure in Industrial Poisons, M(oscow), 1947.

Smorodintsev, I., Myasnaya i molochnaya prom. (Meat and Milk Industry), 1947, No. 9, p. 12.

Shadurskaya, V. S., Farmakol. i toksikol. (Pharmacology and Toxicology), 1953, No. 3, p. 48.

Emanuel', N., Knorre, D., Lyaskovskaya, Yu. and others, Myasnaya industriya (Meat Industry), 1955, No. 6, p. 47.

Idem., ibid., 1958, No. 2, p. 52.

Allen, Sh. C., De Eds, F. J., Am. Oil Chem. Soc., 1951, v. 28, p. 304.

Boehm, E., Williams, R., Quart. J. pharm., 1943, v. 16, p. 232.

Burr, G. O., Barnes, R. H., Physiol. Rev., 1943, v. 23, p. 256.

Deichmann, W. B., Clemmer, J. J., Rakoczy, R. and others, Arch. Industr. Health, 1955, v. 11, p. 93.

Goldacre, P. L., Galston, A. W., Arch. Biochem. 1953, v. 43, p. 169.

Greenberg, S. M., Frazer, A. C., J. Nutrition, 1953, v. 50, p. 421.

Johnson, O. C., Perkins, E., Sugal, M. and others, J. Am. Oil Chem. Soc., 1957, v. 34, 0. 594.

Kaunitz, H., Slanetz, C., Johnson, R. E., Ibid., 1956, v. 33, p. 630.

Lavik, P. S., Baumann, C. A., Cancer Res., 1941, v. 1, p. 181.

Idem., ibid., J. 1943, v. 3, p. 749.

Lehmann, A. J., Fitzugh, O. G., Helson, A. A. and others, Advances in Food Research, 1951, v. 3, p. 197.

Orten, J. M., Knyper, A. C., Smith, A. H., Food Technology, 1948, N. 2, p. 308.

Quackenbush, F. W., Oil and Soap, 1945, v. 22, N. 12, p. 336.

Roffo, A. H., Bol. Inst. de med. exper. para el estud. y trat. d. cancer, 1938, v. 15, p. 837.

Roffo, A. H., Ibid., 1938, v. 15, p. 407.

Roy, B. R., Indian J. M. Res., 1953, v. 41, p. 201, 207.

Sporn, A., Peretianu, J., Igiena, 1956, v. 1, p. 52.

Takata, R., Nagata, T., Vitamins (Japan), 1952, v. 5, p. 203 - 205.

Tappel, A. L., Marr, A. G., J. Agricult. Food Chem., 1954, v. 2, p. 554.

Tollenaar, E. D., Vos, H. J., Fette, Seifen, Austrichmittel, 1956, v. 58, s. 112.

Wilder, O. H. M., Ostby, C., Gregory, R., Publ. Am. Meat Inst. Foundation, 1956, Bull. N. 29, p. 38.

Witting, L. A., Nishida, T., Johnsohn, O. C. and others, J. Am. Oil Chem. Soc., 1957, v. 34, p. 421.

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Кровь всех трех подопытных групп животных показала понижение активности пероксидазы (количество секунд, идущее на окисление субстрата, увеличивалось). Активность пероксидазы крови контрольных животных за время опыта колебалась в пределах 56—86 секунд, в то время как в группе, получающей ВНА, она составила к 42-му дню 105—147 секунд и к концу опыта — 91—135 секунд. Группа животных, получавших ВНТ, показала время 83—116 секунд к концу опытного периода, а группа, получавшая пропилгаллат, — 84—110 секунд.

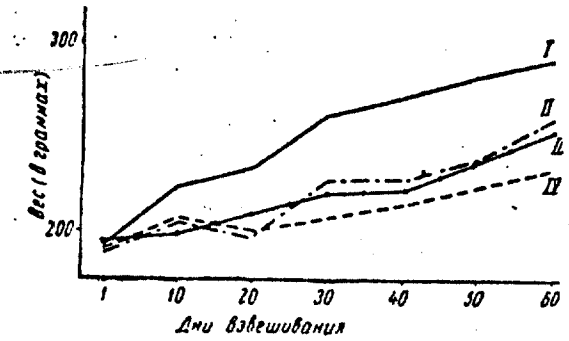

Рис. 1. Изменение веса крыс в течение опытного периода.

I — вес контрольных животных; *II* — вес животных, получавших пропилгаллат; *III* — бутилоксианизол; IV — бутилокситолуол.

Понизилась активность холинэстеразы у животных, в рацион которых примешивался жир с ВНТ и PG.

К концу опыта активность была следующей: у контрольных животных — 19,7—25,4% разрушенного ацетилхолинхлорида; в группе, получавшей ВНТ, — 10,4—16,3%; в группе, получавшей PG, — 13,2—16,3%; в группе, получавшей ВНА, не отличалась от контрольной. Каких-либо отклонений в морфологическом составе крови и при исследовании мочи подопытных животных отмечено не было. Протромбиновое время всех животных подопытных групп не отличалось от такового контрольной группы.

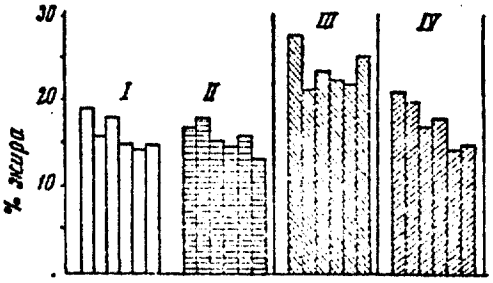

Рис. 2. Содержание жира в печени (на сухое вещество).

I — контрольная группа; *II* — животные, получавшие бутилоксианизол; *III* — получавшие бутилокситолуол; *IV* — получавшие пропилгаллат.

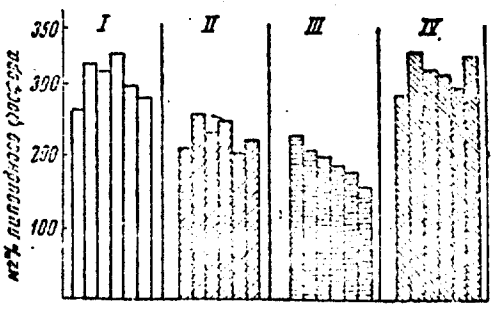

Рис. 3. Содержание липоидного фосфора в печени (на сухое вещество). Условные обозначение те же, что и на рис. 2.

При вскрытии животных грубых патологоанатомических изменений обнаружено не было. Вес печени крыс, получавших ВНА и ВНТ, был выше, чем контрольных, и группы, получавшей PG. При гистологическом исследовании внутренних органов (головного мозга, сердца, легких, желудка, двенадцатиперстной кишки, тонкого кишечника, слепой и ободочной кишки, печени, селезенки, почек, мочевого пузыря, половых органов) все три подопытные группы не дали сколько-нибудь значительных изменений сравнительно с контрольными животными. Только у одной крысы (№ 15) из группы пропилгаллата имелся отек стромы семенника с прекращением сперматогенеза. У другой крысы (№ 17) этой же группы ворсинки тонкого кишечника были отечны с резко расширенными лимфатическими капиллярами. Патоморфологические исследования были проведены в Отделе пищевой гигиены Института питания АМН СССР Ю. П. Кукелем.

Effect of Phenolic Antioxidants of Alimentary Fats on the Animal Organism and Their Hygienic Evaluation

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Fats, which represent a highly valuable product of nutrition, are subject to rapid spoilage, which, basically, is the result of oxidizing processes. Oxidation, at low temperatures, is somewhat inhibited, but it is not discontinued; for this reason, even under refrigerating conditions, fats may be preserved no longer than half a year; this cannot satisfy the needs of the food industry.

In fats, during oxidation, there is a disintegration, in the first place, in the fat-soluble vitamins and in the polyunsaturated fatty acids that are require for the normal life activity of the organism. For this reason, the problem concerned with preserving (keeping) fats fresh over a longer period of time also represents a hygienic problem.

In recent years, a proposal has been made to employ chemical methods for protecting fats from spoilage; these consist in introducing into fats substances that have received the designation of antioxidants (antioxidants) ((the latter term in parentheses is an Anglicized version of the word immediately preceding, which bears the same definition -- Translator))

Oxidizing processes in fats proceed in accordance with the chain reaction typ The mechanism involved in the action of antioxidants, apparently, consists in their capacity to break (cut off) the chain reaction; as a result of this, the oxidation process shows a marked inhibition.

The most effective are acknowledged to be phenolic antioxidants, and among them are: butyloxyanisol, butyloxytoluol and gallic acid esters. For an inhibiting action small amounts of the substances are sufficient (0.01 - 0.02%); this makes it possible to increase the storage time of fats by 4 - 10 times. In connection with the proposal to utilize butyloxyanisol, butyloxytoluol and propy gallate for the stabilization of rendered fats and vegetable oils, the problem arose in the Soviet Union with respect to harmlessness and suitability of such

fats for human nutrition.

There are no domestic studies (papers) on the study of the toxic properties of butyloxyanisol, butyloxytoluol and propylgallate.

The U.S.A., England, Holland, Japan, India and Rumania have been preoccupied with the study of the toxicity of phenolic antioxidants.

Familiarity with these studies (papers) has shown that the LD 50 of butyloxyanisol, butyloxytoluol and of the gallates, in the case of their enteral administration, is a dose above 1000 milligrams/kilogram of the animal's weight; this attests to the comparatively weak toxicity of these compounds. In feeding large amounts (close to 1%, on conversion to the weight of the feed) of these phenolic antioxidants, over a period of 1 - 3 months, the authors have noted an impairment in the growth and development of the animals. Feeding of propylgallate brought about a diminution in the hemoglobin and morphological changes in the tubular apparatus of the kidneys. The introduction of octyl- and dodecylgallates into the feed of nursing female rats brought about an impairment in lactation and, as a result of this, a deteriorated development of the younger generation.

The majority of researchers, who have conducted experiments on a prolonged feeding of butyloxyanisol, butyloxytoluol and gallates, have come to the conclusion that even that amount of antioxidants, which is 100 - 200 times greater than the one proposed for the introduction of antioxidants into fats, is harmless. However, the conclusions of various authors with regard to the toxicity or harmlessness of the compounds do not always coincide.

Lehman and his co-workers did not observe any harmful effect of propylgallate on rats when feeding it in a dose of 1% in the feed; whereas Orten, Kaiper and Smith consider this concentration to be toxic.

Roy confirms that with a concentration of ethylgallate in fat in an amount of 1%, the animals do not react, whereas Sporn and Peretianu, in the case of a 7.5% content of ethylgallate in the diet's fat, have disclosed a disturbance in the growth of the experimental (test) animals, and a reduction in the utilization of nitrogen in the nutritional diet. The authors have disclosed dystrophic changes in the thalamus and in the liver.

Consequently, existing literature on the study of the toxic properties of butyloxyanisol, butyloxytoluol and propylgallate does not yield the fully comparative characteristics of these compounds.

In connection with the inconsistency of the literature data, the present paper should have given an answer to the possibility of utilizing butyloxyanisol, butyl oxytoluol and propylgallate for the stabilization of alimentary fats. This was resolved by the following methods.

1. An investigation was made of the organoleptic properties of the products prepared with fat containing antioxidants.

2. Biological experiments were conducted on animals that included determining the degree of toxicity of the preparations in acute experiments, a study of their toxicity in subacute experiments while feeding large doses, and an investigation of the possible harmful effect on the organism of animals of alimentary fat containing butyloxyanisol, butyloxytoluol and propylgallate in the case of prolonged feeding.

Organoleptic investigations were carried out with the purpose of disclosing the possibility of changes in the external appearance, color, odor and taste of the product, brought about by the addition of the preparation. In the event of disclosing any signs of product spoilage, the problem could be raised as to the possibility of utilizing these compounds in alimentary fats. An undisclosed sampling (tasting) of four samples of fried potatoes, prepared with rendered lard with the addition of 0.02% butyloxyanisol, butyloxytoluol and propylgallate, and without any antioxidants, did not reveal any differences in the organoleptic properties of the potatoes which had been fried with a control sample of fat, and also, with fat having an addition of antioxidants.

The acute toxicity on the part of the antioxidants, was established for white mice and rats.

The antioxidants were administered to the animals in a solution of sunflower oil directly into the esophagus. 8 rats and 10 mice were inoculated with each dose. Altogether, in the acute experiment, there were used 176 rats and 140 mice.

Having carried out an inoculation of the animals with various doses of the

preparations under study, we established the toxicity of these substances from the minimal toxic dose to the absolutely fatal one.

To the extent that it was most convenient to make use of LD 50 for the characteristic of the substance's degree of toxicity, we made a determination of it.

As a rule, the mean lethal dose is determined by means of calculations.

In our investigations, we made a determination of the LD 50 of the preparation under study by means of the three most widespread methods: by the methods of Berens, Rid-Mench, and by the method of equalizing along a straight line, in ord to clarify whether there would be large disparities between the magnitudes calcu ated by the various methods, inasmuch as many toxicologists and immunologists mak use of methods of integration (those of Berens andRid-Mench), whereas statistica specialists, in particular, Boyarskii, criticize these methods because of the fact that, in integration, there is impaired the randomness (chance) in the sele tion of animals and a systematic error is introduced.

Our results indicate that the lethal doses, for the same compound, calculated by the various methods, do not differ to a marked extent from one another, and i is possible to agree with Prof. Merkov that, for practical purposes, this differ ence has hardly any significance. To the extent that the method of equalizing along a straight line represents the most strictly valid one from the theoretica point of view, and what is most important, does not appear to be more labor-consuming than the methods based on integration, we believe that in experimental studies, the method of equalizing (smoothing out) should be given preference.

The results of acute experiments have shown that the antioxidants under stud cannot be reckoned among highly toxic substances. The LD 50 of all three preparations, for rats and mice, were above 1000 milligrams/kilogram of animal weig

Subacute experiments were set up for the purpose of clarifying on which syst and organs of the animals the antioxidants may have an effect in the case of their daily admission into the organism in large amounts. In the event of disclosing any deviations in the activity of any organs, in these experiments, we wanted to fix particular attention on such tests in chronic experiments in the definitive resolution of the problem concerned with the possibility of introduc

antioxidants into alimentary fats.

Four groups of rats were used in the subacute experiments. Three groups were experimental (test) ones; to them, daily, there were administered, in the feed, antioxidants in an amount of 1/5 of LD 50, i. e., 500 - 600 milligrams/kilogram of the animal's weight. The fourth group was a control one and received pure fa

Fat with antioxidants was fed to animals over a period of 2.5 months; during this period of time, the animals received from 2 to 7 grams of the compounds und investigation.

In the course of the experiment, daily observation was applied to the animals a determination was made of the weight, and a morphological investigation of the blood was carried out; a study was made of the activity of the blood enzymes (of the catalase, peroxidase, and of the cholinesterase); a determination was made o the prothrombin time and a study was made of the urine. At the end of the exper ments the animals were destroyed by decapitation, were dissected, and their internal organs were subjected to a histological investigation. The liver, in addition to a histological investigation, was subjected to a chemical study. In it was determined the content of dry substances, the amount of glycogen, fat and lipoid phosphorus.

The indices of the weight and of the biochemical investigations of the blood and of the liver in the animals were statistically treated; a calculation was made of the degree of reliability in the difference of the indices that had bee determined in the case of the experimental (test) and control animals. Weighin of the rats indicated that the introduction, into the diet, of fat with butylox toluol brought about, by the 22nd day of the experiment, a lag in the weight of the animals in this group and it was maintained to the end of the experiment. B the 31st day of the experiment, the rats of all three experimental (test) group (butyloxyanisol, butyloxytoluol and propylgallate) were lagging in weight in comparison with the control group (the difference has a statistical reliability

In the animals of all three experimental (test) groups there was disclosed a reduction in the activity of the blood enzymes. In animals that had received

butyloxyanisol, there was a reduction in the activity of the catalase and of the peroxidase. In rats that had been fed butyloxytoluol, there was a decrease in the activity of all three enzymes under study: of the catalse, peroxidase and of the cholinesterase. The propylgallate showed an inhibiting action on the peroxidase and of the cholinesterase. The propylgallate showed an inhibiting action on the peroxidase and on the cholinesterase.

A reduction in the activity of the oxidation-reduction enzymes in animals that had been fed antioxidants is interesting in view of the fact that these compounds interrupt (discontinue) the oxidation reaction in nutritive (alimentary) products and, apparently, when given in large amounts, manifest a similar action on the flow of the oxidizing processes in the live organism.

In biochemical investigations of the liver, one's attention is drawn to the results of investigations in the butyloxytoluol group. The animals of this group had, in comparison with the control group, a greater liver weight, a greater content of fat in it, and a decrease in the amount of lipoid phosphorus (the reliability has been confirmed statistically). In the animals of this group, apparently, under the influence of administering butyloxytoluol to the organism, there was brought on a disturbance in the synthesis of phospholipids (phosphatides), and, as a result of this, an accumulation of fat in the liver.

There was a somewhat diminished amount of lipoid phosphorus in the liver of rats that had received butyloxyanisol, even though without any retention of fat in this organ.

The histological investigations carried out on the internal organs did not indicate, in the tissues, any changes that could be associated with the administration, into the organism of the animals, of phenolic antioxidants. Only in one rat, that had received propylgallate, was there disclosed an edema of the stroma in a testicle, with the discontinuance of spermatogenesis.

The remaining indices under study, such as the morphological composition of the blood, the prothrombin time, and a study of the urine, did not indicate any changes.

The greatest attention, in the study, was devoted to a study of the possible

toxic action of fats containing antioxidants in chronic experiments. They were carried out on two types of animals: rats and dogs, which remained with the experiment for one year.

As in subacute experiments, the animals were subdivided into four groups. Three of the groups were fed fat with one of the antioxidants, and the fourth group served as a control.

To the extent that with respect to the majority of poisons, a human being is more sensitive than experimental animals, we permitted some aggravation in the chronic experiments (by 10 - 20 times, i. e., an increase in the dose in comparison with the one that may enter the organism of a human being, maximally, with a daily diet).

A feeding that is not fully adequate makes the animals usually more sensitive to the administration (introduction) of harmful agents. For this reason, for a still greater aggravation of the experiment, milk and meat were excluded from th feed diet of rats twice in the course of the year-long experiment (the 7th and 11th months) for 14 days.

Considering the fact that the harmful effect of the substance may show not on the first generation, but on the subsequent ones, we obtained two generations from the principal experimental group, which, prior to attaining a month's growt were separated from the mother and were placed in the experiment. The rats of the second and third generations were included in the experiment for 6 months.

Experiments connected with reproduction are interesting in that, in such physiological states as pregnancy, the sensitivity to poisons increases. By observing females during the period of pregnancy and lactation, we were able to track down the action of antioxidants on the organism, where increased requirements were imposed on it.

In this manner, the possible toxic action of phenolic antioxidants in chroni experiments was studied on 104 rats of three generations and on 8 dogs. Over the extent of the entire experiment, both the rats, as well as the dogs, outwar appeared to be healthy, active (lively), reacted actively to the environment, and had a good appetite.

The weight of the experimental (test) animals did not differ from the weight of the animals in the control group. Even during that period when the organism was artifically weakened by the elimination of meat and milk from the feed, the change in weight in the experimental (test) animals did not differ from the one in the control group.

We approached the study of the blood enzymes with particular interest; they, before other tests, reacted to the administration, into the organism of the animals, of large amounts of antioxidants, in subacute experiments. These investigations did not reveal any noticeable deviations in the animals that had received butyloxyanisol and butyloxytoluol. In rats to whom propylgallate had been fed, sometimes in an entire group, and sometimes in individual animals, it was possible to detect an increase in the peroxidase activity, in the serum chol inesterase in rats of the first generation, in that of the peroxidase -- in animals of the second generation, and in the catalase -- in the third generation

These investigations showed that the feeding of comparatively small doses of propylgallate increases the activity of the blood enzymes; this appears to be un desirable and should be considered a harmful action of the preparation on the organism of the experimental (test) animals.

With the purpose of disclosing any possible impairments in the function of th liver, relative to the synthesis of albumin (protein), an investigation was made of the overall content of albumin in the blood serum, and also, a Takata flocculation test and a Vel'tman (Weltman) coagulation test were set up. No changes were detected by these tests in the amount of serum albumin, in the albumin blood fractions, nor in the electrolytic blood threshold of the experimental (test) animals. Investigations of the remaining nitrogen in the blood of the dogs likewise indicated no pathology in the deaminating and ureagenetic fun tions of the liver nor any impairments in the excretory capacity of the kidneys.

A study of the indices, which characterize the functional state of the liver, such as its glycogen content, and that of fat and lipoid phosphorus, did not disclose any impairments (disturbances) in the activity of this organ in animal that had received butyloxyanisol and butyloxytoluol. Somewhat different result

were obtained in the propylgallate group. In studying the liver of the animals in this group, in two rats there was noted a reduction of the liver (a weight of less than 5 grams), in three there was a reduction in the glycogen content, and i another three -- of the lipoid phosphorus. Changes in the liver in the form of a diminution in its weight, disturbances in glycogen-formation, or in the synthesis of phospholipids (phosphatides) were noted in 5 animals out of 12 that were participating in the experiment. These observations indicate that the introduction of fat into the diet of rats, along with propylgallate, had a harmful effect on the liver function of part of the experimental (test) animals. Similar changes in dogs had not been disclosed.

The feeding of fat with antioxidants did not indicate any effect on the reproduction of the progeny. Offspring (progeny) were produced both by the experimental(test) as well as by the control animals. Observation of such physiologica indices as the amount of young ones in a litter, their weight on birth, recordin the day of appearance of the incisors, the hairy (furry) coat, and opening of th eyes, disclosed no distinctions in the development of the young generation in a single one of the experimental (test) groups.

A six-month feeding of fat with antioxidants to two generations likewise did not have any influence on their overall state, weight and indices under study, with the exception of changes in the enzymatic activity of the blood; the latter (changes) have already been discussed.

Pathological-anatomical and histological investigations carried out on the internal organs did not reveal any regular (natural) changes that could be considered a result of the administration of antioxidants into the organism of the animals, with the exception of the changes that were disclosed in the sexual system of the males. Atrophy in the testes was disclosed in one animal of the "butyloxyanisol, butyloxytoluol and control" groups. Among rats that had receiv propylgallate, atrophy of the testes was noted in three males. And even though the changes disclosed could be associated with the random (chance) selection of animals with atrophic processes in the tests in the propylgallate group, these data once againput us on guard relative to the harmful action of propylgallate.

Thus, the experimental investigations carried out on a prolonged feeding of fat with phenolic antioxidants to three generation of rats and dogs did not indicate any harmful action on the part of butyloxyanisol and butyloxytoluol on the live organism, whereas propylgallate was not neutral (indifferent) withrespect to the experimental (test) animals.

Our data, relative to propylgallate, have something in common with the recentl terminated investigations of Profs. Budagyan and Smirnova, who, having studied the assimilability of nutritive (alimentary) fat, containing the same antioxidants as in our experiments, have indicated that the assimilability of fat is some what reduced upon including, in the diet, fat with propylgallate, whereas the introduction, into the feed, of butyloxyanisol and butyooxytoluol, did not manifest any effect.

The study thus carried out makes it possible to draw the conclusion that the introduction of butyloxyanisol and butyloxytoluol in the alimentary fats, with the purpose of inhibiting, in them, the oxidizing processes, finds no objections in hygienic attitudes, whereas the use of propylgallate cannot be resolved.

It should be also noted that the introduction of antioxidants into fats may be resolved at those enterprises in which a laboratory or appropriate institute may carry out a daily control over the amount of preparation introduced.

The introduction of antioxidants into fats should not in any wise reflect on carrying out and searching for new measures for preventing the oxidation of fats in the course of production and storage. To the same degree as previously, attempts should be made not to permit any prolonged storage of raw material, to pressurize (seal hermetically) the fat-rendering processes to the greatest extent possible, and carry out the storage of fat at a low temperature. Only these measures, in conjunction with antioxidants, will make it possible to increase considerably the storage time of fats without reducing their full physiological value.

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A Study of the Enzyme Secretory Function of the Small Intestine and Pancreas Mucosa in Rats Receiving Phenol Antioxidants in Their Diet

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The prdection of alimentary fats and fat-containing products from oxidation by means of introducing antioxidants is receiving constantly greater circulation both in our country as well as abroad. Some of the proposed antioxidants have b studied from the toxilogical aspect and have been authorized for use in the food industry.

All the same, many aspects of the organism's activity, upon introducing antioxidants with food have not yet been studied. The problem as to what influence is had by food containing antioxidants on the enzymatic activity of the gastrointestinal tract has not yet been clarified sufficiently. It is known that the enzyme scretory function of the digestive glands is closely associated with the character of nutrition (1 - 4); in varying the chemical composition of food, the glands are capable of finely (delicately) regulating the amount and composition the secretions being produced(5).

The purpose of the present investigation has been to study the effect of a number of antioxidants of the phenolic series on the activity of the enterokinas and of the alkaline phosphatase in the mucosa (mucous membrane) of the small intestine, where their concentration is the greatest, and also on the secretion of the pancreatic enzymes (lipase and amylase).

A study was made of 5 antioxidants (butyloxyanisol, butyloxytoluol, propyl- and dodecylgallates) and of a mixture of antioxidants (butyloxyanisol with propylgallate).

For the experiment there were taken 160 white male mice with an initial weigh of 80 - 90 grams. The experiments were carried out in 4 series. In the I and I

series the antioxidants were fed over a period of 1 month, and in the III and IV series -- 3 months. In the experiment, altogether, there were 12 experimental and 4 control groups. The experimental (test) animals received, daily, 15 grams of a No. 1 nutritive feed concentrate(cracked wheat flour, and also, cracked oatmeal and millet flour, dry wheat seeds, hulled sunflower oil cakes, defatted fish flour, defatted dry bone meal, blood meal, egg powder, skimmed dry milk, hydrolyzed irradiated yeast, molasses), 10 milliliters of whole (unskimmed) milk, 3 grams of carrots and 1 gram of rendered lard, freshly melted, with a peroxide number of 0.006% iodine. The enumerated diet, with respect to caloricity, comprised 20% albumen, 25% fat, and 55% carbohydrates.

In the I and II series of experiments, the animals of the 1st group were given, in their diet, along with the rendered lard, butyloxyanisol (BOA); those of the 2nd group -- butyloxytoluol (BOT); those of the 3rd group -- dodecylgallate (DG); the animals of the 4th group represented the control (K). In the II and IV series of experiments,the animals of the 1st group received in their diet ethylgallate (EG; those of the 2nd group -- propylgallate (PG); those of the 3rd group -- a mixture of butyloxyanisol and propylgallate (BOA plus PG); the 4th group represented the control (K). The corresponding antioxidants were included in the diet of a fixed group, calculating 4 milligrams for 1 kilogram of body weight of the animal (a tenfold aggravation). A mixture of BOA and PG was fed in the same dose, but in a ratio of 2 : 1 (the most effective ratio for protecting alimentary fats and products from oxidation). The animals of the control group received the same diet, but the rendered lard, which was included in the feed, was free from antioxidants. The difference between I and III, II and IV series consisted merely in the duration of feeding. The duration of the experiments in the I and II series was 1 month, and in the III and IV series it was 3 months.

The activity of the enterokinase and alkaline phosphatase was determined in a scraping from the mucosa of the small intestine. For this purpose, following the experiment, the animals were destroyed by decapitation; from the upper section

(superior portion) of the small intestine a section was removed that had a lengt of 10 centimeters. After washing off the intestine from its contents by means c the polished (ground) edge of a (microscope) slide, a scraping was made from the mucous membrane (mucosa). Having weighed it, it was placed in a glass homogeniz in which there was prepared a water extraction diluted to 1 : 20. The extractic continued for 2 hours at room temperature. From the centrifugate thus obtained (centrifugal separation of 10 - 15 minutes at 3000 revolutions per minute) there was prepared a dilution of 1 : 1000, which was employed for determining the ente kinase and alkaline phosphatase. The enterokinase content was determined by the method proposed by G. K. Shlygin and it was expressed in arbitrary units for 1 gram of weight.

The activity of the alkaline phosphatase was determined in accordance with th method recommended by L. S. Fomina, and it was likewise expressed in arbitrary units for 1 gram of weight. The enzymatic activity of the pancreas was studied by means of determining the amylase content in the blood and the lipase (content in the pancreas tissue. The amylase was investigated by the method of Engel'ga (Engelhardt) and Gerchuk in the animals, while still alive, prior to the beginn of the experiment, and in the 15, 30, 45 - 50 and 85 - 95th day of the experimental period, and its activity was expressed in milligrams for 1 milliliter.

The lipase content was determined in the tissue of the pancreas immediately after decapitation and in the dissection of the abdominal cavity of the animals. Following extraction, the gland was weighed and pulverized in a mortar with distilled water (dilution of 1 : 20) with the addition of finely ground glass. Th extraction continued for 30 minutes at room temperature. Following a 5-minute centrifuge separation, at the rate of 1500 revolutions per minute, there was pr pared a dilution of 1 : 20000, which served as an initial one for determining the lipase in accordance with the method of G. K. Shlygin, L. S. Fomina and Z. P Pavlova. The lipase activity was expressed in arbitrary units for 1 gram of weight. The results thus obtained were treated by the method of statistics of variations.

During the period of the experiments, the animals appeared healthy, neat

(clean), and consumed the entire feed. The increase in weight in the experiment and control groups was practically identical.

In Tables 1 and 2 are set forth the results of determining the content of the enterokinase in the mucous membrane (mucosa) of the small intestine while introducing, into the feed of the animals, over a course of 1 and 3 months, fat with antioxidants.

((Two tables on pg. 22 of text))

Table 1

Enterokinase Activity in a Scraping from the Mucous Membrane (Mucosa) of the Superior Portion of the Small Intestine in Rats That Received, Over the Course of 1 Month, in Their Diet, Fat with Antioxidants

Series of Experiments	Group of Animals	Antioxidant	M	sigma	m_M	t	P
I	1st	Butyloxyanisol	((See Table 1 on pg. 22 of text for all figures pertaining thereto))				
	2nd	Butyloxytoluol					
	3rd	Dodecylgallate					
	4th	Control					
II	1st	Ethylgallate	((See Table 1 for figures, etc.))				
	2nd	Propylgallate					
	3rd	Butyloxyanisol plus					
	4th	Control					

Table 2

Activity of Enterokinase in a Scraping of Mucosa from the Superior Portion of the Small Intestine in Rats Receiving in Their Diet, Over a Period of 3 Months, Fat with Antioxidants (in units/gram)

Series of Experiments	Group of Animals	Antioxidant	M	sigma	m_M	t	p
III	1st	Butyloxyanisol	((See Table 2 on pg. 22 of text for all figures pertaining thereto)				
	2nd	Butyloxytoluol					
	3rd	Dodecylgallate					
	4th	Control					

((Table continued on the next page))

((Table continued from preceding page)

IV	1st	Ethylgallate	
	2nd	Propylgallate	
	2a-	Control with PG	((See Table 2 for figures, etc.))
	3rd	Butyloxyanisol plus propylgallate	
	4th	Control	

((text continued)): The enterokinase activity in the control groups comprised 2600 plus or minus 300 units/gram (I series) and 3400 plus or minus 340 units/gr (II series). In animals that had received antioxidants, the enterokinase activi was approximately within the same range: in the I series, 2600 - 2750 units/gram and in the II series, 3500 - 3900 units/gram. There was somewhat of an increase in our opinion, in the enterokinase activity in the group of animals that had received PG (M = 4000 units/gram), but in this group there is a rather high arra (series) dispersion (sigma = plus or minus 1300) and the difference, on the whol with respect to the group, is unreliable (P is greater than 0.25).

((Graph on pg. 23 of text)) ((in the upper left-hand corner of this graph)): units/gram; ((within this graph)): BOT (butyloxytoluol); K (control); ((underneath this graph)): Fig. 1. Enterokinase content in the scraping from the mucos of the superior portion of the small intestine in control rats and in those that had received, over a period of 3 months, fat with butyloxytoluol. BOT -- animal that had received fat with butyloxytoluol; K -- control animals that had receive pure fat. Dark circles -- enterokinase activity in individual animals. Here, and in Figs. 2, 3: columns with numbers -- arithmetical mean (M) for each group.

((text continued)):

A three-month introduction, in the feed, of a majority of the antioxidants under study, also showed no noticeable effect on the enterokinase content. Stati tically, a reliable decrease in the amountof this enzyme has been noted in the group of animals that had received BOT (the enterokinase activity in the contro group was 3100 plus or minus 200 units/gram; in the experimental group, 2500 pl or minus 130 units/gram). In spite of the fact that in statistical treatment reliable results had been obtained, one may assume that there is no great diffe ence in the activity of the enzyme (Fig. 1).

In determining the activity of the alkaline phosphatase in the scraping from the mucosa of the small intestine, with a single-month feeding of antioxidants, no distinctions were disclosed between the experimental and control groups of animals (Table 3). ((Table on pg. 23 of text))

Table 3

Activity of the Alkaline Phosphatase in a Scraping from the Mucosa of the Superior Portion of the Small Intestine in Rats that had Received, over the Period of 1 Month, in Their Diet, Fat with Antioxidants (in units/gram)

Series of Experiments	Group of Animals	Antioxidant	M	sigma	m_M	t	P
I	1st	Butyloxyanisol	((See Table 3 on pg. 23 of text for all figures pertaining thereto))				
	2nd	Butyloxytoluol					
	3rd	Dodecylgallate					
	4th	Control					
II	1st	Ethylgallate	((See Table 3 for figures))				
	2nd	Propylgallate					
	3rd	Butyloxyanisol plus propylgallate					
	4th	Control					

((text continued)): A three-month administration of antioxidants into the organism of animals had no noticeable reflection on the activity of the alkaline phosphatase in the mucosa of the superior portion of the small intestine (Table 4). It is only in the group of animals that had received EG (ethylgallate) that we noted a statistically reliable reduction in the activity of the alkaline phosphatase.

((Table on pg. 24 of text))

Table 4

Activity of the Alkaline Phosphatase in a Scraping from the Mucosa of the Superior Portion of the Small Intestine in Rats that had Received, in the Course of 3 Months, in Their Diet, Fat with Antioxidants (in units/gr

((Table continued on next page))

((table continued from the preceding page))

Series of Experiments	Group of Animals	Antioxidant	M	sigma	m_M	t	P
III	1st	Butyloxyanisol	((See Table 4 on pg. 24 of text for all figures pertaining thereto))				
	2nd	Butyloxytoluol					
	3rd	Dodecylgallate					
	4th	Control					
IV	1st	Ethylgallate	((See Table 3 for figures))				
	2nd	Propylgallate					
	2a -	Control with PG					
	3rd	Butyloxyanisol plus propylgallate					
	4th	Control					

((text continued)): In this manner, a month's administration of antioxidants into the organism of animals showed no influence on the enterokinase activity nor on that of the alkaline phosphatase.

With a 3-month administration of antioxidants only in the group of animals that had received EG (ethylgallate), there was noted a reduction in the activity of this enzyme. The difference disclosed in the enterokinase activity in the BOT group can hardly be taken into consideration in view of the fact that, by proceeding from the concept concerning the limit of physiological fluctuations in the activity of this enzyme, we cannot consider this difference as being an essential one.

((Graph on pg. 24 of text)) ((in the upper part of this graph, reading from left to right and then down and again from left to right)): units/gram; I Series, II Series; BOA (butyloxyanisol); BOT (butyloxytoluol); DG (dodecylgallate; K (Control); EG (ethylgallate); PG (propylgallate); BO A plus PG; K (Control; ((alongside of this graph, at lower right)): Fig. 2. Lipase activity in the tis of the pancreas in rats in whose diet, over the course of 1 month, there was included fat with antioxidants.

((text continued)): Investigations carried out on the blood amylase have shown that the activity of this enzyme, which has been determined over the course of a number of days in succession, in various animals, and even in the same animal

shows a very marked variation. In the control animals, which had not been subjected to a possible harmful effect of antioxidants, the amylase activity was from 60 to 180 milligrams/milliliter. The same rat from the control group, over an extension of several days, yielded the following indices for the amylase activity of the blood: 62 - 90 - 180 - 83 milligrams/milliliter.

In all the experimental groups, the fluctuation limit in individual animals was also very high. In connection with this, we believe that this index, which was not constant in its quantitative characteristic, represents no particular interest for us.

((Graph on pg. 25 of text)) ((within this graph, at the top, reading across and then down and across again)): units/gram; I Series; II Series; BOA; BOT; DG; K (control); EG; PG; BOA plus PG; K (control); ((to the left of this graph, at the lower left)): Fig. 3. Lipase activity in the tissue of the pancreas in rats that had received, over the course of 3 months, in their diet,fat with antioxidants. ((text continued)):

In Figs. 2 and 3 are shown the results of determining the lipase activity in the tissue of the pancreas while feeding antioxidants over a period of 1 and 3 months. The lipase content in the individual groups is given in the form of averages (means). An analysis of this material attests to the fact that there is no significant difference in the experimental groups in comparison with the control (one); this is confirmed also by the results of the statistical treatment of the material. The existing difference in some groups should be considered as immaterial (in all cases, P is greater than 0.25).

F. E. Budagyan and L. I. Smirnova (6) have shown that the inclusion of antioxidants in the diet of the animals brings about some reduction in the assimilability of nutritive substances, especially of fats (the difference was not very substantial -- 0.5 - 0.9%). The authors, in their paper, did not explain the mechanism of the possible diminution in the assimilability. In setting up the present study, we wished to track down whether there was not a reduction in the absorption of the nutritive substances by the intestinal wall as a result of a disturbance in the secretory function of the digestive glands. As has been shown

by the investigations carried out, the majority of the antioxidants that we have studied (BOA, PG, DG (dodecylgallate), BOA plus PG), administered per os in the course of 1 and 3 months, do not indicate any influence on the enterokinase content nor on that of the alkaline phosphatase in the mucosa of the small intestine.

Some reduction in the activity of the alkaline phosphatase, which was noted in the study, in animals that had received EG (ethylgallate), and of the enterokinase in animals to whom BOT (butyloxytoluol) had been administered, is possible associated with the fact that the inclusion, in the food, of these antioxidants, which changing the usual chemical composition of the food, brings about, by reflex, a compensatory change in the enzyme secretory function of the intestine.

The lipase content in the tissue of the pancreas did not vary in a single one of the 12 experimental groups of animals. These investigations made it possible to reject the assumption with respect to the reduction in the assimilability of the fat that contained antioxidants, as a result of diminishing the secretion of the lipolytic enzyme in the pancreas.

A determination of the amylase activity in the blood of rats indicated a very high variability in this index in all groups, including those under control. We assume that the index, which yields high physiological fluctuations, cannot be employed as a sensitive test for studying the possible toxic action of various chemical additions on the organism.

Conclusions

1. The introduction into the diet, in the course of 1 and 3 months, of some antioxidants of the phenol series has indicated no effect on the enterokinase activity in the mucosa of the superior portion of the small intestine in white rats.

2. The content of the alkaline phosphatase in the mucosa of the small intestine showed no variation in animals that had received antioxidants with their feed over a period of 1 month. The feeding of ethylgallate, over a period of 3 months, led to some diminution in the activity of the alkaline phosphatase in the mucos of the small intestine. The remaining 5 antioxidants under investigation did not indicate any such action.

3. The activity of the pancreatic enzymes (lipase and amylase) showed no significant variation in the case of feeding antioxidants over a period of 1 an 3 months.

Literature (Bibliography)

1. Shlygin, G. K., Ter. arkh. (Therapeutic Archives), 1956, Issue No. 1, p. 39.

2. Idem., Vestn. AMN SSSR (Bulletin of the Academy of Sciences of the U.S.S.R.), 1964, No. 3, p. 21.

3. Idem., Intestinal Enzymes in the Norm and in Pathology, L(eningrad), 1967.

4. Mikhlin, S. Ya., Labor. delo (Laboratory Work), 1966, Issue No. 10, p. 597.

5. Babushkina, L. M., Fomina, L. S. Faltova, E., Fiziol. zh. SSSR (Journal of Physiology of the U.S.S.R.), 1961, No. 11, p. 1404.

6. Budagyan, F. E., Smirnova, L. I., Vopr. pitaniya (Problems in Nutrition), 1962, No. 2, p. 47.

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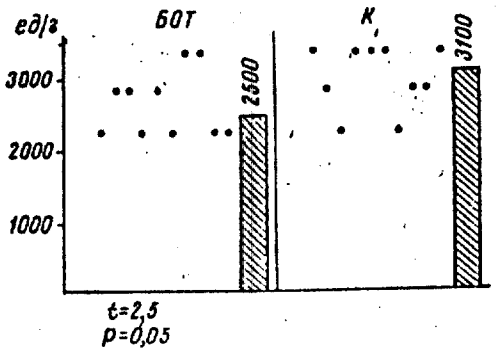

Рис. 1. Содержание энтерокиназы в соскобе со слизистой оболочки верхнего отдела тонкого кишечника у контрольных крыс и получавших в течение 3 месяцев жир с бутилокситолуолом.

БОТ — животные, получавшие жир с бутилокситолуолом; *К* — контрольные животные, получавшие чистый жир. Темные кружки — активность энтерокиназы у отдельных животных. Здесь и на рис. 2, 3: столбики с цифрами — средняя арифметическая (*M*) по каждой группе.

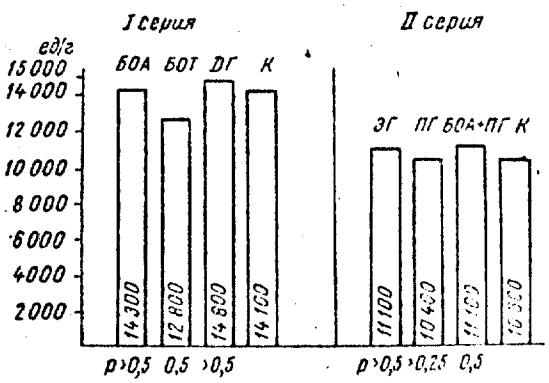

Рис. 2. Активность липазы ткани поджелудочной железы у крыс, в рационе которых в течение 1 месяца включали жир с антиокислителями.

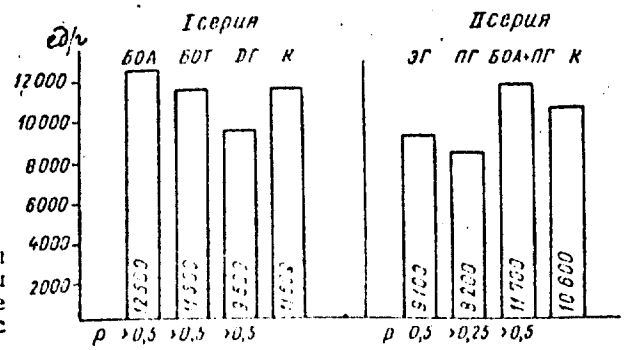

Рис. 3. Активность липазы ткани поджелудочной железы у крыс, получавших в течение 3 месяцев в рационе жир с антиокислителями.

Effect of Propylgallate on the Extraintestinal Digestion of Phytohelminths

S. G. Myuge

(Academy of Sciences of the U.S.S.R., Trudy Gel'mintologicheskoi laboratorii (Proceedings of the Helminthological Laboratory, Vol. XX , 1968)

It has been shown that phytohelminths possess an extraintestinal digestion, secreting a number of hydrolytic enzymes into the plant tissue (Dieter, 1955 - 1956; Zinov'ev, 1956; Myuge, 1956). The proteolysis of the albumin (protein), in the given case, is accomplished by means of cathepsins; the activity of the latter depends on the presence, in the medium -- the plant cell -- of their activators. The cathepsins of phytohelminths are activated by hydrogen donors; at the same time, various types of helminths require definite activators (gluthatione, ascorbic acid, cysteine and others). The presence, in the plant-host, of cathepsin activators, which are specific for the parasite, is responsible for the possibility of helminth existence in the plant (Myuge, 1965). To the extent that the hydrogen donors play a definite part in the oxidation-reduction processes of the plant, therefore, with theinvasion of phytohelminths, there takes place a certa rearrangement of these processes, in view of the fact that a new oxidizing facto is introduced into the cell -- a helminth cathepsin. This phenomenon is disclos by a change in the activity of a number of respiratory enzymes in the plant, and in some cases, by an increase in the inorganic phosphorus and in the generation heat; this, along with an increase in the respiratory activity, indirectly (obli quely) points to the dissociation of respiration and phosphorylation.

Evidently, in the plant, however, there are included "accidental" oxidizing systems, and the latter phenomenon, some time after the invasion, disappears.

By exerting an influence on the oxidizing processes of the plant, it seemed to be possible to inhibit the proteolytic activity of helminths, bringing about, at the same time, a reduction in their pathogenic action on the host and the sta vation of the parasites themselves (Myuge, 1964).

This influence may be attained either by rearranging the oxidizing process of the plant in such a manner that the hydrogen donors, which take part i

cannot activate the SH-groups of the Nematoda (round-worm) cathepsin, in view of the fact that the latter is not adapted to them, or else, by the normalization of the changes brought about by the helminths in the chain of oxidizing processes in the plant.

For the purpose of verifying the first hypothesis, we made use of inhibitors of some oxidizing chain reactions, in particular, propylgallate (PG). With the prupose of investigating the effect of propylgallate on the feeding and on the enzymatic activity of phytohelminths, which are parasites on vegetative organs, we sprayed, with 0.01% PG, the leaves of a fern that had been infected by a leaf nematode, Aphelenchoides olisistus. The external manifestation of the invasion of this nematode is evinced in the necrosis of the blades (laminas); in such case, the rapidity of the necrosis spread (brown spots on the leaf) is proportional to the amount of the particular nematodes and their enzymatic activity.

For the experiment were selected the leaves of a fern (Pteris) with approximately the same invasion foci (nuclei). The experimental leaves were sprayed with a 0.01% solution of PG, and the control ones -- with tap water. Sections of the necrotized tissue were measured and compared (see table).

The leaves, after the experiment, were passed through a Berman funnel. In the control leaves, Nos. 1 and 2, there was disclosed a large amount of live nematodes, and in leaf No. 3, there was a greater amount of dead nematodes and only a few were moving about weakly.

No live nematodes were disclosed in the experimental leaves. This may be explained by the destruction of the nematodes from hunger, in view of the fact that, by means of special experiments, it has been established that propylgallate in a given concentration, is not toxic for worms.

((Table on pg. 138 of text))

Disease Focus Area of Fern Leaves Infected by a Leaf Nematode (in square centimeters)

	No. of leaf	Day of Experiment								
		1	3	5	10	15	18	25	30	35
Control	1									Died
	2				(See table on pg. 138 of					
	3				text for all figures					Died entire-
Experiment	4				pertaining thereto)					ly
	5									
	6									

((text continued)): The presence of dead individuals in the control leaf is explained by the fact that at the moment of taking samples, the entire leaf died, and phytohelminths are capable of feeding only on live plant tissue. Simultaneously with the described experiment, we also carried out a parallel one, spraying underneath the root with a solution of PG. A reduction in the invasion, in this case, was not observed.

In other series of experiments it has been shown that the treatment of plants with PG inhibits the proteolytic activity of a number of sedentary phytohelminths (Myuge, 1962, 1963, 1964).

It is known that propylgallate reacts most actively with enzymes that contain SH-groups (Agatova, Vartanyan, Emanuel', 1963).

Consequently, along with other enzymes, which were found in the plant cells, an effect was had by cathepsin, which was secreted by the nematodes. However, the inhibition of proteolysis and of other enzymes in plant cells, containing SH-groups, brought about an intensified synthesis of the latter or elsec an adaptive synthesis of the enzymes, catalyzing reactions analogous to those that were realized by inhibited enzymes. This is associated with the tendency of a cell to stabilize the biochemical state which has been disturbed by any endogenous factor (Moied, 1964; Umbarger, 1964, and Chans, 1964). On the other hand, it was noted that the proteolytic activity of the phytohelminths, removed from the plant,

Для опыта были выбраны листья папоротника (*Pteris*) с примерно одинаковыми очагами инвазии. Опытные листья опрыскивались 0,01%-ным раствором ПГ, а контрольные — водопроводной водой. Участки некротизированной ткани замерялись и сравнивались (см. таблицу).

Листья после опыта были пропущены через воронку Бермана. В контрольных листьях № 1 и 2 было обнаружено большое количество живых нематод, в листе № 3 было большее количество мертвых нематод и линии единицы были слабо подвижны.

В опытных листьях живых нематод обнаружено не было. Это можно объяснить гибелью нематод от голода, так как специальными опытами установлено, что пропилгаллат в данной концентрации для червей не токсичен.

Площадь очага поражения листьев папоротника листовой нематодой (в см²)

	№ листа	День опыта								
		1	3	5	10	15	18	25	30	35
Контроль	1	0,25	0,30	0,36	0,64	1,2	2,4	4,0	10	Погибли
	2	0,25	0,35	0,40	0,78	1,4	2,8	4,5	12	
	3	0,30	0,35	0,45	0,66	2,0	2,6	4,3	Весь погиб	
Опыт	4	0,25	0,26	0,30	0,50	0,55	0,55	0,55	0,60	0,6
	5	0,25	0,28	0,30	0,50	0,50	0,55	0,58	0,60	0,6
	6	0,30	0,32	0,36	0,56	0,60	0,65	0,70	0,70	0,7

Наличие мертвых особей в контрольном листе объясняется тем, что к моменту взятия пробы весь лист погиб, а фитогельминты способны питаться только живой растительной тканью.

Одновременно с описанным опытом мы провели и параллельный, с поливом раствора ПГ под корень. Уменьшение инвазии в этом случае замечено не было.

В других сериях опытов показано, что обработка растений ПГ ингибирует протеолитическую активность и ряда седентарных фитогельминтов (Мюге, 1962, 1963, 1964).

Известно, что пропилгаллат наиболее активно реагирует с ферментами, содержащими SH-группы (Агатова, Вартанян, Эмануэль, 1963).

Следовательно, наряду с другими ферментами, находящимися в растительных клетках, связывался и катепсин, выделенный нематодами. Однако ингибирование протеолиза и других ферментов растительных клеток, содержащих SH-группы, приводило к усиленному синтезу последних или к адаптивному синтезу ферментов, катализирующих реакции, аналогичные тем, которые осуществлялись ингибированными ферментами. Это связано с тенденцией клетки стабилизировать биохимическое состояние, нарушенное каким-либо эндогенным фактором (Мойед, 1964; Умбаргер, 1964, Чанс, 1964).

С другой стороны, было замечено, что протеолитическая активность извлеченных из растения фитогельминтов уменьшается со временем.

Аналогичные данные были получены В. Г. Зиновьевым (1957). Поэтому мы поставили перед собой задачу выяснить, не влияет ли ингибирование экзоферментов нематод, выделенных в растительную клетку, на темп выделения этих ферментов нематодой.

Для опыта растения свеклы, зараженные *H. schachtii*, обрабатывались пропилгаллатом. Заражение производилось личинками примерно одного

diminishes with time.

Analogous data were obtained by V. G. Zinov'ev (1957). For this reason, we posed a problem for ourselves of clarifying whether or not there is exerted an influence by the inhibition of the nematode exoenzymes, secreted into the plant cell, on the secretion rate of these enzymes by the nematode.

For the purpose of the experiment, garden beets, infected with H. schachti were treated with propylgallate. The infection was carried out by larvae of approximately the same age. After fixed intervals of time, the nematode was removed from the roots (about 50 specimens from the root) and transferred to a physiological solution for the extraction of the enzymes. At the same time, the hydrolysis of the albumin (protein) was determined histochemically in the radicles.

The activity of the proteolytic enzymes secreted by the worms depended on the inhibition of these enzymes in the plant; this points to a bond between the activity of the enzymes in the medium (and consequently, also the feeding of the parasite) and the rate of their secretion.

In this manner, PG inhibits both the cathepsin of nematodes, as well as other enzymes, containing SH-groups that exist in the plant. However, it influences their synthesis in a different way. By stimulating the synthesis of the cellular enzymes, PG does not interest with the enzymatic glands of the parasite, reduce the formation rate of cathepsin.

Literature (Bibliography)

Agatova, A. I., Vartanyan, L. S., Emanuel', N. M., 1958, Mechanisms in the Interaction of Free Radicals Formed from Inhibitors of Radical Processes with SH-groups of Albumins (Proteins), -- Doklad. AN SSSR (Proceedings of Academy of Sciences of the U.S.S.R., 1[illegible], No. [illegible] : 527 - 530.

Zinov'ev, V. G., 1958, Enzymatic activity of Some Nematode Exoenzymes and Biochemical Changes in Parts of the Plant Damaged by Them -- In the collection "Problems of Parasitology," Proceedings of the II Scientific Conference of Parasitologists in the USSR (Ukrainian S.S.R.), Kiev, pp.[illegible]-[illegible].

Zinov'eve, V. G., 1957, Enzymatic Activity of Plant Nematode Parasites, -- Zool. zh. (Journal of Zoology), 36, Issue No. 4, pp.617-620.

Moied, G.S., 1964, Some Problems in Regulating the Activity of Enzymes by the End Product. In the book: "Regulatory Mechanism of Cells," Publication "Mir", pp. 417-428.

Myuge, S.G., 1956, Comparative Analysis of Phytonematodes, -- In the collec tion: "Problems of Parasitology," Proceedings of the II Scientific Conference o Parasitologists in the USSR (Ukrainian S.S.R.) Kieve, pp.300-301.

Myuge, S.G., 1962-1963, Effect of the Inhibitors of Free-Radical Reactions Galloformation and the Development of Phytohelminths, -- Helmithologia,4: 344-34

Myuge, S.G., 1964, Parasitological Nematodes of Plants, Publication "Kolos, 75 pages.

Myuge, S.G., 1965, Physiological Specificity of Phytohelminths, -- Proceedi of the Helminthological Laboratory of the AN SSSR (Academy of Sciences of the U.S.S.R.), 13, pp. 81 - 92.

Umbarger, G., 1964 Suppression (Inhibition) of the Enzyme Activity by the End Product (Inhibition by the End Product) as a Mechanism of Feedback, -- In th book: "Regulatory Mechanisms of the Cell, Publication "Mir," pp.390-407.

Chans, B., 1964, Regulatory Mechanisms of Enzyme Systems, Regulatory Mecha- nisms of the Cell, Publication "Mir," pp.371-389.

Dieter, A., 1955- 1956, Vergleichende experimentells Untersuchungen an neophagen und phytophagen Nematode, -- Wiss. Z. Martin-Lutter-Univ., Halle- Wittenberg. Math.-Naturwiss. Reihe, 5(2) : 157 - 185.

(Translated by Carl Derrick Associates,Inc/LB/t)

(From German publication "Die Nahrung" 9(1):155, 1965)

Effect of Antioxidants on the Liberation of Fatty Acids from Adipose Tissue

The mechanism of fatty acid liberation in vitro from adipose tissue into th albumin medium and its adrenalin activation have, according to the literature, been explored experimentally (1, 2). Although under normal conditions predominantly only saturated fatty acids are released in these processes, also the unsat ated representatives may play a certain part, namely in particular in the transf of fatty acid, for example, to the adipose cell membrane as a structure constituent, to the phospholipids in their participation in lipid transport. In the course of these reactions, the liberated unsaturated fatty acids may be oxidized and form degradation products of lipoperoxidation (3). As the antioxidants inhi autoxidation, we are of the opinion that they are able to influence the liberatio of the fatty acids from adipose tissue for the reasons stated.

There has been used in the experiments epididymis adipose tissue of a group of Wistar rat not fed for 16 hours - about 100 mg - which was incubated for 60 m at 38°C in 1.5 ml of an incubation medium (Krebs-Ringer phosphate buffer pH 7.4, without calcium, 2% albumin, possibly 10 mcg adrenalin per 1 ml incubation mediu while shaking continuously. After heptane extraction, the liberated fatty acids are determined titrimetrically. In another series the antioxidants are added to the incubation medium in buffer solution or in ethanol solution; the final conce tration is 10^{-3} mole. With this test arrangement, the influence of the animal and of the solvent of the antioxidants is eliminated. With the various antioxidants 24-44 determinations have been carried out (Table 1).

Table 1

Effect of antioxidants on the liberation of fatty acids from adipose tissue

AO = Antioxidant; K = Control without antioxidant; MG = Methyl gallate; PG = Propyl gallate; OG = Octyl gallate; AP = Ascorbyl palmitate; NDGA = Nordihydroguaiaretic acid; BHT = Butyl hydroxytoluol; MBI = Mercaptobenzimidazole.

$/^{u}val$ = quantity of liberated fatty acid in /uval/1 g adipose tissue.

Statistical significance for comparison K:AO: * = $p<0.05$; ** - $p<0.01$;

*** - p < 0.001.

The values shown in parentheses represent the mean deviations.

AO	Without adrenalin		With adrenalin		Activation by adrenalin (%)
	/uval	%	/uval	%	
MG	2.59(0.34)	79.9	4.90 (0.81)	87.5	189.1
K	3.24(0.07)	100	5.60 (1.17)	100	172.8
PG	3.45(0.47)	85.8	5.43 (0.63)	79.9	157.3
K	4.02(0.73)	100	6.79 (1.01)	100	168.9
OG	2.43(0.48)	60.4	1.86 (0.31)***	18.3	75.6
K	4.07(0.93)	100	10.15 (0.83)	100	249.4
AP	3.70(0.25)***	100.5	3.24 (0.34)	100.0	87.5
K	2.05(0.23)	100	3.21 (0.25)	100	156.5
NDGA	1.34(0.35)*	62.0	1.28 (0.38)**	35.5	95.5
K	2.16(0.19)	100	3.61 (0.49)	100	167.1
BHT	2.32(1.31)*	65.1	2.51 (0.59)**	31.3	95.8
K	4.02(0.93)	100	8.01 (1.43)	100	199.2
MBI	3.57(0.41)	87.7	1.90 (0.44)***	18.7	53.2
K	4.07(0.93)	100	10.15 (0.83)	100	249.4

(Translated by Carl Demrick Associates,Inc/LH/t)

UDK (Universal Decimal Classification): 613.29(439.1)

Study of the Effect on the Organism of Warmblooded Animals Produced by Admixtures and Additions (additives) Contained in the Domestic Food Products of Hungary

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The assessment of the effect on the organism of admixtures and additions (of foreign substances), which are contained in food products, from the point of view of nutritional hygiene, is becoming an ever more urgent (daily) problem.

In Hungary, the material concerned with this problem has been unified (combined) in the paper of Tarjan (1). These problems have also been clarified partially in the reports of Cieleszky (2,3,4), Sandi (5,6), Denes (7), and Truhau (8,9). Truhaut and Druckrey even assume the presence of an inter-relationship between the increase in the use of foreign substances and malignant diseases (10).

The data that exist in the literature illuminate the problem of the effect of some single foreign substance or of only a single group of foreign substances. Thus, Hecht (11) is preoccupied with the toxicology of coloring materials in food products, and Souci and Mergenthaler (12) -- with preservatives. However, we have not encountered any data in the literature that would illuminate the joint effect of a number of foreign substances that find their way into the organism along with the nutritive products. In our opinion, this problem represents one of the unsolved and very important ones inasmuch as it is known that in daily life, a large number of foreign substances of all kinds of chemical structures find their way into the organism and exert an influence on it. For this reason, the goal of our paper was, on the one hand, to study which foreign substances most frequently are present in food products, that is, speaking more accurately, to establish in what amount these substances find their way into the human organism, and, on the other, we made an attempt to investigate the joint effect on the organism of a number of foreign substances upon their being fed to several generations of animals.

Foreign substances, which are found in nutritive products, are divided into 2 groups. To one group pertain substances that have been intentionally added to food products, that is, so-called supplementary substances -- additions (for example, aromatizing agents, coloring materials, preservatives, and others), and to the other group -- admixtures that contaminate food products, and which find their way into them, for example, in the course of the technological processes. Legislation on hygiene (sanitary measures) limits the selection of food additions (additives), their applicable amounts, and the maximally permissible content of admixtures in nutritive products.

With the purpose of studying the consumption of foreign substances by human beings, we made use of statistical data that oriented us with respect to the amount of food products being used, and also, of literature data (13 - 20) with respect to the amount of admixtures in individual food products. From official statistical data it is not always possible, however, to establish the quality and amount of consumed food products. Thus, for example, it is known that in Hungary for each person, there is attributed 100 kilograms of flour per year. But only part of the consumed flour contains such substances as ammonium carbonate, sodium bicarbonate and tartaric acid, i.e., those substances that are employed in the bread-baking industry.

In such and similar cases, it is only possible to make an approximate assumption with regard to the amount of foreign substances. Relative to flour, we established that the contamination of flour products with foreign substances is equal to 10%.

For meat, phosphate is characteristic, which is employed in cooked sausage (bologna) products; nitrite, which is encountered in marinated meat products; and also, admixtures of tin and lead (in a minimum amount), which are contained in cannned meat foods. In our opinion, 30% of the entire amount of meat consumed is processed, and in this manner, it comes to the purchaser containing foreign substances.

With respect to fat, approximately 80% of its consumed amount contains a

propyl gallate in the capacity of an antioxidant, and in the capacity of an admixture, in lard, there is encountered DDT; close to 20% fat comes to the consumers in the form of margarine that has been colored with an "Annatto" coloring material, and which contains, in the capacity of an admixture, some amount of nic

With reference to sugar, we have made the assumption that 20% of it is employed in the form of candy, jams, and also, fruit preserves or in the form of fruit drinks. These products contain various coloring materials, aromatic substances and benzoic acid as a preservative, and also, tartaric acid. We assume that all milk products are contaminated with DDT, and, in addition to this, 5% of them are consumed in the form of melted cheese, and this means that 5% of the consumed milk products contain phosphates and sodium salts of citric and tartaric acids, which are added in the melting of the cheeses.

Vegetables and fruits may contain the residues of insectofungicides, and canned vegetables and fruit products also contain preservatives.

Table 1 was drawn up on the basis of the abovementioned concepts, and also, by taking into account the consumption of food products in Hungary during 1961.

On the basis of the calculations set forth in Table 1, we made an approxima determination of the amount of foreign substances that are attributable to 1000 kilograms of nutritive products.

Toxicological experiments were carried out on rats, adding to the customary feed of rats foreign substances in tenfold amount. The uniformity of mixing the foreign substances in the feed was established by means of a quantitative determination of copper.

The composition of the feed for rats, and also, the amount of foreign substances that were added to 100 kilograms of feed, are set forth in Table 2.

((Table on pg. 13 of text))

Table 1

Amount of Foreign Substances that Find Their Way into the Organism with Food Products

Food Products	Amount of Product for 1 person per year (in kilograms)	Amount of the Given Product in 1000 kilograms of all the products consumed (in kilograms)	Amount of the Given Product Containing Foreign Substances		((Headings of this table contained across below))
			in kilograms	in %	
Flour					
Meat					
Fat					
Sugar					
Milk		((See Table 1 on pg. 13 of text for all			
Oil and others		other figures pertaining thereto))			
Vegetables					
Fruits					
Potatoes					
Eggs	12 (240 pieces)				
Total.....	712	998			

Foreign Substances	Amount of Foreign Substances in 1 kilogram of the given product (in milligrams)	Amount of Foreign Substances in 1000 kilograms of all the food products consumes (in grams)
$(NY_4)_2CO_3$		
$NaHCO_3$		
Tartaric Acid	((See Table 1 on pg. 13 of text for all	
Phosphates	figures pertaining thereto))	
Nitrite		
Tin		
Lead		
gallate		

DDT

"Annetto: Coloring Material

Nickel

Coloring Materials

Aromatic Substances

Benzoic Acid

Tartaric Acid

DDT

Phosphates

DDT

TMTD (Tetramethylthiuram disulfide)

Cyneb

Copper

Salicylic Acid

DDT

TMTD

Cyneb

Copper

Benzoic Acid

Sorbic Acid

Coloring Materials

((text continued)): It was assumed that if a rat, with a live weight of 1 kilogram, consumed daily 100 grams of feed with the composition indicated in Table [illegible] then it received 1[illegible] milligrams of ammonium carbonate, 1[illegible] milligrams of sodium [illegible] carbonate, 1[illegible] milligrams of tartaric acid, and so forth, per day, calculating [illegible] kilogram of live weight. In contrast with rats, a human being consumes daily [illegible] 33.5 grams of food, on an average, calculating on 1 kilogram of body weight. [illegible] means that rats consume 3[illegible] times as much food (calculating on 1 kilogram of [illegible] weight) as do people. Consequently, experimental (test) rats received, on [illegible] average, 33 times more foreign substances than find their way into the food of [illegible]

urged to investigate its copyright status.

Таблица 1

Количество чужеродных веществ, попадающих в организм с пищевыми продуктами

Пищевые продукты	Количество продукта на 1 человека в год (в *кг*)	Количество данного продукта в 1000 *кг* всех потребленных продуктов (в *кг*)	Количество данного продукта, содержащее чужеродные вещества		Чужеродные вещества	Количество чужеродных веществ в 1 *кг* данного продукта (в *мг*)	Количество чужеродных веществ в 1000 *кг* всех потребленных пищевых продуктов (в *г*)
			в *кг*	в %			
Мука	100	140	14	10	$(NH_4)_2CO_3$	1 000	14,0
					$NaHCO_3$	1 000	14,0
					Виннокаменная кислота	1 000	14,0
Мясо	70	98	29,5	30	Фосфаты	2 000	60,0
					Нитрит	150	4,4
					Олово	100	
					Свинец	5	0,15
Жир	20	28	22,4	80	Пропилгаллат	100	2,2
					ДДТ	1,5	0,035
			5,6	20	Краситель «Аннатто»	100	0,56
					Никель	1,0	0,006
Сахар	30	43	8,6	20	Красители	100	0,86
					Ароматические вещества	10	0,09
					Бензойная кислота	1 000	8,6
					Виннокаменная кислота	15 000	130,0
Молоко	200	280	280	100	ДДТ	0,045	0,013
Масло и др.			14	5	Фосфаты	3 000	42,0
Овощи	100	140	70	50	ДДТ	0,2	0,014
					ТМТД	1,0	0,07
					Цинеб	1,0	0,07
			14	10	Медь	10,0	0,14
			2,8	2	Салициловая кислота	1 000	2,8
Фрукты	100	140	70	50	ДДТ	1,0	0,07
					ТМДТ	1,0	0,07
					Цинеб	1,0	0,07
					Медь	10,0	0,7
			14	10	Бензойная кислота	500	7,0
					Сорбиновая кислота	500	7,0
					Красители	100	1,4
Картофель	80	112					
Яйца	12 (240 шт.)	17					
Итого...	712	998					

Предполагалось, что если крыса с живым весом 1 *кг* ежедневно потребляла 100 *г* корма с составом, указанным в табл. 2, тогда она получала 14 *мг* карбоната аммония, 14 *мг* бикарбоната натрия, 144 *мг* виннокаменной кислоты и т. д. в день, в расчете на 1 *кг* живого веса. В отличие от крыс человек употребляет ежедневно только 28,5 *г* пищи в среднем в расчете на 1 *кг* веса тела. Значит крысы потребляют в 3½ раза больше пищи (в расчете на 1 *кг* веса тела), чем люди. Следовательно, подопытные крысы получили чужеродных веществ в среднем в 35 раз больше, чем их попадает в пищу человека, так как человек ежедневно потребляет на 1 *кг* веса тела в среднем 0,4 *мг* карбоната аммония, 0,4 *мг* бикарбоната натрия, 4,1 *мг* виннокаменной кислоты и т. д.

Опыты были проведены на белых крысах-самках, которые были разведены в нашем институте селективным, ротационным методом. В опытные (т. е. получающие чужеродные вещества) и контрольные группы мы выбирали животных из одного рода. В каждую клетку (площадью 3000 *см*²) помещали по 8 самок и к ним подсаживали

human being, in view of the fact that a human consumes daily, for 1 kilogram of body weight, on an average, 0.4 milligram of ammonium carbonate, 0.4 milligram of sodium bicarbonate, 4.1 milligrams of tartaric acid, and so forth.

Experiments were carried out on white female rats that were bred in our institute by a selective, rotational method. For the experimental (i.e., those receiving foreign substances) and control groups, we selected animals of one typ In each cage (with an area of 3000 square centimeters) were placed 8 females, an they were mated with one male having a high capacity for impregnation (fecunditi who was older than the females by 1 - 2 months. In the cages, at the same time, there were found no more than 9 animals; this was necessary from the point of vi of the biology of multiplying the population density, according to Lane-Petter(2

((Table on pg. 14 of text))

Table 2

Composition of the Diet for Experimental (Test) Animals

(Calculating on 100 kilograms of feed)

Mixed Feed (in kilograms)		Mixture of Salts (in grams for 1 kilogram of the salt mixture)		Mixture of Vitamins		Mixture of Foreig Substances (in grams for 1 kilo-gram of the mixt of foreign substances)	
Wheat	18	$MnSO_4$	35	Vitamin A	600000 ME for 1 kilogram of the vitamin mixture	Ammonium Car-bonate	1
Barley	18	$MgSO_4$	50			Sodium Bicar-bonate	1
Rye	14						
Oats	14	$FeSO_4$	5			Tartaric Acid	1
Corn	4	$CuSO_4$	2.5				
Milled Sunflower Seeds	8	$ZnSO_4$	0.4	Vitamin B_1 (product of the Ri-koin plant)	300 milli-grams	Phosphates	1
		KJ	0.2			Nitrites	1.
						Tin	
Alfalfa Flour	2	NaCl Bram	500 Up to 1000	Vitamin B_2 (product of the Roch plant)	300 milli-grams	Lead	1.
						Propylgalli-	2.
						DDT	1.
Fish Flour	5						

Casein	5
Defatted Milk Powder	4
Dry Yeast	4
Phoscal (Product of the "Filaksiya" plant)[1]	2
Mixture of Salts	1
Mixture of Vitamins	1

Niacin	3000 milligrams
Calcium Salt of Pantothenic Acid (product of the Pfizer plant)	1200 milligrams
Vitamin D_2	100000 ME
Vitamin E	6000 milligrams
Cholinchloride	200 grams
Methionine	400 grams
Ethoxymethylquino-	12.5 grams
Bran (siftings) were added up to	1000 grams

"Annatto" Coloring Material	0.33
Nickel	0.00[illegible]
Coloring Materials[2]	2.3[illegible]
Benzoic Acid	15.3
TMTD Tetramethylthiuram	0.14
Copper	0.3[illegible]
Salicylic[illegible]	2.8
Sorbic Acid	7
Bran (Siftings) were added up to	100[illegible]

---

[1] Secondary calcium orthophosphate

[2] Mixture of coloring materials (n grams): neucoccin, 0.50; amaranth, 0.50; Tartrazine, 0.50; brilliant black, 0.50; indigocarmine, 0.13; acid yellow, 0.13.

((text continued)): Investigations were started of 46 experimental (test) and [illegible] 45 control female rats. For coupling (pairing) there were used 13 males who, [illegible] ar 8 months, were replaced. The males were alternated between the 4 experimental [illegible] and 3 control groups after every 2 weeks, in accordance with the method of [illegible] exchange coupling (pairing).

The female rats were fed with the above-mentioned feed for 10 months, and [illegible] the extent of this period, we observed a particular F generation from a mixed [illegible] brood. For further investigations, out of 1[illegible] experimental (test) animals of the F generation, we held aside [illegible] of animals (6[illegible] females and 13 males), [illegible] of 1[illegible] control animals -- altogether [illegible] (11 females and 13 males).

Daily, the animals received a feed once that was mixed in water. [illegible] mixed water [illegible] at 11 [illegible]. The pregnant females were placed in [illegible]

cages. The animals from the experimental group, during the period of being with young, birth and feeding, continued to receive a feed supplemented with foreign substances.

In addition to the clinical observation of the animals, under the influence of their chronic feeding with a mixture of foreign substances, observations were also carried out of the reproduction of the animals, and of the viability and development of their progeny (offspring).

It is known that the organism becomes accustomed to the elements of the environment which harm it to a relatively negligible extent. The adaptation mechanisms prevent harm to the organism. It is assumed, however, that in such cases, the organism may protect itself less successfully against new effects of the environment as a result of the overloading of the adaptation mechanism. In connection with these prerequisites for clarifying the difference between the experimental (test) and control animals, both groups were subjected to a physical burden (swimming) and also, to toxic action (poisoning with tetrachloromethane).

The rats swam in a tank with a surface of 0.3 square meter and with the temperature of the water being 10 deg. At the same time, no more than 10 animals were placed in the tank, with approximately the same body weight (with a difference of plus or minus 10 grams). In each case, the same number of rats were swimming from the experimental and control groups. The duration of the swimming (from the beginning to the first immersion of the animal in the water) was measured by means of a stopwatch (timer). The rats that were pulled out of the tank were dried with a dry cloth and were placed under a lamp with infrared rays. After 8 days the procedure with swimming was repeated, and after that, the animals were decapitated, their organs were weighed and were placed in fixing agents, which is necessary for histochemical investigations. Similar investigations were carried on the other 2 groups of experimental (test) and control rats, but prior to swimming, the animals were poisoned daily, over a period of 5 days, with [illegible] chloromethane, which was administered by means of a stomach probe (0.[illegible] - [illegible] milliliter for 100 grams of body weight).

The organs of 30 experimental (test) and of 27 control rats were investigated histologically. The fresh organs were placed in a Carnoy fixing solution, i.e., in anhydrous acetone with a temperature of 4 deg. After pouring over with paraffin, we stained the liver with hematoxylin-eosin, as well as the kidneys, small intestine, pancreas, lungs, and also, a reaction was carried out for an alkaline phosphatase in the kidneys and for a reaction for an esterase in the pancreas and in the bowels (intestines). After fixing the suprarenal (adrenal) glands in a 4% solution of formalin, over a period of 48 hours, we prepared from them frozen sections with dimensions of 15 - 20 microns, and after that, we deposited on them glycerin-gelatine and made a study, by means of a polarizing microscope, of the amount of birefringent (double-refracting) lipoids in the individual layers of the suprarenal (adrenal) glands.

The biometric treatment of the data was accomplished in accordance with the methods set forth in Weber's book (22). The statistical evaluation of the amount of animals that succumbed from poisoning with tetrachlormethane was compiled according to the method of Heath and Irwin (23), and after that, calculated according to the method of Van der Waerden and Nievergelt (24).

Out of 92 females 90 brought forth offspring several times, and only 2 of the control females were pregnant 1 time. The animals in the group that had received foreign substances became pregnant 207 times (i.e., each female in the amount of 4.9 times). In 4 females from the group of rats that had received foreign substances, and in 11 females from the group of control animals, the young ones succumbed. In the majority of animals in the experimental group the young ones of two groups of offspring grew up to maturity, and in the control group, only one. The number of females whose young ones succumbed was practically the same in both groups. The number of all the abortions in the experimental group was equal to 30, and in the control one -- 50. In the course of 18 months of the experiment, 1573 live young ones were born from females that had received foreign substances, i.e., 35.8 animals, on an average. In the control group,

there were born 1374 young ones, i.e., 20.9 animals. In this manner, the females that had consumed foreign substances brought forth, on an average, 8.9 young ones in their brood, and the females in the control group -- 9.8, on an average.

The data set forth in Table 3 illustrate the development of the progeny.

In the live weight of the animals withdrawn from their mothers, in the mature animals, no considerable difference was observed between the experimental and control group. The development of the progeny, in both groups, was satisfactory.

In Table 4 are set forth data that had been obtained in burdening, with swimming, the experimental (test) and control animals.

((Two tables on pg. 16 of text))

Table 3

Live Weight (in grams) of Animals Withdrawn from their Mothers and of Mature Animals of the F Generation that had Consumed Foreign Substances, and of the Control Ones

	Experimental (Test) Animals	Control Animals
Live Weight of Animals withdrawn from Their Mothers:		
males	64.3	65.1
females	63.9	63.4
Live Weight of Mature Animals Prior to Swimming:		
males	332.4	371.3
Females	222.6	232.2

Table 4

Results of Burdening, with Swimming, the Experimental (test) and Control Animals

	Experimental (Test) Animals		Control Animals	
	number of animals	duration of swimming (in minutes, seconds)	number of animals	duration of swimming

(continued) (in min to second

1st swimming of the mothers

2nd swimming of the mothers (see Table 4 on pg. 16 of text for all figures pertaining thereto)

Swimming of the mothers following poisoning with tetrachlormethane

1st swimming of the progeny:
males

females

2nd swimming of the progeny:

males

females

Swimming of the progeny following poisoning with tetrachlormethane.....

Total.....

(text continued): The data obtained attest to the fact that the duration of swimming of the experimental (test) and control animals was practically the same. Nor was there observed any noticeable difference even in the case of introducing (administering) tetrachlormethane. Under the influence of tetrachlormethane, out of the number of animals that had received a mixture of foreign substances, [illegible] succumbed, and out of the control ones -- 30%. This difference is negligible. If we should enumerate the succumbed animals according to the method of Hatch and Irwin (28), in the order of their dropping out, and should note the experimental (test) animals with the letter A, and that of the control animals with the letter B, then we would receive the following series:

in the mothers -- B B B A A B; in the progeny -- A B A B B A B A A A A A B B A A.

Relative to the weight of the organs, there likewise was not observed any distinction between the animals in the experimental and control groups.

In comparing the organs of the experimental (test) and control animals, it was not possible to establish any significant distinction between them from the

ttention is drawn to the possibility that this text may be copyrighted. The reade
s urged to investigate its copyright status.

В течение 10 месяцев опыта от самок, получавших чужеродные вещества, родилось 1573 живых детеныша, т. е. в среднем по 35,8 животного. В контрольной группе родилось 1374 детеныша, т. е. по 29,9 животного. Таким образом, самки, потреблявшие чужеродные вещества, принесли в среднем 8,9 детеныша в выводке, а самки контрольной группы — в среднем 9,3.

Данные, приведенные в табл. 3, иллюстрируют развитие потомства.

В живом весе животных, отлученных от матерей, и взрослых животных значительной разницы между опытной и контрольной группой не наблюдалось. Развитие потомства в обеих группах было удовлетворительным.

Таблица 3

Живой вес (в г) животных, отлученных от матерей, и взрослых животных F поколения, потреблявших чужеродные вещества, и контрольных

	Подопытные животные	Контрольные животные
Живой вес отлученных от матерей животных:		
самцы	64,3	65,1
самки	63,9	63,4
Живой вес взрослых животных перед плаванием:		
самцы	322,4	371,9
самки	222,6	232,2

В табл. 4 представлены данные, полученные при нагрузке плаванием подопытных и контрольных животных.

Полученные данные свидетельствуют о том, что продолжительность плавания подопытных и контрольных животных была практически одинаковой. Не наблюдалось заметной разницы и при введении тетрахлорметана. Под воздействием тетрахлорметана из числа животных, получавших смесь чужеродных веществ, погибло 48%, а из контрольных — 30%. Эта разница является незначительной. Если мы перечислим погибших животных по методу Heath и Irwin [23] в порядке их падежа и отметим подопытных животных буквой А, а контрольных животных буквой Б, то получим следующий ряд:

у матерей — Б Б Б А А Б; у потомства — А Б А Б Б А Б А А А А А Б Б А А.

Таблица 4

Результаты нагрузки плаванием подопытных и контрольных животных

	Подопытные крысы		Контрольные крысы	
	количество животных	продолжительность плавания (мин., сек.)	количество животных	продолжительность плавания (мин., сек.)
1-е плавание матерей	33	11,19	32	11,19
2-е плавание матерей	16	11,48	20	11,42
Плавание матерей после отравления тетрахлорметаном	17	13,55	14	13,07
1-е плавание потомства:				
самцы	19	16,55	13	16,44
самки	24	10,00	11	10,00
2-е плавание потомства:				
самцы	8	18,48	6	19,12
самки	9	12,49	5	12,42
Плавание потомства после отравления тетрахлорметаном	4	13,30	4	14,36
Итого	130	12,53	105	12,46

point of view of histology and histochemistry. In the liver and kidneys both of the experimental (test), as well of the control animals, which had been poisoned with tetrachlormethane, there were observed, indicated to the same degree, adiposity, hemorrhages, necrobiosis and micronecrosis.

Thus, on the basis of the observations carried out, it is possible to draw the following conclusion: a daily administration, with the diet, in a chronic experiment, to 2 generations of white rats, of a mixture of all the foreign substances that are found in the nutritive products consumed by the population of Hungary, with an aggravation of the ingredients going into the mixture by 35 times as against their usual content in products (on conversion to 1 kilogram of body weight), did not reflect in any essential manner on the state of the experimental (test) animals.

No considerable distinctions between the animals that had received foreign substances and the control ones had been disclosed either in the addition of weight, or in the data concerned with reproduction, or in the physical burdening of the animals (with swimming), or under the influence of poisoning with tetrachlormethane. The weight of the organs, and the histological and histochemical investigations likewise did not reveal any distinction between the experimental and control group.

Literature (Bibliography)

1. Barjan, R., Egészségtudomány, 1960, vol. 4, p.77.

2. Cieleszky, V., Pintér, M., Élelmiszervizsgálati Közlemények, 1[illegible], vol. [illegible] p. 38.

3. Cieleszky, V., Nevezéktan[illegible], 1[illegible], vol. [illegible], p. [illegible]1.

4. [illegible], Labor[illegible], 1[illegible], Bd. [illegible], S. 301.

5. [illegible], Z., [illegible], [illegible], 1[illegible], vol. 1[illegible], p. [illegible].

6. [illegible], Élelmiszervizsgálati Közlemények, 1[illegible], vol. p. 1[illegible].

7. [illegible], [illegible], [illegible], 1[illegible], Bd. [illegible], S. [illegible].

8. [illegible], Bull. Soc. sci. hyg. alim., 1[illegible], v. [illegible], p. [illegible].

9. Idem., Ann. Falsific. Fraudes, 1957, c. 15, p. 112.

10. Truhaut, R., Druckrey, H, Presse med., 1958, v. 66, p. 1180.

11. Hecht, G., Toxikologische Daten von Farbstoffen und ihre Zulassung fur Lebensmittel in verschieden Landern, Mitteilung 6 der "Deutschen Forschungsgemeinschaft," Wiesbaden, 1957.

12. Souci, S. W., Morgenthaler, E., Fremstoffe in Lebensmitteln mit besonderer Berucksichtigung der Konservierung, Muchen, 1958.

13. Druckrey, H., Kopfmuller, D., Z. Naturforsch., 1948, Bd.36, S. 254.

14. Day, P., Am. J. clin. Nutr., 1961, v. 9. p.288.

15. Goodman, R. N., Ibid., 0. 289.

16. Hazleton, L. W., J. Agric. Food Chem, 1957, v. 5, p.336.

17. Tarjan, R., Lindner, K., Elelmezesegeszsegugyi azebkonyv (Tapanyagtablazat), Budapest, 1963.

18. Tarjan, R., Nahrung, 1963, Bd. 7, S. 21.

19. Radas, I., Nepegeszsegugy, 1963, vol. 10, p. 84.

20. Tarjan, R., Hann, G., Szalay, B., Egeszsegtudomany, 1963, vol. 2, p. 1.

21. Lane-Petter, W., Brit. J. of Animal Behaviour, 1953, v. 1. p. 1[illegible].

22. Weber, E., Grundriss der biologischen Statistik, Jena, 1953.

23. Heath, D. F., Irwin, J. O., Nature, 1960, v. 181, p. 1128.

24. Van der Waerden, B. L. , Nievergelt, E., Tafeln zum Vergleich zwei u Stichproben mittels X-test und Zeichentest, Berling, 1956.

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METABOLIC PATHWAYS OF THE PHENOLIC ANTIOXIDANTS

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INTRODUCTION

The addition of phenolic antioxidants to fats and foods containing fats intended for human consumption is practised in many countries throughout the world today. Their purpose is to prevent edible fats and oils from becoming rancid by autoxidation. The compounds that are at present being used, their permitted levels in the fat, and the number of countries using them are set out in Table 1. Over 1,000 tons of the antioxidants BHA, BHT and PG were added to food and animal fodders in the U.S.A. during 1956; over 400 tons of this amount were incorporated in foods for human consumption. Of these phenolic compounds, the most commonly used are BHA (55%), BHT (25%) and PG (20%) while THBP is the most recently developed of them and at the time of writing has been approved for use only in the U.S.A. Legislative approval for the use of any of the antioxidants listed in Table 1 has not yet been given in New Zealand.

Table 1: The phenolic antioxidants, their permitted maximum allowable concentrations and the number of countries that have approved their use in foods (as at Dec., 1959).

Antioxidant, accepted abbreviation	*Chemical name*	*Maximum allowable concentration in fats (%)*	*Number of countries permitting their use*
BHA[1]	2-*t*-butyl-4-methoxyphenol (85%) and 3-*t*-butyl-4-methoxyphenol (15%)	0.02	16
BHT[2]	2:6-di-*t*-butyl-4-methylphenol	0.02	8
PG	*n*-propyl gallate	0.01	19
OG	octyl gallate	0.015	16
DG	dodecyl gallate	0.02	
NDGA[3]	4:4'-(2:3-dimethyltetramethylene)-dipyrocatechol	0.01	12
THBP	2:4:5-trihydroxybutyrophenone	0.01	1

[1] Also known as 3-*t*-butyl-4-hydroxyanisole (major isomer) and 2-*t*-butyl-4-hydroxyanisole (minor isomer).
[2] Also known as 3:5-di-*t*-butyl-4-hydroxytoluene.
[3] Commonly called nordihydroguaiaretic acid.

This important article was not included in the Propyl Gallate abstracts sent to us, but we found mention of it in ref. no. 196.

Extensive toxicity studies have been carried out on all these compounds using experimental animals. These include acute toxicity tests, particularly for the determination of the LD_{50},* and both short- and long-term toxicity tests which include usually a histopathological examination of the test species. The recommended species for this testing are the rabbit, rat and dog.

An antioxidant is at present regarded as safe for human use if it satisfies two conditions. First, the LD_{50} must be not less than 1,000 mg/kg animal body weight; second, it must not have any significant effect on growth when fed to experimental animals in their total diet for long periods (up to two years) at a level one hundred times that proposed for fats for human consumption. All the approved antioxidants have, of course, passed these tests. Quinol (hydroquinone), one of the first phenolic compounds to be used as an antioxidant, has a LD_{50} well below this safe level and is not now used. The approximate LD_{50}'s of the phenolic antioxidants compared with phenol, quinol and *p*-cresol are set out in Table 2.

Is the acute and chronic toxicity study a sufficient criterion for assessing the safety of these compounds? The joint FAO/WHO expert committee on food additives recently considered this question (World Health Organization, 1958). In addition to the routine toxicity tests, the report recommended that additional knowledge of the metabolic and biochemical effects of these additives should be provided for their evaluation. It is necessary to have as much information as possible about an additive before it appears in our food.

The Toxicology Research Unit of the Medical Research Council of New Zealand was formed in 1954 to study the possible dangers to health arising from the increasing use of chemicals as food additives. Because of the growing uneasiness among those people responsible for establishing the safety for use of food additives, a research programme was planned for an investigation of the toxicity and metabolic fate of some of the phenolic antioxidants. Until we commenced this work, virtually nothing was known on how the body copes with these compounds. From the point of view of public health protection, information was required to supply the answers to the following:

* LD_{50} (Lethal Dose 50%) is a measure of toxicity—*i.e.*, the quantity of the compound, preferably expressed in mg/kg of animal body weight, which when administered to experimental animals will be 50% fatal. Toxicity may also be expressed as LD_{25}, LD_{90}, etc.

Table 2: The approximate LD_{50} (mg/kg) by oral administration to rats, mice and rabbits of the phenolic antioxidants, compared with phenol, quinol and *p*-cresol.

	Approximate LD_{50} (mg/kg)		
Antioxidant	*Rats*	*Mice*	*Rabbits*
BHA	2,200	1,250	
	2,900	2,000	
	4,130 (non-fasted)		
	>5,000 (fasted)		
BHT	1,700 (female)	2,000	2,100-3,200
	1,970 (male)		
	2,450		
PG	2,600	1,700	
	3,600	2,000	
	3,800	3,500	
OG	4,500		
DG	6,500		
NDGA	>2,000	>2,000	
	5,500	4,000	
THBP	3,200-6,400	800-1,600	
Phenol	440	395	420
	450	520	
	1,500		
Quinol	320	400	
p-Cresol	1,800		620

(i) What effects on animals have very high doses of antioxidants? It is not sufficient to find the dose level at which some adverse effect is produced; it is necessary to know precisely what the effect is and how it is produced.

(ii) How does the animal's body deal with the strange chemical? This involves a study of the chemical changes that the antioxidant undergoes in the animal's body.

(iii) If the dose is repeated daily, will the material accumulate in the animal body and in one way or another produce cumulative effects?

This paper summarizes the results of investigations that have been carried out in the last six years in an attempt to answer the above questions. It is hoped that metabolic studies of this kind will provide the essential additional evidence required for assessing the safety for use of these antioxidants in our foods.

DETOXICATION PATHWAYS OF PHENOLIC ANTIOXIDANTS

Phenolic compounds are detoxified in the body by conjugation with various compounds involving both the hydroxyl group and the benzene ring system. In certain cases other groups attached to the phenolic ring may undergo some metabolic reactions also. These conjugation reactions involve glucuronic acid in glucuronide formation, sulphuric acid in ethereal sulphate formation, glycine in hippuric acid formation, and rarely *O*-methylation or hydroxylation of the aromatic ring.

The first section of this account considers the origin of the principal compounds in the body that are available to conjugate with the phenolic antioxidants to form the metabolites that are ultimately excreted in the urine. The second section outlines the forms in which the phenolic antioxidants take on conjugation—*i.e.*, the structural patterns of the metabolites of which the antioxidant constitutes the aglycone portion.

A. Enzymic pathways for the conjugation of the phenolic antioxidants with glucuronic acid, sulphuric acid and glycine.

When phenols are administered to animals, the main portion of a dose can be accounted for by the excretion in the urine of the corresponding aryl-sulphuric acid and β:D-glucosiduronic acid. Aromatic and phenolic acids are also excreted by conjugation with sulphuric and glucuronic acids, and a portion of the dose may be combined with glycine as the hippuric acid.

1. Glucuronide synthesis.

Within the last few years, the steps in the enzymic synthesis of glucuronides have been fairly well established. The steps in the formation of a phenolic glucuronide, which takes place in the liver, are shown in the following scheme:

glycogen
↓
α:D-glucose-1-phosphate + uridine-5-triphosphate
↓ *uridyl transferase*
uridine diphosphate-α:D-glucose + pyrophosphate
↓ DPN$^+$ *UDPG-dehydrogenase*
uridine diphosphate-α:D-glucosiduronic acid + DPNH
↓ Ar-OH *glucuronyl transferase*
Ar-β:D-glucosiduronic acid + uridine diphosphate
(Ar-OH = phenolic antioxidant)

The important point to note is that at no time does glucuronic acid occur in the free state; it is produced from the glucose of uridine diphosphate-α:D-glucose by oxidation. The conjugated glucuronic acids of the phenolic antioxidants are all β:D-glucuronides and in the last step in their synthesis, which is catalysed by glucuronyl transferase, a Walden inversion reaction probably takes place. The same enzyme systems are involved in the formation of the ester-linked type of glucuronide (conjugation through a carboxy acid group) as distinct from the ether-linked type (conjugation through the phenolic hydroxyl group).

2. Ethereal sulphate synthesis.

It is now quite clear that the enzymic synthesis of the aryl sulphates takes place in the liver also. A process of sulphate activation is brought about which requires both ATP and magnesium ions. Sulphate then is transferred enzymically from the active sulphate (PAPS) to the phenolic antioxidant to form the ethereal sulphate as shown in the following tentative scheme:

Adenosine-5′-triphosphate (ATP) + SO_4^{--}
↓ *ATP-sulphurylase*
Adenosine-5′-phosphosulphate (APS) + pyrophosphate
↓ ATP *APS-kinase*
Adenosine-3′-phospho-5′-phosphosulphate (PAPS) + ADP
↓ Ar-OH *phenol sulphokinase*
Ar-SO_4^- + 3′:5′-diphosphoadenosine (PAP)
(Ar-OH = phenolic antioxidant)

3. Hippuric acid synthesis.

The synthesis of hippuric acids occurs in the liver and the kidney in some animals. The process requires an activated form of the benzoic acid derivative which appears to be a benzoyl-coenzyme A. The pathway can be represented as follows:

Benzoic acid derivative + ATP ⟶ Adenylbenzoate + pyrophosphate
Adenylbenzoate + CoA ⟶ Benzoyl-CoA + adenylic acid
Benzoyl-CoA + glycine ⟶ Hippuric acid derivative

This enzymic synthesis involves a phenolic carboxy group only.

B. Structural patterns of the metabolites of the phenolic antioxidants.

The study of the metabolism of these compounds is essentially an investigation of the urinary excretion following administration of

the phenolic antioxidant to the animal. An initial analysis of the urine for the different conjugates has been carried out for the majority of them. The percentage excretion of the urinary metabolites of the phenolic antioxidants (including quinol) is summarized in Table 3.

Table 3: Urinary metabolites of the phenolic antioxidants
(Results expressed as a percentage of the dose)

Antioxidant:	*BHA*		*BHT*	*PG*	*THBP*		*Quinol*
Species used:	Rabbit	Rat	Rabbit	Rabbit	Dog	Rat	Rabbit
Dose level:	1g	0.08-0.1g	0.8-1.6 g/kg	1g	0.3g/kg	0.4g/kg	0.1-0.2 g/kg
Metabolites:							
Glucuronide conjugate	46.4	71.5	35.9	72.0	30	23	42.9
Ethereal sulphate	9.2	13.5	8.0	—	45	52	30.0
Unconjugated phenol	5.8	5	8.4	6.7	2.3	6.3	+
Glycine conjugate	0	—	1.8	—	—	—	—
Total recovery:	61.4	90.0	54.1	78.7	77.3	81.3	>72.9

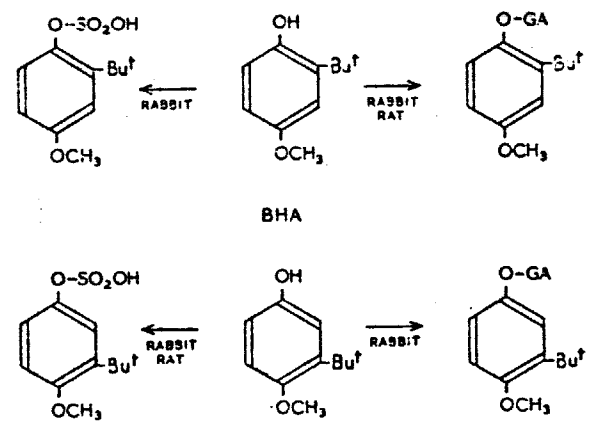
The next section outlines the structures of the urinary metabolites of the individual antioxidants as determined by analysis, by degradation and in some cases by their synthesis, which naturally follows after their isolation from urine.

1. Metabolites of BHA.

BHA was the first phenolic antioxidant to be studied in any detail. The metabolism of BHA and of its isomers (2-*t*-butyl- and 3-*t*-butyl-4-methoxyphenol) in the rabbit was investigated by Dacre *et al.* (1956). They found BHA to be conjugated with both glucuronic acid and sulphuric acid. A small part of the BHA was excreted unchanged in the urine. The glucuronide conjugates of the two isomers were isolated by these workers as the crystalline barium salts; the free glucuronides were found to have the structures 2-*t*-butyl- and 3-*t*-butyl-4-methoxyphenyl-β:D-glucosiduronic acid. From the urine of rats after the administration of BHA, the glucuronide of the 2-*t*-butyl isomer was isolated as the crystalline triacetyl methyl ester (Dacre, 1958). Astill *et al.* (1959) found that rats metabolized the 2-*t*-butyl isomer chiefly to the glucuronide and the 3-*t*-butyl isomer to the corresponding

ethereal sulphate. The ethereal sulphates of the two isomers of BHA were synthesized and shown to be constituted as 2-*t*-butyl- and 3-*t*-butyl-4-methoxyphenyl hydrogen sulphate. Both the sulphates were isolated from rat urine and shown to be identical with the synthetic compounds. These workers also presented evidence for the probable formation by demethylation of 2- (or 3-*t*-butyl-4-hydroxyphenyl hydrogen sulphate.

The metabolic pathways of BHA are set out in Scheme 1.



Scheme 1.

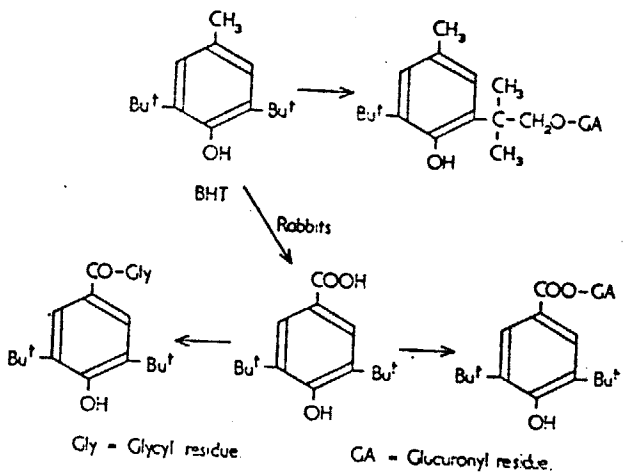
2. Metabolites of BHT.

The metabolism of BHT in the rabbit was investigated by Dacre (1960a, 1960b). This antioxidant presents an unusual metabolic problem to the animal because the hydroxyl group is sterically blocked by the two large *t*-butyl groups in the 3 and 5 positions. Conjugation of this hindered group with glucuronic acid and sulphuric acid in the conventional manner cannot, of course, take place. How then does the animal manage to cope with this antioxidant? The compound was found to be metabolized by oxidation along two separate pathways: firstly, one of the *t*-butyl groups is converted by ω-oxidation to the 2:2-dimethyl-2-ethyl structure; and, secondly, the methyl group in position 1 is oxidized to the carboxy acid. The conjugates are then formed through one of these two new groups.

Four metabolites of BHT were isolated and characterized by Dacre (1960a) from the urine after feeding BHT to rabbits. The major portion of the dose of BHT was conjugated with glucuronic acid as both ester-linked and ether-linked glucuronides; some was

excreted as a glycine conjugate: and some was present in an unconjugated but oxidized form. The two glucuronides were isolated as the crystalline triacetyl methyl ester derivatives. Acid and enzyme hydrolysis experiments showed that these conjugates have the structures 3:5-di-*t*-butyl-4-hydroxybenzoyl-β:D-glucosiduronic acid and β:β'-dimethyl-β''-(2-hydroxy-3-*t*-butyl-5-methylphenyl) -ethyl-1-β:D-glucosiduronic acid. Similar hydrolysis experiments on the glycine conjugate show that it is formulated as 3:5-di-*t*-butyl-4-hydroxyhippuric acid. The free phenol was identified as 3:5-di-*t*-butyl-4-hydroxybenzoic acid. Analysis of the urine indicated some excretion of BHT as an ethereal sulphate but nothing is known at present about the structure of this metabolite. The same benzoyl-β:D-glucosiduronic acid and hippuric acid conjugates were found in rabbit urine following the oral administration of 3:5-di-*t*-butyl-4-hydroxybenzoic acid in the amounts 84.0% and 8.8% respectively of the dose (Dacre, 1906b).

The metabolic pathways of BHT are set out in Scheme 2.



Scheme 2.

The nature of the cause of death when relatively large, repeated doses of BHA and BHT were given orally to experimental animals was studied by Denz and Llaurado (1957). The fatal effects of large doses in rabbits are due to a gross disturbance of water and salt balance. There is an increased urinary excretion of sodium, potassium and aldosterone, associated with a fall in serum and muscle potassium, a rise in muscle sodium, and a fall in extracellular fluid volume. Large doses of BHA do not produce in the rat the

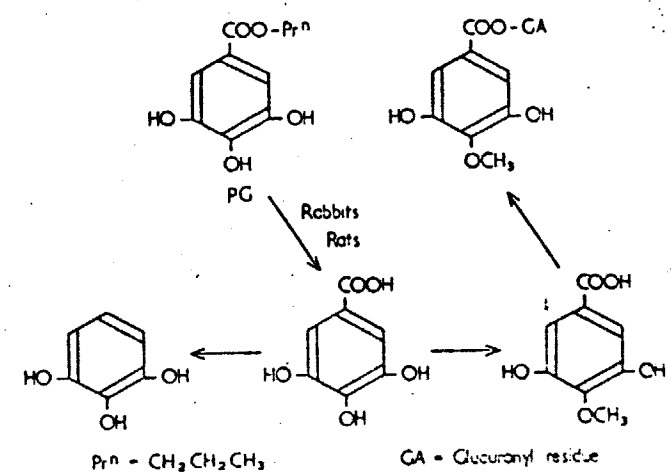
large increase in potassium excretion that is found in the rabbit. It is well known that phenolic compounds are toxic in large doses but this is the first evidence of the way in which they produce their toxic effects.

3. *Metabolites of PG.*

It is only recently that the metabolic fate of gallic acid and its related compounds, propyl gallate and lauryl gallate, was investigated (Booth *et al.* 1959). When PG was given to rats by stomach tube, the metabolites identified chromatographically in the urine were mainly 4-*O*-methyl gallic acid and smaller amounts of gallic acid. Some evidence for a conjugate of 4-*O*-methyl gallic acid was found, when this acid was given to rats.

The metabolism of PG was studied by Dacre (unpublished results). The initial analysis of rabbit urine for conjugates following the administration of PG is summarized in Table 3. A paper chromatographic analysis for metabolites in the urine showed the presence of a glucuronide as the major urinary metabolite. Other metabolites identified were 4-*O*-methyl gallic acid, gallic acid and pyrogallol. From its chemical reactions, the glucuronide conjugate almost certainly has the structure 4-*O*-methyl galloyl-β:D-glucosiduronic acid. It seems clear then that the main pathway is by cleavage of the ester linkage followed by methylation of the hydroxyl group in the 4 position and/or conjugation with glucuronic acid.

The metabolic pathways of PG are set out in Scheme 3. There are no studies reported on the metabolism of OG and DG in animals.

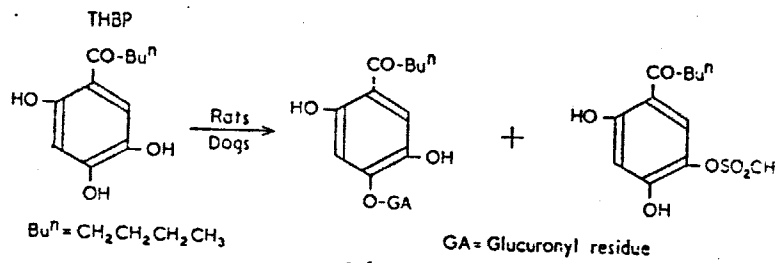


Scheme 3.

4. Metabolites of THBP.

The metabolism of the antioxidant THBP in the rat and dog was recently investigated by Astill *et al.* (1959). They showed that this compound was metabolized largely by ethereal sulphate formation, with a lesser amount of conjugation with glucuronic acid. The butyryl side-chain appeared to survive intact and no evidence of oxidation to more complex phenols was obtained. The ethereal sulphate conjugate was isolated as its potassium salt and shown to have the structure 5-butyryl-2:4-dihydroxyphenyl hydrogen sulphate. The glucuronide of THBP was not isolated but experiments showed that its structure was 4-butyryl-2:5-dihydroxyphenyl-β:D-glucosiduronic acid. There was also some evidence for a third conjugate (an ethereal sulphate) of THBP in both rat and dog urine. Small quantities of THBP were also excreted unchanged in the urine.

The metabolic pathways outlined above are set out in Scheme 4.



Scheme 4.

5. Metabolites of Quinol.

This was the first phenol to be used as an antioxidant. Garton and Williams (1949) studied its metabolic fate in the rabbit as part of their extensive investigations of the detoxication of benzene. Small doses were excreted, chiefly as the glucuronide and to a lesser extent as the ethereal sulphate conjugate. The glucuronide was isolated as the crystalline triacetyl methyl ester and shown to have the structure *p*-hydroxyphenyl-β:D-glucosiduronic acid. The ethereal sulphate could not be isolated from rabbit urine but their experiments showed that it is almost certainly the monosulphate of the compound—*i.e.*, it is *p*-hydroxyphenyl hydrogen sulphate. Some of the dose of quinol is excreted uncombined in the urine.

6. Metabolites of NDGA.

Nothing is known of the metabolism of this antioxidant.

There is very little known about other methods of excretion of the antioxidants in experimental animals. There is some excretion in the faeces of animals but the evidence so far obtained shows little or no storage of them in organs and tissues. The further fact that recovery of these compounds in the urine is by no means completely accounted for (see Table 3) indicates the need for further studies. Other metabolic pathways may possibly be found.

SUMMARY

1. The metabolic pathways of the phenolic antioxidants in experimental animals have been outlined.

2. The studies have shown that these compounds are metabolized along well-known and recognized routes—*i.e.*, by conjugation with glucuronic acid, with sulphuric acid or with glycine.

3. In the case of BHT conjugation takes place only after the compound has undergone oxidation along two different pathways; in the case of PG only after it has undergone ester cleavage and *O*-methylation.

4. No evidence has so far been found which suggests that these antioxidants produce adverse biochemical or metabolic effects in the animal body. The studies provide additional evidence for establishing the safety for use of these food additives.

REFERENCES

Astill, B. D., Fassett, D. W., and Roudabush, R. L. (1959): *Biochem. J.*, *72*: 451-9.

Astill, B. D., Fassett, D. W., and Roudabush, R. L. (1960): *Biochem. J.*, *75*: 543-51.

Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., and DeEds, F. (1959): *J. Biol. Chem.*, *234*: 3,014-6.

Dacre, J. C. (1958): In *Ann. Rep. Med. Res. Council of N.Z.*, p.32.

Dacre, J. C. (1960a): *Proc. Univ. Otago Med. Sch.*, *38*: 1-2.

Dacre, J. C. (1960b): *Biochem. J.*, *77* (in the Press).

Dacre, J. C., Denz, F. A., and Kennedy, T. H. (1956): *Biochem. J.*, *64*: 777-82.

Denz, F. A., and Lauradó, J. G. (1957): *Brit. J. Exp. Path.*, *38*: 515-28.

Garton, G. A., and Williams, R. T. (1949): *Biochem. J.*, *44*: 234-8.

World Health Organization (1958): In *Procedures for the Testing of Intentional Food Additives to Establish Their Safety For Use* (Second report of the joint FAO WHO expert committee on food additives), pp. 1-19. *Tech. Rep. Ser. No. 144.*