isolated DFTM attractant and of synthetic (*Z*)- and (*E*)-6-heneicosen-11-ones were prepared by reaction with *m*-chloroperbenzoic acid. By using a 1.2 m by 2 mm (inner diameter) column of 10 percent Apolar 10C on Gas-Chrom Q (Applied Science Laboratories) the isomeric *Z* and *E* derivatives were readily separated; the derivative obtained from the natural attractant corresponded to the *Z* isomer.

To synthesize both isomers of 6-heneicosen-11-one (**1** and the corresponding *E* isomer) we used as starting material 2-decyl-1,3-dithiane prepared from undecanal (*8*) and 1,3-propanedithiol (*11*) by the method of Fieser (*12*). The lithium derivative of this dithiane, prepared by using *n*-butyllithium (*13*), was alkylated with 1-chloro-4-decyne (*8*), and the carbonyl function of the resulting product was regenerated by using cupric chloride and cupric oxide (*14*) to yield 6-heneicosyn-11-one. The acetylenic bond of this compound was reduced over P-2 nickel catalyst (with ethylenediamine) (*15*) to yield (*Z*)-6-heneicosen-11-one. To prepare the *E* isomer from 6-heneicosyn-11-one it was necessary first to prepare the corresponding acetylenic alcohol by lithium aluminum hydride reduction; the acetylenic bond of this compound was then reduced with sodium in liquid ammonia (*16*). Finally, the olefinic alcohol obtained in this manner was oxidized with chromium trioxide–pyridine complex (*17*) to yield (*E*)-6-heneicosen-11-one.

The laboratory bioassay procedure was designed to provide qualitative, yes-or-no evaluations of test samples. A wind tunnel (60 by 60 by 170 cm) containing 0.5-liter cylindrical adhesive traps baited with test samples was used. Usually two but sometimes three traps including a blank control were exposed during a test. Test samples in methylene chloride solution were impregnated on filter paper or small disks of cotton wick for placement in the traps. The rate of air flow through the wind tunnel was regulated at 7 cm/sec.

For a sample to be judged attractive, DFTM males had to fly upwind toward a sticky trap baited with the sample, land on the trap, and enter it through the opening, which was 3 cm in diameter. Positive responses were generally clear-cut, with 50 percent or more of 30 to 50 DFTM males (1 to 5 days old) exhibiting this behavior. Bioassays were carried out between 4 and 6 p.m. to correspond with the period of maximum flight activity in the field (*2*). The caged males were exposed to no more than one attractive sample within a 24-hour period.

Both synthetic *Z* and *E* isomers are strongly attractive to DFTM males in laboratory bioassays. When a choice of a 200-ng sample of the *Z* isomer and an extract of five DFTM female abdominal tips was presented, DFTM males exhibited a 4:1 preference for the synthetic attractant. In a similar test DFTM males showed a 5:1 preference for female extract when the alternative was 200 ng of the synthetic *E* isomer.

Adhesive traps baited with as little as 400 ng of the synthetic *E* isomer have successfully captured DFTM males under field conditions. Owing to initial incorrect assignment of *E* stereochemistry to the natural pheromone, only this isomer was available for field trial during the 1974 flight season. Results of laboratory tests leave little doubt that the synthetic *Z* isomer will be even more effective in the field.

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Butylated Hydroxytoluene Inactivates Lipid-Containing Viruses

Abstract. *Butylated hydroxytoluene (BHT) is widely used as a food preservative for its antioxidizing property. This small, hydrophobic molecule has been found to be a potent inactivator of lipid-containing mammalian and bacterial viruses.*

Butylated hydroxytoluene (BHT) (Fig. 1) is one of several antioxidants that are commonly added to foods to maintain freshness and prevent spoilage by oxidation. It is frequently used in dried cereals, cooking oils, canned goods, and various animal foods. The average daily intake of BHT per person in the United States has been estimated at 2 mg (*1*) and in the United Kingdom as 1 mg (*2*). It is not readily excreted however, and measurements on 12 U.S. residents revealed a concentration of 1.30 ± 0.82 parts per million (ppm) in the body fat (*3*). For U.K. residents, the value was 0.23 ± 0.15 ppm. This represents an accumulation factor of approximately 45 over the daily intake in the United States and about 16 in the United Kingdom. Although BHT is generally recognized as safe by the Food and Drug Administration (*4*), there are varying reports as to the effects of this compound on organisms. It has been found that BHT causes bile duct proliferation in mice (*5*), is toxic to developing insect larvae (*6*), and reduces the growth rate of cultured mammalian cells (*7*). Some individuals show chemical intolerance

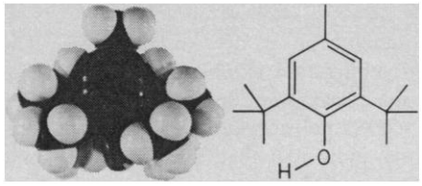

Fig. 1. Structure of BHT.

to this compound (*8*). On the other hand, data show that BHT reduces chemical carcinogenesis (*9*), protects against damage due to choline deficiency (*10*), protects against poisoning by carbon tetrachloride (*11*), and, under certain conditions, retards aging in mice (*12*).

The effectiveness of BHT as an antioxidant for certain foods is probably due in good part to its high solubility in fats and its extremely low solubility in aqueous environments (*13*). This places the BHT molecule in regions where its antioxidant activity is most effective. These same hydrophobic characteristics, when considered for living systems, suggest that BHT may have strong interactions with the hydrocarbon zones of membrane structures, and perturbing effects of BHT on membrane-associated functions have, in fact, been observed (*14*). The interaction of BHT with membranes and the results of this interaction are very likely distinct from and independent of its antioxidizing action.

Because of these considerations and the importance of BHT as a food additive, we have studied the effects of BHT on the infectivity of several viruses, some that have a membrane as part of their structure and some that do not (*15*). Samples of a virus preparation were exposed to varying concentrations of BHT for 30 minutes, diluted, and plated for plaques on appropriate indicator host cells. Figure 2 shows results for two membrane-bound viruses, herpes simplex virus (HSV) and ϕ6, and for poliovirus, which contains no lipids. From data such as these, the approximate concentration of BHT resulting in 50 percent inactivation for a 30-minute exposure was estimated for all the viruses studied. These results are summarized in Table 1.

In every case, viruses that contain lipids were readily inactivated by BHT, whereas viruses that contain no lipids were comparatively insensitive to BHT at the concentrations used here. The most sensitive by far was ϕ6, a bacterial virus that is bound by a rather loose membrane structure. The virus ϕ23-1-a, which infects the same host as ϕ6 (*15*), contains no lipids and is not inactivated. Wild-type HSV and the syncytia-forming mutant *syn* 20 derived from it are inactivated, as is bacteriophage PM2, but no effect of BHT on polio virus was observed.

We believe that some membrane-bound viruses in addition to the ones listed here may be sensitive to BHT in the concentrations used here, while others having the same general features could be resistant. Further experimentation will elucidate the general significance of BHT as an antiviral drug.

Some comparisons between the concentrations of BHT that inactivate virus in vitro and the concentrations present in body fat seem appropriate. For U.S. residents, the level of 1.3 ppm corresponds to approximately $0.6 \times 10^{-5} M$. This concentration, for our experimental conditions, inactivates ϕ6, but about ten times this concentration is required for a significant effect on HSV and PM2. In considerations of this sort, it may be noted that (i) in vivo, the partition coefficient of BHT between the virus and its surroundings may influence the degree of inactivation; (ii) in vivo, viruses would be exposed to BHT for periods of time much longer than the 30 minutes used in these experiments; (iii) BHT levels much greater than 1.3 ppm could easily be achieved by ingestion of larger amounts of BHT; and (iv) other viruses may have greater or lesser sensitivity to BHT than do those studied here.

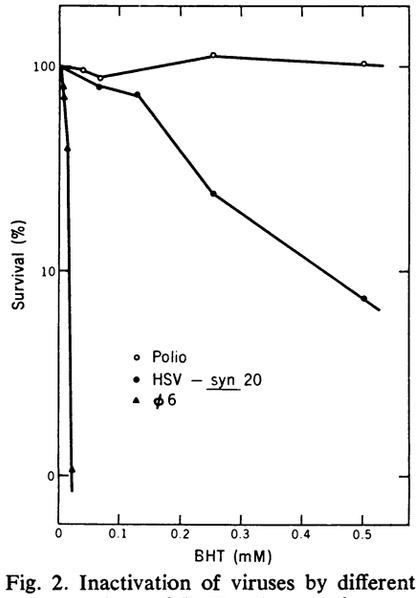

Fig. 2. Inactivation of viruses by different concentrations of BHT. Virus stocks were diluted to approximately (2 to 4) $\times 10^7$ plaque-forming units per milliliter in appropriate buffered solutions. A 0.1-ml portion of a BHT solution in 95 percent ethanol was added to 5 ml of buffer, and this was added, after mixing, to a 5-ml sample of the virus. After 30 minutes, the samples were diluted and assayed for plaque-forming units on appropriate host cells. The abscissa indicates the BHT concentration present during the 30-minute exposure.

Table 1. Effect of BHT on virus infectivity. Numbers in parentheses refer to references.

Virus	Lipid content (%)	BHT for 50 percent inactivation (*M*)
ϕ6	25 (*15*)	10^{-5}
HSV	22 (*16*)	0.7×10^{-4}
HSV–*syn* 20	22 (*16*)	1.6×10^{-4}
PM2	13 (*17*)	10^{-4}
ϕ23-1-a	None	$> 5 \times 10^{-4}$
Polio	None	$> 5 \times 10^{-4}$

Our observations and these considerations suggest that in vitro studies of the mechanism of inactivation as well as in vivo studies of the potential antiviral activity of BHT with laboratory animals may be appropriate.

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Reconstitution of Rh (D) Antigen Activity from Human Erythrocyte Membranes Solubilized by Deoxycholate

Abstract. *Proteins were selectively solubilized from human erythrocyte membranes with deoxycholate. After ultracentrifugation and preliminary fractionation procedures, the detergent was removed from the soluble membranes by Bio-Beads SM-2 and dialysis against 5 millimolar magnesium chloride. Reaggregation took place with the apparent formation of vesicles which showed serologically specific Rh antigen activity.*

Since their initial description (*1*), the Rh antigens have been found on the surface of erythrocytes of man and other primates. The Rh antigenic sites are molecularly dispersed in a random two-dimensional array on the surface of the erythrocyte (*2*) and appear related to the problem of erythrocyte membrane integrity (*3*). Attempts to completely solubilize membrane Rh antigens, by techniques known to solubilize other membrane proteins and carbohydrate antigens, have been unsuccessful (*4*). The loss of Rh activity caused by some of these procedures (*5*) is due in part to loss of phospholipid which appeared bound to a Rh antigen-related membrane protein and required for the antigen activity to be manifested.

We now report the selective solubilization of the human erythrocyte membrane proteins with recovery of Rh antigenic activity by the following solubilization procedure. Erythrocyte membranes (*6*) were solubilized in a 20 m*M* sodium phosphate buffer (*p*H 7.5) containing 0.8 percent (weight to volume) NaCl, 10 m*M* dithiothreitol, 1 m*M* ethylenediaminetetraacetate, 20 percent (weight to volume) glycerol (*7*), and 0.9 percent (weight to volume) deoxycholate at a concentration of 4.5 mg of dry membranes per milliliter. The mixture was stirred for 30 minutes at 4°C and then centrifuged at 151,000*g* (average) for 90 minutes to sediment the unsolubilized material. Protein determinations of the mixture and of the supernatant fraction were used to calculate the percentage of membrane protein solubilized (*8*). The supernatant fraction was treated with Bio-Beads SM-2 (*9*) to remove most of the deoxycholate and then dialyzed for 2 to 3 days against water buffered to *p*H 7.5 with tris base (1 mg/liter), or buffered water containing 5 m*M* $MgCl_2$ or $CaCl_2$. The dialyzed supernatants were then centrifuged at 151,000*g* (average) for 60 minutes. The pellets were either suspended in phosphate-buffered saline, *p*H 7.4, or lyophilized; the Rh antigenic activity was then determined (*10*).

Solubilization of erythrocyte membranes by this procedure produced supernatant fractions which contained 55 to 70 percent of the membrane protein, while the pellets contained less than 1 percent of the original Rh antigenic activity on a dry weight basis. The solubilization exhibited some degree of specificity, since sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis indicated that spectrin and some polypeptides contained in bands 3, 4, and 5 (*11*) were not solubilized. More protein was pelleted when the samples were dialyzed against magnesium or calcium than when they were dialyzed against buffered water (Table 1). Each type of dialysis showed recovery of Rh antigenic activity, but maximum reactivation per milligram of protein occurred with dialysis against $MgCl_2$.

Table 1. Distribution of protein and Rh antigen activity after membrane solubilization and reconstitution with dialysis. After treatment with Bio-Beads SM-2, the soluble preparations were dialyzed against water, 5 m*M* $MgCl_2$ or 5 m*M* $CaCl_2$. The samples were then centrifuged, and the reconstituted pellets were assayed for Rh antigenic activity (*10*) and protein. Samples retained activity for at least 1 week when stored at 4°C, either as pellets or suspensions in phosphate-buffered saline, but lost activity within 1 day when lyophilized. The numbers in parentheses are the number of experiments.

Cation	Pellet	
	Protein* (%)	Rh antigen† (%)
None	45–60 (4)	30–60 (2)
Magnesium	70–90 (4)	50–240 (6)
Calcium	81–86 (4)	25–75 (6)

* Percent of total protein after dialysis. † Percent of original membrane protein.

To rule out any possibility of nonselective absorption of antiserum (anti-D) to the D antigen during the titer assays, membranes that were Rh(D)–negative, c-positive were run as controls. The Rh(D)–negative membranes do not have D antigen specificity, but do exhibit c antigen specificity, among others. The amount of protein solubilized, and the distribution of protein after dialysis and centrifugation were the same as with D-positive membranes. Assay of preparations from D-negative membranes with anti-D showed no binding of anti-D to the samples, as would be expected. These same samples, however, showed c antigen activity. Therefore, the absorption of anti-D by the D-positive samples was completely specific. In addition, the conditions used to reconstitute the D antigen were also favorable for the reconstitution of the c antigen.

Maximum Rh antigenic activity was obtained with dialysis against $MgCl_2$. To study this, we performed phospholipid analysis, SDS polyacrylamide gel electrophoresis, and electron photomicroscopy on representative reconstituted samples. Total phospholipid content was the same and ranged from 16 to 17 percent on a dry weight basis compared with erythrocyte membranes which contain 32 percent phospholipid. The 50 percent reduction in phospholipid content was presumably due to removal of phospholipid-deoxycholate mixed micelles by Bio-Beads SM-2. Phospholipid distribution on thin-layer chromatography plates was unchanged. The SDS polyacrylamide gel electrophoresis of samples reconstituted in each of the three ways showed the same number of protein peaks in approximately the same ratios. This data, plus the data in Table 1, suggested that samples reconstituted with Mg and Ca ions differed from the water samples because the proteins had reaggregated