The psychotropic effects of chlorpromazine in humans have been reported to be maintained for 1 to 2 weeks after its discontinuation (5), and excretion of the drug in trace amounts in the urine may last for weeks (5, 12). Salzman and Brodie reported a relatively slow rate of biotransformation of chlorpromazine in dogs, and attribute the slow rate partly to extensive localization of the drug in various organs with consequent lesser immediate availability of the drug to metabolizing enzymes (6).

The studies on the organ distribution of methotrimeprazine in the rat (13) indicate that there is also rapid uptake of the drug by organ depots, especially parenchymatous tissues, and this is consistent with findings on most basic compounds (15). In the case of methotrimeprazine, organ levels of the drug may still be appreciable 12 hr. after its administration. Ultimately, however, the drug is extensively biotransformed. Less than 1% of the unchanged drug is excreted in the urine, but a variety of metabolic products, including sulfoxide metabolites, are detectable in varying amounts (13); similar findings have been reported for humans (5).

To what extent the biotransformation products of methotrimeprazine may contribute to the sum total of pharmacologic effects remains a question. The prolonged half-life of the drug indicates that adequate amounts of the original substance are available for eliciting drug effects. A reasonable degree of parallelism was noted for the curve for concentration of drug in the brain and that for coordinated motor ability. Such a correlation suggests that the parent substance is principally mediating the pharmacologic effects under observation, but whether loss of motor coordination is accompanied by analgesia cannot be answered by our experiments.

The failure to obtain a good correlation with "analgesia" resulted from the inability to establish a clear-cut response with methotrimeprazine by the test procedure. This is not surprising and was in part anticipated. Agents other than the narcotic analgetic type yield erratic effects in prolonging the tail reaction time to thermal stimulus and in our hands methotrimeprazine proved to be no exception to the rule. In view of the established effectiveness of the compound as a potent analgetic clinically (2–4), the tail flick procedure obviously would be a poor screening method for other compounds in this series. These findings are not consistent with an earlier report (14), and we have no explanation for the discrepancy. There is still clearly a need to establish a suitable test procedure for surveying non-narcotic type analgetics.

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Effect of γ Radiation on Selected Pharmaceuticals

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Aqueous solutions of chlorobutanol, theophylline, and sodium carboxymethylcellulose (CMC) were irradiated with γ radiation from a ^{60}Co source. Solutions were irradiated in glass and polyethylene containers. Chlorobutanol solutions were analyzed for appearance, pH changes, chlorobutanol degradation, and production of chlorine, acetone, and hydrogen peroxide. Theophylline solutions were analyzed for appearance, pH changes, theophylline degradation, and production of hydrogen peroxide. CMC solutions were analyzed for viscosity changes. All systems showed pronounced changes, even at low radiation levels.

THE ABILITY of γ radiation to destroy microorganisms without an appreciable temperature rise in the substrate offers an attractive means of sterilizing pharmaceuticals. The possibility of side effects must be considered, however, as γ radiation has the capacity to create highly reactive chemical species in any matter through which it passes. This can lead to an alteration of the physical properties of materials and to the production of numerous chemical changes.

Willis (1) and Proctor and Goldblith (2) have published review articles on the effects of radiation on pharmaceuticals. Although much valuable information has appeared in the literature, the majority of the published reports are confined

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to the study of the active ingredients in the system, and usually only indicate which products may or may not be successfully radiation sterilized. Reports concerning the effects of radiation on pharmaceutical adjuncts, as for example, preservatives or suspending agents, are few. Detailed studies showing degradation curves and degradation products are also lacking.

Few reports have been published which investigate the effects of the type container used in the systems studied. Hills and Johnson (3) have shown that the type container used in radiation studies may influence the final result. For irradiation of distilled water with γ radiation, they reported that the samples became more acid, an effect which was greater in polyethylene containers than in glass. They also noted that with dextrose and fructose solutions the coloration produced was more pronounced for the solutions packaged in glass containers.

The object of this investigation was to study the effect of γ radiation from a ^{60}Co source on aqueous solutions of chlorobutanol, theophylline, and sodium carboxymethylcellulose packaged in glass and polyethylene containers.

EXPERIMENTAL

Equipment—The irradiation facility used in this investigation was a ^{60}Co source.[1] A calibration certificate supplied by the company indicated a dose rate of 5.5×10^5 rad/hr. on May 17, 1962. The measuring technique was by ferrous chemical dosimetry. A check of the calibration was made using the ASTM Tentative Method of Test for Absorbed Gamma Radiation Dose in the Fricke Dosimeter (4).

A Beckman DU,[2] model 2400, spectrophotometer with attached Beckman DU power supply was used for colorimetric and ultraviolet analysis.

A Will 80–100 sec. 2-ml. Ostwald viscometer[3] was used for all viscosity determinations, except the 2% sodium carboxymethylcellulose (CMC) solution. For this CMC solution, the viscometer employed was a Haake Rotovisco,[4] with an attached Haake Ultra-Thermostat, type NBS.

A Beckman Zeromatic pH meter,[2] number 9500, with standard electrodes was used for all pH measurements.

Containers—Two types of containers were used in this investigation. One was a 4-oz., 0.95 d., amber polyethylene container.[5] The second type was a 4-oz. amber glass container.[6] Screw type lined, plastic caps were used for both containers.

PROCEDURES

The solutions were prepared, packaged in glass and polyethylene containers, irradiated for varying lengths of time, and analyzed within 24 hr. Irradiation was performed at room temperature. Air was not evacuated from the containers.

Analysis of chlorobutanol was by the colorimetric method of Rehm and Mader (5). Hydrogen peroxide was determined by the colorimetric method of Eisenberg (6). Acetone produced in chlorobutanol solutions was determined by the method of Greenberg and Lester (7). Chlorine was determined with *o*-tolidine reagent using the procedure described by Boltz (8). Theophylline was determined by the spectrophotometric method of Comer and Hiltz (9).

All viscosity determinations were made at 100° F. For the Ostwald viscometer 4-ml. aliquots of the solution were pipetted into the lower bulb of the viscometer, and allowed to stand 15 min. for the temperature to equilibrate. The liquid was then drawn into the second bulb and to a position about 5 mm. above the first timing mark. The time required for the meniscus to pass from the first timing mark to the second was then measured with a stopwatch.

The Haake viscometer was used to follow the change in the characteristic flow curve of the 2% CMC solution. The measurements were made at 100° F., using the MV-11 cup and bob system.

The increase in the viscosity of a polymer solution over that of the pure solvent can be expressed as specific viscosity (10)

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0}$$

where η = viscosity of the solution,
η_0 = viscosity of the pure solvent,
η_{sp} = specific viscosity.

If η_{sp}/concentration is plotted against concentration, the point of intercept at zero concentration is known as the intrinsic viscosity $[\eta]$. The intrinsic viscosity is related to the molecular weight by two constants (K and a) in the equation $[\eta] = KM^a$ (11), so any change in the intrinsic viscosity should be indicative of a change in the molecular weight.

For the molecular weight changes, a 0.25% solution of sodium CMC was irradiated for 0, 5, 10, and 20 min. Each sample was then diluted 5, 10, 50, and 100 times, and the viscosities were determined with the Ostwald viscometer, as described previously. The η_{sp} was then calculated and plots were made of η_{sp}/C *versus* C.

RESULTS AND DISCUSSION

Chlorobutanol Solutions—Visual observation of the chlorobutanol solutions showed the development of an amorphous, yellow precipitate during irradiation. No attempt was made to identify the composition of the precipitate.

Analysis for chlorine showed that no chlorine was produced in a 0.5% chlorobutanol solution irradiated up to 6.8×10^6 rads.

Measurement of pH changes showed that irradiated chlorobutanol solutions suffered severe drops in pH after very short periods of irradiation. Table I presents the change in pH of a 0.5% chlorobutanol solution. It can be seen that a dose of only 6.1×10^3 rads causes a pH drop of more than 1 unit. Measurements of irradiated distilled water

[1] Gammacell 200. Atomic Energy of Canada Limited, Commercial Products Division, Ottawa, Ontario, Canada.
[2] Beckman Instruments, Inc., Fullerton, Calif.
[3] Will Scientific, Inc., New York, N. Y.
[4] Brinkmann Instruments, Inc., Great Neck, N. Y.
[5] Monsanto Co., Packaging Division, Hartford, Conn.
[6] Armstrong Co., Lancaster, Pa.

showed no change in pH over the same dose range as the chlorobutanol solution. This indicates that the drop in pH is due to the degradation of the chlorobutanol. Similar pH drops have been reported by others for heated chlorobutanol solutions (12, 13).

TABLE I—pH OF 0.5% CHLOROBUTANOL SOLUTION

Total Dose, rads	pH
0	5.78
6.1×10^3	4.55
3.1×10^4	3.40
9.1×10^4	2.90
1.8×10^5	2.68
3.7×10^5	2.37

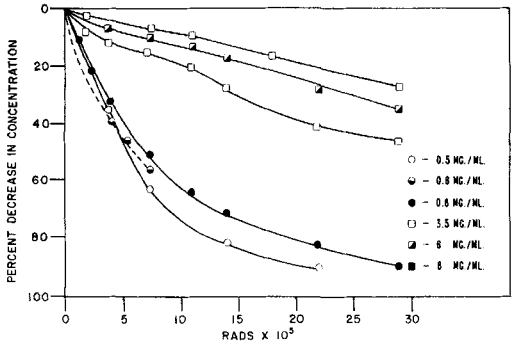

Fig. 1—Per cent decrease of chlorobutanol solutions.

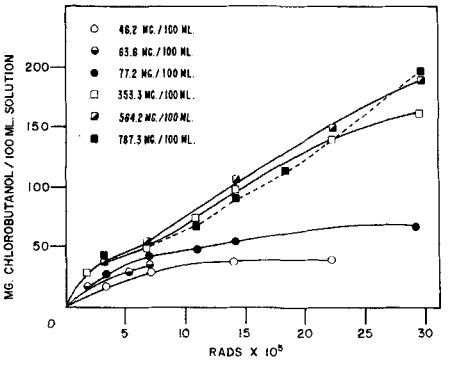

Fig. 2—Milligram loss curve for chlorobutanol solution.

Figure 1 shows the per cent decrease in irradiated chlorobutanol solutions, and Fig. 2 presents the total milligram loss for the same solutions. Figure 1 shows that those solutions containing a higher concentration of chlorobutanol have less per cent decomposition, and this appears to indicate that the more concentrated solutions are more stable to γ radiation. If, however, the total milligram loss is plotted for each solution of chlorobutanol, it is found that the total milligram loss at first increases as the concentration increases, until a limiting factor is reached. These results support the indirect action theory which states that in dilute aqueous solutions the major effect of radiation is on the water molecules, and the degradation of the solution is due to a secondary reaction. Since the number of water molecules remains relatively constant, the same amount of chlorobutanol should be degraded in each case. The apparent discrepancy which occurs in the chlorobutanol solutions of low concentration may be due to the fact that a large percentage of the chlorobutanol is degraded, and this in turn limits the total milligram loss.

The production of hydrogen peroxide in chlorobutanol solutions presents a most unusual result (Fig. 3). The data show that more concentrated solutions produce higher levels of hydrogen peroxide. However, in every instance the level of hydrogen peroxide increases rapidly, reaches a peak, and then suffers a reduction in concentration. The point of maximum hydrogen peroxide concentration occurs at about the time the precipitate becomes evident. One possible explanation for this unusual result is that the peroxide may be used up during formation of the insoluble material. It is impossible, however, to suggest appropriate mechanisms without knowing all the degradation products produced during irradiation.

Analysis of the data for acetone production (Fig. 4) shows that as the concentration of chlorobutanol increases, so does the level of acetone. The amount of acetone produced is less than would be expected if each mole of chlorobutanol degraded formed 1 mole of acetone.

For all the assay procedures used, there was no difference in results due to the container.

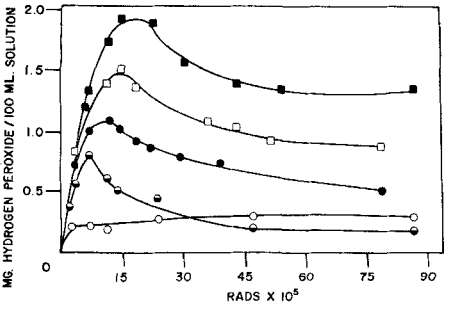

Fig. 3—Hydrogen peroxide levels of chlorobutanol solutions and distilled water. Key: ○, distilled water; ◒, 0.5 mg./ml.; ●, 1.5 mg./ml.; □, 2.5 mg./ml.; ■, 5.0 mg./ml.

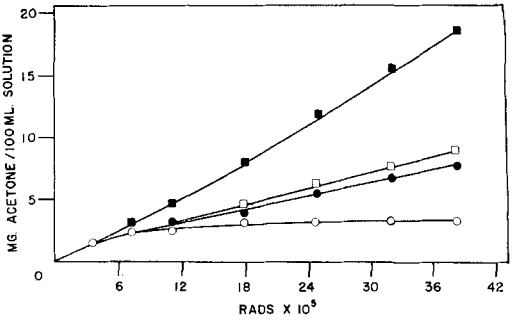

Fig. 4—Acetone levels of chlorobutanol solutions. Key: ○, 0.5 mg./ml.; ●, 1.5 mg./ml.; □, 2.5 mg./ml.; ■, 5.0 mg./ml.

TABLE II—pH OF THEOPHYLLINE SOLUTION (0.1 Gm./L.)

Total Dose, rads	pH
0	6.50
8.8×10^4	6.45
1.8×10^5	6.40
2.6×10^5	6.50
3.5×10^5	6.50
5.3×10^5	6.50
7.0×10^5	6.50
1.1×10^6	6.50
1.4×10^6	6.48
2.1×10^6	6.55

TABLE III—pH OF THEOPHYLLINE SOLUTION (5.0 Gm./L.)

Total Dose, rads	pH
0	6.58
3.9×10^6	6.40
7.7×10^6	6.39
1.2×10^7	6.42
1.5×10^7	6.49
2.0×10^7	6.40

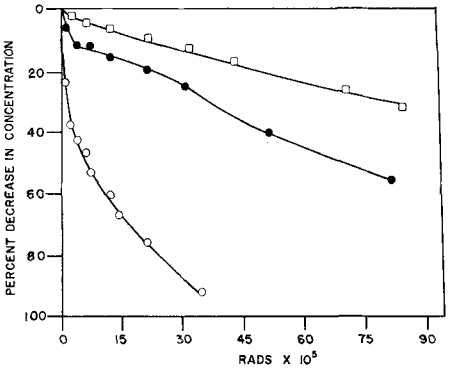

Fig. 5—Per cent decrease curve of theophylline solution. Key: ○, 0.1 Gm./L.; ●, 0.5 Gm./L.; □, 1.0 Gm./L.

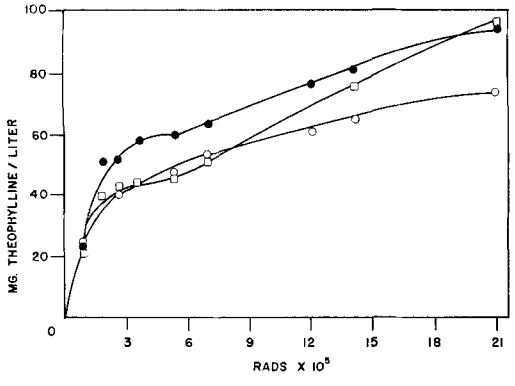

Fig. 6—Milligram loss curve for theophylline solutions. Key: ○, 0.1 Gm./L.; ●, 0.5 Gm./L.; □, 1.0 Gm./L.

Theophylline Solutions—Visual observations of theophylline solutions showed that irradiation causes a slight yellowing to develop in the solution. This yellow tint was more pronounced the higher the concentration of the solution, and the longer the irradiation time.

Unlike chlorobutanol solutions, the pH of theophylline solutions remain relatively constant, even after prolonged periods of irradiation (Tables II and III).

The degradation of theophylline solutions is very similar to chlorobutanol solutions (Figs. 5 and 6). The more concentrated solutions have less degradation, on a per cent basis, than the more dilute solutions. But, once again, it may be seen that the milligram loss for all solutions remains relatively constant. This, as explained previously, is an indication of an indirect effect on the theophylline molecule.

The curves for hydrogen peroxide levels (Fig. 7) are of a much simpler nature than those for chlorobutanol solutions. For more concentrated solutions of theophylline, there is a higher production

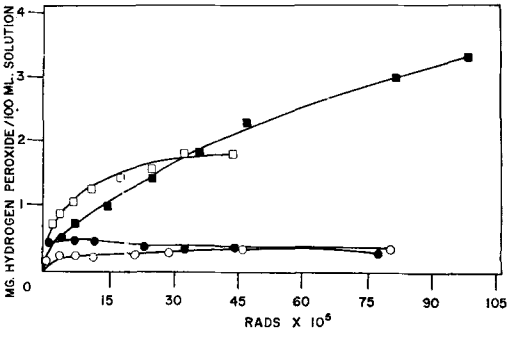

Fig. 7—Hydrogen peroxide levels of theophylline solutions. Key: ○, distilled water; ●, 0.1 Gm./L.; □, 0.5 Gm./L.; ■, 5.0 Gm./L.

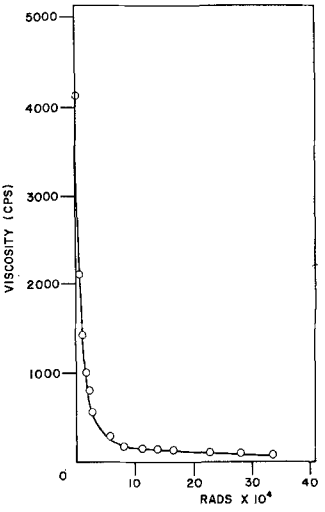

Fig. 8—Viscosity decrease of 0.5% sodium carboxymethylcellulose at 100° F.

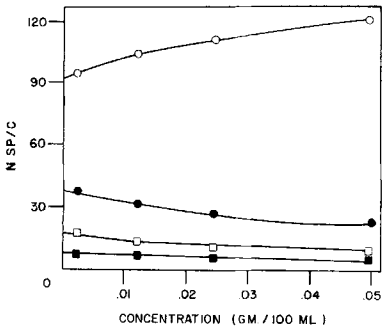

Fig. 9—Effect of irradiation on η_{sp}/C versus C curves for 0.25% sodium carboxymethylcellulose at 100° F. Key: ○, control; ●, 2.8 × 10⁴ rads; □, 5.7 × 10⁴ rads; ■, 1.1 × 10⁵ rads.

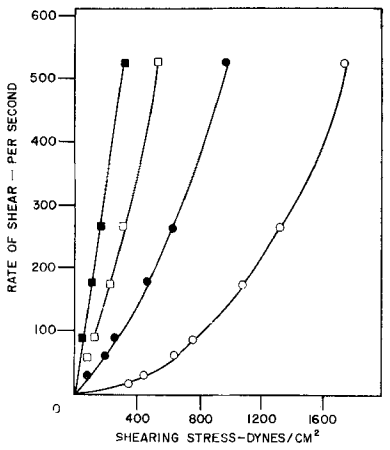

Fig. 10—Effect of irradiation on flow curves of 2% sodium carboxymethylcellulose at 100° F. Key: ○, control; ●, 2.8 × 10⁴ rads; □, 5.7 × 10⁴ rads; ■, 8.5 × 10⁴ rads.

of hydrogen peroxide, but there is no decrease in the level after a certain point, as was the case with chlorobutanol solutions.

There was no difference in effects due to the containers used for the theophylline solutions.

Sodium Carboxymethylcellulose Solutions (CMC)—Ostwald viscometer measurements of 0.125%, 0.25%, and 0.5% CMC solutions indicated that irradiation with γ rays causes a severe and immediate drop in viscosity. As little as 5.7 × 10^3 rads caused a decrease in viscosity of 48 to 66% for the solutions. Figure 8 presents the decrease for the 0.5% solution. The curves for the 0.125% and 0.25% solutions were very similar.

The results of intrinsic viscosity determination (Fig. 9) indicate a profound reduction in molecular weight. As discussed previously, the intrinsic viscosity is related to the average molecular weight.

Analysis of the total flow curve for a 2% CMC solution shows that irradiation causes the curve to change from a pseudoplastic flow curve to a nearly Newtonian system, after a dose of 8.5 × 10^4 rads (Fig. 10). This loss of pseudoplastic behavior is another indication of the reduction in length of the polymer chain which occurs during irradiation.

No container effect was noted for the CMC solutions.

CONCLUSIONS

Gamma irradiation of aqueous solutions of chlorobutanol, theophylline, and sodium carboxymethylcellulose caused severe degradation in all three systems.

These results indicate that γ irradiation is not a suitable method for sterilizing aqueous solutions of these products.

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