Influence of Cobalt (Dietary), Cobalamins, and Inorganic Cobalt Salts on Phenytoin- and Cortisone-Induced Teratogenesis in Mice

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Abstract □ Various cobalt-containing agents (cyanocobalamin, sodium cobaltinitrite, and cobaltous chloride), which formerly had been shown to prevent the onset of cleft palate in CF-1 mice injected with cortisone, were studied to determine whether they would afford similar protection against phenytoin. Phenytoin, however, failed to cause cleft palate in the mouse fetus when given to pregnant animals alone; and cortisone, on the contrary, induced this anomaly in the presence of the so-called cobalt antagonists as well as when administered in their absence. It is suggested from these results that high dietary intake of cobalt prevents cleft palate caused by phenytoin challenge and also negates the protective effects associated with the acute administration of cobalt compounds. Therefore, it is concluded that these well-known teratogens inhibit palatal closure in mice by different mechanisms.

Keyphrases □ Cobalt-containing agents, various—effect on phenytoin- and cortisone-induced teratogenesis in mice □ Teratogenesis, phenytoin and cortisone induced—effect of various cobalt-containing agents in mice □ Phenytoin-induced teratogenesis—effect of various cobalt-containing agents in mice □ Cortisone-induced teratogenesis—effect of various cobalt-containing agents in mice

Phenytoin (diphenylhydantoin) was first used therapeutically to palliate the seizures of *grand mal* epilepsy and later was used to mitigate the cortical dysrhythmias associated with psychomotor forms of this disease (1). Its clinical use has been extended to the treatment of the schizophrenias (2), delirium tremens (3), peripheral neuropathies (4), and ventricular dysrhythmias (5). Such diversification eventually brought attention to previously unrecognized side effects including megaloblastic anemia (6), osteomalacia (7), gingival hyperplasia (8), and various congenital malformations such as hare-lip and cleft palate (9).

Kuenssberg and Knox (10), after reviewing the findings of a prospective study involving more than 15,000 pregnancies, noted that five offspring of 48 deliveries were born with anomalies (hydrocephalus, hypospadias, and anal defects) to mothers who had received antiepileptic therapy (phenytoin and/or other drugs) during their gestation periods. Massey (11) reported the appearance of cleft palate in A/Jax mice after phenytoin was employed unsuccessfully as an antagonist to cortisone-induced cleft palate. Gibson and Becker (12), assuming that Massey's results might have been due to strain specificity, gave phenytoin to Swiss-Webster mice and succeeded in causing cleft palate along with decreased fetal weight, increased resorptions, and long bone deformities. Coincident with Massey's work with phenytoin and cortisone, Kasirsky *et al.* (13, 14) injected cobalt salts in CF-1 mice and produced a low incidence of this anomaly. However, when a cobalt salt was injected simultaneously with teratogenic doses of cortisone, the occurrence of the palatal defect was significantly inhibited.

Mann and Gautieri (15) reported a decreased incidence of cortisone-induced cleft palate with another cobalt-containing agent, cyanocobalamin (vitamin B_{12}). They proposed that other agents with a cobalt moiety might also inhibit the onset of the most prominent teratological manifestation associated with phenytoin administration in mice and humans, cleft palate. Therefore, the principal objectives of this study were to ascertain: (*a*) if cyanocobalamin and its congener, hydroxycobalamin, could prevent this and other anomalies from arising after phenytoin administration in mice, and (*b*) if cobaltous chloride and sodium cobaltinitrite could protect against phenytoin teratogenesis as each agent did against cortisone challenge under comparable experimental conditions. The latter observation, if affirmative, would substantiate the pharmacological similarities presumed to exist between the steroidal hormone and phenytoin at fundamental levels of activity.

Finally, the clinical benefit to be derived from this investigation, if indeed cortisone and phenytoin proved to be alike in this respect, is that pregnant patients who require phenytoin therapy can minimize the likelihood of giving birth to malformed children through the simultaneous administration of either cyanocobalamin or its congener, hydroxycobalamin, during their gestation periods.

EXPERIMENTAL

Animals—CF-1 albino mice[1] were utilized. The females were confined in groups of 10–15 to aggregate cages and were not mated until they weighed at least 20 g. Males were placed individually in metal cages (12.5 × 15 × 10 cm) with a wire-mesh front and floor[2]. All mice were maintained on a mouse/rat diet[3] (6% fat) and tap water *ad libitum*. The room housing the mice was protected from exposure to natural sunlight and was equipped with an electrical lighting system[4], which allowed 12 hr of light (7:00 am–7:00 pm) and 12 hr of darkness. The temperature was maintained between 22 and 26°.

Breeding Procedure and Dosage Regimen—The breeding procedure was described previously (16). Gravid mice were assigned to 29 experimental groups, each consisting of a minimum of six animals. The various groups employed are listed in Table I according to treatment regimen.

Suspected phenytoin antagonists were given intramuscularly in a fixed volume of 0.42 ml, except for the cobalt salts (cobaltous chloride and sodium cobaltinitrite) which were injected intraperitoneally in a volume according to body weight. The cobalt salts were prepared in distilled water in concentrations of 0.25% (cobaltous chloride) and 0.5% (sodium cobaltinitrite), which permitted delivery of the required milligrams per kilogram dose in a volume of 0.01 ml/g. Thus, each animal received a volume of solution no greater than 0.42 ml/injection.

Phenytoin was administered subcutaneously in a volume that corre-

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] RD-T unit, Norwich Wire Works, Inc., Norwich, N.Y.
[3] Teklad Standard Diets, Division of ARS/Sprague-Dawley, Monmouth, Ill.
[4] Astronomic dial time switch with "skipper," model V-45073, International Register Co., Spring Grove, Ill.

sponded to the volume required for delivery of the estimated dose of this agonist (50-mg/kg dose as 0.005 ml of a 1.0% solution/g). At a single injection, each animal received a volume of this agonist no greater than 0.26 ml. Cortisone acetate[5] was given intramuscularly in a fixed dose of 2.5 mg (0.1 ml of a 2.5% suspension). All doses given subcutaneously were made beneath the loose skin of the back, while intramuscular doses were injected in close proximity to the semimembranous muscle. When multiple injections were required, alternate leg sites were used and an aspiration was performed to avoid intravenous administration.

The three dosage regimens were as follows:

1. According to volume per gram of body weight: physiological saline[6], 0.005 ml/g, and propylene glycol[7], 70% in saline, 0.005 ml/g.
2. In a fixed dose in a volume of 0.42 ml: physiological saline and cyanocobalamin[8], 0.25% (1.05 mg), 0.5% (2.1 mg), and 1.0% (4.2 mg); and hydroxycobalamin[9], 0.25% (1.05 mg), 0.5% (2.1 mg), and 1.0% (4.2 mg). In a volume of 0.1 ml: cortisone acetate, 2.5% (2.5 mg).
3. On a milligram per kilogram basis: cobaltous chloride[10], 0.25% (25 mg/kg); sodium cobaltinitrite[11], 0.5% (50 mg/kg); and phenytoin[12], 1.0% (50 mg/kg).

Preparation of Solutions—A 70% concentration of propylene glycol (in saline) was chosen as the solvent for phenytoin, because it was less irritating than higher concentrations when given parenterally[13] and the lower pH (9.39–9.95), approaching the physiological range, was less likely to cause fetal anomalies because of extreme alkalinity compared to the commercial preparation (pH 12).

The phenytoin solution was freshly prepared prior to injection by agitating the drug in the propylene glycol vehicle for approximately 20 min[14]. Because agitation causes an exothermic reaction, the solution was allowed to equilibrate before administration. Determinations of pH were made prior to injection[15].

Cyanocobalamin and hydroxycobalamin solutions were freshly prepared by weighing[16] and dissolving each agent in physiological saline. Cobaltous chloride and sodium cobaltinitrite were similarly weighed and dissolved in distilled water immediately before injection. Cortisone acetate was available as a commercially prepared suspension in a multiple-dose vial. All injections were accomplished with a glass syringe[17].

Examination of Fetuses—Following treatment, which ended on Day 14, gestation was allowed to continue to Day 18, 12–24 hr prior to expected delivery. At this time, each pregnant mouse was weighed[18] and sacrificed by cervical dislocation. A laparotomy was performed extending from the vaginal orifice to an area just below the xiphoid process along the linea alba to expose the uterine horns. The number and position of fetuses and resorption sites (metrial glands) were determined and recorded. The fetuses were removed and stimulated mechanically with a blunt probe to determine viability. Then they were blotted dry, weighed to the nearest hundredth of a gram[18], examined for gross external defects, and sexed on the basis of gross observations.

The specimens were then prepared for macroscopic examination. Because it was desirable to evaluate structural changes of bone and soft tissues, two methods of preparation were employed. For skeletal examination, every third fetus was prepared according to the method of Staples and Schnell (17). The other fetuses were fixed and decalcified in Bouin's solution in preparation of freehand razor blade sectioning according to the technique of Wilson (18). This method permits the evaluation of a large number of animals quickly and efficiently. All specimens were examined for bone and soft tissue defects[19].

Statistical Methods and Analysis—The degree of significance of observed variations among the experimental groups was determined by the Student t test and the uncorrected χ^2 test for binomial populations (19). All t values and χ^2 calculations were performed on a computer[20].

[5] Cortone, lot 0772N, Merck Sharp and Dohme, West Point, Pa.
[6] 0.9% Sodium Chloride Injection USP, lots G261A3 and G282X8, Baxter Laboratories, Division of Travenol Laboratories, Deerfield, Ill.
[7] Lot 766340, Fisher Scientific Co., Fair Lawn, N.J.
[8] Code 28625, courtesy of Dr. Walter J. Bagdon, Merck Sharp and Dohme, West Point, Pa.
[9] Lot E85428/70444, courtesy of Dr. Walter J. Bagdon, Merck Sharp and Dohme, West Point, Pa.
[10] Lot 713262, Fisher Scientific Co., Fair Lawn, N.J.
[11] Lot 22658, J. T. Baker Chemical Co., Phillipsburg, N.J.
[12] Dilantin sodium, lots W508K and PE338, Parke-Davis Co., Detroit, Mich.
[13] As determined from a pilot study.
[14] Vortex Genie mixer, model S8223.
[15] Beckman Expandomatic SS-2 pH meter.
[16] Ainsworth substitution balance, type 28N.
[17] B-D 1-ml tuberculin syringe with 1.27-cm, 26-gauge needle.
[18] Torbal torsion balance, model PL-800.
[19] Bausch & Lomb stereoscopic dissecting microscope, model ASZ30L2.
[20] Wang 2200.

Table I—Treatment Regimen of Experimental Groups

Group	Treatment[a] (Day)
1	Untreated controls
2	Saline (11) + saline (11–14)
3	Saline (11) + 70% I in saline (11–14)
4	Saline (11) + II (11–14)
5	III, 4.2 mg (11), + II (11–14)
6	III, 2.1 mg (11), + II (11–14)
7	III, 1.05 mg (11), + II (11–14)
8	Saline (10) + saline (11–14)
9	Saline (10) + 70% I in saline (11–14)
10	Saline (10) + II (11–14)
11	III, 4.2 mg (10), + II (11–14)
12	III, 2.1 mg (10), + II (11–14)
13	III, 1.05 mg (10), + II (11–14)
14	IV (11) + II (11–14)
15	V (11) + II (11–14)
16	IV (11) + cortisone (11–14)
17	V (11) + cortisone (11–14)
18	IV (11) + saline (11–14)
19	V (11) + saline (11–14)
20	VI, 4.2 mg (11), + II (11–14)
21	VI, 2.1 mg (11), + II (11–14)
22	VI, 1.05 mg (11), + II (11–14)
23	VI, 4.2 mg (11), + saline (11–14)
24	VI, 2.1 mg (11), + saline (11–14)
25	VI, 1.05 mg (11), + saline (11–14)
26	III, 2.1 mg (11), + saline (11–14)
27	III, 4.2 mg (11), + saline (11–14)
28	Saline (11) + cortisone (11–14)
29	III, 4.2 mg (10), + saline (11–14)

[a] I is propylene glycol, II is phenytoin, III is cyanocobalamin, IV is cobaltous chloride, V is sodium cobaltinitrite, and VI is hydroxycobalamin.

The probability, p, was determined for the t and χ^2 values using standard probability tables.

RESULTS AND DISCUSSION

Maternal Effects of Drugs—Injections of cobaltous chloride, sodium cobaltinitrite, and cortisone elicited slight lethargic responses in gravid mice that lasted approximately 5 min after each administration. This reaction is in agreement with the responses of malaise, weakness, and fatigue previously observed following the administration of cobalt salts (20). Spontaneous seizures caused by increased brain excitability attributed to cortisone were not seen (21). Physiological saline, propylene glycol, phenytoin, and the cobalamins did not induce central stimulation, although, according to Massey (11), a dose of 50 mg of phenytoin/kg produced restlessness and agitation in mice followed by lethargy.

Although previous studies utilizing propylene glycol (100%) as the solvent for phenytoin did not report skin reactions (11), this investigation revealed that the minimal concentration of this vehicle (70%) required to dissolve the antiepileptic agent caused local irritation, alopecia, and epidermal lesions in some mice several days after the initial subcutaneous injection. No deaths in gravid animals resulted from drug administration.

Table II presents the mean values for maternal and fetal deviations of the test groups in relation to their respective saline controls. Each group contained a minimum of six litters and approximately 120 fetuses.

Maternal Weights—Significantly higher mean terminal weights occurred in Groups 3, 4, 7, 9, 21, and 22 whose animals received low and intermediate doses of the cobalamins or saline followed by propylene glycol.

Fetal Ratio—Minor variations in the fetal ratio (the number of fetuses in the right uterine horn *versus* the number of fetuses in the left horn) were noted in eight groups; the occurrence appeared to be of a random nature.

Fetal Resorptions and Sex Ratios—There were no differences in the fetal resorption and sex ratios among the various groups.

Mean Fetal Weight—A significant decrease in mean fetal weight was observed in Groups 16 and 17 compared to the saline control. This response is in disagreement with that observed by Kasirsky *et al.* (13), who reported that cobaltous chloride prevented the reduction in mean fetal weight caused by cortisone. The decrease in mean fetal weight was significant when either saline control value (Group 2 or 8) was compared to that of the untreated group. This effect was probably due to injection trauma.

A significant increase in mean fetal weight occurred when cyanoco-

Table II—Mean Values of Test Groups

Treatment Group[a]	Maternal Weight Ratio, Start/Terminal	Fetal Ratio, Right/Left	Resorption Ratio, Right/Left	$\bar{X}$ Fetal Weight, g	Sex Ratio, Male/Female	Soft Tissue Abnormalities	Skeletal Abnormalities
1	27.5/50.4	6.4[b]/4.3[b]	0.4/0.1	1.24	5.4/5.3	2.4	0.5
2	27.9/44.9	4.2/7.6	0.2/0.2	1.05[b]	6.0/5.5	2.5	0.7
3	29.8/53.1[b]	5.7/6.9	0.0/0.0	1.13	6.7/5.7	7.7[b]	0.9
4	30.9/51.8[b]	5.3/6.7	0.7/0.0	1.07	6.3/5.7	2.0	0.7
5	29.7/51.2	5.9/5.3[b]	0.4/0.0	1.17	6.1/4.8	3.0	1.1
6	30.6/43.9	6.7[b]/5.2	0.0/0.0	0.95	6.0/5.5	10.0[b]	1.0
7	28.4/51.5[b]	5.2/6.2	0.0/0.1	1.23[b]	5.9/5.5	6.0	1.5[b]
8	25.3/42.9	4.8/5.0	0.3/0.0	1.01[b]	5.5/4.2	9.3	1.5
9	30.6[b]/51.3[b]	5.0/4.8	0.2/0.5	1.18[b]	4.7/5.2	8.2	1.7
10	25.8/45.7	6.0/5.2	0.2/0.0	1.06	6.2/5.0	10.3	0.5
11	26.2/47.1	5.2/5.7	0.2/0.0	1.19[b]	6.0/4.8	8.0	1.0
12	27.1/47.9	5.3/6.0	0.3/0.3	1.16[b]	6.3/5.0	10.1	1.1
13	26.1/44.0	5.7/5.2	0.3/0.2	1.06	6.0/4.8	4.0	0.8
14	25.4/43.7	3.8/4.8	0.2/0.5	1.18	4.3/4.3	2.8	0.3
15	27.0/45.8	4.1/5.3	1.1/0.9	1.16	4.0/5.4	4.7	0.1[b]
16	29.2/40.9	3.8/3.4[b]	1.0/0.5	0.87[b]	4.8/4.2	8.2[b-d]	2.8[b]
17	28.0/43.7	4.3/5.0	0.4/1.0	0.86[b]	5.8/5.2	11.4[b-d]	5.0[b]
18	27.6/49.5	3.7/5.5	0.7/0.7	1.18	4.8/4.3	1.3	1.2
19	27.0/48.1	4.2/6.3	0.0/0.7	1.11	5.5/5.0	3.2	1.3
20	30.6/49.1	4.5/4.0[b]	0.2/0.0	1.25[b]	3.8/4.3	2.5	1.0
21	29.8/51.8[b]	4.3/7.0	0.3/0.0	1.13	5.5/5.8	2.3	0.2
22	28.9/51.7[b]	5.3/6.8	0.2/0.0	1.17	6.3/5.8	1.5	0.7
23	27.1/47.5	5.2/4.8[b]	0.3/0.5	1.13	5.7/4.3	1.2	0.7
24	28.9/50.6	5.2/5.7	0.8/0.5	1.18	5.0/5.8	1.2	0.5
25	29.3/50.2	4.7/6.5	0.3/0.5	1.14	6.2/5.0	1.7	0.5
26	25.7/50.4	5.3/5.3[b]	0.3/0.2	1.25[b]	5.2/5.5	2.8	0.5
27	26.7/48.4	4.7/5.0[b]	0.2/0.7	1.27[b]	5.3/4.3	1.7	0.2
28	29.9/44.0	3.3/4.2[b]	1.2/0.6	0.90	4.0/5.0	6.4[b-d]	3.6[b]
29	30.5[b]/53.8[b]	6.0/6.7	0.3/0.0	1.18[b]	7.2/5.5	2.2[b]	1.7

[a] See Table I. [b] As compared to saline (Day 10 or 11) + saline (Days 11–14), p less than 0.05 (minimum of six litters per group). [c] Litter incidence of cleft palate. [d] Fetal incidence of cleft palate.

balamin was substituted for saline before the phenytoin administration. With this treatment regimen, doubling or halving the 2.1-mg dose of the vitamin given on Day 11 or 10 of gestation prior to phenytoin caused this response in Group 7 or 11, respectively. Intermediate and large doses of the cobalamins followed by saline (Groups 26, 27, and 29) also caused significantly greater mean fetal weights than those of their saline counterparts. Groups 9, 12, and 20 likewise showed significantly greater mean fetal weights than did the saline group.

Production of Soft Tissue and Skeletal Anomalies—The various soft tissue and skeletal malformations representing significant deviations from their respective saline groups in their occurrence and nature are presented in Tables III and IV, respectively.

It was assumed at the onset of this study that cortisone and phenytoin produced cleft palate by a similar mechanism, because both agents had been shown to cause this defect in several strains of mice (11, 12, 22). Also, because cortisone induced cleft palate in CF-1 mice (13, 14), it was presumed that phenytoin would elicit similar results in this strain and that the prophylactic administration of cobalt salts would prevent the appearance of this anomaly in response to phenytoin challenge. However, in this study, the antiepileptic agent did not cause the palatal defect in either the cobalt salt- or saline-pretreated groups, whereas cleft palate occurred after cortisone administration in both of these pretreated groups, contrary to previous findings (13, 14).

A plausible explanation for these unexpected results may be the factor of diet, a major variable that could explain the earlier observations of Massey (11) and Kasirsky *et al.* (13, 14). The former investigator may have inadvertently revealed the important effect of food in her teratogenic studies when she reported the results of using two different kinds of diet in two otherwise similar experiments. The incidence of cleft palate among the fetuses of phenytoin-treated gravid mice fed a diet rich in cobalt and cyanocobalamin was lower than that among fetuses of such mice fed a diet poor in these constituents. Even greater amounts of cobalt and cyanocobalamin were contained in the diet utilized in this study than in that employed by Massey, and all animals received this enriched regimen immediately upon arrival at this facility.

Therefore, with all other factors being equal, it is suggested that the chronic intake of large quantities of dietary cobalt is the principal means by which cleft palate production caused by phenytoin is inhibited. In fact, when gravid animals were given this diet, neither cobaltous chloride nor sodium cobaltinitrite injection alone produced this anomaly in the offspring or, in conjunction with challenge by cortisone, prevented its onset as previously described by Kasirsky *et al.* (13, 14). Consequently, it is concluded that a relatively high dietary intake of cobalt is the principal means by which phenytoin-induced cleft palate is prevented in CF-1 mice and that, because cortisone teratogenesis referable to the appearance of this palatal defect remains unaltered under these dietary conditions,

Table III—Significant Occurrence and Nature of Soft Tissue Anomalies

Treatment Group[a]	Number of Litters	Number of Fetuses with Defects[b]									
		C.P.	Mal. P.	C.T.T.	Hyd.	Cryp.	Ano.	6 D	Ex.	EcO.	C. Tpa.
2	6	0	4	4	0	0	4	0	0	0	0
3	7	0	18	11	1	2	0	3	0	0	0
6	6	0	10	1	3	4	0	0	0	0	5
16	6	24	0	8	0	4	0	1	1	1	0
17	6	29	0	11	8	2	0	2	0	0	0
28	6	22	0	2	3	3	0	1	0	0	0
8	6	0	3	17	1	6	0	8	0	0	6
29	6	0	0	3	0	6	0	3	0	0	0

[a] See Table I. [b] C.P. = cleft palate, Mal.P. = malformed palate, C.T.T. = connective tissue beneath temporal lobe(s), Hyd. = hydronephrosis (uni- or bilateral), Cryp. = uni- or bilateral cryptorchidism, Ano. = anophthalmia (uni- or bilateral), 6 D = sixth digit on forepaw(s), Ex. = exencephaly, EcO = ectopic ovary, and C.Tpa. = connective tissue in pelvic area.

Table IV—Significant Occurrence and Nature of Skeletal Anomalies

Treatment Group[a]	Number of Litters	Number of Fetuses with Defects[b]						
		Mal.St.	F.St.	Cr.St.	Rud.St.	D.O.St.	ExR.	S.O.
2	6	2	1	0	0	0	1	0
7	11	6	0	1	2	6	2	0
15	7	0	1	0	0	0	0	0
16	6	0	1	1	0	0	1	11
17	6	0	6	0	0	0	2	17
28	6	0	1	2	0	3	0	12

[a] See Table I. [b] Mal.St. = malformed sternebrae, F.St. = fused sternebrae, Cr.St. = crankshaft sternebrae, Rud.St. = rudimentary sternebrae, D.O.St. = delayed ossification of sternebrae, ExR. = extra rib, and S.O. = split occiput.

different mechanisms of action for cortisone and phenytoin must be involved in the inhibition of palate closure in this strain of mice. Additional studies involving high and low dietary intake of cobalt to confirm this hypothesis are being contemplated.

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Differences in Antibacterial Activity of Benzalkonium Chloride

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Abstract □ Benzalkonium solutions obtained from different manufacturers were shown to have different activities. This difference in activity was related to the composition of the benzalkonium chloride. The potential seriousness of this situation is emphasized, and a recommendation is made that the official monographs on benzalkonium chloride be amended appropriately, noting the apparently superior antibacterial activity of the tetradecyl (C_{14}) homolog.

Keyphrases □ Benzalkonium chloride—various commercial solutions, antibacterial activity evaluated and related to composition □ Antibacterial activity—evaluated in various commercial solutions of benzalkonium chloride, related to composition

The International Pharmacopoeia (IP 1967) (1) allows the alkyl chain of benzalkonium chloride to be a mixture of the alkyls from C_8H_{17} to $C_{18}H_{37}$. USP XIX (2) states that the alkyl chain may represent a mixture including all or some of the group beginning with C_8H_{17} and extending through higher homologs, with the $C_{12}H_{25}$ homolog representing not less than 40%, on the anhydrous basis, and the $C_{14}H_{29}$ homolog representing not less than 20% of the total alkylbenzyldimethylammonium chloride content; the two homologs together must comprise not less than 70% of the total alkylbenzyldimethylammonium chloride content.

BP 1973 (3) allows a mixture of alkylbenzyldimethylammonium chlorides, without specifying any particular homolog or percentage composition.

This permitted variation in the alkyl chain length may contribute to the variation in benzalkonium chloride activity (compare Refs. 4 and 5 and Refs. 6 and 7). Therefore, the comparative antibacterial activity of four commercial benzalkonium solutions was investigated to determine whether differences occur in the antibacterial activity due to variations in the number of carbon atoms in the alkyl radical and the relative percentage of particular chain lengths in a given benzalkonium chloride. Such investigations should also indicate whether a given formulation preserved with benzalkonium chloride might have different preservative capacities, depending on the commercial source of benzalkonium chloride.

Two benzalkonium solutions had approximately 50% tetradecyl (C_{14}) derivative, and the other two products had approximately 65% dodecyl (C_{12}). Therefore, with respect to the proportion of the C_{12} and C_{14} homologs, the four products were divided into two pairs. Other differences in composition, however, also existed among the four solutions.