not hydrolyzable by aryl sulfatase. The identity of the hydrolysis product was confirmed by inverse isotope dilution and TLC. The nature of the glucuronide bonding has not been determined.

Inverse isotope dilutions on the urine of three rats that had received seclazone-2-^{14}C indicated the presence of malonic acid in an amount that accounted for 2–14% of the urinary radioactivity. In addition, succinic acid, a known metabolite of malonic acid, was present and represented 1–3% of the urinary radioactivity. The presence of these two compounds suggests that the carbon dioxide-^{14}C present in respired air (Table I) originates either from the direct decarboxylation of malonic acid or from succinic acid through the tricarboxylic acid cycle. In any event, the radioactive carbon dioxide arises through metabolic cleavage of the isoxazoline ring of seclazone.

The metabolic fate of seclazone is summarized in Scheme I.

SUMMARY

Seclazone-9-^{14}C is readily absorbed after oral administration to the rat, beagle hound, and rhesus monkey. The half-lives of blood radioactivity in the three species were found to be 10, 8.5, and 6 hr., respectively. In the rat, about half of the ^{14}C given orally was excreted in the urine within 24 hr. Significant amounts of radioactivity were also found in the carcass, liver, and GI tract. Seclazone is almost completely metabolized to 5-chlorosalicylic acid in each of these three species. The latter compound was excreted either uncombined or conjugated with glucuronic acid or glycine. Studies

with seclazone-2-^{14}C suggested that carbons 2, 3, and 3A of seclazone are metabolized to malonic acid.

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Displacement of Sulfonylureas from Human Serum Proteins by Coumarin Derivatives and Cortical Steroids

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Abstract ☐ Several coumarin derivatives (ethyl biscoumacetate, phenprocoumon, anisindione, acenocoumarol, and diphenadione) were examined for their abilities to displace sulfonylureas (acetohexamide, chlorpropamide, and tolbutamide) from human serum albumin, utilizing equilibrium dialysis for the estimation of protein binding. All of the coumarin compounds caused reduction in binding of the sulfonylureas, although there was no clearcut pattern with variation of pH. At lower pH values, ethyl biscoumacetate caused the greatest reduction in binding of sulfonylureas; but at pH 8.4, diphenadione was the most potent displacing agent. Acetohexamide was tested for its ability to displace cortical steroids from human serum albumin and human serum fraction α-globulin IV-4, the latter containing transcortin. Of the steroids examined (cortisone, cortisol, prednisone, prednisolone, and corticosterone), the binding of cortisol was the most reduced with human serum albumin as the protein; but if α-globulin fraction IV-4 was the protein in the system, prednisone was most displaced. The data establish that coumarin derivatives are capable of interfering with the binding of sulfonylureas to human serum albumin, and acetohexamide causes reduction of binding of the steroids tested to human serum albumin and α-globulin fraction IV-4. These observations may be the mechanism for interactions between these drugs.

Keyphrases ☐ Sulfonylureas—displacement from human serum proteins by coumarin derivatives and cortical steroids ☐ Serum protein binding, sulfonylureas—displacement by coumarins and cortical steroids ☐ Coumarin derivatives—displacement of sulfonylureas from human serum proteins ☐ Cortical steroids—displacement of sulfonylureas from human serum proteins ☐ Acetohexamide—displacement of cortical steroids from human serum proteins

Reports in the recent literature have cited interactions of sulfonylureas and various drugs including coumarin derivatives (1–9). Although there have been apparently no reports of displacement of cortical steroids by sulfonylureas, compounds such as phenylbutazone have been shown to displace cortical steroids bound to human serum proteins; since competition has been demonstrated between phenylbutazone and sulfonylureas for binding sites on human serum proteins, one could conclude that similar competition should exist between cortical steroids and sulfonylureas in serum protein bindings (10). Sellers and Koch-Weser (11) recently pointed out that, although the clinical importance of metabolic interactions between drugs has become increasingly clear, not enough work has been done to demonstrate the mechanisms of drug interactions by *in vitro* laboratory experiments including studies of displacement of drugs bound to plasma proteins. Such data would make it possible to predict clinically important drug interactions.

Table I—Characteristics of Inhibition of Albumin Binding of Sulfonylureas by Ethyl Biscoumacetate

Sulfonylurea	Concentration of Ethyl Biscoumacetate (× 10⁻⁸ mole)	Percent Reduction of r at pH:					
		6.5	Coefficient of Correlation[a]	7.4	Coefficient of Correlation	8.4	Coefficient of Correlation
Acetohexamide[b]	0	—		—		—	
	12	6.08		0.19		-4.93	
	24	20.01		9.10		2.09	
	48	21.53		32.07		25.57	
	216	80.11	0.9751	77.93	0.9607	72.95	0.9704
Chlorpropamide[c]	0	—		—		—	
	12	30.15		12.00		22.84	
	24	39.27		24.67		42.30	
	48	63.89		65.12		64.86	
	216	91.32	0.8343	88.10	0.8849	93.19	0.8250
Tolbutamide[c]	0	—		—		—	
	12	11.51		11.23		8.09	
	24	19.27		18.20		13.97	
	48	46.00		38.36		52.04	
	216	86.29	0.9225	89.22	0.9415	89.01	0.9022

[a] Coefficient of correlation (Pearson's) calculated for plot of r values versus concentration of sulfonylurea. [b] A total of 11.88 × 10⁻⁸ mole present in system in all experiments involving coumarin derivatives. [c] A total of 12 × 10⁻⁸ mole present in system in all experiments involving coumarin derivatives.

Table II—Characteristics of Inhibition of Albumin Binding of Sulfonylureas by Phenprocoumon

Sulfonylurea	Concentration of Phenprocoumon (× 10⁻⁸ mole)	Percent Reduction of r at pH:					
		6.5	Coefficient of Correlation	7.4	Coefficient of Correlation	8.4	Coefficient of Correlation
Acetohexamide	0	—		—		—	
	12	5.31		1.97		-0.09	
	24	6.73		7.60		8.87	
	48	15.29		16.65		14.24	
	216	48.53	0.9928	44.71	0.9918	51.86	0.9903
Chlorpropamide	0	—		—		—	
	12	6.15		7.61		2.45	
	24	8.74		27.74		16.41	
	48	32.36		40.42		54.08	
	216	82.69	0.9612	74.77	0.9372	78.51	0.9343
Tolbutamide	0	—		—		—	
	12	3.91		1.36		12.69	
	24	7.91		4.56		23.70	
	48	15.74		13.81		34.99	
	216	61.96	0.9912	66.33	0.9928	61.26	0.9638

Within this context, it seemed valuable to study: (a) the ability of several coumarin derivatives to displace the three major sulfonylurea compounds in clinical use from human serum albumin and (b) the ability of acetohexamide to displace several cortical steroids from human serum proteins.

EXPERIMENTAL[1]

Stock solutions of the coumarin derivatives were made in pH 8.4 tromethamine buffer (0.067 M) except diphenadione which was dissolved in pH 9.5 tromethamine buffer. The stock solutions of

the cortical steroids were made with absolute ethanol as the solvent. Binding and displacement of bound drugs were studied utilizing equilibrium dialysis as described previously (10). The specific activities of the sulfonylurea derivatives and concentrations of stock solutions were also as previously described. The specific activities of the cortical steroids used were as follows: corticosterone-1,2-³H, 36 c./mmole; prednisone-T (G), 2.5 c./mmole; cortisone-1,2-³H, 500 mc./mmole; prednisolone-³H (G), 2.5 c./mmole; and tritium-labeled cortisol-1,2, 2.0 c./mmole. Serum proteins were dissolved in tromethamine buffer of appropriate pH and placed in one side of the dialysis cell. The compound, the binding of which was being studied, and the competitor, if any, were placed in the other side of the cell. A total of 1.0 ml. was contained in each side of the cell. Other details of the procedure were identical with those previously published (10).

The amounts of proteins in the systems were as follows. Albumin, either as the sole protein or together with α-globulin fraction IV-4, was present in the amount of 5.797 × 10⁻⁷ mole. α-Globulin fraction IV-4 as the sole protein was present in the amount of 5.714 × 10⁻⁸ mole (assuming an average molecular weight of 70,000); when in combination with albumin, 7.15 × 10⁻⁸ mole of fraction IV-4 was present.

Although data relevant to possible clinical implications of com-

Table III—Characteristics of Inhibition of Albumin Binding of Sulfonylureas by Anisindione

| Sulfonylurea | Concentration of Anisindione ($\times 10^{-8}$ mole) | Percent Reduction of r at pH: | | | | | |
		6.5	Coefficient of Correlation	7.4	Coefficient of Correlation	8.4	Coefficient of Correlation
Acetohexamide	0	—		—		—	
	12	10.23		6.99		6.29	
	24	18.89		11.94		10.42	
	48	38.03		36.79		39.81	
	216	77.32	0.9502	75.62	0.9571	80.66	0.9468
Chlorpropamide	0	—		—		—	
	12	22.24		10.85		13.22	
	24	22.78		16.78		24.79	
	48	53.44		38.79		51.62	
	216	87.72	0.8982	80.60	0.9475	78.06	0.9288
Tolbutamide	0	—		—		—	
	12	10.77		7.22		5.00	
	24	21.42		16.45		14.49	
	48	41.41		39.64		45.41	
	216	83.87	0.9297	79.79	0.9458	88.59	0.9519

Table IV—Characteristics of Inhibition of Albumin Binding of Sulfonylureas by Acenocoumarol

| Sulfonylurea | Concentration of Acenocoumarol ($\times 10^{-8}$ mole) | Percent Reduction of r at pH: | | | |
		7.4	Coefficient of Correlation	8.4	Coefficient of Correlation
Acetohexamide	0	—		—	
	12	8.24		9.50	
	24	4.74		8.08	
	48	20.88		32.03	
	216	49.37	0.9950	70.92	0.9683
Chlorpropamide	0	—		—	
	12	13.39		25.03	
	24	19.49		29.62	
	48	30.62		67.47	
	216	71.38	0.9634	88.10	0.8749
Tolbutamide	0	—		—	
	12	3.05		−2.41	
	24	11.03		4.58	
	48	31.07		25.87	
	216	71.78	0.9674	76.72	0.9713

Table V—Characteristics of Inhibition of Albumin Binding of Sulfonylureas by Diphenadione at pH 8.4

Sulfonylurea	Concentration of Diphenadione ($\times 10^{-8}$ mole)	Percent Reduction of r	Coefficient of Correlation
Acetohexamide	0	—	
	12	9.43	
	24	22.40	
	48	60.89	
	216	97.93	0.8385
Chlorpropamide	0	—	
	12	19.79	
	24	34.83	
	48	76.13	
	216	97.63	0.7659
Tolbutamide	0	—	
	12	7.94	
	24	27.74	
	48	63.84	
	216	91.33	0.8577

petitive protein binding could be obtained at pH 7.4, determinations were made also at pH 6.5 and 8.4. One objective of accumulating data at two pH values outside of the physiological range was to determine from a more basic viewpoint whether pH affects the binding process and competition between bound molecules. Furthermore, in the case of several compounds with solubilities limited to certain pH ranges, it was necessary to work at a nonphysiological pH. This was true, for example, with diphenadione which was insoluble at pH values below 8.4.

RESULTS AND DISCUSSION

Over the range of concentrations of the various coumarin compounds used, substantial reduction in binding of the three sulfonylureas resulted (Tables I-V). Diphenadione was studied at pH 8.4 only because of solubility problems at values below 8.4. For the same reason, acenocoumarol could not be tested at pH 6.5. The other derivatives were examined at the three pH values indicated, and the final concentrations were adjusted to be comparable to that found in plasma when dicumarol is used in therapy (12-17). Although the abilities of the various coumarin compounds to displace sulfonylureas from human serum albumin varied with the type of coumarin compound as well as the pH, there appeared to be no discernible clearcut pattern in terms of the effect of pH on displacement.

Table VI—Comparison of Highest Percent Reduction in r Values of Sulfonylureas Caused by Coumarin Derivatives

Coumarin Derivative in System	Acetohexamide pH 6.5	7.4	8.4	Chlorpropamide pH 6.5	7.4	8.4	Tolbutamide pH 6.5	7.4	8.4
Ethyl biscoumacetate	80.11	77.93	72.95	91.32	88.10	93.10	86.29	89.22	89.01
Phenprocoumon	48.53	44.71	51.86	82.69	74.77	78.51	61.96	66.33	61.26
Anisindione	77.32	75.62	80.66	87.72	80.60	78.06	83.87	79.79	88.59
Acenocoumarol	—	49.37	70.92	—	71.38	88.10	—	71.78	76.72
Diphenadione	—	—	97.93	—	—	97.63	—	—	91.33

Table VII—Potency of Coumarin Derivatives to Displace Sulfonylureas from Human Serum Albumin

Sulfonylurea	pH	Decreasing Reduction in r Values of Sulfonylureas
Acetohexamide	6.5	Ethyl biscoumacetate > anisindione > phenprocoumon
	7.4	Ethyl biscoumacetate > anisindione > acenocoumarol > phenprocoumon
	8.4	Diphenadione > anisindione > ethyl biscoumacetate > acenocoumarol > phenprocoumon
Chlorpropamide	6.5	Ethyl biscoumacetate > anisindione > phenprocoumon
	7.4	Ethyl biscoumacetate > anisindione > phenprocoumon > acenocoumarol
	8.4	Diphenadione > ethyl biscoumacetate > acenocoumarol > phenprocoumon > anisindione
Tolbutamide	6.5	Ethyl biscoumacetate > anisindione > phenprocoumon
	7.4	Ethyl biscoumacetate > anisindione > acenocoumarol > phenprocoumon
	8.4	Diphenadione > ethyl biscoumacetate > anisindione > acenocoumarol > phenprocoumon

Table VIII—Characteristics of Inhibition of Serum Protein (Albumin) Binding of Steroids by Acetohexamide

Steroid[a]	Concentration of Acetohexamide (× 10^{-8} mole)	r (× 10^{-4})	Percent Reduction of r	Coefficient of Correlation
Prednisone	0	5.11	—	
	9.88	5.08	0.59	
	19.76	4.69	8.22	
	39.52	4.24	17.03	
	197.6	3.35	34.44	0.9423
Prednisolone	0	4.47	—	
	9.88	4.48	0	
	19.76	3.81	14.77	
	39.52	3.51	21.48	
	197.6	3.02	32.44	0.8257
Cortisone	0	6.45	—	
	9.88	5.72	11.32	
	19.76	6.19	4.03	
	39.52	5.00	22.48	
	197.6	4.01	37.83	0.9018
Cortisol	0	5.13	—	
	9.88	4.07	20.66	
	19.76	4.71	8.19	
	39.52	4.43	13.65	
	197.6	2.58	49.71	0.9364
Corticosterone	0	9.41	—	
	9.88	9.49	0	
	19.76	8.98	4.57	
	39.52	8.37	11.05	
	197.6	7.19	23.59	0.9421

[a] Total steroid concentration was 10^{-8} mole in the experimental system throughout.

Table IX—Characteristics of Inhibition of Serum Protein (α-Globulin Fraction IV-4) Binding of Steroids by Acetohexamide

Steroid	Concentration of Acetohexamide (× 10^{-8} mole)	r	Percent Reduction of r	Coefficient of Correlation
Prednisone	0	1.02×10^{-3}	—	
	9.88	1.35×10^{-3}	0	
	19.76	1.20×10^{-3}	0	
	39.52	4.73×10^{-4}	53.63	
	197.6	1.28×10^{-4}	87.45	0.8372
Prednisolone	0	9.34×10^{-4}	—	
	9.88	9.49×10^{-4}	0	
	19.76	9.84×10^{-4}	0	
	39.52	7.15×10^{-4}	23.45	
	197.6	4.01×10^{-4}	57.07	0.9429
Cortisone	0	1.45×10^{-3}	—	
	9.88	1.31×10^{-3}	9.66	
	19.76	1.26×10^{-3}	13.10	
	39.52	8.91×10^{-4}	38.55	
	197.6	3.17×10^{-4}	78.14	0.9597
Cortisol	0	1.64×10^{-3}	—	
	9.88	1.54×10^{-3}	6.10	
	19.76	1.28×10^{-3}	21.95	
	39.52	1.15×10^{-3}	29.88	
	197.8	3.77×10^{-4}	77.01	0.9788
Corticosterone	0	3.21×10^{-3}	—	
	9.88	3.56×10^{-3}	0	
	19.76	3.12×10^{-3}	2.80	
	39.52	2.54×10^{-3}	20.87	
	197.6	1.50×10^{-3}	53.27	0.9397

As estimated by percent reduction in r value[2], phenprocoumon showed less displacement of acetohexamide at all three pH values

[2] The r value refers to moles of steroid bound per mole of protein at the concentrations of steroid and protein present in the system.

and acenocoumarol showed similar lower displacement of acetohexamide at pH 7.4. The other coumarin derivatives produced a 70–80% reduction of r values in all other instances. A comparison of the percent reduction in r values of the sulfonylureas is tabulated in Table VI. In Table VII, the coumarin derivatives are ranked in decreasing order of reduction in r values obtained. At the two lower

Table X—Characteristics of Inhibition of Serum Protein (Albumin + α-Globulin Fraction IV-4) Binding of Steroids by Acetohexamide

Steroid	Concentration of Acetohexamide $(\times 10^{-8}$ mole)	$r (\times 10^{-4})$	Percent Reduction of r	Coefficient of Correlation
Prednisone	0	5.03	—	
	9.88	5.06	0	
	19.76	4.66	7.36	
	39.52	4.52	10.14	
	197.6	3.06	39.17	0.9884
Prednisolone	0	3.91	—	
	9.88	4.22	0	
	19.76	4.03	0	
	39.52	3.32	15.09	
	197.6	2.42	39.11	0.9373
Cortisone	0	5.08	—	
	9.88	5.19	0	
	19.76	5.36	0	
	39.52	4.90	3.54	
	197.6	3.04	40.16	0.9933
Cortisol	0	4.73	—	
	9.88	4.81	0	
	19.76	4.23	10.57	
	39.52	4.53	4.23	
	197.6	2.92	38.27	0.9583
Corticosterone	0	9.20	—	
	9.88	8.72	5.22	
	19.76	8.53	7.28	
	39.52	8.43	8.37	
	197.6	6.61	28.15	0.9938

pH values studied, ethyl biscoumacetate was the most potent derivative in terms of ability to displace sulfonylureas from human serum albumin; but at the higher pH value (8.4), diphenadione was the most potent displacing agent with phenprocoumon the least potent except in the case of chlorpropamide. At physiological pH (7.4), ethyl biscoumacetate would appear to be the coumarin derivative most likely to potentiate the action of a sulfonylurea compound. These results clearly indicate that the coumarin derivatives studied do displace sulfonylureas from human serum albumin, and this could easily be the mechanism by which coumarin derivatives could cause hypoglycemia when administered concurrently with sulfonylureas. A clinical report of this interaction was published by Kristensen and Hansen (3), involving potentiation of the effects of tolbutamide by dicumarol.

Cortical steroids have been shown to bind primarily to a specific blood protein, with corticosteroid binding globulin (18) or transcortin until the latter is saturated and then the excess steroid is is bound to albumin. Albumin is known to bind a variety of compounds, and the binding of cortical steroids to albumin is not specific as it is to corticosteroid binding globulin. Since both of these blood proteins bind cortical steroids, they were both included in the study. Cohn fraction IV-4 α-globulin contains the cortical binding globulin (18). The results obtained are tabulated in Tables VIII-X. The r values are rather low, but these are not to be considered as true r values. For an estimation of true r values, it would be necessary to study a variety of concentrations of the steroids up to satura-

tion of the protein; in these studies, the concentrations of steroids were adjusted to that normally found in plasma when cortical steroids are used in therapy (19-25).

Only one sulfonylurea compound, acetohexamide, was chosen for this study as a representative of the group. Acetohexamide, as well as chlorpropamide and tolbutamide, was added in concentrations typical of blood levels attained in therapy (26-29). Acetohexamide caused a greater reduction in the r value when α-globulin fraction IV-4 was the protein studied than in the case of albumin or a combination of albumin and fraction IV-4. The greater displacement from fraction IV-4 is more significant clinically probably in the sense that binding of cortical steroids would be greatest to the α-globulin fraction with normal plasma steroid concentrations. Acetohexamide caused the greatest reduction in binding to human serum albumin in the case of cortisol and the least with corticosterone. With human serum α-globulin fraction IV-4, the greatest reduction in binding was seen with prednisone and least with corticosterone. When both proteins were present in the system, the binding of cortisone was the most reduced and the binding of corticosterone was the least reduced. On the basis of the data in Tables VIII-X, specifically the percent reduction in r values, it would seem that with human serum albumin as the protein in the system, the several steroids showed a decreasing percent reduction in r value in the presence of acetohexamide as follows: cortisol, cortisone, prednisone, prednisolone, and corticosterone. Apparently, one cannot consider the r value obtained in the absence of acetohexamide as an indication of binding capacity, because one does not observe an inverse relationship between binding capacity and percent reduction in r value in the presence of a displacing agent.

Table XI indicates r values in the absence of acetohexamide and this inverse relationship does not seem to hold except in the case of corticosterone. This was equally true when human serum α-globulin fraction IV-4 was the protein in the system, once again with the exception of corticosterone. The lack of an inverse relationship between binding capacity and ease of displacement by acetohexamide could very well be due to the lack of a proper index of binding capacity. The r values in Table XI do not represent true r values in the sense that saturation of the protein was not nearly achieved with the very low concentrations of steroids employed. It probably would be more realistic to use such values as n (number of binding sites) or k_a (binding constant). In future work, it is planned to make a comparison utilizing the latter indexes of binding capacity and binding strength.

For the data in Tables I-V and VIII-X, coefficients of correlation were calculated for each set of concentrations of binding inhibitors and resulting r values. In almost all cases, the coefficients of correlation were significant to a level of confidence of 90%, although a somewhat lower level of confidence is indicated in a very few cases. The statistical values suggest that there is a reasonable quantitative relationship between concentrations of the inhibitors and amounts of organic molecule bound.

These results indicate that acetohexamide is capable of displacing cortical steroids from plasma proteins and potentially could result in the equivalent of an overdosage of steroid if sulfonylureas and cortical steroids are administered concurrently. It is interesting that there are apparently no reports in the clinical literature of this sort of drug interaction and perhaps it is of no clinical consequence. It would be interesting to note whether diabetics with rheumatic or arthritic conditions experience an improvement in the latter conditions upon introduction of sulfonylurea therapy. If such cases do exist, they may be examples of a beneficial drug interaction.

Table XI—Comparison of Steroids in Terms of Moles Bound per Mole of Human Serum Albumin and α-Globulin Fraction IV-4

Protein	Steroid[a] and r Value[b]				
	Prednisone	Prednisolone	Cortisone	Cortisol	Corticosterone
Human serum albumin[c]	5.11×10^{-4}	4.47×10^{-4}	6.45×10^{-4}	5.13×10^{-4}	9.41×10^{-4}
Human serum α-globulin fraction IV-4[d]	1.02×10^{-3}	9.34×10^{-4}	1.45×10^{-3}	1.64×10^{-3}	3.21×10^{-3}

[a] The amount of steroid in the system in all cases was 10^{-9} mole, and thus r does not represent a true r value indicating total binding capacity of the protein. [b] The r value refers to moles of steroid bound per mole of protein at the concentrations of steroid and protein present in the system. [c] Steroids ranked in order of decreasing r values: corticosterone > cortisone > cortisol > prednisone > prednisolone. [d] Steroids ranked in order of decreasing r values: corticosterone > cortisol > cortisone > prednisone > prednisolone.

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Toxicological Studies of 2-Mercaptoethanol

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Abstract ☐ The toxicity of 2-mercaptoethanol, a potential reaction product between residual ethylene oxide and sulfides in rubber medical devices sterilized by ethylene oxide, was investigated. This report includes LD$_{50}$ determinations for mice by both the intraperitoneal and oral routes of administration, as well as subchronic dosage studies. Both ethanol and sodium pentobarbital significantly decreased the acute toxicity potential of 2-mercaptoethanol, although pretreatment with atropine and with a combination of metal ions had no beneficial effects. 2-Mercaptoethanol was found to be more toxic to all tissues than ethanol but showed a marked decrease in such activity upon dilution.

Keyphrases ☐ 2-Mercaptoethanol—toxicity profile ☐ Toxicity profile—2-mercaptoethanol ☐ Sterilization residues, toxic—profile of 2-mercaptoethanol as potential reaction product between ethylene oxide and sulfides ☐ Ethylene oxide sterilization—toxicity profile of 2-mercaptoethanol as potential residue

The contemporary procedure of sterilization by ethylene oxide of rubber and plastic medical devices has provoked a number of pertinent questions as to potential health hazards such devices might propound. Bronsted *et al.* (1) demonstrated that ethylene oxide will react with a wide variety of nucleophilic agents, thus introducing the likelihood of production of reaction products from chemical contaminants in the steri-

lized articles. One such reaction product, the extremely toxic chlorohydrin 2-chloroethanol, was investigated by Guess (2). Guess and O'Leary (3) found another reaction product of ethylene oxide sterilization, 2-(2-hydroxyethylmercapto)benzothiazole, to be quite toxic to cells in culture and to mice.

A number of rubber devices, including tracheotomy tubes, in-dwelling catheters, multidose vial stoppers, bottle cap liners, and gloves are commonly sterilized with ethylene oxide. During the vulcanization of rubber with sulfur, it is likely that sulfides, including hydrogen sulfide, are formed. The formation of 2-mercaptoethanol by the mechanism proposed by Bronsted *et al.* (1) is then possible. The aforementioned medical materials come into both direct and indirect contact with various body tissues, including mucous membranes, epithelial and endothelial cells, muscle, and ocular tissues. Therefore, if these devices are to be used with safety, the toxic potential of any possible contaminants must be determined.

A search of the literature revealed a paucity of information on the systemic and specific tissue toxicity of 2-mercaptoethanol. Finley and Carlson (4) speculated that the LD$_{50}$ by intraperitoneal injection in mice was approximately 195 mg./kg. A patent authored by Utrumi