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# Detection of Drugs Using XAD-2 Resin. II: Analysis of Liver in Medical Examiner's Cases

Toxie agents tend to accumulate in body tissues as a result of the chemical nature of the substance and the biological composition and function of the tissue. Since liver is the primary site for biotransformation, drugs tend to concentrate there in quantities generally greater than those found in blood or other body compartments [*1-8*]. Because of its dynamic nature and the availability of sizable quantities as a result of medicolegal autopsies, liver has become an important component of a comprehensive toxicologic investigation. Classical liquid-liquid extraction techniques [*9-17*] for the isolation of drugs from liver are time-consuming and cumbersome, require large quantities of both tissue and organic solvents, are often limited in the scope of compounds detected, and are not easily adaptable to the simultaneous processing of a large number of samples. Amberlite® XAD-2, a nonionic polystyrene divinylbenzene resin, has been recently applied with success as a general adsorbent for the extraction of drugs from urine [*18-28*] and to a more limited extent from other specimens [*29-34*]. Preliminary studies concerning choice of resin, column and chromatographic conditions, recovery, and other studies have been reported [*35*]. This paper will discuss the application of XAD-2 resin techniques to the comprehensive analysis of liver for drugs.

## Materials and Methods

Resin preparation, column and chromatographic parameters, and analytical methods for ultraviolet spectroscopy (UV) and thin-layer (TLC) and gas chromatography (GC) were as previously reported [*35*].

### *Preparation of Aqueous Liver Phase*

Liver tissue was obtained from medical examiner's cases that were negative for drugs in blood, bile, or urine. Liver (20 g) was homogenized with 2*N* hydrochloric acid (pH 2) (100 ml), 2% hydrochloric acid (pH 3) (100 ml), or 3% tartaric acid (pH 4) (100 ml). A steam bath hydrolysis of the homogenate was conducted for 30 to 40 min. After cooling, the pH was adjusted to neutrality with 40% sodium hydroxide (approximately 20 ml) and then to pH 8 with solid sodium bicarbonate (2 to 3 g). The homogenate was centrifuged and the supernatant filtered over glass wool. The tissue residue was resuspended in

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water (100 ml), centrifuged, and filtered as before. The combined buffered supernatant was then poured onto the XAD-2 resin column. The eluting solvent was a mixture of ethyl acetate/1,2-dichloroethane (3:2) (100 ml). After evaporation of the solvent, drugs were analyzed by TLC and quantitated by either UV or GC techniques [*35*].

*Analysis of Blood*

Blood (5 ml) was extracted with chloroform (50 ml) and analyzed for barbiturates by the Broughton modification of the procedure of Goldbaum [*36*]. Basic drugs were extracted from blood (2 ml) with *n*-butyl chloride (6 ml) after adjustment to pH 8.5 to 9. The *n*-butyl chloride fraction was separated and extracted with 1*N* sulfuric acid (3 ml) and further analyzed by GC [*35*].

*Analysis of Liver for Acidic Drugs*

Liver (10 g) was homogenized with water (10 ml) according to the procedure of Sunshine et al [*37*]. The homogenate was extracted with chloroform (50 ml) and analyzed for barbiturates by the Broughton modification of the procedure of Goldbaum [*36*].

*Analysis of Liver for Basic Drugs*

Liver (2.5 g) was homogenized with water (10 ml) and sodium bicarbonate (100 mg). The homogenate was extracted with three times its volume of *n*-butyl chloride. The organic solvent was extracted with 1*N* sulfuric acid (3 ml) and analyzed with GC techniques [*35*].

## Results and Discussion

*Effect of Hydrolysis*

The effect of hydrolysis with various pH conditions on the recovery of pentobarbital, butalbital, codeine, meperidine, and morphine from liver is shown in Table 1. All cases studied were submitted for toxicologic analysis by medical examiners and therefore were biologically constituted with drugs. A pH of 2 was obtained by homogenizing the liver tissue with 2*N* hydrochloric acid, a pH of 3 by homogenizing with 2% hydrochloric acid, and a pH of 4 by homogenizing with 3% tartaric acid. In eight of nine cases, hydrolysis at pH 4 resulted in similar or lower recoveries than hydrolysis at pH 2 or 3. Barbiturate recoveries at pH 2 were 80 to 96% of those performed at pH 3. Codeine and meperidine recoveries were essentially the same at both pH 2 and pH 3. However, in the case of morphine a considerable increase in recovery was observed with pH 2, that is, 2*N* hydrochloric acid.

The major pathway for the detoxification of morphine is conjugation with glucuronic acid; consequently, morphine concentrates in tissue primarily as morphine glucuronide. It has been reported [*26*] that hydrolysis of biological samples liberates approximately 75% of the morphine present as the glucuronide. A stronger acid will hydrolyze the morphine glucuronide more effectively. Since codeine and meperidine are not concentrated primarily as glucuronides, the effect of the hydrolysis with a stronger acid is not as obvious as in the case of morphine. Therefore, 2*N* hydrochloric acid was selected for the preparation of the deproteinated aqueous phase. Pranitis et al [*31*] reported an increase in the UV adsorption spectrum of propoxyphene following acid hydrolysis. However, a steam bath hydrolysis of 20 min did not result in any significant increase in the UV absorption of propoxyphene. The increase was observed when the hydrolysis was performed for 60 min or longer.

TABLE 1—*The effect of hydrolysis at different degrees of acidity on the concentration of drugs recovered from liver.*[a]

| Case | Drug | Concentration, mg/100 g | | |
|---|---|---|---|---|
| | | pH 2 2*N* Hydrochloric Acid | pH 3 2% Hydrochloric Acid | pH 4 3% Tartaric Acid |
| 1 | pentobarbital | 3.8 | 4.7 | 4.4 |
| 2 | pentobarbital | 11.5 | 11.8 | 11.2 |
| 3 | pentobarbital | 6.5 | 7.1 | 6.3 |
| 4 | pentobarbital | 6.5 | 7.2 | 5.9 |
| 5 | butalbital | 3.5 | 4.1 | 3.5 |
| 6 | codeine | 1.5 | 1.3 | 1.3 |
| 7 | codeine | 1.2 | 1.2 | 0.9 |
| 8 | meperidine | 2.0 | 2.0 | 2.0 |
| 9 | morphine | 0.7 | 0.1 | 0.1 |

[a]Results of a minimum of two analyses for each case. Values corrected for losses determined for the procedure.

*Efficiency of XAD-2 Resin in Extracting Drugs from Liver*

Table 2 shows the percentage of recovery of drugs added to water, aqueous liver extract, and liver homogenate. When drug standards were added to the supernatant aqueous liver extract rather than to the liver homogenate, recoveries of pentobarbital, phenobarbital, codeine, morphine, meperidine, and methadone were very close to the aqueous standard recoveries. These comparisons do not include corrections for TLC and resin losses. However, when drugs were added to the liver homogenate an additional loss, varying from 22 to 55%, was observed for the four drugs tested, indicating that significant amounts of drugs have become bound to the tissue pellets. Repeated washing with water of the tissue pellets (more than twice) did not improve recovery and caused difficulty in centrifugation of the particulate matter suspended in the water. Even when the remaining tissue was rehydrolyzed with 2*N* hydrochloric acid instead of simply being washed with water, recovery did not improve. Niyogi et al [*38*] obtained similar results with codeine and strychnine. The recovery of drugs added to minced liver was 62% for codeine and 39% for strychnine while recovery for codeine and strychnine added to the liver extract obtained after centrifugation of liver homogenate was found to be 92 and 95%, respectively.

Table 3 shows the recovery of three basic and four acidic drugs during the XAD-2 resin extraction of liver homogenates. Large quantities of drug were shown to be trapped in the tissue pellets with losses ranging from 22 to 55%. Other losses, ranging from 10 to

TABLE 2—*Percent recovery of drugs added to water, aqueous liver extract, and liver homogenate.*

| Drug | Water | | Aqueous Liver Extract | | Liver Homogenate | |
|---|---|---|---|---|---|---|
| | Mean | *s* | Mean | *s* | Mean | *s* |
| Pentobarbital | 65 | 10.1 | 63 | 6.8 | 32 | 8.6 |
| Phenobarbital | 47 | 4.1 | 44 | 2.9 | ... | ... |
| Codeine | 71 | 7.8 | 64 | 5.6 | 49 | 11.3 |
| Morphine | 56 | 7.8 | 56 | 6.4 | 38 | 9.6 |
| Meperidine | 61 | 6.7 | 60 | 1.7 | 20 | 4.9 |
| Methadone | 24 | 3.3 | 21 | 3.3 | ... | ... |

TABLE 3—*Losses of drugs at different stages during the XAD-2 resin extraction of liver.*

| | Amobarbital | | Pentobarbital | | Phenobarbital | | Secobarbital | | Morphine | | Codeine | | Meperidine | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | *s* | Mean | *s* | Mean | *s* | Mean | *s* | Mean | *s* | Mean | *s* | Mean | *s* |
| Amount added, μg | 100 | ... | 100 | ... | 100 | ... | 100 | ... | 100 | ... | 100 | ... | 100 | ... |
| Trapped in tissue pellets, μg | 44[a] | 7.8 | 53[a] | 5.6 | 55[a] | 9.1 | 47[a] | 8.2 | 36[a] | 10.9 | 22[b] | 6.7 | 41[b] | 2.1 |
| Other losses,[c] μg | 22 | ... | 22 | ... | 18 | ... | 28 | ... | 24 | ... | 10 | ... | 23 | ... |
| Total losses, μg | 66 | ... | 75 | ... | 73 | ... | 75 | ... | 60 | ... | 33 | ... | 64 | ... |
| Amount recovered,[d] % | ... | ... | 32 | 8.6 | ... | ... | ... | ... | 38 | 9.6 | 49 | 11.3 | 20 | 4.9 |
| Corrected recovery, % | ... | ... | 107 | ... | ... | ... | ... | ... | 98 | ... | 82 | ... | 84 | ... |

[a]Losses determined by RIA.
[b]Calculated losses.
[c]Adsorption, desorption, and TLC loss.
[d]Average of a minimum of twelve analyses.

24%, were similar to the combined adsorption and desorption losses, ranging from 11 to 24%, seen with aqueous solutions [*35*]. Corrected recoveries for pentobarbital, morphine, codeine, and meperidine were calculated by adding total losses at various steps of the procedure to the amount recovered from the spiked liver homogenate. The correction factors were 3.1, 2.6, 2.0, and 5.0 for pentobarbital, morphine, codeine, and meperidine, respectively.

*Scheme for Drug Detection*

A scheme for the detection of drugs in liver with XAD-2 resin is presented in Fig. 1. The plastic columns supplied in the Brinkmann mass screening system are convenient for the analysis of urine or bile where the sample size does not exceed the 25-ml reservoir.

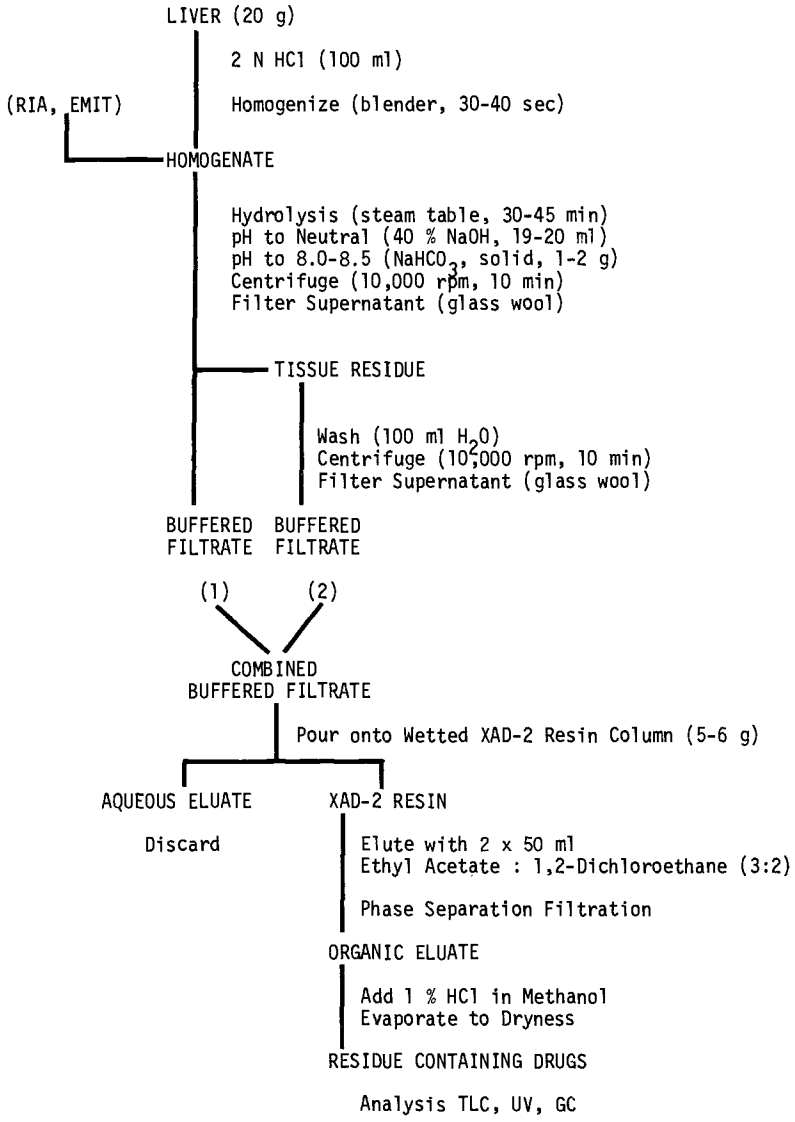


FIG. 1—*Scheme for detection of drugs in liver with XAD-2 resin.*

However, in liver analysis, the sample volume of the aqueous phase is generally about ten times this amount, and glass columns with 200-ml reservoir capacity were found to be more practical. A mild steam bath hydrolysis has been reported to improve drug recoveries [*31*]. Hydrolysis of the liver homogenate for 30 to 45 min in 2*N* hydrochloric acid did not affect the drugs tested (morphine, codeine, meperidine, propoxyphene, amobarbital, secobarbital, pentobarbital, and phenobarbital). The adjustment of pH prior to centrifugation of the liver homogenate was time-saving since additional particulate matter may become suspended in the aqueous phase if the pH is adjusted after centrifugation, thus requiring a second centrifugation step. Adsorption of drugs onto XAD-2 resin was effected at pH 8.0 to 8.5 since it is generally accepted that this pH is optimum for the simultaneous extraction of acidic, basic, and neutral drugs [*21,23,24,26*]. The polarity of the eluting solvent ethyl acetate/1,2-dichloroethane (3:2) was found to be optimum for desorption of drugs from the resin since it resulted in good drug recoveries (60% and above) and relatively clean extracts [*35*]. The sensitivity of the XAD-2 resin extraction procedure can be increased by using a larger amount of tissue. Preliminary studies have indicated that EMIT® urine assays can be applied directly to the liver homogenate.

This XAD-2 resin method is recommended as a general, comprehensive screening procedure for drugs in liver tissue. The method is rapid, does not require any specialized equipment, and is suitable for the simultaneous processing of a large number of samples. The small quantity of tissue used requires only a small quantity of the organic solvents. The XAD-2 resin is relatively inexpensive and once prepared can be used for at least a year. More than 20 acidic, basic, and neutral drugs commonly encountered in forensic toxicology have been effectively detected by the procedure. Drug losses during the preparation of the aqueous liver extract are rather large, ranging from 22% (estimated standard deviation $s = 6.7$) for codeine to 55% ($s = 9.1$) for pentobarbital (Table 3). Only small amounts of drugs (0 to 5%) are not adsorbed by the resin, and losses of 6 to 40% were observed during the elution of the different drugs from the resin (Table 3). Therefore, if the procedure is to be used for quantitative purposes, it is necessary to determine a correction factor that would take into consideration all the losses resulting during the procedure for each drug. Alternatively, a standard liver sample can be carried through the procedure to determine a correction factor for a given drug, or one may resort to a more specialized method for quantitation.

### *XAD-2 Resin Extraction Method Compared to Other Procedures*

Although the losses reported for different drugs during the various steps of the XAD-2 extraction procedure seem rather large, this is not unusual when one reviews recovery studies conducted with other extraction procedures. Table 4 presents recoveries of drugs from liver with the XAD-2 resin and a number of different extraction procedures. Results with the XAD-2 resin method have been corrected for the losses determined for the procedure. Four drugs, pentobarbital, morphine, codeine, and meperidine, are compared. In many cases, the procedure reported in the literature was developed for a single drug rather than as a comprehensive screening procedure [*6,39,40*], although some procedures were designed for groups of drugs [*41-43*]. Bonnichsen et al [*44*] and Moghrabi and Curry [*42*] describe procedures for the extraction of barbiturates, but successful results have not been obtained by applying the same procedures to the analysis of basic drugs. On the other hand, the method of Abernethy et al [*41*] was useful for basic drugs only. Berman and Wright [*45*] reported satisfactory recoveries for codeine, but morphine, meperidine, and methadone were not easily detected.

Recovery for pentobarbital (107%) is similar in six of the eight cases reported, with values ranging from 19 to 97%. Reported recoveries for morphine ranged from 26 to 98% in eight different procedures. Only three of these values were close to the morphine recovery by the XAD-2 resin procedure (98%). Codeine recovery with XAD-2 resin (82%)

TABLE 4—*Comparison of the recovery of drugs using the XAD-2 resin method with recoveries using other extraction procedures reported in the literature.*

| Drug | Recovery, % | | Reference |
|---|---|---|---|
| | XAD-2 Method | Other Methods | |
| Pentobarbital | 107 | 97 | *42* |
| | | 86 | *42* |
| | | 19 | *42* |
| | | 96 | *42* |
| | | 97 | *42* |
| | | 30 | *43* |
| | | 81 | *43* |
| | | 91 | *43* |
| Morphine | 98 | 26 | *46* |
| | | 37-46 | *46* |
| | | 50 | *54* |
| | | 80 | *12* |
| | | 98 | *39* |
| | | 95 | *40* |
| | | 75-80 | *41* |
| | | 92 | *31* |
| Codeine | 82 | 76 | *45* |
| | | 62 | *38* |
| | | 75-80 | *41* |
| Meperidine | 84 | 75-80 | *41* |
| | | 94 | *6* |

was somewhat better than any of the values resulting from the three other procedures. Meperidine recovery (84%) compared favorably to those recoveries reported by Abernethy et al [*41*] and Kazyak [*6*]. Most of the procedures reported in Table 4 require large amounts of liver, 100 to 400 g [*12,38,41,46*], which consequently require large amounts of organic solvents for extraction. For example, the method of Curry and Phang [*12*] requires more than 1.0 litre of ethanol for the extraction of 250 g of liver. It should be noted that most of the reports cited do not indicate whether the resulting recoveries represent corrected or uncorrected values.

### *Medical Examiner's Cases*

The following drugs and metabolites were detected in liver by the XAD-2 resin extraction procedure in 45 medical examiner's cases collected over a period of twelve months: amitriptyline (nortriptyline), amobarbital, butalbital, caffeine, chlordiazepoxide, codeine, diazepam, diphenylhydantoin, doxepin (nordoxepin), glutethimide, imipramine, meperidine (normeperidine), lidocaine, meprobamate, methadone, methaqualone, morphine, nicotine, pentazocine, pentobarbital, phenobarbital, propoxyphene (norpropoxyphene), quinine, secobarbital, thiopental, and thioridazine. This represents a wide variety of drugs commonly abused and encountered, including narcotics, analgesics, hypnotics, sedatives, and tranquilizers.

Table 5 shows the concentrations of barbiturates found in twelve liver samples with the XAD-2 resin method and the direct extraction of tissue with chloroform, as well as blood levels and liver/blood ratios. Pentobarbital was detected in Cases 1 through 5. Concentrations found with the XAD-2 resin procedure were 1.7 to 5.4 times greater than with direct chloroform extraction. In Cases 6 and 7 the combined concentration of amobarbital and secobarbital was determined, and the XAD-2 resin results were found to be 3.2 and 15 times greater than those found with chloroform extraction. Cases 8 and 9 involved amo-

TABLE 5—*Liver and blood concentrations*[a] *and liver/blood ratios of barbiturates in medical examiner's cases.*

| Case | Drug | Blood | XAD-2 | | Direct Chloroform Extraction | |
|---|---|---|---|---|---|---|
| | | | Liver[b] | Liver/Blood Ratio | Liver | Liver/Blood Ratio |
| 1 | pentobarbital | 2.0 | 25.5 | 12.7 | 5.9 | 2.9 |
| 2 | pentobarbital | 1.6 | 10.8 | 6.7 | 6.5 | 4.1 |
| 3 | pentobarbital | 1.2 | 8.5 | 7.1 | 4.0 | 3.3 |
| 4 | pentobarbital | 2.5 | 8.1 | 3.2 | 1.5 | 1.1 |
| 5 | pentobarbital | 1.3 | 5.0 | 3.6 | 1.4 | 1.1 |
| 6 | amobarbital and secobarbital | 2.1 | 10.3 | 5.0 | 3.2 | 1.5 |
| 7 | amobarbital and secobarbital | 0.7 | 3.0 | 4.3 | 0.2 | 0.3 |
| 8 | amobarbital | 0.2 | 1.1 | 5.5 | 0.3 | 1.5 |
| 9 | butalbital | 1.2 | 5.2 | 4.5 | 2.6 | 2.2 |
| 10 | phenobarbital | 2.9 | 10.7 | 3.4 | 5.1 | 0.7 |
| 11 | phenobarbital | 3.7 | 5.6 | 1.5 | 3.4 | 0.9 |
| 12 | phenobarbital | 1.5 | 3.1 | 2.1 | 1.4 | 0.9 |

[a]Blood in mg/100 ml and liver in mg/100 g.
[b]Concentrations corrected for losses determined for the procedure.

barbital and butalbital, respectively. Concentrations with XAD-2 resin extraction were 3.7 and 2.0 times greater than those resulting from chloroform extraction. Phenobarbital was detected in the last three cases. Concentrations found with XAD-2 extraction were 1.6 to 2.2 times those of chloroform extraction. Overall, the corrected concentrations found with the XAD-2 resin procedure were 1.6 to 5.4 times greater than the results of direct chloroform extraction, except in Case 7, in which the XAD-2 procedure resulted in a value 15 times greater than that of direct chloroform extraction. The relatively lower results obtained with direct chloroform extraction may be attributed to the fact that no hydrolysis was included in this procedure.

The liver/blood ratios for barbiturates ranged from 3.2 to 12.7 with a mean of 5.0 and standard deviation of 2.9 when the XAD-2 resin extraction procedure was used. With the direct chloroform extraction the liver/blood ratios ranged from 0.3 to 4.1 with a mean of 1.7 and standard deviation of 2.9. Liver/blood ratios reported by Sunshine et al [*37*] with the direct chloroform extraction method ranged from 2.2 to 9.3 with a mean of 5.1 and standard deviation of 2.9. The liver always shows higher concentrations of barbiturates than blood, usually at least twice and sometimes as much as a 20-fold increase [*1*]. It has been suggested that high liver/blood ratios (greater than 4) prove recent intake, that is, ingestion within 5 h prior to death [*47*]. Of the twelve cases presented in Table 5, five cases (1, 2, 3, 6, and 10) were defined as acute barbiturate intoxications. Four cases (4, 5, 9, and 11) were multiple drug intoxication deaths. In Case 7, death was attributed to probable adverse reaction from combined drug therapy, and in Cases 8 and 12 death was caused by factors other than drugs.

The liver/blood ratios (XAD-2 resin) for phenobarbital were lower than those of the other barbiturates. For phenobarbital the values ranged from 1.5 to 3.5 with a mean of 1.8 and a standard deviation of 0.4; amobarbital and secobarbital gave values ranging from 4.3 to 5.5 with a mean of 4.9 and a standard deviation of 0.6; for pentobarbital the range was 3.2 to 12.7 with a mean of 6.7 and standard deviation of 3.8. The lower ratios for phenobarbital can be attributed to the fact that it is six to eight times less lipid-soluble than the other barbiturates. Liver/blood ratios should be interpreted with caution since they may vary with the laboratory method and technique used.

Table 6 compares the results obtained by analyzing liver for basic drugs with the

TABLE 6—*Liver and blood concentrations*[a] *of basic drugs in medical examiner's cases.*

| Case | Drug | Liver | | Blood |
|---|---|---|---|---|
| | | XAD-2[b] | *n*-Butyl Chloride | |
| 13 | codeine | 0.9 | 0.3 | 0.2 |
| 14 | codeine | 0.5 | 0.1 | 0.4 |
| 15 | codeine | 0.4 | 0.3 | 0.9 |
| 16 | codeine | 1.0 | 0.1 | ... |
| | morphine | 1.4 | ... | ... |
| 17 | meperidine | 1.2 | ... | 0.7 |
| 18 | meperidine | 1.4 | ... | 0.7 |
| 19 | propoxyphene | 1.5 | 1.7 | 0.2 |
| 20 | propoxyphene | 5.4 | 2.1 | 1.1 |
| 21 | propoxyphene | 0.5 | 0.4 | 0.1 |
| 22 | propoxyphene | 0.6 | 0.9 | 0.1 |
| 23 | propoxyphene | 3.0 | 1.2 | 0.7 |
| 24 | amitriptyline | 0.7 | 0.1 | 0.1 |
| 25 | amitriptyline | 1.0 | 0.7 | 0.1 |
| 26 | amitriptyline | 0.2 | 0.6 | 0.1 |
| 27 | amitriptyline | 0.1 | 0.1 | ... |
| 28 | imipramine | 0.2 | 0.6 | 0.1 |
| 29 | methaqualone | 0.1 | 0.1 | 0.4 |
| 30 | lidocaine | 0.1 | 0.6 | ... |
| 31 | phencyclidine | 0.1 | 0.1 | ... |
| 32 | pentazocine | 0.2 | ... | 2.1 |

[a]Blood in mg/100 ml and liver in mg/100 g.

[b]Concentrations in Cases 13 to 28 were corrected for losses determined for the procedure. Cases 29 to 32 were not corrected.

XAD-2 resin procedure and direct *n*-butyl chloride extraction. Blood concentrations are included when available. Direct extraction of liver tissue was performed with *n*-butyl chloride since chloroform extracts significant concentrations of extraneous biological artifacts, producing chromatographic responses that interfere with GC quantitation of drugs [*48*]. The results in the first six cases (four codeine, one morphine, and two meperidine) were corrected for the losses determined for the XAD-2 procedure [*35*]. By using corrected results, meperidine and codeine were on the average four times (range, 1.3 to 10) greater than those obtained with direct *n*-butyl extraction. In the cases involving propoxyphene, amitriptyline, and imipramine, the correction factor was determined based on the values obtained from liver standards that were analyzed by the same procedure concomitantly with the test samples. Only 10% of each of the three drugs was recovered when added to the liver homogenate, thus necessitating the use of a high correction factor.

Corrected concentrations of propoxyphene resulting from XAD-2 resin extraction ranged from 0.5 to 5.4 mg/100 g with a mean of 2.2 mg/100 g ($s = 2$). In three of the five corrected propoxyphene cases XAD-2 resin results were on the average twice as high as *n*-butyl chloride values and in the other two cases the XAD-2 resin values were 1.3 times lower than the *n*-butyl chloride values. Postmortem liver concentrations for propoxyphene reported in the literature vary. Cravey et al [*49*] report levels ranging from 0.5 to 55 mg/100 g. In 16 cases where death was attributed to propoxyphene, McBay et al [*50*] report liver concentrations ranging from 1.5 to 20 mg/100 g, with a majority ranging from 5 to 12 mg/100 g. Thompson et al [*51*] found postmortem propoxyphene concentrations in 17 cases to range between 3.1 and 12.6 mg/100 g. McBay [*52*], in six propoxyphene fatalities, reports liver levels ranging from 0.57 to 22.94 mg/100 g.

Corrected amitriptyline concentrations with XAD-2 resin ranged from 0.2 to 1.0 mg/100 g with a mean of 0.6 mg/100 g ($s = 0.4$). Two out of three amitriptyline cases

showed results 1.5 and 20 times greater than those with *n*-butyl chloride extraction, while in one case the XAD-2 resin result was three times lower than the *n*-butyl chloride value. Postmortem liver concentrations of amitriptyline reported by Bonnichsen et al [*3*] ranged from 2.6 to 45 mg/100 g.

Robinson and Williams [*53*] have shown that liver concentrations of morphine in fatal heroin or morphine intoxications ranged from 0.1 to 2.1 mg/100 g. Felby et al [*7*] report concentrations in a similar range of 0.04 to 1.8 mg/100 g. Since XAD-2 resin extraction recovers approximately 40% of the morphine present, detection of low concentrations of this drug can be difficult. Whenever the presence of morphine was suspected a preliminary liver screening test using EMIT® or radioimmunoassay (RIA) techniques was included. The high sensitivity of RIA (about ten times that of EMIT or TLC) was useful in the detection of trace amounts of morphine in liver. In seven cases in which morphine was detected in bile or urine, its presence in liver could be demonstrated only by RIA.

The XAD-2 resin extraction procedure can be successfully applied to detection of acidic, basic, and neutral drugs in liver in medical examiner's cases. Barbiturate recoveries were satisfactory when compared to direct chloroform extraction. Quantitation of barbiturates should be performed with the use of an appropriate correction factor. Because of the low recoveries of certain basic drugs, the XAD-2 resin method is not the method of choice for quantitation of these drugs. However, the method is sufficiently sensitive to detect toxic and lethal concentrations that are of concern in medicolegal cases and may be used quantitatively with caution.

## Summary

Liver tends to concentrate drugs in quantities generally higher than those found in blood or other body compartments. This fact as well as the general availability of liver in postmortem cases makes it an important specimen for comprehensive toxicologic investigation. A scheme for the analysis of liver for drugs with tissue hydrolysis, XAD-2 resin extraction, and TLC has been developed and the parameters affecting recovery have been studied. The hydrolysis of liver specimens at various pH conditions resulted in an improved recovery for morphine by using pH 2 (2*N* hydrochloric acid). Recoveries of barbiturates, codeine, and meperidine were essentially the same at pH 2 and pH 3. A considerable loss (22 to 55%) was observed for four drugs (pentobarbital, morphine, codeine, and meperidine) as a result of drug binding to the tissue pellets during the process of centrifuging the liver homogenates. This method is recommended as a comprehensive screening procedure for drugs in liver tissue. For quantitative purposes, however, it is necessary to determine a correction factor for all the losses occurring at the various steps of the procedure. This procedure compared favorably with other procedures for liver analysis reported in literature.

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