for the determination of the low nanogram concentrations of I in plasma following administration of therapeutic doses to humans (6, 7). However, none of the reported procedures can approach the simplicity, sensitivity, and speed of radioimmunoassay. We now report a specific and sensitive radioimmunoassay for I that permits its quantitation directly in plasma or blood.

To obtain antibodies to I, an immunogen first was prepared by covalently coupling 3-hemisuccinyloxyflunitrazepam to bovine serum albumin using the mixed anhydride procedure of Erlanger *et al.* (8). The resulting conjugate consisted of 18 moles of the hapten coupled to 1 mole of albumin. Rabbits were immunized intradermally, and the antiserum with the highest titer (1:3000 dilution) of antibodies to I was used.

The radioligand used for the assay was [*methyl*-^{3}H]-flunitrazepam[2] with a specific activity of 87.5 Ci/mmole. Prior to use, radiolabeled I was purified by TLC on silica gel with chloroform–acetone (4:1) as the solvent system.

The radioimmunoassay was carried out in a manner identical to that described recently for diazepam (9). A logit–log calibration curve for I was linear from 15 to 1000 pg/tube; thus, a working limit of sensitivity of 0.15 ng/ml was obtained using 0.1 ml of plasma. This value represents about a fivefold increase in sensitivity compared to electron-capture GLC techniques. However, for routine analysis of plasma and blood from subjects receiving chronic therapeutic doses of I, such sensitivity is unnecessary and a 10-μl sample is more appropriate for analysis. The intra- and interassay coefficients of variation did not exceed 10% over a range of 1.65–10 ng of I/ml in a selection of random clinical samples.

The antiserum specificity was determined initially by its cross-reactivity with the known metabolites of I present in blood and with the benzodiazepine drugs, diazepam, *N*-desmethyldiazepam, flurazepam, and nitrazepam. For each compound tested, cross-reactivity was <1% relative to I (100%); this finding demonstrated that the 1-methyl, 7-nitro, and 2′-fluoro groups on the hapten were potent antigenic determinants and indicated that the antiserum was highly specific for I.

Further evidence for the specificity of the radioimmunoassay procedure was obtained by comparison with an electron-capture GLC method (7). The joint determinations for I in 20 plasma and 12 whole blood samples from subjects who received a 2-mg dose of the drug were subjected to linear regression analysis by a method that takes into account differences in the precision of both analytical procedures (10). The correlation coefficient, regression line slope, and y-intercept were 0.98, 0.93, and 0.13, respectively, over a range of 0.5–18 ng/ml. Furthermore, the two groups of data were not significantly different ($p > 0.05$) using a t test. These statistical parameters indicate that the radioimmunoassay and electron-capture GLC methods yield equivalent results.

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Calcium Binding by Arteriographic Contrast Media

Keyphrases □ Arteriographic contrast media—calcium binding, effect of additives in media on calcium binding *in vitro* □ Calcium—binding in plasma by arteriographic contrast media, effect of additives in media □ Diatrizoate meglumine sodium—arteriographic contrast medium, effect on calcium binding □ Binding—calcium to ionic arteriographic contrast media, effect of additives

To the Editor:

Selective coronary arteriography with common ionic contrast media is associated with a decrease of myocardial force, which was suggested to be due to decreased ionized calcium levels in the blood perfusing the myocardium (1, 2). This depressant action has been attributed exclusively to the potent calcium-binding ligands added to contrast media for anticoagulation, stabilization, and buffering (1–3). The calcium-binding properties of contrast media have not yet been reported.

To investigate the basis for the reduction of ionized calcium levels, fixed quantities of diluted contrast media were titrated with increasing amounts of calcium chloride *in vitro*. The following contrast media were evaluated: ioxaglate meglumine sodium[1] (59% sodium and methylglucamine *N*-(2-hydroxyethyl)-2,4,6-triiodo-5-{2-[2,4,6-triiodo-3-(*N*-methylacetamido)-5-(methylcarbonyl)-benzamido]acetamido} isophthalamic acid, I), lysine diatrizoate[2] [80% L-lysine 3,5-bis(acetamido)-2,4,6-triiodobenzoate, II], diatrizoate meglumine sodium[3] [76% sodium and methylglucamine 3,5-bis(acetamido)-2,4,6-triiodobenzoate, III], and diatrizoate meglumine sodium[4] with 0.32% sodium citrate and 0.04% edetate disodium [(ethylenedinitrilo)tetraacetic acid disodium salt] (IV).

[1] Hexabrix, Byk Gulden, Konstanz, West Germany.
[2] Peritrast-400, Dr. Franz Köhler Chemie, Alsbach, Bergstrasse, West Germany.
[3] Urografin-76, Schering AG, Berlin/Bergkamen, West Germany.
[4] Renografin-76, Squibb, Princeton, N.J.

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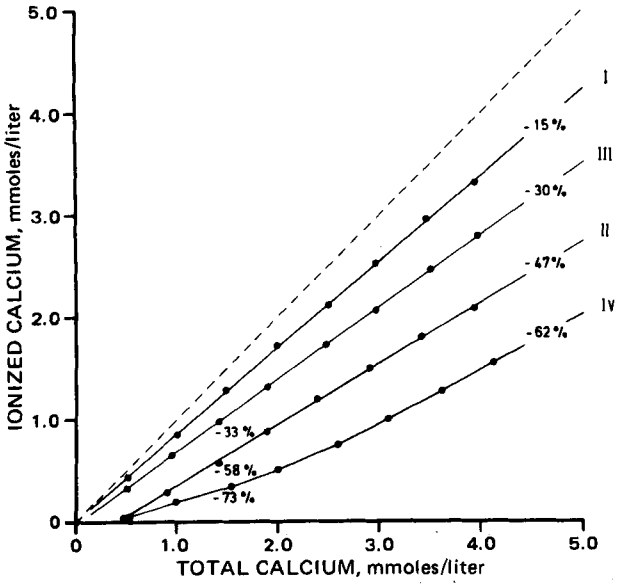

Figure 1—*Binding of added ionized calcium by contrast media (two parts) in a physiological saline solution (0.9% NaCl, seven parts). The roman numerals refer to contrast media (see text). Percentage values denote the reduction of ionized calcium levels below the total calcium content of 1.5 and 4.0 mmoles/liter. The expected line (i.e., no binding of ionized calcium) is shown as a dashed line with a slope of 1.*

The presence or absence of additives and their amounts were not analyzed; the compositions are given as presented in the manufacturer's data.

Total calcium content was determined by the fluorometric method[5] with correction for any edetate disodium present. Ionized calcium levels were measured by a calcium-sensitive flow-through electrode[6]. The reduction of ionized calcium levels below the expected line (Fig. 1) is consistent with calcium binding.

The shift in the lysine diatrizoate (II) titration curve, as well as the shift and the bend at 2 mmoles of total calcium/liter in one of the diatrizoate meglumine sodium (IV) curves, is believed to be due to the saturation of the calcium-chelating additives present in these contrast media (*e.g.*, 3.2 mg of citrate/ml and 0.4 mg of edetate disodium/ml in IV). The near-zero intercepts on the abscissa of the titration curves for ioxaglate meglumine sodium (I) and diatrizoate meglumine sodium (III) suggest a lack of large amounts of potent calcium-binding ligands in these contrast media. Deviations of the slopes of individual titration curves from the expected line after saturation of disodium edetate and citrate binding sites, or without any chelating additives present, indicate that calcium chelation is primarily an inherent property of ionic contrast media and not that of the additives.

The association constants (K_0) of radiopaque ligands (L) with calcium have been calculated on the basis of a 1:1 association by:

$$K_0 = \frac{[CaL]}{[Ca][L]} \qquad \text{(Eq. 1)}$$

Log K_0 values were 0.36 for the diatrizoate–calcium complex and 0.28 for the ioxaglate–calcium complex. However, the calculations do not necessarily signify the stability constants for well-defined chemical structures since the stoichiometry is not known. Values are below the known stability constants of human plasma protein–calcium complexes [log $K_0 \simeq$ 2–7 (4)]. Nevertheless, when the opacifying bolus is injected intracoronarily, the contrast molecules are more concentrated than the protein fraction in plasma and thus cause a marked reduction of plasma ionized calcium levels.

[5] Corning calcium analyzer 940, Vogel, Giessen, West Germany.
[6] Nova 2, Union Carbide, Newton, Mass.

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Effect of Sampling Probe Size on Dissolution of Tableted Drug Samples

Keyphrases □ Dissolution rates—effect of sampling probe size on dissolution rate of tableted drug samples, USP paddle method □ Dissolution testing systems—various sampling probe sizes evaluated for effect on dissolution rate of tableted drug samples □ Hydrodynamics—dissolution rates affected by sampling probe size, USP paddle method

To the Editor:

When *in vitro* dissolution rates were determined using the USP paddle method (1), some tablet formulations consistently gave higher dissolution rates when sampled with an automated sampling system than when sampled manually. This difference was traced to turbulence caused by the filter-tipped probes used in automated sampling. With the automated system used in this laboratory, the probes are suspended in the dissolution medium during the entire test.

Table I—Effect of Sampling Probes on Dissolution Rate

Probe	Probe Volume, mm^3	Dissolution[a], % of label claim	Increase in Dissolution, %
None	—	41.4	—
1	44	41.4	0
2	177	43.0	1.6
3	466	45.1	3.7
4	706	46.4	5.0
5	877	48.4	7.0

[a] Average of 12 tablets.

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