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Distribution of Morphine and Related Compounds in Human Tissues and Biological Fluids Using Radioimmunoassay Techniques

The utilization of chromatography, fluorometry, and spectrophotometry for the qualitative identification and quantitative determination of drugs and their metabolites in biological materials is well established [*1,2*]. The need to develop an even more sensitive and reliable procedure for the detection of narcotics, primarily morphine and related compounds, has never been so urgent as in the recent past.

The development of immunologic methods for assaying polypeptides, hormones, and drugs has, in the last few years, provided an invaluable tool for the determination of these constituents in biological specimens in nanogramic amounts [*3,4*].

Spector and Parker [*5*] made use of the ability of morphine to inhibit the binding of dihydromorphine-^{3}H to anti-carboxymethyl methyl morphine. Similar techniques were also advanced by Ryan et al [*7*]. Van Vounakis et al [*8*] developed a radioimmunoassay (RIA) for morphine that utilized a multivalent copolymer-carboxymethyl morphine conjugate in which the tyrosine of the copolymer is labelled with ^{125}I. This radioimmunoantigen was shown to be 10 to 1000 times more effective for detection of antimorphine than ^{14}C morphine. Adler and Liu [*9*] developed a similar technique based on hemagglutination inhibition. Spector and Flynn [*10*] have also reported on the development of a radioimmunoassay that can measure as little as 5 ng of barbiturate.

The purpose of this investigation is to apply radioimmunoassay procedures for the detection of narcotics (morphine and related compounds) in autopsy materials. The method described in this investigation is a modification of the assay previously described by Spector and Parker [*5*].

Materials and Methods

Assay Tubes

Assay tubes were obtained from Hoffmann LaRoche Pharmaceutical Co. in Nutley, N.J., and consisted of a series of 10 by 75 glass tubes containing the following ingredients: 0.1 ml of normal goat serum, 0.1 ml 1:50 dilution goat morphine antibody, 0.04 ml of dihydromorphine-^{3}H (approximately 4000 cpm), and 0.16 ml of phosphate buffered

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saline at pH 7.4. The assay tubes were refrigerated and used as needed. Reagents maintained at refrigeration temperature are stable for at least 4 to 6 months.

Preparation of the Organ Homogenates

Five grams of tissue were homogenized with 25 ml of normal saline in a Lourdes electric homogenizer, at maximum speed for 5 min. An aliquot of the homogenate extract was centrifuged in a table-model GL-International Centrifuge for 5 min. The supernatant was used directly for assay. Biological fluids such as blood, urine, and vitreous humor were assayed directly. Bile and highly viscous blood samples were diluted 1:5 with normal saline prior to the assay.

Radioimmunoassay Procedure

100 μl obtained from a tissue supernatant or biologic fluid were added to a separate assay tube using an Eppendorf pipet. The mixture was then vortex mixed and allowed to incubate at room temperature for 60 min. 0.5 ml of saturated ammonium sulfate solution was then added to each tube, and the precipitate vortex was mixed and incubated in the refrigerator (at approximately 10°C) for 15 min. Specimens were centrifuged for 10 min at 3000 rpm (International Co., Model DU). A 0.5-ml aliquot from the supernatant was then added to a liquid scintillation vial containing 12.0 ml of Bray's counting solution and mixed well. The radio activity was measured in a liquid scintillation spectrometer (Packard-Tricarb).

Standard Graphs and Recovery Studies

Morphine dilutions ranging from 12.5 to 200 ng/ml were added to the supernatants obtained from the various tissue homogenates or biological fluids. Figure 1 shows graphs which were obtained by plotting cpm versus morphine concentrations.

A linear relationship exists between morphine, in concentrations up to 100 ng/ml, and cpm. Above 100 ng/ml this linearity was not maintained and the possibility of error should be considered.

For recovery experiments, morphine sulfate, in concentrations ranging from 25 ng/ml to 200 ng/ml, was added to 5 ml of various tissue homogenates. Samples were mixed vigorously using a vortex and incubated at room temperature for 6 h. Mixtures were centrifuged and the supernatant was assayed for morphine. Table 1 shows the percent of recovered morphine from the tissue homogenates.

It was noted that highly viscous materials (some bile specimens), highly hemolized blood specimens, and biological fluids with acid reaction, showed higher cpm. No significant variations were noted when specimens were incubated at room or refrigeration temperatures and when the initial incubation period was reduced from 60 min to 30 min. It is important that resuspension of the precipitated protein does not take place after the final centrifugation.

Results and Discussion

Figure 1 shows the base value of the various biological fluids and tissues used in this study. Nearly all curves show a linear relationship in concentrations ranging from 0 to 100 ng/ml. Above this value the relationship is not linear (Fig. 2), thus introducing a certain degree of error.

The data in Table 1, on the percent recovery of morphine that was added to tissues in different concentrations, are self-explanatory. Values shown were obtained after triplicate assay carried over a period of three days and single saline extraction.

TABLE 1—*Amount of morphine spiked in liver, kidney, and brain homogenate and percent recovery from their supernatants.*

Organ	Unspiked Sample, cpm	S.E.M.[a]	Amount of Morphine Spiked, ng/ml	Spiked Sample, cpm	S.E.M.[a]	Amount of Morphine Recovered in Supernatant, ng/ml	S.E.M.[a]	Percent Recovery
Liver	1033	±50	200	2526	±54	160	±5.4	80
	1033	±50	100	1960	±60	87	±6.3	87
	1033	±50	50	1440	±20	38	±2.0	76
	1033	±50	25	1243	±13	20	+1.3	80
Kidney	1062	±47	200	2498	±65	150	±6.5	75
	1062	±47	100	1904	±17	94	±1.7	94
	1062	±47	50	1472	±58	46	±5.8	92
	1062	±47	25	1196	±29	15	±2.0	60
Brain	1160	±21	200	2627	± 5	148	±5.0	74
	1160	±21	100	1966	±17	86	±1.7	86
	1160	±21	50	1521	±40	38	±4.0	76
	1160	±21	25	1205	±35	12	±3.5	48

[a] S.E.M. = standard error of mean.

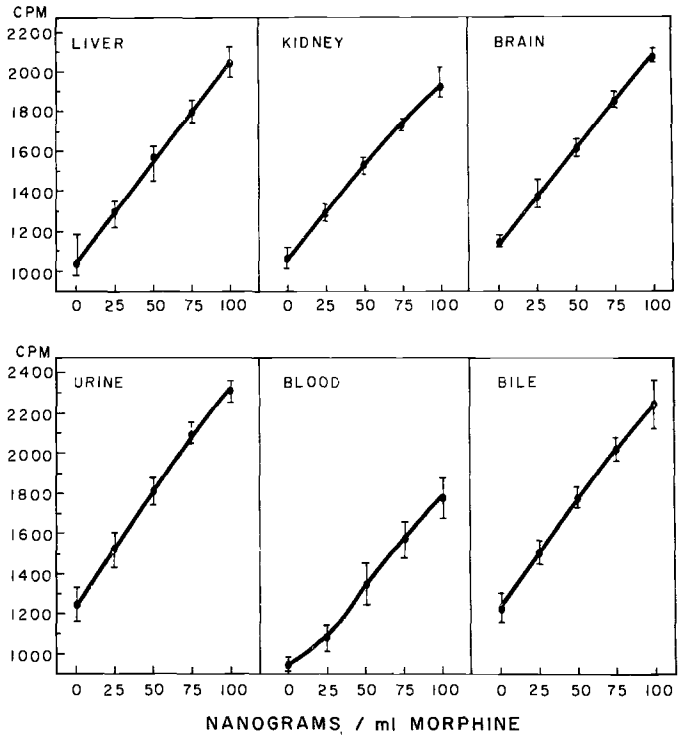

FIG. 1—*Standard calibration curves for tissues and biological fluids—linear relationship.*

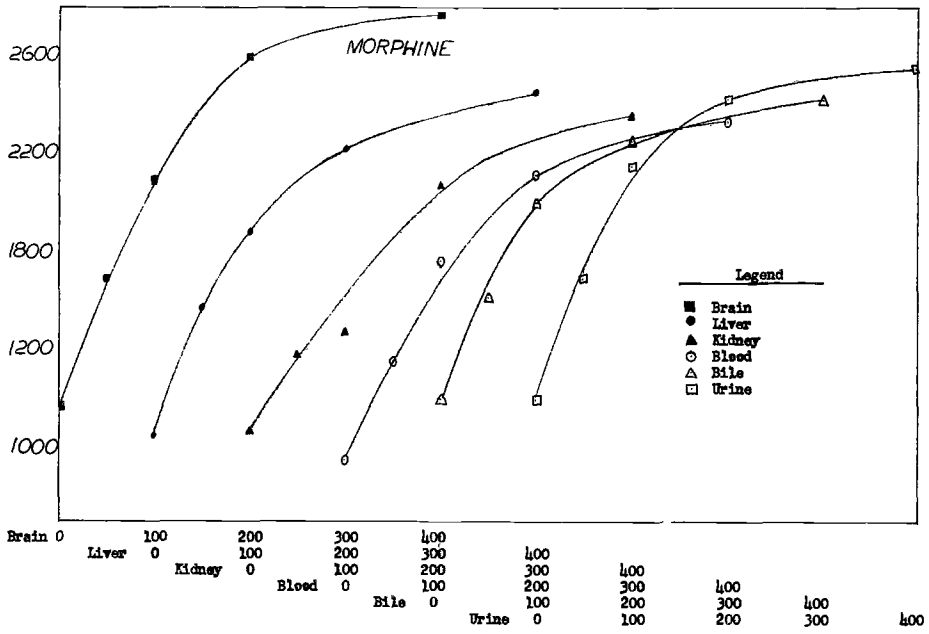

FIG. 2—*Standard calibration curves for tissues and biological fluids—nonlinear relationship—above 100 mg/ml.*

Table 2 reveals the levels of morphine distribution in the various organs and fluids. No definite significance can be attached to levels of morphine in the heart, lung, skin, spleen, and vitreous humor, due to insufficient number of specimens analyzed. Bile remains the specimen of choice in the detection of morphine, followed by kidney or liver or both. An examination of Table 3 indicates levels of morphine established by RIA technique in 66 cases submitted for toxicological analysis. Positive morphine identification was obtained using thin-layer chromatography (TLC), fluorometry, and chemical crystalloscopy. Negative results were obtained in another 100 toxicological cases using RIA, TLC, and fluorometry procedures. In addition, 16 other toxicological cases identified as positive by RIA were identified as codeine, methadone, meperidine, and phenothiazines, in concentrations of 200, 100, 300, and 300 ng, respectively.

TABLE 2—*Morphine distribution levels in organs and biological fluids.*

Sample	Number of Cases Analyzed	Concentration[a]
Bile	19	621
Urine	6	153
Kidney	29	93
Blood	20	84
Vitreous	2	74
Spleen	2	73
Liver	44	72
Lungs	3	62
Heart	1	37
Brain	43	35
Skin	4	20

[a] Bio-fluids, μg/ml; tissues, μg/100 g.

TABLE 3—*Concentration of morphine in μg percent in organs and biological fluids.*

No.	Sex	Age	Liver	Kidney	Brain	Blood	Bile	Other Organs and Fluids
1	M	20	81	75	19	41	450	urine 118, heart 37
2	M	20	76	90	49	60	...	
3	M	24	77	60	18	67	...	
4	M	21	90	115	62	72	...	
5	M	20	...	117	50	200	637	
6	M	25	92	96	76	61	...	
7	M	30	78	...	36	30	...	urine 187, skin 12
8	M	31	103	106	27	175	687	
9	M	2½	18	15	Neg.	...	240	
10	M	32	102	122	88	175	...	
11	M	18	83	99	...	105	700	
12	M	28	45	125	...	115	...	lung 15, spleen 67
13	M	27	77	89	...	75	639	urine 162, vitreous 75/73, skin 28
14	M	19	71	104	14	...	...	spleen 79, skin 31
15	M	28	59	97	6	...	...	
16	M	22	...	...	6	112	700	
17	M	3	15	21	trace	...	...	
18	M	20	125	150	53	...	...	
19	M	19	137	63	Neg.	43	...	
20	M	23	125	175	30	...	...	urine 200
21	F	17	65	125	64	...	...	
22	M	19	...	225	200	70	...	lung 150
23	M	29	36	67	8	...	...	skin 8
24	M	26	50	...	Neg.	...	...	
25	M	22	63	150	46	...	...	
26	M	36	75	90	8	...	...	
27	M	27	17	10	...	...	...	
28	M	44	...	...	...	...	697	
29	M	23	...	...	40	65	...	
30	M	24	30	...	Neg.	...	...	
31	M	35	...	...	...	...	165	
32	M	55	...	...	...	...	80	
33	M	21	...	...	...	...	...	urine 125
34	M	29	...	...	...	...	125	
35	M	20	...	...	...	...	...	urine 125
36	F	41	85	...	Neg.	...	...	
37	M	26	61	...	Neg.	...	...	
38	M	27	112	...	...	...	...	
39	M	19	...	...	...	...	117	
40	M	26	86	...	...	...	...	lung 23
41	M	30	...	...	...	...	550	
42	M	18	...	...	...	175	...	
43	M	30	62	...	18	...	...	
44	F	24	9	7	7	...	...	
45	M	18	76	...	66	...	...	
46	M	20	50	39	...	...	...	
47	F	37	...	...	...	38	...	
48	M	22	...	...	...	...	195	
49	M	26	125	...	21	...	...	
50	F	25	...	...	31	...	...	
51	F	19	...	...	...	18	...	
52	F	21	...	...	41	...	...	
53	M	12	26	...	40	...	...	
54	M	22	...	...	...	...	52	
55	M	20	6	...	Neg.	...	5254	
56	M	25	150	...	42	...	...	
57	M	40	18	5	trace	...	...	
58	M	27	200	150	20	...	...	
59	F	17	70	...	4	...	...	
60	M	21	17	125	Neg.	...	...	
61	F	16	...	...	...	Neg.	125	
62	F	43	7	...	Neg.	...	...	
63	M	17	77	...	16	...	...	
64	M	26	150	...	125	...	...	
65	M	17	...	...	...	...	250	
66	F	23	...	...	...	...	150	

The value of RIA as another analytical tool for the forensic toxicologist is evident. The assay is definitely rapid, simple, reliable, and unquestionably sensitive. It provides a direct assay method for biological fluids, and a simple saline extraction when tissues are investigated for narcotics and related drugs.

In this study it was noted that bile viscosity and blood hemolysis resulted in higher morphine levels. However, this interference can be eliminated if proper dilutions are made.

Although RIA lacks specificity, it certainly provides sensitivity; morphine can be detected in picogramic levels; and it has invaluable application in medicolegal cases with well-established histories of narcotic addiction. A negative RIA, no matter how reliable the case history may be, cannot justify the often used term: "Death due to acute intravenous narcotism."

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