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Tissue Redistribution of Fentanyl and Termination of Its Effects in Rats

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The kinetics of fentanyl elimination from plasma suggest its rapid and extensive uptake by tissues. The authors determined the relationships between tissue and plasma concentrations of fentanyl. Six rats injected iv with ^{3}H-fentanyl citrate (50 μg/kg) were sacrificed at each of the following times: 1.5, 5, 15, 30, 60, 120, and 240 min after injection. Tissues were analyzed for unchanged ^{3}H-fentanyl citrate and for total ^{3}H-radioactivity. Fentanyl effects were evident 10 s after injection; recovery began at 5 min and was complete within 60 min. Fentanyl concentrations in brain, heart, and lung equilibrated with that in plasma before 1.5 min and declined at the same rate ($t_{1/2\alpha}$ = 8 min, $t_{1/2\beta}$ = 45 min). Fentanyl uptake by muscle and fat was slower and equilibration with plasma occurred by 120 min. Muscle accumulated 56 per cent of the dose within 5 min by which time brain fentanyl levels had declined by 90 per cent. Only 6 per cent of the dose was in fat at 5 min but this increased to a maximum of 17 per cent at 30 min. Fentanyl was extensively metabolized; metabolites represented 25 per cent of body ^{3}H-radioactivity at 15 min, and 80 per cent at 4 h. The authors conclude that the short duration of fentanyl effect is due to its rapid redistribution from sites of action in the brain to sites of storage (muscle and fat) and biotransformation (liver). The elimination of fentanyl from the body is governed by its reuptake from storage sites and its metabolism in the liver. Most of the dose is ultimately excreted in the form of fentanyl metabolites in urine. (Key words: Analgesics: fentanyl. Anesthetics, intravenous: fentanyl. Biotransformation (drug). Pharmacokinetics: distribution, models.)

DETAILED STUDIES of the pharmacokinetics of ^{3}H-fentanyl citrate in plasma of dogs given an intravenous dose revealed the following facts.[1,2] 1) There was a close correlation between the intensity of ventilatory depression and the log-concentration of fentanyl in plasma. 2) Approximately 98 per cent of the dose was eliminated from plasma in the first 5 min after injection. 3) The ultimate elimination of fentanyl from plasma was a slow process ($t_{1/2\beta}$ = 3.3 h, range 2.3–5.0 h) and accumulation of the drug and of its respiratory depressant effects occurred after repeated intravenous doses. 4) Fentanyl was efficiently and extensively biotransformed to metabolites which accounted for most of the dose recovered in urine.

Pharmacokinetic analysis of the data led to the following hypotheses. 1) The initially rapid elimination of fentanyl from plasma is the result of its extensive uptake by body tissues. 2) The release of fentanyl from some tissues is slow and limits the rate of biotransformation which is necessary for the ultimate elimination of this lipophilic drug from the body. 3) The pharmacokinetics of fentanyl can be summarized by a three-compartment model and we speculate that the central compartment corresponded to the vessel-rich tissues (*e.g.*, brain, heart, lung, liver, kidney), and that the two peripheral compartments represented muscle and fat among other tissues. Fentanyl was thought to be eliminated from the central compartments primarily by biotransformation.

The objectives of the present study were to evaluate the validity of the previously described model of fentanyl disposition and to identify the tissues belonging to each compartment.

Materials and Methods

Unanesthetized male rats‡ weighing 152–294 g (215 ± 37 SD) were given a 50 μg/kg dose of tritium-labeled fentanyl citrate (hereafter referred to as "fentanyl") through the tail vein.§¶ Six rats were sacrificed by decapitation at each time interval (1.5, 5, 15, 30, 60, 120, and 240 min) following the injection. Blood flowing from the body was collected in beakers previously rinsed with heparin (1,000 units/ml) and dried. The brain was removed from the skull. The thorax and abdomen were incised and the liver, heart, lung, and both kidneys were removed. In addition, subcutaneous fat and muscle were excised from each thigh. The tissues were immediately wrapped in aluminum foil, frozen on dry ice, and stored in a freezer until the time of analysis.

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‡ CFE strain Sprague-Dawley rats, purchased from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

§ Muscular rigidity developed within 10–15 s of the intravenous injection in all animals in which the injection was satisfactory. When the drug solution extravasated in the tail the reaction did not develop and the animals were not included in the study.

¶ Doses and concentrations in this paper are expressed in terms of fentanyl citrate (mol. wt. 528.6); they can be converted to equivalents of fentanyl base (mol. wt. 336.5) by multiplying them by 0.64. ^{3}H-Fentanyl citrate was uniformly labeled with tritium on the aniline ring (specific activity 105 nCi/μg) and was generously supplied by Janssen Pharmaceutica, Beerse, Belgium.[1]

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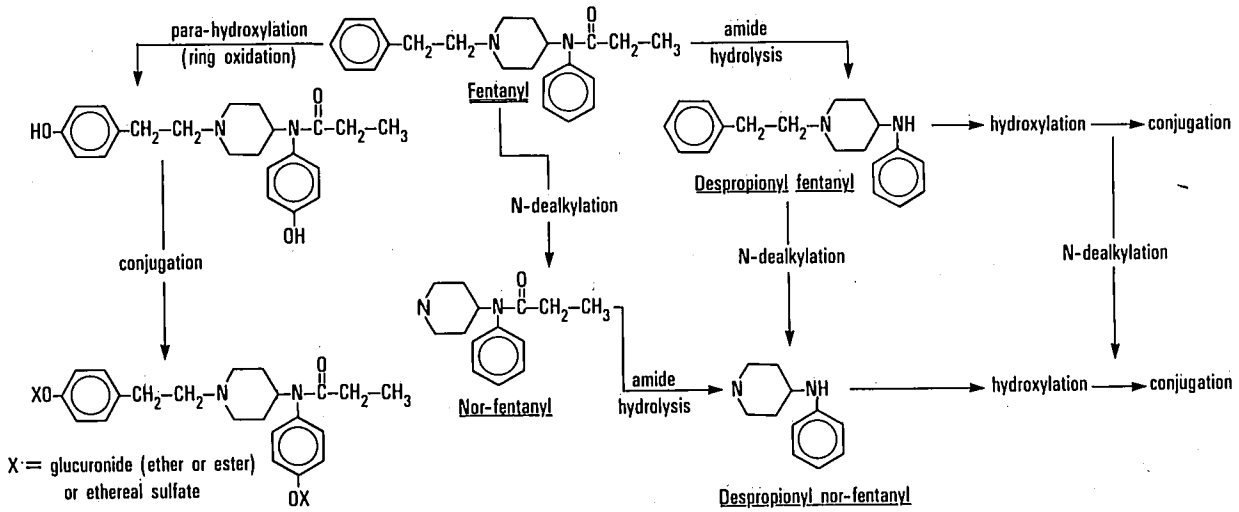

FIG. 1. Theoretical pathways of fentanyl biotransformation. Despropionyl fentanyl is formed by hydrolysis of the amide bond in fentanyl. Norfentanyl results from the N-dealkylation of the piperidine ring. Despropionyl norfentanyl could be the result of both reactions.

Tissues were thawed while wrapped in foil, weighed and placed in homogenizing tubes for analysis. Ten ml of 0.5 N HCl were added to the tubes containing brain, liver, kidney, or lung; they were homogenized using a grinder pestle. Five ml distilled water were added to tubes containing fat, heart, or muscle; the tissues were disrupted with a Polytron® sonicator** and then 5 ml 1 N HCl were added. The contents of each tube were thoroughly mixed (Vortex mixer) and duplicate 4-ml aliquots were pipetted to round bottomed bottles containing 1 ml nonradioactive fentanyl (0.1 mg/ml). After adjustment of the *p*H to 9–10 with approximately 1.5 ml of 2.5 N NaOH, the samples were analyzed for unchanged ^{3}H-fentanyl by extraction into toluene.[1] To determine total ^{3}H-radioactivity, 0.2-ml aliquots of the tissue homogenate were placed in empty scintillation vials to which 10 ml BBS-3-Dilufluor® were subsequently added.[1] Plasma samples were refrigerated and then analyzed for unchanged ^{3}H-fentanyl and for total ^{3}H-activity.[1] Recovery of known quantities of ^{3}H-fentanyl added to homogenates of tissues from rats that received no drug injections averaged 96 ± 2.5 SD per cent. The difference between total radioactivity and fentanyl concentrations was taken as an estimate of the concentration of metabolites in each sample. Data are presented as the mean (±SEM) concentrations determined in the tissues obtained from six rats.

Fentanyl levels expressed as "per cent of dose" were calculated as the product of fentanyl concentration and the mass of tissue in the body of a normal, lean adult rat (200 g),[3] divided by the dose and multiplied by 100.

** Model no. PT10-35, Brinkmann Instruments, Westbury, N. Y.

Per cent of Dose

$$= \frac{\text{Fentanyl (ng/g)} \times \text{Tissue mass (g)}}{\text{Dose (50 } \mu\text{g/kg} \times 0.2 \text{ kg)} \times 10^3} \times 100$$

Identification of Metabolites in Toluene Extracts of Liver and Kidney

A specimen of liver from each of two rats sacrificed at each time interval after injection was extracted into toluene according to the procedure described above for the analysis of unchanged ^{3}H-fentanyl. Similar toluene extracts were prepared from the combined six kidneys for animals sacrificed at 30, 60, and 240 min. Each of the toluene extracts was evaporated to dryness under vacuum at 40°C. The residue was dissolved in methanol and an aliquot containing at least 3,000 cpm was applied to Whatman No. 3 paper that had previously been soaked in 0.2 M phosphate buffer (*p*H 6.0) and dried.[4] Authentic samples of fentanyl citrate, despropionyl fentanyl, norfentanyl, and despropionyl norfentanyl were applied to each paper as standards (fig. 1). The paper was equilibrated in the solvent system overnight, the chromatogram developed the next day in either the acidic or basic solvent systems, and the radioactivity and authentic compounds localized as described previously.[5]

Pharmacokinetic Analysis

Following intravenous injection, the fall in the concentration of unchanged fentanyl in plasma was biphasic. A curve could be fitted to the data points by nonlinear least-squares analysis and could be described by a biexponential equation as follows[1]:

$$C_{p(t)} = A \exp^{-\alpha t} + B \exp^{-\beta t}$$

where C_p = concentration of unchanged fentanyl in plasma at any time (t) after an intravenous injection, A and B = the ordinal intercepts of the individual exponential lines composing the log-concentration/time curve computed from the least-squares analysis of the data, α and β = slopes of the individual exponential lines and the first-order rate constants determined by the least-squares analysis of log-concentration *vs.* time.

Results

The effects of fentanyl were evident within 10 s after its intravenous injection as a transient muscular rigidity followed by relaxation at 30 s and unresponsiveness at 2 min. A few rats became apneic for a period of 1–2 min and received thoracic compression to assist their ventilation during that period. All animals survived, began spontaneous movement at 5 min, regained their righting reflex at 16 ± 2 min (range 7–38 min), and appeared to have recovered at 29 ± 2 min (range 13–60 min) following the injection.

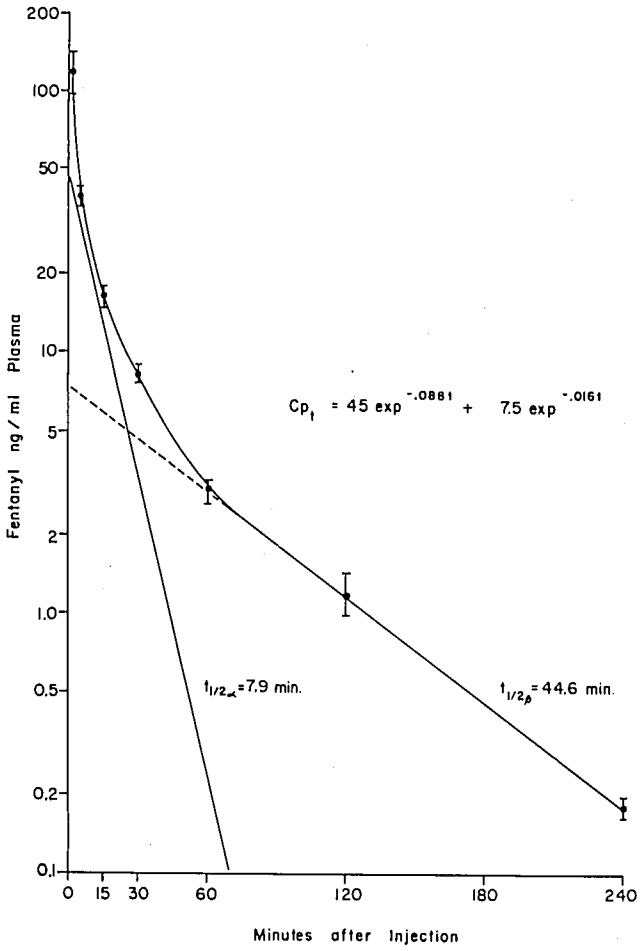

FIG. 2. Plasma levels of unchanged ^{3}H-fentanyl after a bolus dose of 50 μg/kg administered intravenously to the rat. Each point and vertical line represent the mean ± SEM for six rats sacrificed at each time. The half-times for the α and β phases are 7.9 and 44.6 min, respectively. The intercepts (A and B) are indicated on the y-axis.

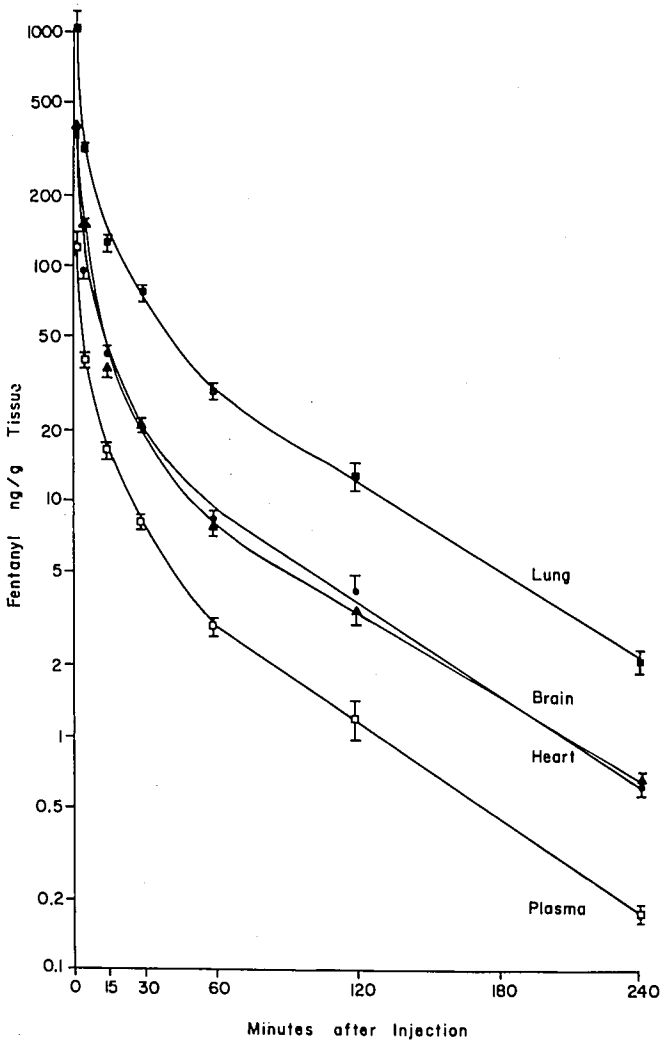

FIG. 3. Concentrations of unchanged ^{3}H-fentanyl in "central group" of tissues and plasma following intravenous injection of 50 μg/kg. Each data point represents the mean ± SEM for six rats.

Plasma levels of unchanged fentanyl fell rapidly and 97 per cent of the dose had left the circulation within 5 min (fig. 2). The half-time of this first phase ($t_{1/2\alpha}$) was 8 min. In the 1- to 4-h interval, the elimination half-time ($t_{1/2\beta}$) was approximately 45 min.

The uptake of the fentanyl by lung, heart, and brain was extremely rapid with maximum levels occurring at or before the first sampling at 1.5 min. Fentanyl elimination from these tissues ("central group") paralleled its elimination from plasma (fig. 3). The actual concentrations of fentanyl were 2–3 times greater in heart and brain than in plasma, and 10 times greater in lung throughout the 4-h period of observations.

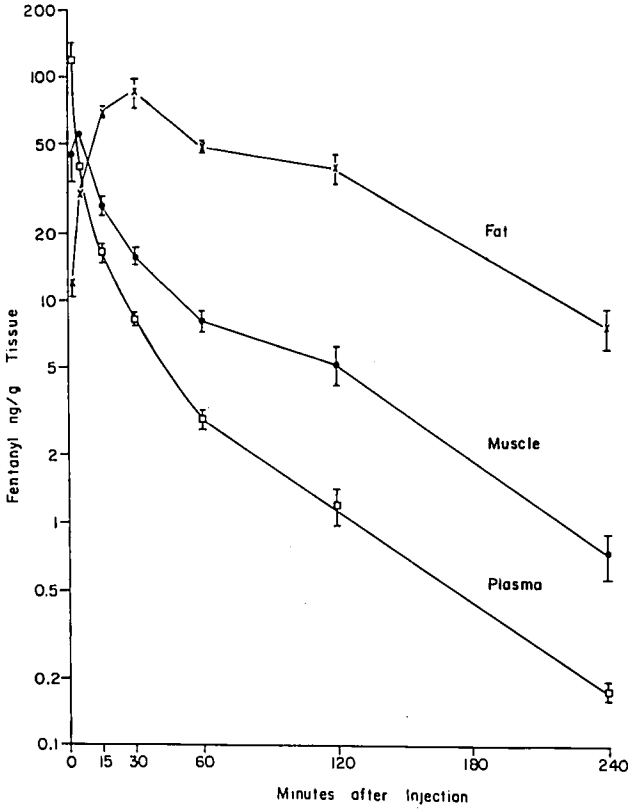

FIG. 4. Concentrations of unchanged ^{3}H-fentanyl in muscle, fat, and plasma following an intravenous injection of 50 μg/kg. Each data point represents the mean ± SEM for six rats.

The uptake of fentanyl by skeletal muscle and subcutaneous fat was slower than that for the "central group" of tissues (fig. 4). Maximum concentrations of fentanyl were found at 5 min in muscle and at 30 min in fat. The rate of elimination of fentanyl from muscle and fat was slower than its elimination from plasma through the first 120 min after injection. Thereafter, fentanyl concentrations in muscle and fat declined in parallel with those in plasma. The actual concentrations of fentanyl were 2–4 times greater in muscle and as much as 35 times greater in fat than in plasma.

Biotransformation of fentanyl appered to be extensive. Metabolites were detected in the earliest samples of plasma (fig. 5) and by 15 min after injection, they accounted for more than 50 per cent of the total ^{3}H-radioactivity in plasma and 25 per cent of that in the body. At fours hours, metabolites contributed 80 per cent of total body ^{3}H-radioactivity and they represented 92 per cent of the tritium in plasma.

The uptake and elimination of fentanyl by the liver and kidneys appeared to be slower than for the "central group" of tissues (fig. 6). However, this is an artifact caused by the accumulation of a metabolite, despropionyl fentanyl, which was extracted into toluene and measured as unchanged fentanyl. Paper chromatograms of the extracts of liver and kidney from rats sacrificed 15 min or later after drug injection revealed two areas of ^{3}H-radioactivity corresponding in R_f to authentic samples of fentanyl and despropionyl fentanyl (figs. 1 and 7). At 60 min despropionyl fentanyl accounted for approximately 60 per cent of the ^{3}H-radioactivity in liver, while 40 per cent represented unchanged fentanyl. Only unchanged fentanyl was detected in the extracts of other tissues in the rat.

Discussion

The overall pattern of fentanyl elimination from plasma of rats was similar to that found in the dog[1,2] and in humans[6]; 95 per cent of the dose was removed within the first 5 min after an intravenous injection. The elimination of fentanyl from plasma in dogs and humans was best described by a triexponential equation; it appeared to be a biexponential process in the rat (fig. 2). There are two possible explanations for this apparent species difference. 1) A very rapid distribution phase could have

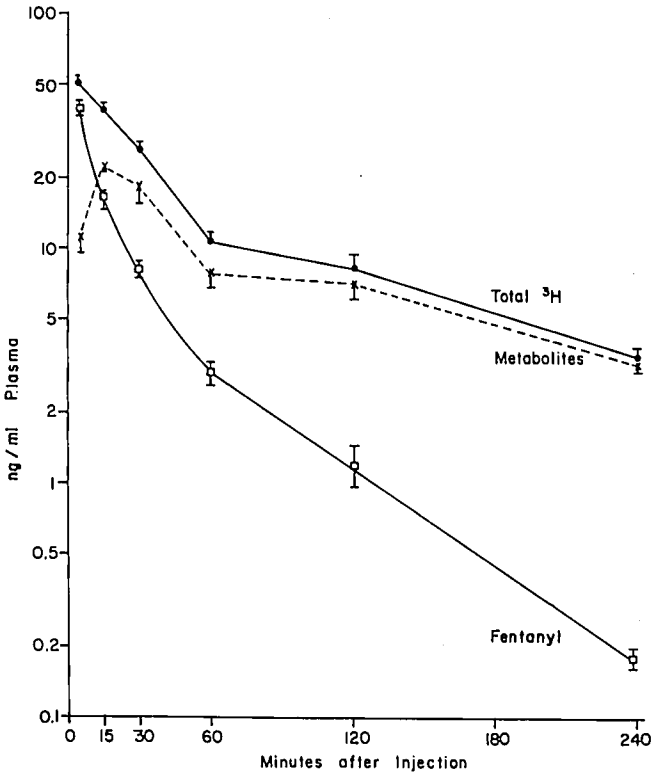

FIG. 5. Plasma levels of total ^{3}H-radioactivity, ^{3}H-metabolites, and unchanged ^{3}H-fentanyl after intravenous injection of ^{3}H-fentanyl, 50 μg/kg, in the rat. Each data point represents the mean ± SEM for six rats. The metabolites were determined as the difference between the total ^{3}H-radioactivity and unchanged fentanyl.

gone undetected because of an insufficient number of sampling intervals at the very early times after intravenous injection in the rat. 2) Rat tissues have a greater perfusion relative to their mass compared to higher species.[7] Hence, differences in the rate of drug uptake between various tissue groups, specifically between skeletal muscle and fat, may be less, and may not be as easily detected in the overall kinetics of fentanyl elimination from plasma.

The half-time of the terminal elimination phase ($t_{1/2\beta}$) for fentanyl in the rat was approximately 45 min and much shorter than the 3.5-h $t_{1/2\beta}$ found in dogs[1,2] and humans.[6] Rodents typically eliminate drugs more rapidly than phylogenetically higher species. The rats appeared to recover from the effects of fentanyl by 60 min when the average concentration of fentanyl in plasma was 2.9 ± 0.3 ng/ml.††

The close parallelism among the concentration *vs.* time curves for fentanyl in the vessel-rich tissues (*i.e.*, brain, heart, and lung) and plasma indicates that fentanyl equilibrated so rapidly between these tissues and plasma that they cannot be distinguished kinetically (fig. 3) and that they all belong to the central compartment in the pharmacokinetic model.[6] The rapid uptake and elimination of fentanyl from the brain also paralleled its effects. The

†† Recovery from the respiratory depressant effects of fentanyl in dogs lightly anesthetized with enflurane and in human volunteers breathing oxygen was noted at concentrations of fentanyl citrate in plasma of 1 to 2 ng/ml.[2,6]

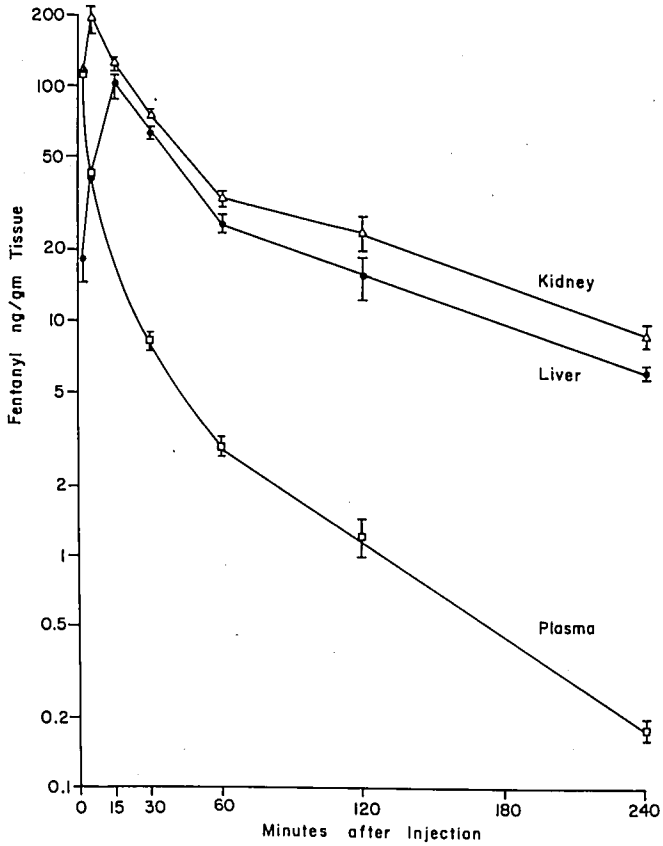

FIG. 6. Concentrations of apparent ^{3}H-fentanyl in the kidney, liver, and plasma of rats following intravenous injection of ^{3}H-fentanyl, 50 μg/kg. Each point represents the mean ± SEM for six animals.

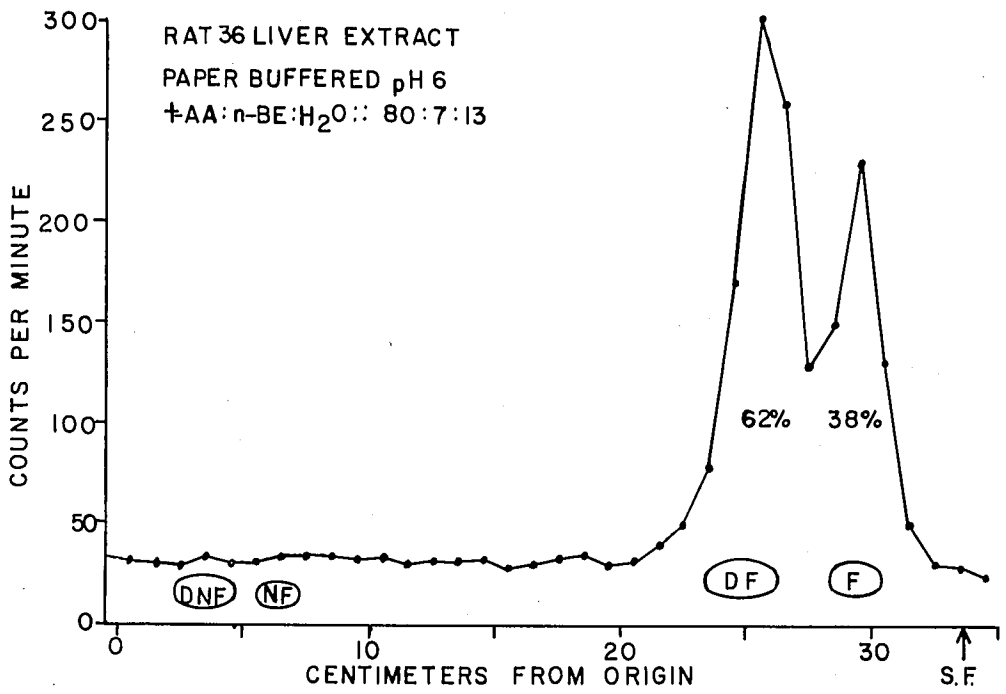

FIG. 7. Chromatograph of the toluene extract of liver tissue in one rat sacrificed 60 min after ^{3}H-fentanyl injected intravenously. DNF, NF, DF, and F represent authentic samples of despropionyl norfentanyl, norfentanyl, despropionyl fentanyl, and fentanyl, respectively, applied to the paper as standards and localized by spraying the chromatogram with iodoplatinate solution. See the Materials and Methods for details. S.F. is the solvent front. The solvent system was *tert.*-amyl alcohol, *n*-butyl ether, and water (80:7:13, v/v).The R_f values for the authentic samples were: F = 0.85, DF = 0.74, NF = 0.19, and DNF = 0.10. The R_f values for the peaks of F and DF were 0.87 and 0.75, respectively.

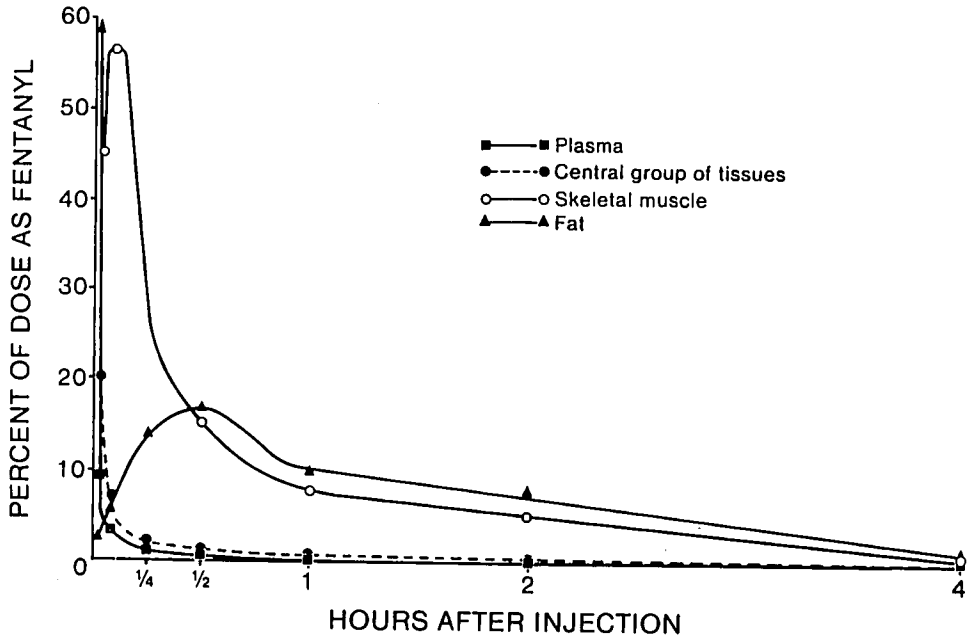

FIG. 8. Fentanyl uptake and elimination in various tissues of the rat following an intravenous injection. The levels of unchanged fentanyl are expressed as "per cent of dose" (see Materials and Methods). "Central" represents the combined content of brain, heart, and lung tissues. All points are the means for six animals.

onset of action was evident within 10 s and the rats appeared normal at 60 min.

Muscle and fat differed considerably from the other tissues in their rates of fentanyl uptake and elimination. The maximum concentrations of fentanyl were not reached until 5 min in muscle and 30 min in fat (fig. 4). Equilibration with plasma did not occur until 120 min, after which time the concentration of fentanyl in both fat and muscle appeared to decline in parallel with that in plasma. Because of the delayed uptake and elimination of fentanyl from muscle and fat, they have been designated as part of the peripheral compartments in the pharmacokinetic model, possibly V_2 and V_3, respectively.

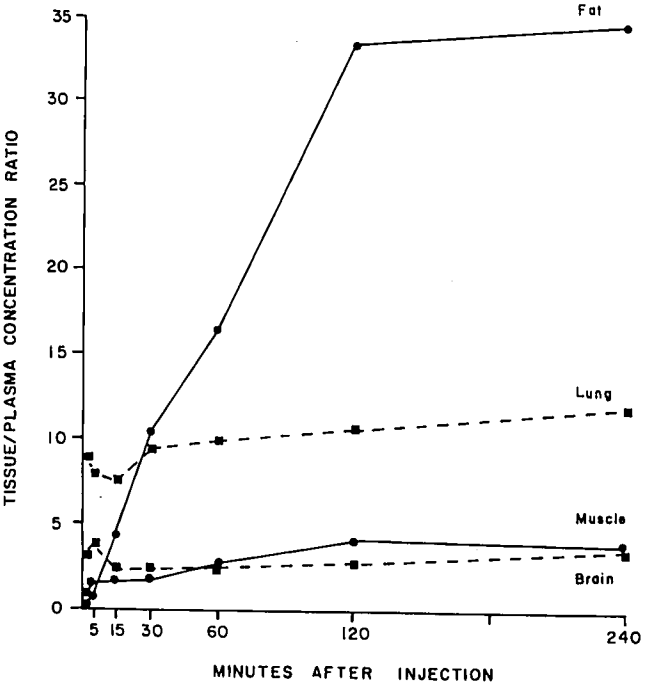

FIG. 9. Tissue/plasma concentration ratios for unchanged ^{3}H-fentanyl in tissues following an intravenous injection of 50 μg/kg.

The impact of muscle is more apparent when it is noted that muscle accounts for approximately 50 per cent of the body weight of the rat,[3] and when its content of fentanyl is expressed in terms of the per cent of the total dose (fig. 8). Immediately after an intravenous bolus injection (time zero), 100 per cent of the dose was in plasma. By 1.5 min, 90 per cent of the dose had left the plasma. Seventy-one per cent of the dose was accounted for as unchanged fetanyl in the vessel-rich group (24 per cent), muscle (45 per cent), and fat (2 per cent). By 5 min, 18 per cent of the injected dose of fentanyl could be accounted for in tissues and plasma as metabolites and 78 per cent as unchanged fentanyl. Only 13 per cent of the unchanged drug was found in the "central" compartment (including plasma), while muscle contained approximately 56 per cent and fat had 6 per cent. The progressive fall in the concentration of fentanyl in the central compartment was accompanied by progressively increasing fentanyl concentrations in muscle and fat (fig. 4) and by its continued biotransformation (fig. 5).

When the tissue/plasma concentration ratios for fentanyl in various tissues are plotted against time after injection (fig. 9), several things are apparent: 1) the rapid equilibration of the central group of tissues with plasma; 2) the slower equilibration between plasma and muscle, which has a slightly higher affinity for fentanyl than

plasma (T/P = 4); the slow equilibration but very great affinity of fat for fentanyl, a lipophilic drug.‡‡ Thus, fat which is assumed to represent only 10 per cent of body mass in these rats has accumulated approximately 17 per cent of the dose within 30 min of its injection (fig. 8). Obviously, greater proportions of a fentanyl dose will likely accumulate in the adipose tissues of obese subjects.

It appears that the large mass of muscle and the high affinity of fat for fentanyl served as a drain on the fentanyl content of the central compartment. No sooner had peak levels of fentanyl occurred in brain than the rapid decline of fentanyl concentrations in plasma occasioned by its uptake into muscle and fat led to a similarly rapid decline in brain concentrations of the drug and to a relatively short duration of action. This pattern of drug disposition resembles that of another highly lipophilic drug, thiopental.[9-11]

Fentanyl is rapidly and extensively metabolized (see fig. 1). As early as 1.5 min after injection, metabolites represented 20 per cent of the radioactivity in plasma and by four hours they accounted for 92 per cent of the tritium in plasma. The concentration of metabolites in plasma reached a maximum at 15 min and then declined at a slower rate than the unchanged drug. A similar pattern was observed in the dog[1] and in humans.[6] It is expected from studies in other species that most of the administered fentanyl was excreted as metabolites in urine.[1,6,12,13] Only small amounts of unchanged fentanyl have been recovered in urine and feces.[6] (We did not collect urine or feces in this study of rats.)

The metabolite detected in liver and kidney has tentatively been identified as despropionyl fentanyl. Maruyama and Hosoya[14] reported 20 per cent of the fentanyl dose recovered as despropionyl fentanyl in the urine of rats but it was not detected in rats by van Wijngaarden and Soudijin.[15] We have not found this metabolite interfering with extracts of plasma or urine from either dogs[1] or humans.[6] None of this metabolite was detected in plasma or in tissues other than liver and kidney. Its concentration in these two tissues makes determination of the actual concentrations of unchanged fentanyl in these tissues impossible without further studies.

In summary, the rapid onset of fentanyl actions is related to the very rapid uptake of this lipophilic drug by the central nervous system. The short duration of action following a single intravenous dose of moderate size is due to its rapid elimination from plasma and brain as a result of extensive uptake of the unchanged drug by skeletal muscle and fat, and rapid conversion of fentanyl to its metabolites. Accumulation of fentanyl in peripheral tissue compartments is extensive because of the large mass of muscle and the high affinity of fentanyl for fat. Biotransformation is necessary for the ultimate excretion of the drug from the body. Biotransformation processes appear to be efficient but the ultimate rate of fentanyl elimination may be limited by its rate of recirculation from muscle and fat to liver and kidney where it is metabolized and excreted. Despropionyl fentanyl is a metabolite of fentanyl in the rat and interferes with the estimation of unchanged fentanyl in the liver and kidneys.

‡‡ The heptane-water partition coefficient for fentanyl is 19.4 compared to 1×10^{-5} for morphine.[8]

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