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INHIBITION OF MORPHINE METABOLISM BY CATECHOL IN THE CHICKEN KIDNEY*

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Abstract—In the Sperber preparation of the chicken morphine is secreted by the renal tubular cells and is metabolized within these cells to morphine ethereal sulfate (MES). When morphine-*N*-$^{14}CH_3$ was infused into a leg vein, high values for the apparent tubular excretion fraction (ATEF) were obtained. Large excesses of ^{14}C morphine and ^{14}C MES were excreted by the kidney on the infused side. The effect of catechol was determined by adding it to the infusion solution. As the molar ratio of catechol to morphine was progressively increased up to 100 to 1, the excretion of ^{14}C MES diminished to zero while proportionate increases in the excretion of ^{14}C morphine were observed. These changes were not accompanied by any change in the ^{14}C ATEF. When ^{14}C MES itself was infused high ATEF values were obtained and these values were unaffected by large doses of catechol. Thus, catechol clearly inhibited the metabolism of morphine to MES within the renal tubular cells and did not affect the renal tubular transport of either morphine or MES. On the other hand, inhibition of metabolism and transport of catechol could not be demonstrated with morphine infused at high rates.

USING the Sperber chicken preparation[1,2] work from this laboratory has shown that morphine is secreted by the cationic transport system in the chicken kidney,[3] and morphine is metabolized within the tubular cell to morphine-3-ethereal sulfate (MES).[4] MES itself is also transported by the kidney, but by the anionic rather than the cationic transport system.[5] Probenecid blocked this transport of extrarenally formed MES but did not block the excretion of MES formed within the renal tubular cell from morphine. Thus the primary site of probenecid action was placed at the peritubular border of the renal cell. Further support for this site of action of probenecid was provided by Hakim and Fujimoto[6] with work on the drug metabolite pair, 5-hydroxytryptamine (5HT)-5-hydroxyindoleacetic acid (5HIAA). A base, 5HT, was transported and metabolized within the cell to 5HIAA, an acid. The transport of 5HIAA was blocked by probenecid when 5HIAA was administered intravenously but not when it was formed intracellularly from 5HT. One advantage of the 5HT-5HIAA pair over that of morphine-MES was that the transport of 5HT could be studied independently of its metabolism by inhibiting monoamine oxidase. In the present paper, we have been able to demonstrate that catechol inhibits metabolism but not transport of morphine, findings that clearly support our model of tubular mechanisms in the chicken kidney.

There were compelling reasons for selecting catechol as an inhibitor of metabolism of morphine. Sperber[7] had shown that catechol was metabolized to an ethereal

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sulfate (as well as a glucuronide) by the chicken, and Quebbemann and Rennick[8] had shown that catechol was not only actively transported by the chicken kidney but was metabolized within the tubular cell. Conceptually, inhibition of morphine ethereal sulfate formation was anticipated because catechol should serve as an alternate substrate to morphine for the sulfate conjugating system.

METHODS

Sources of materials. Morphine-*N*-$^{14}CH_3$ hydrochloride, 57 mc/m-mole and catechol-^{3}H (ring labeled), 50 mc/m-mole, were obtained from Amersham/Searle Corp. Tetraethyl-1-^{14}C ammonium bromide, 1·15 mc/m-mole, was purchased from New England Nuclear Corp. Morphine-*N*-$^{14}CH_3$-3-ethereal sulfate (0·25–0·5 mc/m-mole) was prepared biologically by methods described in an earlier publication.[5]

Animal preparations. All studies of renal tubular transport and metabolism were carried out in hens prepared in a manner similar to that originally described by Sperber.[1] Details of the technique as we have been using it are given in a recent publication.[5] Tygon collecting tubes with foam rubber cuffs were sutured over each ureteral opening and were perfused continuously with distilled water to prevent clogging by the viscous urine. A 23 G needle, at the end of a PE 90 polyethylene cannula, was inserted into either of the saphenous veins. All drugs were infused through this cannula. The basic infusion solution consisted of *p*-aminohippuric acid (PAH), 8 mg %, and inulin, 30 mg %, in 0·9% sodium chloride. The agent being studied was dissolved in the basic infusion solution and was infused at a constant rate of 0·42 ml/min for periods ranging from 40 to 90 min before the first urine samples were collected. Following these periods, urine from the two kidneys was collected separately for six to eight control periods of 5 min each. Agents being evaluated as inhibitors of metabolism or transport were added to the control infusion solution and infused for a 20–30 min period before urine samples were again collected. Urine samples were diluted to a constant volume of 5 ml. In some experiments progressively increasing concentrations of the blocking agent were infused, while in others the reversibility of the inhibition was evaluated by reverting to the control infusion solution for up to 2 hr before recommencing the urine collections.

In the chicken, blood from the saphenous vein perfuses the renal portal system of the ipsilateral kidney before it reaches the systemic circulation. The active secretory transport of a substance may be demonstrated by infusing that substance into one saphenous vein. If net secretion occurs the substance will appear in excess in the urine elaborated by the kidney ipsilateral to the infusion site. If renal metabolism of the drug occurs, the metabolite will also appear in excess in the urine of the infused kidney.

The extent to which a particular substance is secreted is quantified by calculating the apparent tubular excretion fraction (ATEF) for that substance. The ATEF is defined by the equation

$$\text{ATEF} = \frac{\text{I} - \text{NI}}{\text{INF}} \times 100,$$

where I is the amount of the substance excreted by the kidney ipsilateral to the site of infusion, NI is the amount excreted by the contralateral (noninfused) kidney, and INF is the total amount of the substance infused during the collection period.

In order to evaluate the relative efficiency of the transport of a given drug, the data are presented as the ATEF for the drug and the ratio of the ATEF for the drug to that of a marker substance. A marker substance is one that is known to be completely cleared from the blood during a single passage of the blood through the peritubular capillary network. In these experiments PAH and ^{14}C tetracthyl ammonium (TEA) were used as marker agents. The use of these agents as markers in the Sperber preparation was recently discussed by Quebbemann and Rennick.[9]

Animals

Three breeds of chickens were used in these studies: Rhode Island Red, White Rock and a black sex-link cross of a Rhode Island Red male and a Barred Rock female. No consistent differences were observed between the three strains. Most of the experiments reported here employed White Rock hens. Laying hens weighing 2·0–3·4 kg were used. The animals were generally fasted for 18–24 hr prior to each experiment. During the experiments, adequate urine flow was maintained by the periodic administration of water or saline through a crop tube.

Analyses. The radioactivity in each urine sample was determined in a Packard Tri-Carb liquid scintillation spectrometer. Samples of 0·2 ml were pipetted into 15 ml of a scintillation cocktail prepared by adding 500 ml of Triton X-100 to 1000 ml of a solution of 0·4% 2,5-diphenyloxazole (PPO) and 0·005% 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in toluene. For single isotope studies the counting efficiencies were 78 per cent for ^{14}C and 40 per cent for ^{3}H. In dual label experiments the counting efficiencies were 46 per cent for ^{14}C and 13 per cent for ^{3}H with 8 per cent ^{14}C to ^{3}H crossover and 0·03 per cent ^{3}H to ^{14}C crossover. The data presented are corrected for these factors. An external standard was used to correct for quenching. In some experiments in which ^{14}C was the only radioactive isotope, 0·2–0·5 ml of urine was plated and counted on a Tracerlab low background system. Counting efficiency was 10 per cent at infinite thinness. PAH was determined by the method of Smith *et al.*[10]

Separation of ^{14}C morphine from ^{14}C MES. Although a countercurrent distribution system for separating morphine from MES was available,[5] a more rapid separation system was developed. One-half ml of urine was added to 1·5 ml of 0·02 M $NaHCO_3$ buffer, pH 8·8. The pH of the mixture was adjusted, if necessary, to pH 8·7–9·0 with 1 N NaOH. The ^{14}C activity of the urine-buffer mixture was determined by counting 0·05 ml (for scintillation counting) or 0·5 ml (for solid sample counting). Then 1·0 ml of the urine-buffer solution was extracted four times with 2·0 ml of a mixture of isoamyl alcohol and chloroform (1:19, v/v). This extraction procedure removed 87 per cent of the morphine but essentially none of the MES from the aqueous phase. The residual ^{14}C activity in the extracted aqueous phase was determined as previously described for the unextracted aqueous phase. The amount of morphine per milliliter of the original buffer solution was calculated from the formula

$$(\text{morphine counts/min/ml}) = \frac{(S_1 - S_2)}{0{\cdot}87} \times k,$$

where S_1 and S_2 are the counts per minute of the aqueous phase before and after extraction respectively, and k is the reciprocal of the sample volume in milliliters. The difference between S_1 and the counts/min/ml of morphine was assumed to be the

counts/min/ml of MES. The accuracy of the method was evaluated by applying it to chicken urine to which ^{14}C morphine and ^{14}C MES had been added in known proportions. The experimentally derived estimate of the MES agreed well with the known values.

When this method was used to estimate MES in the urine from chickens given ^{14}C morphine, it was assumed that the urine contained only morphine and MES. In reality, what is being called the MES fraction contained other polar metabolites. The data to be presented here along with that in earlier publications[4,5] indicate that when ^{14}C morphine is injected into a chicken, MES is the major metabolite and that non-MES metabolites do not account for more than a few per cent of the total radioactivity appearing in the urine. These non-MES metabolites were designated as "X" and "Y" by Fujimoto and Haarstad.[4] Although it might have been desirable to quantitate each of these metabolites in the present experiments, we saw little advantage in undertaking such a complex analytical task in view of the conclusive nature of the results obtained. In certain cases where the percentage of MES was near zero relative to total radioactivity in the urine samples from chickens being treated with catechol, negative values were obtained for per cent MES. This finding indicated that a slight error was present when our procedure was applied to urine samples containing large amounts of morphine and small amounts of MES; the error probably arises from the correction factor 0·87. However, the error was at most 5 per cent, and the validity of the analysis was not compromised.

An additional countercurrent distribution system was also developed to separate drugs from their metabolites. In this system, 30 mg of tris buffer was dissolved in 3 ml of urine, and the pH adjusted to 8·8 with 1 N NaOH. Two ml of the buffered urine were used as the aqueous phase of tube 0 in a countercurrent system in which the mobile (aqueous) phase was 0·1 M tris, pH 8·8, and the stationary phase was ethyl acetate. This system was useful because it could separate catechol from its metabolite(s) when a six-tube (five-transfer) system was used and could also separate morphine from MES using a nine-tube system. The nine-tube system was used in experiments in which ^{14}C morphine and ^{3}H catechol were infused concurrently. The method of Way and Bennett[11] was used to calculate the relative proportions of parent compounds to their metabolites.

Thin layer and column chromatography. Methods described earlier[4] were adapted to separate ^{14}C morphine from its metabolites. Five to 50 ml of dilute urine were applied to an Amberlite XAD-2 column ($\frac{1}{4} \times 6\frac{1}{2}$ in. bed volume). The column was washed with 5 ml distilled water and eluted with methanol. The first 3 ml of eluate appearing after the addition of methanol contained very little radioactivity and were discarded. The next 5 ml were collected in a small beaker and evaporated to dryness at approximately 90° in an oven. The organic fraction of the resulting residue was redissolved in 0·25 ml of methanol, and 50–200 λ of this solution were applied as a 3 cm streak on Gelman ITLC^{R} silica gel sheets. The chromatograms were developed in a solvent system consisting of *n*-butanol–glacial acetic acid–water (35:3:10, by vol.). The developed chromatograms were dried at 90°, and radioactive peaks were located with a Tracerlab 4 π scanner. Morphine and MES were also identified by spraying the chromatograms with iodoplatinate reagent.[12] The combined Amberlite–TLC procedure yielded chromatograms that clearly corroborated the countercurrent distribution and multiple extraction analyses.

Since ^{3}H catechol was infused into some chickens another thin layer chromatography system was used to distinguish between catechol and its metabolites. Urine from these chickens and also urine to which ^{3}H catechol was added were spotted or streaked on Gelman ITLC^{R} silica gel sheets, and the chromatograms were developed in cyclohexane–glacial acetic acid (9:1, v/v). After drying, the chromatograms were scanned for radioactivity with a Tracerlab 4 π instrument and sprayed with 5 per cent $FeCl_3$ to test for free phenols.[13]

RESULTS

Figure 1 summarizes the results of five experiments in which the effect of catechol on morphine metabolism was determined. It must be emphasized that the data in this figure express the proportion of the radioactivity in the urine which was accounted for as MES. For illustration, experiment 4 may be examined. During the infusion

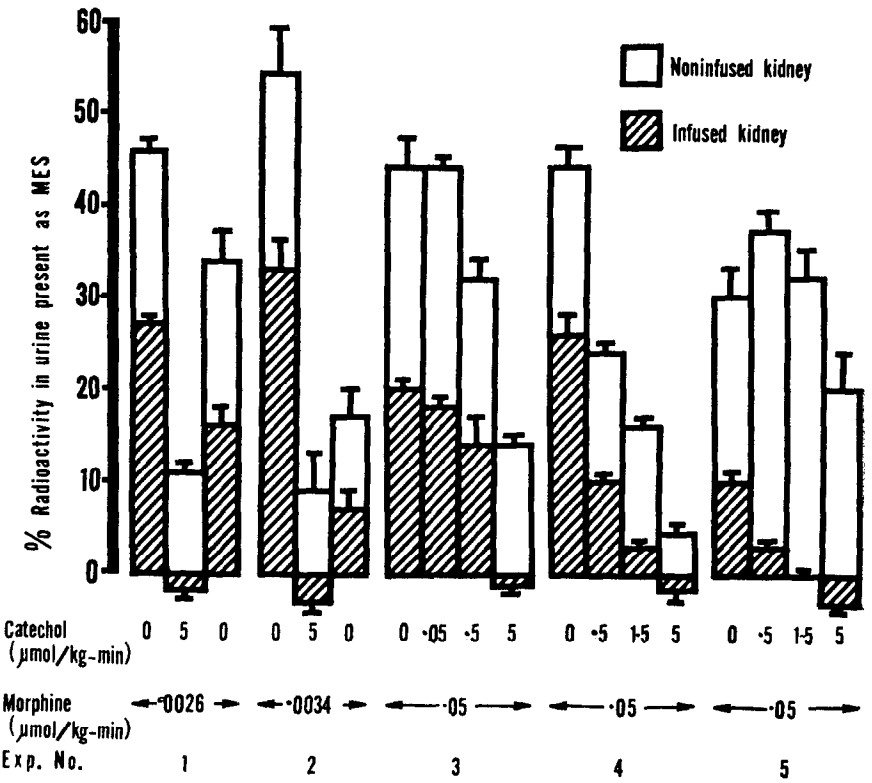

FIG. 1. Results of five experiments showing the inhibitory effect on morphine metabolism in the chicken when catechol is added to infusion solutions containing ^{14}C morphine. In all five experiments for both kidneys the addition of catechol produced a decrease in excretion of ^{14}C MES (expressed as per cent of the total ^{14}C radioactivity in each urine sample). Mean $\pm$ S.E.

of 0·05 μmoles/kg/min of ^{14}C morphine, MES accounted for 26 per cent of the radioactivity appearing in the urine from the infused kidney. The noninfused, contralateral kidney excreted radioactive products of which 44 per cent was MES. These percentages do not indicate anything about the total quantity of radioactivity that

was being excreted by each kidney. (These data will be presented later.) In experiment 4, when catechol, 0·5 μmoles/kg/min, was infused, the percentage of MES dropped to 10 per cent for the infused side and 24 per cent for the noninfused side. As the rate of catechol infusion increased to 5 μmoles/kg/min the per cent MES declined to near zero values for both kidneys. Although the dose response relationships were not as apparent in the other experiments as in experiment 4, it is clear that the infusion of 5 μmoles/kg/min of catechol completely inhibited the excretion of MES by the infused kidneys and always produced a significant decrease in the excretion of MES by the noninfused kidney. This effect on the noninfused kidney was probably a manifestation of the passage of substantial quantities of catechol into the general circulation and access of catechol to other sites of MES formation. It can also be seen in Fig. 1 that the inhibitory effect of catechol was reversible. In experiments 1 and 2, the solution containing ^{14}C morphine, but not catechol, was infused during the control periods and again after the catechol infusion was terminated. In both experiments, the percentage of MES was substantially higher in the postcatechol periods than in the periods when catechol was infused.

For corroborative purposes, the analytical results were verified qualitatively in each experiment by thin layer chromatography. Urine from the six collection periods in each treatment condition was pooled and processed through the Amberlite XAD-2

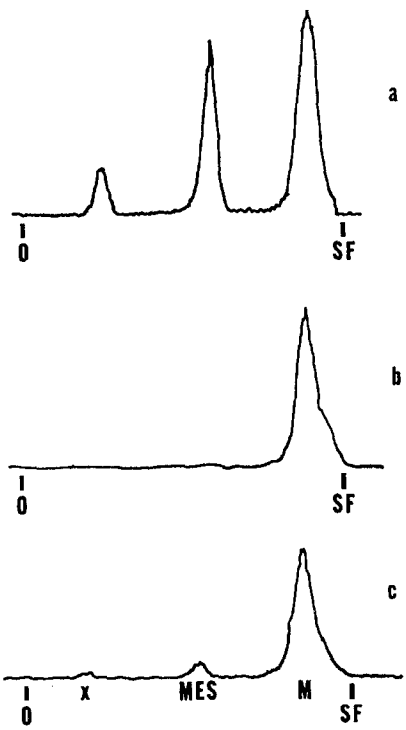

FIG. 2. Radiochromatograms of urine collected from a chicken being infused with ^{14}C morphine. Panel (a) shows the chromatogram obtained when ^{14}C morphine was infused. Peaks corresponding to free morphine (M), MES and an unknown metabolite, *X*, are seen. (b) When catechol (5 μmoles/kg/min) was added to the infusion solution, the peaks for MES and *X* do not appear. (c) Twenty min after discontinuing the catechol infusion MES and *X* begin to reappear.

resin columns. The methanol eluate was chromatographed on silica gel thin layer sheets. An example is shown in Fig. 2 which shows results from the infused kidney in experiment 2. During the control period (Panel a), three distinct peaks corresponding to morphine, MES and an unidentified metabolite, *X*, are seen. Panel (b) shows the chromatogram of the urine collected during catechol infusion. The peaks from MES and *X* are now gone and only morphine is present. These results support the hypothesis that catechol inhibited the metabolism of morphine. Panel (c) shows that after the 20 min washout period both MES and *X* began to reappear in the urine. No attempt was made to characterize metabolite *X* because it usually represented a much smaller fraction of the total radioactivity than in the experiment shown. The R_f of *X* closely approximates that of morphine-3-glucuronide, although no direct comparison of *X* with authentic morphine-3-glucuronide was made.

Earlier studies[5] have shown that morphine is metabolized to MES within the renal tubular cell. Therefore, the observed decrease in the excretion of MES produced by catechol could conceivably occur if catechol blocked either the transport of morphine into the cell or the egress of MES from the cell into the luminal fluid. These possibilities were evaluated by examining the ATEF values calculated from the experiments discussed above. The data presented in Table 1 indicate that there was no important effect of catechol on the transcellular transport of ^{14}C activity when ^{14}C morphine was infused. More specifically, in three of the five experiments, catechol produced no decrease in the ATEF of ^{14}C. In the other two experiments, a decrease in the ^{14}C ATEF was observed at low catechol infusion rates, but when the high dose

TABLE 1. LACK OF EFFECT OF CATECHOL ON THE RENAL TUBULAR TRANSPORT OF ^{14}C MORPHINE

Expt. no.	Catechol infusion rate (μmoles/kg/min)	Average ATEF*			Recovery*(%)	
		^{14}C†	PAH	$\frac{^{14}C}{PAH}$	^{14}C	PAH
1	5·0	45	58	0·80	96	103
		48	69	0·70	104	106
		31	41	0·76	104	106
2	5·0	31	42	0·75	80	102
		35	44	0·81	85	92
		37	34	0·76	95	100
3		32	45	0·73	76	81
	0·05	13	32	0·52	77	81
	0·5	12	17	1·12	54	50
	5·0	32	48	0·62	82	91
4		26	42	0·56	81	74
	0·5	13	26	0·44	95	88
	1·5	37	52	0·71	110	106
	5·0	31	48	0·66	112	107
5		38	53	0·80	80	92
	0·5	40	53	0·73	85	96
	1·5	60	63	0·98	103	106
	5·0	50	50	0·96	99	99

* Each value is the mean of six periods, each 5 min long.

† Tracer morphine was infused only in experiments 1 and 2. Carrier was added in experiments 3, 4 and 5 to bring the infusion rate of morphine to 0·05 μmoles/kg/min.

of catechol was infused, the ^{14}C ATEF was equal to or greater than the control values. It should also be noted that no significant decrease in the ATEF ratio of ^{14}C/PAH occurred in any of the five experiments. The failure of catechol to lower significantly the ATEF of ^{14}C or the ATEF ratio is interpreted to mean that catechol does not block the transport of morphine into the renal tubular cell. The data also indicate that catechol did not cause a decrease in the per cent recovery of ^{14}C activity. Since neither the ^{14}C ATEF nor the ^{14}C per cent recovery was decreased by catechol, it seems unlikely that catechol could have any effect on the movement of MES from the cell into the lumen. This possibility was tested directly by the experiments summarized in Table 2. ^{14}C MES ($1{\cdot}5 \times 10^{-3}$ μc/min) was infused for 45–90 min before

TABLE 2. LACK OF EFFECT OF CATECHOL ON THE RENAL TUBULAR TRANSPORT OF EXTERNALLY FORMED ^{14}C MORPHINE ETHEREAL SULFATE (MES)

Expt. no.	Agents and dose (μmoles/kg/min)			Average ATEF*			Metabolized (%)		Recovery (%)	
	^{14}C MES	Morphine	Catechol	^{14}C	PAH	$\frac{^{14}C}{PAH}$	I†	NI†	^{14}C	PAH
6	0·005			62	‡	‡			96	‡
	0·005		5	60	‡	‡			98	‡
		0·00011	5	41	54	0·77	−3	8	76	89
		0·00011		34	48	0·70	12	22	92	106
7	0·0036			55	55	1·01			98	100
	0·0036		5	54	45	1·18			90	84
		0·00012	5	41	45	0·97	6	10	64	92
		0·00012		72	82	0·91	25	56	85	106

* Each value is the mean of six or eight periods, each 5 min long.

† I and NI refer to the percentage of radioactivity excreted by the infused and noninfused kidneys respectively that was accounted for as MES.

‡ Missing data.

the first collections were made to determine the ATEF of ^{14}C. The effect of catechol on MES transport was determined by adding catechol (5 μmoles/kg/min) to the infusion solution 30 min before collecting six 5-min urine samples. Following this, the catechol infusion was continued, but ^{14}C morphine (15×10^{-2} μc/min) was infused in place of ^{14}C MES. Thirty min were allowed for the ^{14}C MES from the previous step to wash out before six urine samples were collected during ^{14}C morphine infusion. Catechol infusion was then discontinued and six more urine samples were collected after a washout period of 60–120 min. In both experiments, the addition of catechol (5 μmoles/kg/min) to an infusion solution containing ^{14}C MES had no significant effect on either the ATEF or per cent recovery of ^{14}C. In experiment 7, catechol had no significant effect on the ATEF ratio of ^{14}C MES/PAH. However, at this infusion rate, catechol was an effective inhibitor of morphine metabolism in both experiments, since the metabolism of morphine to MES was minimal during catechol infusion but increased severalfold after the catechol infusion was terminated. This inhibition of metabolism is indicated in Table 2 by the low percentage of urinary radioactivity present as MES in the periods when catechol was infused with ^{14}C morphine. The effect was apparent in both the infused (I) kidney and noninfused (NI) kidneys.

Clearly, the effect of catechol is to inhibit the metabolism of morphine to MES. Catechol has no effect on the transport of MES.

The effect of morphine on the metabolism of catechol. Several experiments were designed to assess the effect of morphine on catechol metabolism and transport. Thin layer chromatography and countercurrent distribution techniques were used to determine the extent of catechol metabolism. In Fig. 3 (a) the region giving the radioactivity peak for ^{3}H catechol turned blue when sprayed with 5 per cent ferric chloride and had the same R_f as unlabeled catechol. Panel (b) of Fig. 3 shows that the urine of

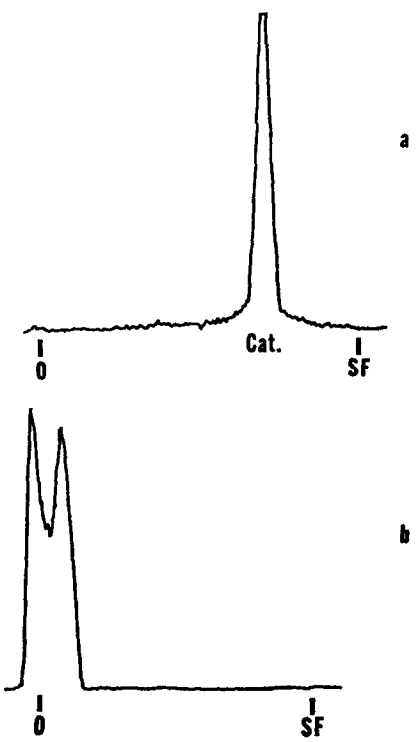

FIG. 3. Radiochromatograms of (a), ^{3}H catechol added to dilute chicken urine, and (b), urine from a chicken infused with ^{3}H catechol. The catechol is completely metabolized to at least two products.

a chicken given ^{3}H catechol contained no unchanged catechol. At least two metabolites appeared to be present. The countercurrent distribution analyses of the radioactivity in the urine of the chicken given ^{3}H catechol showed that 97 per cent of the radioactivity from the urine was in a metabolite fraction that had a partition coefficient of 0·08. These results led to the same conclusion as with the TLC results. The finding that the metabolism of catechol is virtually complete confirms the work of Quebbemann and Rennick.[8]

Table 3 summarizes two experiments that tested the potential inhibition of catechol metabolism and transport by morphine. The experimental protocol used in these experiments was similar to that used in experiments 1 and 2 (Table 1). Countercurrent distribution of pooled urine samples was used to determine the extent of

TABLE 3. LACK OF EFFECT OF MORPHINE ON THE RENAL TUBULAR TRANSPORT AND METABOLISM OF ^3H CATECHOL

Expt. no.	Agents and dose (μmoles/kg/min) Catechol	Morphine	Metabolized (%) I†	NI†	Average ATEF* ^3H	PAH	$\frac{^3H}{PAH}$	Recovery* (%) ^3H	PAH
8	5×10^{-5}		100	100	39	50	0·78	84	102
	5×10^{-5}	2	100	100	22	28	0·77	74	91
	5×10^{-5}		100	100	43	52	0·83	100	104
9	2×10^{-5}		99	99	12	15	0·82	92	102
	2×10^{-5}	5	99	100	11	21	0·50	74	93
	2×10^{-5}		100	100	24	39	0·64	109	112

* Each value is the mean of six periods, each 5 min long.
† I and NI refer to the percentage of radioactivity excreted by the infused and noninfused kidneys respectively that was accounted for as catechol metabolites.

catechol metabolism by the infused and noninfused kidneys. Morphine had no detectable effect on catechol metabolism *in vivo* when morphine was infused at 2 or 5 μmoles/kg/min. In these experiments, the molar ratio of morphine/catechol was 40,000 250,000 respectively. A higher dose level of morphine (10 μmoles/kg/min) was tried in one chicken but proved to be fatal.

It also appeared that morphine did not affect catechol transport even though these latter data are more difficult to interpret. In experiment 8, the ATEF's of both ^3H and PAH were decreased but to an approximately equal extent so there was no significant change in the ATEF ratio. In experiment 9, the control ATEF's for both ^3H and PAH were low and nothing can be stated about transport.

The effect of morphine on PAH and TEA transport. The experiments summarized in Table 4 evaluated the effect of morphine on the renal tubular transport of PAH and

TABLE 4. EFFECT OF MORPHINE ON RENAL TUBULAR TRANSPORT OF ^{14}C TEA

Expt. no.	Morphine (μmoles/kg/min)	ATEF* ^{14}C TEA†	PAH	$\frac{TEA}{PAH}$	Recovery (%) TEA	PAH
10		68	77	0·89	105	107
	5·0	−1·4	49	−0·03	64	102
		29	46	0·64	90	108
11		54	50	1·08	99	102
	5·0	5·1	38	0·14	89	113
		22	31	0·73	108	101

* Each value is the mean of five or six periods each 5 min long.
† ^{14}C TEA infused at $7{\cdot}5 \times 10^{-3}$ μmoles/min.

TEA. Both PAH and TEA were infused for 1 hr prior to collecting the five or six control samples. Morphine was added to the infusion solution 30 min before collecting the next group of samples. After switching back to the control infusion solution,

90 min (experiment 10) or 35 min (experiment 11) passed before the final groups of samples were collected. In both experiments, the most marked effect of morphine was to decrease the ATEF of TEA. There was also a decrease in the ATEF of PAH ($P < 0{\cdot}02$ in both experiments, control versus morphine period), but this was small in comparison to the effect of TEA. Also of interest, is that effect of morphine on the TEA ATEF was partially reversed after the morphine washout period, although the effect of morphine on the ATEF of PAH was not reversed. The interpretation of the effect of morphine on the PAH ATEF is complex and will be given in the discussion. It is felt that the important conclusion to be derived from the TEA transport data is that morphine blocks the renal tubular transport of TEA.

DISCUSSION

The study of drug metabolism in an *in vivo* system is complicated by many factors, not the least of which are transmembrane and transcellular transport. When inhibitors are included in *in vivo* studies, it becomes critical to distinguish between blockade of transport and inhibition of metabolism.

In the presentation of the data from the experiments that determined the effect of catechol on morphine metabolism, we have demonstrated that the data are consistent with the interpretation that catechol inhibited the biotransformation of morphine to MES. Morphine was actively transported from the blood to the luminal fluid, and was partially converted within the tubular cell to MES which was also excreted in the urine. These results confirmed earlier findings.[3, 4] When catechol was added to the infusion solution, the excretion of MES declined sharply. Catechol could conceivably have acted in several ways to produce this decrease in the excretion of MES. Certainly if catechol inhibited morphine transport into the tubular cell, less morphine would be metabolized to MES, and MES excretion would decrease. If this were the case, the ratio of MES to morphine in the urine would have remained the same or possibly increased. Furthermore, there would have been a decrease in the ^{14}C ATEF and also a decrease in the recovery of ^{14}C. The data indicated, however, that the ratio of ^{14}C MES to ^{14}C morphine in the urine decreased sharply. Also, there was no decrease in either the ATEF or the recovery of ^{14}C. The hypothesis that catechol might interfere with morphine transport was therefore rejected.

Another explanation of the catechol-induced decrease in MES excretion might be that catechol or its metabolites blocked the transport of the intracellularly formed MES from the cell into the lumen. There are several reasons for discarding this hypothesis. This action by catechol would also have produced a decrease in both the ^{14}C ATEF and the ^{14}C recovery. Furthermore, if catechol or its metabolites did block the egress of MES from the cell at the luminal border, this action could presumably have been demonstrated on infused ^{14}C MES as well as that formed intrarenally when ^{14}C morphine was infused. The data presented indicated that catechol had no effect on the ATEF or recovery of infused ^{14}C MES. The hypothesis that catechol might interfere with MES transport was also rejected.

The bulk of the evidence indicates that catechol has no effects on the renal tubular transport of either morphine or MES. Clearly then, the primary action of catechol in this system is the inhibition of the metabolic conversion of morphine to MES.

Because morphine and catechol are both metabolized to ethereal sulfates in the kidney the *a priori* speculation was that competition between the two substances for

the sulfokinase enzyme could be demonstrated. The readily reversible inhibition of morphine metabolism by catechol favors the hypothesis of a competitive interaction, but our failure to demonstrate inhibition of catechol metabolism by morphine argues against such a mechanism. In light of some of the constraints imposed by our *in vivo* system, it would be premature to reject the hypothesis of a competitive mechanism on the basis of the data presented here. Administering morphine at doses much higher than ones reported caused death of our animals. Had it been possible to increase the dose of morphine, perhaps inhibition of catechol metabolism would have been demonstrated. It should also be noted that although the molar ratio of morphine to catechol in experiments 8 and 9 was apparently quite high (4×10^4 and $2{\cdot}5 \times 10^5$), these values really refer to the molar ratios in the infusion solutions. Both catechol and morphine are actively transported across the tubular cell. The possibility exists, therefore, that catechol is concentrated within the tubular cell to a greater extent than is morphine. This and other factors could act separately or in concert to inflate erroneously the apparent affinity of catechol for the sulfokinase enzyme.

The data presented here and in other publications suggest that the Sperber preparation can be used to good advantage in making the distinction between inhibition of metabolism and blockade of transport. We have shown that the metabolism of morphine to MES can be inhibited in the absence of any effect on the transport of either morphine or MES. Similarly, Hakim and Fujimoto[6] have shown that the metabolism of 5HT to 5HIAA can be inhibited in the absence of any effect on the transport of 5HT or 5HIAA. The importance of making this distinction between inhibition of metabolism and blockade of transport was illustrated in another publication in which Fujimoto and Hakim[14] reported on experiments that examined the possibility of inhibiting morphine metabolism in the chicken with compounds of the SKF 525A type. These agents were ineffective as inhibitors of morphine metabolism but manifested pronounced effects on morphine transport.

Our finding that morphine blocked the renal tubular transport of TEA was not surprising in light of the earlier work of May *et al.*[3] showing that morphine was a substrate for the base transport system. Therefore morphine would be expected to compete with TEA for the base transport carrier. The effect of morphine to decrease the ATEF of PAH in these experiments was less prominent, but nevertheless quite consistent. Although this decrease could have resulted from an interaction of morphine with the PAH carrier, the data support equally well an alternative explanation. If morphine caused a reduction in renal portal flow, the clearance of PAH would be reduced, and this would be reflected by a lower ATEF of PAH. Blood flow through the renal portal system is controlled by a muscular valve, the renal portal valve, that is located within the lumen of the external iliac vein. If this valve were relaxed by the high doses (over 300 mg/hr) or morphine used in these experiments, the result would be a shunting of blood around the peritubular capillary network and a decrease in the ATEF of any substance being secreted. It cannot be determined from the data from these experiments if morphine was interacting weakly with the PAH carrier or if it was acting nonspecifically on the renal portal valve. Even if it is assumed that morphine did affect the valve our conclusion that morphine blocks the base transport system is still valid, because in both experiments the ATEF of ^{14}C TEA was reduced essentially to zero while the ATEF of PAH was only slightly reduced. If the only effect of morphine were to relax the renal portal valve there would have been equal

reductions in the ATEF's of both substances. Parenthetically, it should be noted that the observed changes in PAH excretion were not related to any changes in urine flow in our experiments. Although morphine has been shown to be an antidiuretic agent in many species, Inturrisi *et al.*[15] have shown morphine to be diuretic in the chicken.

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REFERENCES

1. I. Sperber, *Nature, Lond.* **158**, 131 (1946).
2. I. Sperber, *Ann. Royal Agriculture College, Sweden* **15**, 108 (1948).
3. D. G. May, J. M. Fujimoto and C. E. Inturrisi, *J. Pharmac. exp. Ther.* **157**, 626 (1967).
4. J. M. Fujimoto and V. B. Haarstad, *J. Pharmac. exp. Ther.* **165**, 45 (1969).
5. W. M. Watrous, D. G. May and J. M. Fujimoto, *J. Pharmac. exp. Ther.* **172**, 224 (1970).
6. R. Hakim and J. M. Fujimoto, *Pharmacologist* **11**, 233 (1969).
7. I. Sperber, *Ann. Royal Agriculture College, Sweden* **16**, 446 (1949).
8. A. J. Quebbemann and B. R. Rennick, *Am. J. Physiol.* **214**, 1201 (1968).
9. A. J. Quebbemann and B. R. Rennick, *J. Pharmac. exp. Ther.* **166**, 52 (1969).
10. H. W. Smith, N. Finkelstein, L. Aliminosa, B. Crawford and M. Graber, *J. clin. Invest.* **24**, 388 (1945).
11. E. L. Way and B. M. Bennett, *J. biol. Chem.* **192**, 335 (1951).
12. J. Cochin and J. W. Daly, *Experientia* **18**, 294 (1962).
13. L. Reio, *Chromatog. Rev.* **1**, 39 (1959).
14. J. M. Fujimoto and R. Hakim, *Pharmacologist* **12**, 292 (1970).
15. C. E. Inturrisi, D. G. May and J. M. Fujimoto, *Europ. J. Pharmac.* **5**, 79 (1968).