Biochemical Pharmacology, Vol. 41, No. 11, pp. 1671–1678, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

IN VITRO STUDIES ON THE DEACETYLATION–REACETYLATION OF ARYLAMIDES AND THE TRANSACETYLATION OF ARYLAMINES BY HUMAN AND RAT WHOLE BLOOD

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(*Received* 5 *November* 1990; *accepted* 4 *January* 1991)

Abstract—Human and rat whole blood were shown to metabolize the aromatic amides acetanilide and phenacetin by deacetylation followed by reacetylation *in vitro*. Derivatives of the parent compounds labelled with deuterium in the *N*-acetyl group produced non-labelled material after incubation. The reaction was monitored by capillary gas chromatographic–mass spectrometric (GC–MS) analysis. There was no significant difference in the acetyl group exchange of these substrates using blood samples donated by non-diabetic volunteers or Type 2 diabetic patients (respective mean ± SEM values = 4.0 ± 0.2% and 4.2 ± 0.3% for trideuteroacetanilide, 6.2 ± 0.6% and 6.1 ± 0.3% for trideuterophenacetin). Increasing the glucose concentration in the incubation medium by 50 mmol/L significantly ($P < 0.01$) increased deacetylation–reacetylation of trideuteroacetanilide in each group (4.6 ± 0.2% and 4.7 ± 0.2% for non-diabetic and diabetic subjects, respectively). In rat blood the amount of deacetylation–reacetylation was much higher: 7.2 ± 0.6% and 8.3 ± 0.7% for trideuteroacetanilide and trideuterophenacetin, respectively. Induction of experimental diabetes using streptozotocin did not significantly change the extent of deacetylation–reacetylation of either deuterated substrate (10.1 ± 2.1% and 9.5 ± 1.1%). Elevation of the incubation glucose concentration by 50 mmol/L produced an increase in acetyl group exchange (for trideuteroacetanilide) in diabetic (14.3 ± 2.2%) and non-diabetic (10.6 ± 1.0%) rats. The donation of acetyl groups (transacetylation) was observed after incubation of blood samples from both diabetic and non-diabetic human subjects and rats with trideuterophenacetin and a molar excess of aniline. This reaction significantly ($P < 0.001$) decreased the acetyl group exchange of trideuterophenacetin (these values were 4.5 ± 0.4% and 3.4 ± 0.6% using samples from non-diabetic human subjects and rats, respectively) and demonstrated the ability of whole blood to catalyse transacetylation (acetyl-CoA-independent acetylation). There was correlation between the amount of (unlabelled) acetanilide produced by acetylation with acetyl-CoA and the percentage present as trideuteroacetanilide. The proportion of trideuteroacetanilide was higher using rat blood (e.g. the values for non-diabetic subjects were 25.5 ± 1.7% vs 8.5 ± 0.3%; $P < 0.001$) although the total amount of acetanilide produced was lower (0.54 ± 0.14 nmol vs 1.82 ± 0.23 nmol; $P < 0.05$) than that observed using human blood.

N-Acetylation of arylamines is catalysed by *N*-acetyltransferase (EC 2.3.1.5) and involves acetyl-coenzyme A (acetyl-CoA) as the acetyl group donor [1]. The enzymes responsible for catalysis of the reverse reaction (deacetylation) belong to the general class of arylacylamide amidohydrolases (EC 3.5.1.13) and are usually referred to as arylamidases or deacetylases [2]. Several studies have reported either acetylation of arylamine drugs and carcinogenic arylamines (for a review see Ref. 3) or deacetylation of the respective arylamides [4–7] both *in vivo* and *in vitro*. Significant tissue differences in acetylating and deacetylating capacities *in vitro* have also been identified [4, 5, 8–10]. The existence of these opposing enzyme systems prompted several groups to investigate the possibility that they both operate *in vivo*. The major difficulty of such studies is determining the source of arylamide at the end of the incubation period, i.e. distinguishing between the parent compound originally present and the product of deacetylation followed by reacetylation ("apparent parent compound"). This problem may be overcome by the use of an arylamide containing either a radioactively-labelled or a stable isotopically-labelled acetyl group. Deacetylation removes the labelled group and the subsequent reacetylation with endogenous (unlabelled) acetyl-CoA gives an unlabelled amide. The extent of acetyl group exchange (i.e. deacetylation followed by reacetylation) of the arylamide can then be determined by measurement of either specific activity or mass spectrometric analysis depending on the isotopic label used. The use of these techniques has established that acetyl group exchange occurs *in vivo* in rats [11, 12], baboons [13] and man [11, 12, 14]. The only published report of *in vitro* acetyl group exchange was observed using ^{14}C-labelled acetylaminofluorene and rat liver slices [15].

Our previous studies on the ability of human blood to acetylate arylamines such as aniline [16], *p*-aminobenzoic acid [17] and sulphamethazine [16, 18] *in vitro* and the several reports on either the

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acetylation or deacetylation capacity of human and rat blood [9, 19–26] prompted us to investigate whether deacetylation–reacetylation of arylamides is also catalysed by blood samples from these species. We chose to use stable isotopically-labelled probes in preference to radiolabels since the analytical methods were more specific. The substrates studied contained *N*-acetyl groups in which the three hydrogen atoms were replaced with deuterium (i.e. trideuteroacetyl groups) and the extent of acetyl group exchange was determined by mass spectrometry. The use of three deuterium atoms enables greater discrimination of the substrate or parent compound from the "apparent parent compound" described above which is three mass units lower.

The second aim of this work was to determine whether human and rat blood also catalysed transacetylation, i.e. an arylamide acting as an acetyl group donor towards an arylamine. Acetyl-CoA-independent acetylation of arylamine drugs has only previously been demonstrated *in vitro* using partially purified pigeon liver *N*-acetyltransferase preparations [27, 28] and rat liver cytosol [29, 30]. However, several studies have shown the ability of various tissues (e.g. liver, kidney, intestine) from rat [29–31], rabbit [32] and hamster [8] to catalyse transacetylation of carcinogenic arylamines such as aminofluorene. In the current study, the use of an acetyl group donor containing a trideuteroacetyl group enables mass-spectrometric discrimination between arylamide produced by acetyl-CoA-dependent acetylation (unlabelled) and that produced by acetyl-CoA-independent transacetylation (trideuterated). Our rationale for investigating deacetylation–reacetylation and transacetylation reactions using samples from diabetic human subjects and streptozotocin-induced diabetic rats is based on the results of our previous studies which demonstrated increased blood *N*-acetyltransferase activities of these groups compared to non-diabetic controls [33, 34]. The effect of artificially increasing the endogenous glucose concentration of samples from all these groups on the above reactions is also reported.

MATERIALS AND METHODS

Materials. Aniline and phenetidine were supplied by the Aldrich Chemical Co. (Poole, U.K.). Streptozotocin and hexadeuteroacetic anhydride were obtained from the Sigma Chemical Co. (Poole, U.K.). The following chemicals were supplied by the BDH Chemical Co. (Poole, U.K.): acetanilide, phenacetin, 2-ethylacetanilide, D-glucose, potassium dihydrogen phosphate and disodium hydrogen phosphate. Diethyl ether and ethyl acetate (both "Pronalysis" grade) were purchased from May and Baker Ltd (Dagenham, U.K.). Methanol (HPLC grade) was obtained from Rathburn Chemicals (Peebleshire, U.K.). Solid carbon dioxide was supplied by the Distillers Company (CO_2) Ltd (Glasgow, U.K.).

Synthesis of trideuteroacetanilide and trideuterophenacetin. Acetanilide and phenacetin in which the three hydrogens of the acetyl group were replaced with deuterium were prepared by adding 10 mmol (1 mL) of hexadeuteroacetic anhydride to 9.5 mmol of aniline and phenetidine, respectively. These mixtures were maintained at 70° for 1 hr and trideuteroacetanilide and trideuterophenacetin were obtained by recrystallizing twice using distilled water and methanol, respectively. The yields were approximately 100% and the purities, as judged by HPLC, were 100%. Melting points were consistent with those previously reported [35] and mass spectrometry confirmed the structures. The chromatographic and UV characteristics of the trideuteroarylamides were identical to those of the unlabelled analogues.

Human subjects. Non-diabetic subjects were staff or students of the Department of Biochemical Medicine at Ninewells Hospital and Medical School. These subjects were not receiving any medication and the mean (range) age was 29 (23–44) years. The diabetic patients were all Type 2 (non-insulin dependent) out-patients attending a monthly diabetic clinic and were not age-matched to the non-diabetic subjects. The mean (range) age of these subjects was 59 (49–67) years and they were being treated by either management of diet alone or with insulin. All the volunteers fasted overnight prior to donating blood. Blood was collected by venepuncture and dispensed into potassium–EDTA anticoagulant containers.

Animals. Adult male Sprague–Dawley rats were purchased from Charles River Ltd (Margate, U.K.). These animals (approximately 400–550 g) were maintained at 20° on 12 hr light/dark cycles in individual cages and were fed SDS rat and mouse No. 1 expanded feed (Special Diet Services Ltd, Witham, U.K.) and water *ad lib.*

Administration of streptozotocin. Streptozotocin was used to induce experimental diabetes in rats fasted overnight. After anaesthetizing the animals with diethyl ether, a single intravenous injection of streptozotocin (50 mg/kg; 0.2 mL/kg body wt) in citrate buffered (pH 4.5) isotonic saline was made into the tail. Five days after administration of streptozotocin, non-fasting rats were killed by stunning and cervical dislocation. Blood was collected into heparinized syringes by cardiac puncture and dispensed into pressure–EDTA anticoagulant containers.

In vitro *assay of deacetylation–reacetylation and transacetylation by human and rat whole blood: incubation and extraction protocol.* Stock solutions (2.5 mmol/L) of trideuteroacetanilide and trideuterophenacetin were prepared by dissolution in methanol–phosphate buffer (66.7 mmol/l, pH 7.4) (5.95) due to the limited solubility of the arylamides in phosphate buffer alone. A volume of 200 μL of whole blood and 100 μL of phosphate buffer containing either trideuteroacetanilide or trideuterophenacetin (10 nmol) were mixed in 10 mL glass bottles. The incubation volume was then increased to 400 μL by adding 100 μL of phosphate buffer. In addition to blank samples (equivolume mixes of whole blood and phosphate buffer), suitably diluted samples containing the trideuteroarylamides only were also prepared to determine if non-enzymic acetyl group exchange occurred during the incubation

period. After incubation at 37° for 24 hr, the reaction was stopped by the addition of 4 mL of diethyl ether. After shaking for 2 min, the samples were centrifuged at 1000 *g* for 10 min then placed on solid carbon dioxide to freeze the aqueous layer. The ether layer was then decanted, split into two aliquots for GC-MS and HPLC analyses and allowed to evaporate at room temperature.

The effect of added glucose and aniline. In a further set of experiments, trideuteroacetanilide (10 nmol) or trideuterophenacetin (10 nmol) in 100 μL of phosphate buffer and either glucose (20 μmol) or aniline (10 or 20 nmol) in 100 μL of phosphate buffer were added to 200 μL of whole blood. These samples were then incubated and extracted as described above.

Gas chromatographic–mass spectrometric (GC-MS) analysis. The extracted residues were reconstituted in 200 μL of ethyl acetate. GC-MS was performed as described previously [12] except that the accelerating voltage of the mass spectrometer was varied by voltage switching and *m/z* values of 135, 138, 179 and 182 monitored (sample time = 50 msec per mass) as appropriate. These *m/z* values correspond to the molecular ions of acetanilide, trideuteroacetanilide, phenacetin and trideuterophenacetin, respectively. The extent of acetyl group exchange was determined using calibration mixtures prepared by mixing known amounts of acetanilide, phenacetin and their respective trideutero analogues to simulate a range of 0–30% acetyl group exchange. The calibration lines were constructed by dividing the height of the peak corresponding to the unlabelled compound by the sum of the heights of the deuterated and unlabelled compounds and plotting this value against the simulated value of acetyl group exchange. The extent of acetyl group exchange in the samples was obtained from these calibration lines by interpolation. A similar procedure was used to calculate the percentage of acetanilide present produced by transacetylation in samples containing aniline and trideuterophenacetin.

HPLC analysis. Phenacetin was measured in all samples incubated with trideuterophenacetin by reverse-phase HPLC. We considered determination of the amount of phenacetin remaining after incubation important since the degree of acetyl group exchange indicates only the relative amounts of deuterated and unlabelled phenacetin present, a parameter which could remain constant at different levels of this arylamide. Similarly, we considered that any stimulatory or inhibitory effects of added glucose or aniline on the deacetylation or reacetylation reactions could alter the amount of phenacetin present. Attempts to develop HPLC methods for aniline and phenetidine were unsuccessful. The acetanilide content of samples co-incubated with aniline and trideuterophenacetin was also determined by HPLC. A Gilson 302 reciprocating pump (Gilson Medical Electronics, Villiers-le-Bel, France) was used to deliver a mobile phase of methanol–phosphate buffer (66.7 mmol/L, pH 7.4) (40:60) at 1 mL/min. Dried extracts were resuspended in 200 μL of HPLC mobile phase containing 2-ethylacetanilide (20 nmol) as external standard prior to injection. Samples were introduced through a Rheodyne injection valve (20 μL, Rheodyne Cotati, CA, U.S.A.) using a Gilson Model 231-401 auto-sampling injector (Gilson Medical Electronics, Villiers-le-Bel, France). Sample components were separated on a Spherisorb ODS2 column (250 mm × 4.6 mm i.d., 5 μm) and detected using a Pye Unicam LC UV detector (Philips Analytical, Cambridge, U.K.) set at a wavelength of 236 nm. The detector signal was monitored by an LDC/Milton Roy CI-10 integrator (Laboratory Data Control, U.K.) interfaced to a NEC PC-8023B-C printer.

Glucose analysis. A Beckman glucose analyser 2 (Beckman-RIIC, High Wycombe, U.K.) employing a glucose oxidase method was used to determine plasma glucose concentrations.

Statistics. Statistical significance was tested by either a two-tailed Student's *t*-test (population variances not assumed to be equal) or a paired *t*-test as appropriate. The level of significance was set at $P < 0.05$.

RESULTS

In vitro *deacetylation–reacetylation by whole blood*

Incubation of phosphate buffer solutions containing only trideuteroacetanilide and trideuterophenacetin at 37° for 24 hr established that non-enzymatic acetyl group exchange of the deuterated compounds did not occur.

Human studies. Unlabelled acetanilide and phenacetin were detected in blood samples incubated with the respective trideuteroacetyl analogues. Acetyl group exchange of trideuterophenacetin was significantly ($P < 0.01$) higher than that of trideuteroacetanilide (Table 1). The extent of deacetylation–reacetylation of either substrate was not significantly different using blood samples donated from non-diabetic and Type 2 diabetic subjects, although the mean fasting plasma glucose concentration of the diabetic group was significantly higher. Increasing the incubation glucose concentration by 50 mmol/L significantly increased the extent of acetyl group exchange by each group with the exception of trideuterophenacetin incubated with blood samples from non-diabetic subjects (Table 1). The total amounts of phenacetin (labelled and unlabelled) remaining after incubation of whole blood with trideuterophenacetin were not significantly different using samples from diabetic and non-diabetic volunteers. This parameter was also unaffected by the addition of glucose to samples from both groups (Table 1).

Acetyl group exchange of trideuterophenacetin was decreased by co-incubation with an equimolar amount of aniline although this effect was only significant using blood samples donated by diabetic subjects (Table 2). Co-incubation of trideuterophenacetin with a molar excess of aniline significantly decreased the proportion of unlabelled to deuterated substrate in both groups. Aniline had no significant effect on the amount of phenacetin recovered (Table 2).

Rat studies. These results are shown in Tables 3 and 4 and can be summarized as follows:

Table 1. The effect of added glucose on *in vitro* acetyl group exchange of trideuteroacetanilide and trideuterophenacetin by whole blood from Type 2 diabetic and non-diabetic human subjects

Subjects (number)	Plasma glucose concentration* (mmol/L)	Substrate	Increase in incubation glucose concentration (mmol/L)	% Acetyl group exchange	Amount of phenacetin present (nmol)
Non-diabetic (11)	5.3 ± 0.1	Trideuteroacetanilide	0	4.0 ± 0.2	—
			50	4.6 ± 0.2§	—
Non-diabetic (8)	4.8 ± 0.2	Trideuterophenacetin	0	6.2 ± 0.6	8.54 ± 0.33
			50	6.9 ± 0.8	8.78 ± 0.24
Type 2 diabetic (8)	10.0 ± 1.0†	Trideuteroacetanilide	0	4.2 ± 0.3	—
			50	4.7 ± 0.2§	—
Type 2 diabetic (8)	9.2 ± 0.6‡	Trideuterophenacetin	0	6.1 ± 0.3	8.00 ± 0.22
			50	6.8 ± 0.4	8.12 ± 0.21

Values represent mean ± SEM. Initial substrate concentration = 25 μmol/L (10 nmol/sample). Incubation time = 24 hr.
* Fasting.
The values are significantly different from the non-diabetic group using Student's *t*-test, †$P < 0.002$, ‡$P < 0.001$.
The values are significantly different from the control samples (no glucose added) by paired *t*-test, §$P < 0.01$.

(a) There was no significant difference in the extent of deacetylation–reacetylation of the deuterated substrates using rat blood samples (Table 3).

(b) The degree of acetyl group exchange of trideuteroacetanilide ($P < 0.01$) and trideuterophenacetin ($P < 0.05$) using rat blood (Table 3) was significantly higher than that observed using samples donated by human subjects (Table 1). The mean amount of phenacetin present after incubation of trideuterophenacetin with rat blood samples was significantly lower ($P < 0.05$) than that obtained using human blood.

(c) As expected, the mean plasma glucose concentration of rats treated with streptozotocin was significantly higher than that of untreated animals. Although the extents of acetyl group exchange catalysed by blood from these animals was higher than that of samples from the non-diabetic rats the differences were not significant (Table 3).

(d) Addition of glucose to the incubation medium significantly increased deacetylation–reacetylation of trideuteroacetanilide by both groups (Table 3).

(e) There was no significant differences in the amounts of phenacetin present either in samples from both groups or after addition of glucose (Table 3).

(f) The extent of acetyl group exchange of trideuterophenacetin was significantly decreased by co-incubation with aniline although the recovery of phenacetin was not significantly changed (Table 4).

In vitro *transacetylation by whole blood*

Trideuteroacetanilide was detected in all blood samples co-incubated with trideuterophenacetin and aniline. The mean total amounts of acetanilide (i.e. labelled and unlabelled) produced and the percentages recovered as trideuteroacetanilide (i.e. the product of transacetylation) in studies using human and rat blood samples are shown in Table 5 and Table 6, respectively. Although the total amount of acetanilide produced using samples donated by human subjects was significantly higher ($P < 0.05$) than by rat blood, the percentage of trideuteroacetanilide present was significantly higher using rat blood samples ($P < 0.001$). The correlation between these two parameters using human and rat blood was significant ($r = 0.88$, $N = 16$, $P < 0.001$ and $r = 0.84$, $N = 14$, $P < 0.001$, respectively). In both species, samples from diabetic and non-diabetic subjects showed no significant difference in either the total amount of acetanilide produced or the amount of trideuteroacetanilide produced by transacetylation. Increasing the incubation aniline concentration to 50 μmol/L significantly increased the amount of acetanilide produced in all cases except for blood samples donated by human diabetic subjects. This treatment did not significantly change the percentage of trideuteroacetanilide present in any case.

DISCUSSION

This is the first reported study to demonstrate deacetylation–reacetylation of arylamides catalysed by human and rat blood *in vitro*. The results of

Table 2. The effect of added aniline on *in vitro* acetyl group exchange of trideuterophenacetin by whole blood from Type 2 diabetic and non-diabetic human subjects

Subjects (number)	Plasma glucose concentration* (mmol/L)	Initial aniline concentration (μmol/L)	% Acetyl group exchange	Amount of phenacetin present (nmol)
Non-diabetic (8)	4.8 ± 0.2	0	6.2 ± 0.6	8.54 ± 0.33
		25	5.2 ± 0.4	8.51 ± 0.21
		50	4.5 ± 0.4§	8.50 ± 0.22
Type 2 diabetic (8)	9.2 ± 0.6†	0	6.1 ± 0.3	8.00 ± 0.22
		25	5.2 ± 0.2‡	7.89 ± 0.23
		50	4.8 ± 0.3§	7.88 ± 0.23

Values represent mean ± SEM. Initial trideuterophenacetin concentration = 25 μmol/L (10 nmol/sample). Incubation time = 24 hr.

* Fasting.

The values are significantly different from the non-diabetic group using Student's *t*-test, †$P < 0.001$.

The values are significantly different from the control samples (no aniline added) by paired *t*-test, ‡$P < 0.01$, §$P < 0.001$.

Table 3. The effect of added glucose on *in vitro* acetyl group exchange of trideuteroacetanilide and trideuterophenacetin by whole blood from non-diabetic and streptozotocin-induced diabetic rats

Animals (number)	Plasma glucose concentration* (mmol/L)	Substrate	Increase in incubation glucose concentration (mmol/L)	% Acetyl group exchange	Amount of phenacetin present (nmol)
Non-diabetic (6)	6.9 ± 0.3	Trideuteroacetanilide	0	7.2 ± 0.6	—
			50	10.6 ± 1.0§	—
Non-diabetic (8)	5.6 ± 0.1	Trideuterophenacetin	0	8.3 ± 0.7	7.30 ± 0.31
			50	8.9 ± 0.9	7.40 ± 0.20
Diabetic (8)	23.0 ± 1.0‡	Trideuteroacetanilide	0	10.1 ± 2.1	—
			50	14.3 ± 2.2§	—
Diabetic (6)	14.0 ± 1.4†	Trideuterophenacetin	0	9.5 ± 1.1	7.26 ± 0.26
			50	11.0 ± 1.9	7.64 ± 0.10

Values represent mean ± SEM. Initial substrate concentration = 25 μmol/L (10 nmol/sample). Incubation time = 24 hr.

* Non-fasting.

The values are significantly different from the non-diabetic group using Student's *t*-test, †$P < 0.01$, ‡$P < 0.001$.

The values are significantly different from the control samples (no glucose added) by paired *t*-test, §$P < 0.01$.

Table 4. The effect of added aniline on *in vitro* acetyl group exchange of trideuterophenacetin by whole blood from non-diabetic and streptozotocin-induced diabetic rats

Animals (number)	Plasma glucose concentration* (mmol/L)	Initial aniline concentration (μmol/L)	% Acetyl group exchange	Amount of phenacetin present (nmol)
Non-diabetic (8)	5.6 ± 0.1	0	8.3 ± 0.7	7.30 ± 0.31
		25	4.2 ± 0.6‡	6.99 ± 0.15
		50	3.4 ± 0.6‡	6.77 ± 0.13
Diabetic (6)	14.0 ± 1.4†	0	9.5 ± 1.1	7.26 ± 0.26
		25	5.7 ± 0.7‡	6.99 ± 0.17
		50	4.9 ± 0.7‡	6.90 ± 0.18

Values represent mean ± SEM. Initial trideuterophenacetin concentration = 25 μmol/L (10 nmol/sample). Incubation time = 24 hr.

* Non-fasting.

The values are significantly different from the non-diabetic group using Student's *t*-test, †$P < 0.01$.

The values are significantly different from the control samples (no aniline added) by paired *t*-test, ‡$P < 0.001$.

Table 5. *In vitro* transacetylation of aniline by trideuterophenacetin catalysed by whole blood from non-diabetic and Type 2 diabetic human subjects

Subjects (number)	Plasma glucose concentration* (mmol/L)	Initial aniline concentration (μmol/L)	Total amount of acetanilide produced (nmol)	% Trideuteroacetanilide present
Non-diabetic (8)	4.8 ± 0.2	0	—	—
		25	1.42 ± 0.30	8.9 ± 0.3
		50	1.82 ± 0.23‡	8.5 ± 0.3
Type 2 diabetic (8)	9.2 ± 0.6†	0	—	—
		25	1.12 ± 0.21	8.7 ± 0.4
		50	1.45 ± 0.31	8.8 ± 0.3

Values represent mean ± SEM. Initial trideuterophenacetin concentration = 25 μmol/L. Incubation time = 24 hr.
* Fasting.
The values are significantly different from the non-diabetic group using Student's *t*-test, †$P < 0.001$.
The value obtained is significantly different from that at an aniline concentration of 25 μmol/L by paired *t*-test, ‡$P < 0.02$.

previous studies reporting either acetylation of arylamines or deacetylation of arylamides by blood samples from these species [9, 16–18, 20–26] must therefore now be considered to represent an equilibrium between these processes. For example, the reported inability of human blood to deacetylate *p*-acetaminobenzoic acid [20] may be due either to an actual deficiency of a deacetylase specific for this substrate or to rapid reacetylation of *p*-aminobenzoic acid liberated by deacetylation. The observed acetyl group exchange of acetanilide and phenacetin may also be of toxicological importance since it involves the initial generation of aniline and phenetidine, respectively. The rate of reacetylation and therefore the lifetime of these highly toxic arylamines is unknown. The amount of phenacetin present was analysed to provide an indirect indication of the maximum amount of phenetidine present. Clearly, specific determination of aniline and phenetidine would be useful in future studies of the above reactions.

Deacetylation–reacetylation of trideuterophenacetin was significantly higher than that of trideuteroacetanilide using whole blood from human subjects (Table 1). Substrate structure also influences the equilibrium between these reactions *in vivo*. For example, the degree of acetyl group exchange of trideuteroparacetamol by rats decreases in the order trideuteroacetanilide, trideuterophenacetin and trideuteroparacetamol after separate administration of these deuterated compounds [11]. Successive substitution of up to two methyl groups on the N^1-pyrimidine ring of acetylsulphadiazine significantly increases acetyl group exchange of this homologous acetylsulphonamide series in man [36]. These results probably reflect altered substrate binding affinity of the enzymes involved [36].

The increased deacetylation–reacetylation capacity of rat blood (Table 3) compared to human blood (Table 1) contrasts the higher *N*-acetyltransferase activity of the latter [20, 24, 34]. This difference could be due to several variables including the activities of *N*-acetyltransferase and deacetylase enzymes and concentrations of both acetyl group donors and acceptors. The decreased arylamide remaining and increased acetyl group exchange observed using rat blood could be explained if reacetylation of the deacetylated amine is rate-limiting. This hypothesis is supported by the significantly lower phenacetin recovery from incubations using rat blood.

The degree of deacetylation–reacetylation was unrelated to the endogenous glucose concentration in either species (Tables 1 and 3). Elevation of the incubation glucose level increased acetyl group exchange although this effect was only significant for samples incubated with trideuteroacetanilide. Previous studies have demonstrated that glucose significantly enhances blood *N*-acetyltransferase activity *in vitro* [20, 24, 33, 34], an effect which we have recently shown to be at least partially mediated by metabolism of glucose to an acetyl group donor [37]. A similar mechanism could account for the increased deacetylation–reacetylation capacity of blood samples supplemented with glucose if the reacetylation reaction is rate-limiting.

Co-incubation of equimolar amounts of aniline and trideuterophenacetin with whole blood reduced acetyl group exchange of the latter compound, an effect which was particularly marked using rat blood (Tables 2 and 4). This reduction was significant in every case when a molar excess of aniline was used. This result could reflect competition for *N*-acetyltransferase between aniline and phenetidine (produced by either deacetylation of trideuterophenacetin or donation of its acetyl group to aniline). We have previously demonstrated partial inhibition of phenetidine acetylation by human and rat blood samples incubated with aniline [38].

The above study also enabled us to specifically demonstrate the ability of human and rat blood to catalyse transacetylation *in vitro* (Tables 5 and 6). The production of trideuteroacetanilide by blood samples from these species incubated with trideuterophenacetin and aniline clearly indicates that trideuterophenacetin may act as an acetyl group donor for the acetylation of aniline. The significant correlations observed between the extents of aniline acetylation (i.e. total acetanilide produced) and aniline transacetylation (i.e. percentage of acetanilide

Table 6. *In vitro* transacetylation of aniline by trideuterophenacetin catalysed by whole blood from non-diabetic and streptozotocin-induced diabetic rats

Animals (number)	Plasma glucose concentration* (mmol/L)	Initial aniline concentration (μmol/L)	Total amount of acetanilide produced (nmol)	% Trideuteroacetanilide present
Non-diabetic (8)	5.6 ± 0.1	0	—	—
		25	0.36 ± 0.09	24.2 ± 1.1
		50	0.54 ± 0.14‡	25.5 ± 1.7
Diabetic (6)	14.0 ± 1.4†	0	—	—
		25	0.48 ± 0.11	25.4 ± 1.2
		50	0.77 ± 0.11‡	28.2 ± 1.8

Values represent mean ± SEM. Initial trideuterophenacetin concentration = 25 μmol/L. Incubation time = 24 hr.
* Non-fasting.
The values are significantly different from the non-diabetic group using Student's *t*-test, †$P < 0.01$.
The value obtained is significantly different from that at an aniline concentration of 25 μmol/L by paired *t*-test, ‡$P < 0.02$.

present as trideuteroacetanilide) in each species may indicate that the same enzyme is involved in both processes. These reactions are known to be catalysed by identical enzymes in rabbit liver [32]. The significantly increased amount of acetanilide produced using human blood samples confirms the higher *N*-acetyltransferase activity compared to rat blood [20, 24, 34]. In contrast, the amount of trideuteroacetanilide produced by blood samples from the two species was not significantly different although the ratio of labelled acetanilide to total acetanilide was significantly higher using rat blood. These differences could reflect the fact that acetylation and transacetylation are acetyl-CoA-dependent and acetyl-CoA-independent processes, respectively. Although several enzymic methods exist for determination of acetyl-CoA in tissues such as rat kidney and liver [39], we have been unable to measure this cofactor in blood samples using spectrophotometric and fluorimetric methods (R. M. Lindsay, A. M. McLaren and J. D. Baty, unpublished results). In the absence of available information on blood acetyl-CoA concentrations in these species, the possibility that differences in this parameter account for the discrepancies in acetylation and transacetylation capacities cannot be excluded.

In conclusion, this study unequivocally demonstrates that whole blood from human subjects and rats catalyses deacetylation–reacetylation and transacetylation of arylamides and arylamines respectively *in vitro*. These results suggest that the interpretation of previous studies on either the acetylation or deacetylation capacity of whole blood is complex and reflects an equilibrium between these reactions. The toxicological implications of these findings in terms of both drug metabolism and activation of carcinogenic arylamines, which are also metabolized by these routes [3], may be important and we are currently investigating the effect of arylamide dose on the extent of these reactions *in vivo*.

Acknowledgements—We thank the Research Initiatives Fund (University of Dundee) for financial support. Dr J. F. Doran of the Department of Biochemical Medicine at Ninewells Hospital and Medical School and Dr N. R. Waugh of the Department of Community Medicine, Tayside Health Board are thanked for collecting blood samples from the non-diabetic volunteers and diabetic outpatients, respectively.

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