a serum albumin concentration of 3 per cent. This agrees well with the previous estimate of 98 per cent bound to pooled human serum [7].

Binding of glipizide to both sites increases with decreasing temperature. The thermodynamic parameters, calculated by standard methods, are given in Table 1. The substantial temperature dependence suggests that ionic forces are not involved in the interaction unlike the binding of sulphonamides [8, 9], tolbutamide and chlorpropamide [3]. The free energy of binding is the result of almost equal contributions by a negative enthalpy change and a positive entropy change. This is consistent with binding by van der Waals forces [10] and is supported by the pH independent binding (Fig. 2). At pH 6·4 where the drug is 76 per cent ionised (pKa = 5·9), binding is almost identical to that at pH 7·4 where 97 per cent is ionised. Thus, albumin possesses equal affinity for both non-ionised and ionised glipizide which further suggests that ionic forces are not involved.

The interaction of glipizide with HSA thus resembles that of glyburide rather than tolbutamide [3]. This is significant in that glyburide is only weakly displaced by drug anions such as phenylbutazone, warfarin and salicylate, whereas tolbutamide is very strongly displaced.* For this reason, it is possible that glipizide may be a suitable sulphonylurea for use in conjunction with anionic drugs to reduce displacement hazards.

Previously, it was shown that tromethamine buffer and chloride greatly reduce the binding to HSA of tolbutamide but not glyburide [4]. From Fig. 2 it can be seen that the interaction of glipizide with HSA is not reduced significantly by tromethamine. Furthermore, the presence of 0·154 M sodium chloride reduces glipizide association constants k_1 and k_2 by only $\frac{1}{2}$ and $\frac{1}{3}$, respectively, compared to a 30-fold reduction in k_1 for tolbutamide [4]. Therefore, in this respect also, binding of glipizide resembles that of glyburide rather than tolbutamide or sulphamethizole [9]. This suggests that, because of the non-ionic nature of the interaction, the choice of buffer for binding studies *in vitro* of glipizide is not as critical as for drugs bound solely as anions.

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Tissue uptake of δ-aminolaevulinic acid

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Acute intermittent porphyria is a genetically inherited disease in which there is an increased hepatic biosynthesis and excessive urinary excretion of prophyrins and their precursors δ-aminolaevulinic acid (ALA) and porphobilinogen. ALA is also present in abnormally high plasma concentrations. The normal plasma ALA level is usually taken to be zero; however, concentrations as high as 24 μg/ml have been reported in acute porphyric attack [1].

It is known that porphobilinogen and the porphyrins are pharmacologically inactive and it is, therefore, unlikely that they play any direct role in the development of the signs and symptoms of acute porphyria [2]. Recently however, there has been increasing evidence that ALA may be an aetiological factor in the production of the clinical manifestations of the disease.

In vitro studies have shown that ALA is capable of inhibiting red cell and brain ATPase, and membrane sodium transport [3, 4]. Such actions of ALA *in vivo* could affect tissue function. To exert such actions however, it would be necessary for ALA to penetrate into these tissues. It is already known that ALA can cross the blood-brain barrier and thereby enter the brain [5].

The present experiment was designed to see if ALA was taken up into tissues other than the brain and if so, how long it remained unchanged in certain tissues.

Since it was necessary to evaluate tissue ALA levels which had been in equilibrium with a blood ALA concentration similar to that found in acute porphyria, ALA was injected intraperitoneally in rats and the resulting blood concentrations were measured. Blood samples were removed from a cannulated carotid artery in the anaesthetized rat and the ALA content was estimated at times after an intra-

peritoneal injection of ALA. It was found that ALA (40 mg/kg) in saline, injected in a volume of 0·5 ml, produced a blood concentration of some 20–30 μg/ml, which remained constant for 15–45 min after injection. The tissue estimations were therefore made after this dose of ALA, and the initial estimates were made 30 min after injection.

Methods

Fifteen male Sprague–Dawley rats (200–250 g) were given i.p. injections of ALA (40 mg/kg) in saline. The ALA had previously been spiked with [^{14}C]ALA and adjusted to pH 6–7 with molar sodium bicarbonate. The volume of injection was <0·5 ml. As controls a further three rats were given i.p. injections of [^{14}C]ALA only; this allowed ALA determination by isotopic methods but the total ALA thus injected was less than 4 μg.

Thirty minutes after injection three of the animals given ALA and the three control animals were injected into a tail vein with technecium (^{99}Tc) labelled serum albumin. This was in order to determine the blood contents of the various tissues. The albumin was allowed to circulate for two minutes at which time the animals were stunned, exsanguinated from the throat, and samples of the following tissues taken; heart, lung, liver, kidney, spleen, gut (ileum), brain, mesenteric fat and blood. The blood ALA content was determined chemically by the method described by Haeger-Aronsen [6] and the ALA content of the other tissues, independent of their blood ALA contents, was determined as previously described [5].

Using the same methods, ALA was determined in the heart, liver, brain and blood of three animals at times 3, 24, 72 and 192 hr after injection.

For each tissue, and at each time interval, a chromatogram was run of the tissue supernate against an ALA standard. By determination of the radioactivity in the test spot which corresponded with the ALA standard, the activity present due to unmetabolized ALA could be ascertained.

The fraction of the total counts on the chromatogram which this represented, was also the fraction of the total tissue counts which was due to unmetabolized labelled ALA. The ALA recovery from all chromatograms was between 85 and 90 per cent.

As the activity of the labelled ALA originally injected in the spiked solution was known, it was relatively simple to calculate the real tissue ALA level from these figures.

Any errors involved in these procedures would be constant throughout, and would tend to underestimate the tissue ALA, rather than overestimate it.

Although chemical methods are not so sensitive as isotopic methods when measuring small quantities, each of the tissue supernates were also assayed chemically for ALA [6]. The results from both methods were in good agreement.

Results

The results obtained 30 min after injection are shown in Table 1. In the control animals it can be seen that the tissue ALA contents were either undetectable or very low. The highest was found to be in the blood where a mean content of 0·25 μg/ml was obtained. After ALA was injected however, all the tissues had high ALA contents compared with their control levels. The relatively greatest increase was seen in the liver, and the lowest in the brain.

Table 1. Mean tissue ALA content (μg/g) 30 min after i.p. injection

	Control (^{14}C ALA) only	Test (40 mg/kg ALA i.p.)
Blood	0·25 ± 0·15	26·66 ± 7·43
Brain	0·046 ± 0·04	1·07 ± 0·41
Heart	0·00	23·53 ± 3·16
Liver	0·06 ± 0·04	162·53 ± 60·71
Kidney	0·21 ± 0·10	23·33 ± 3·70
Spleen	0·00	74·10 ± 17·26
Gut	0·00	72·97 ± 3·48
Fat	0·00	54·70 ± 10·30

Each result is expressed as the mean of three experiments ± S.D.

The change of ALA tissue content with time is shown in Table 2. The blood concentration of ALA remained elevated above normal for up to 24 hr after injection; at 72 hr it had returned to normal. The liver, heart and brain tissues however, maintained ALA levels greater than normal even after the blood level had returned to normal. Thus at 72 hr all these tissues showed ALA levels greater than the control values and at 192 hr after injection, although the brain content was normal, the heart and liver continued to do so.

Discussion

These results show that in rats exposed to an equilibrium blood concentration of ALA similar to that found in an acute porphyric attack, there is passage of ALA in to the various tissues studied. The fact that the ALA content of the liver, heart and brain can remain greater than the control contents, even when the blood concentration has returned to the control value, suggests that in these tissues at least ALA is not in a diffusion equilibrium with the blood, but is either bound or maintained there actively by the expenditure of metabolic energy.

Acute porphyria is clinically characterized by gastrointestinal, cardiovascular, neurological and psychological abnormalities. Thus the fact that ALA can penetrate the gut, heart, brain and other tissues, at concentrations occurring in porphyria, together with the fact that it demonstrates some pharmacological activity, is of interest. Although in this study the tissue uptake was studied in the acute situation, it is important to note that in acute porphyria the tis-

Table 2. Time after injection

		3 hr	24 hr	72 hr	192 hr
Tissue ALA (μg/g)	Blood	3·80 ± 0·51	0·83 ± 0·18	0·30 ± 0·10	0·27 ± 0·06
	Brain	0·49 ± 0·10	0·14 ± 0·05	0·12 ± 0·05	0·04 ± 0·01
	Heart	2·62 ± 0·54	1·03 ± 0·10	1·22 ± 0·35	0·50 ± 0·10
	Liver	82·73 ± 7·16	3·49 ± 0·57	3·18 ± 0·40	0·38 ± 0·91

Each result is expressed as the mean of three experiments ± S.D.

sues are likely to be chronically exposed to elevated circulating ALA levels, as there is elevated urinary excretion even in latent asymptomatic cases. Therefore quite high tissue contents of ALA are probably reached, which will be maintained over long periods of time.

These results then contribute further to the accumulating evidence that ALA could be a factor in the production of the clinical manifestations of acute intermittent porphyria.

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Sex difference in the phospholipid composition of rat liver microsomes

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Male rats have a higher level of drug-metabolizing activity in the liver than females [1–10]. There is also a sex difference in phospholipid metabolism of the rat liver [11–15]. Since there is an abundance of evidence to demonstrate that phospholipids play a role in drug metabolism [16–23], it seems possible that the sex difference in drug-metabolizing activity and phospholipid metabolism might be related. However, a sex-dependent association between microsomal phospholipids and drug metabolism has not yet been studied. In previous papers [16, 17, 24], we have demonstrated that changes in drug-metabolizing processes are accompanied by corresponding and parallel alterations in microsomal phospholipids. We decided, therefore, to study whether or not a difference exists in the level and composition of microsomal phospholipids between male and female rats which may be similar to changes of drug metabolism induced by drugs [16, 17, 24].

Two groups of four male and four female Wistar albino rats of approximately equal age and weighing 132–141 g and 105–118 g, respectively, were killed on two separate days. Liver microsomes were prepared [16] and analyzed for protein [25] and phospholipid content [16]. Extraction and separation of the individual phospholipids by thin-layer chromatography were carried out as described previously [16].

The activity of MT-ase* was determined in a medium (2·0 ml) containing Tris buffer, pH 8, 250 μmoles; adenosine triphosphate sodium, 8 μmoles; $MgCl_2$, 20 μmoles; L-[^{14}C-Me]methionine, 0·8 μCi (sp. act. 33·3 μCi/μmole); microsomes, 0·3 ml (protein content 4·5 to 7·4 mg/ml) and supernatant, 0·3 ml (protein content 12·0 to 16·5 mg/ml) equivalent to 75 mg wet liver tissue. Incubation was carried out at 37°, and the reaction was stopped after 0, 10 and 20 min by the addition of a 0·5-ml aliquot to 9·5 ml chloroform–methanol mixture (2:1, by volume) containing 2·0 ml of 0·73% saline; the [^{14}C-Me]-labeled phospholipid was then

* Abbreviations used: MT-ase, *S*-adenosyl-methionine microsomal-phospholipid methyl transferase; PA, phosphatidic acid; PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PDE, phosphatidyldimethylethanolamine, PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; LPC, lysophosphatidylcholine; and SM, sphingomyelin.

Table 1. Measurements of liver weight, hepatic microsomal protein and phospholipid of male and female rats*†

Sex	Relative liver wt (% of body wt)	Microsomal Protein (mg/g liver)	Microsomal Phospholipid (μmoles P/g liver)
Male	5·04 ± 0·04	17·54 ± 0·97	9·60 ± 0·21
Female	5·07 ± 0·10	17·10 ± 1·05	8·22 ± 0·20‡

* Description of animals and methods is given in the experimental section.
† Results represent the mean ± S. E. of eight rats in each group.
‡ Values differ significantly from males, $P < 0·05$.