TABLE 1. RADIOACTIVE WATER IN URINE SAMPLES FROM RATS, COLLECTED OVER SUCCESSIVE TIME INTERVALS AFTER ORAL ADMINISTRATION OF 10 MG/KG OF ORPHENADRINE-^{3}H HCl (I)

Time interval of collection in hr	Radioactivity present as water (Each value represents the mean of two rats) percentage of administered dose	percentage of total radioactivity in urine
0– 8	0·06	0·76
8–24	0·20	0·76
24–48	0·38	15·38
48–72	0·32	46·38

From these figures it can be concluded that a volatile radioactive product is involved, the biological half-life of which amounts to several days. Since this is in agreement with that reported in the literature for tritiated water in the rat,[3] little doubt would remain that tritiated water is actually present. The question arises of how it is formed. Chemical instability of the label is unlikely; on theoretical grounds the tritium atom can be regarded as being bound very stable. The alternative explanation is a metabolic attack on the side chain, setting free the label. A possibility might be that *N,N*-didemethyl orphenadrine, one of the metabolites of orphenadrine in the rat,[2] would be subject to an oxidative deamination. This is consistent with the literature on related compounds such as amitriptyline[4] and chlorpromazine[5] for which an oxidative deamination *in vivo* has been proposed on the ground of the structure of metabolites found. Moreover, a reaction starting from *N,N*-didemethyl orphenadrine would explain the quantitatively minor importance of this side-chain degradation, which will be limited by the concentration of the intermediate metabolite, itself formed in small amounts only.

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Effects of halothane, trichloroethylene, pentobarbitone and thiopentone on amino acid transport in the perfused rat liver

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EVIDENCE[1, 2] has recently been presented that the liver plays an important role in the regulation of blood amino acid levels. Since various anaesthetics have been found to have effects on membrane functions of liver cells,[3] experiments were conducted to find whether the anaesthetics also affect amino acid transport in the perfused rat liver.

METHODS

The isolated, perfused rat liver preparation used in this work was similar to that described by Fisher and Kerly,[4] but adapted for the use of Krebs–Ringer bicarbonate buffer instead of rat blood as perfusion medium. The method will be described in full elsewhere.[5]

The anaesthetics used in this work were halothane (1:1:1-trifluoro-2-bromo-2-chloroethane, Fluothane, I.C.I.), trichloroethylene (Trilene, I.C.I.), pentobarbitone sodium (veterinary Nembutal, Abbott Laboratories) and thiopentone sodium (Pentothal, May & Baker). Their administration to the animals is described elsewhere.[3]

The effects of the anaesthetics on the perfusate levels of the amino acids were tested by adding the anaesthetic to the perfusate after 90 min of blank perfusions and measuring changes in the amino acid concentrations.

The gaseous anaesthetics, halothane and trichloroethylene were added to the perfusate by passing the anaesthetic vapour into the perfusate oxygenator with the O_2/CO_2 (95:5) stream for a 10 min period, and conditions were so arranged that the perfusate was saturated with the vapour for that period. The methods used are described in more detail elsewhere.[3] Data on the solubility in water of these two anaesthetics[6, 7] suggest that the perfusate concentration of halothane during the administration period was 15–20 times that found in the blood of rats under deep halothane anaesthesia[8] and the concentration of trichloroethylene was 5 times that to be expected in the blood of anaesthetised rats (data extrapolated from measurements in man,[9] since no figures are available for rats). Pentobarbitone and thiopentone were added to the perfusate reservoir in amounts equal in each case to that required to anaesthetise the donor rat (4·8 mg of pentobarbitone in 0·08 ml of aqueous solution per 100 g body wt., and 7·5 mg of thiopentone in 0·3 ml of freshly prepared solution per 100 g body wt.).

In each experiment the same anaesthetic was used to produce anaesthesia for removal of the liver from the donor rat as was added to the perfusate in the subsequent perfusion.

Perfusate amino acid concentrations were measured chromatographically using the Technicon automic amino acid analyser, 20-hr run system (Technicon Instruments, Amino Acid Analyser handbook, 2nd issue). Perfusate samples were acidified with 1/20 vol. of 1·0 N HCl and immediately transferred to the analyser column, without further treatment. The quantity of protein present was not enough to interfere.

RESULTS AND DISCUSSION

The isolated perfused rat liver will regulate the perfusate concentration of the amino acids, except valine, leucine and isoleucine, the branched chain group, to characteristic levels by net transport into or out of the cells.[2] Thus, when the liver is perfused with a medium initially containing no amino acids, the acids are given out by the liver until the 'equilbrium level' of each is reached. Thereafter this level is maintained, in many cases against large concentration gradients. On the other hand, the branched chain amino acids behave differently as a group.[2] They are continuously transported out of the liver cells at rapid rates even against considerable concentration gradients, their concentrations not tending towards characteristic levels. This pattern of events can also be seen in the perfusions, results of which are shown in Figs. 1 and 2, before addition of the anaesthetics.

The effects on perfusate amino acid levels of adding halothane and trichloroethylene to the perfusion medium are shown in Fig. 1. Both anaesthetics tended to cause a flow, from the liver into the perfusate, of those amino acids normally maintained at perfusate concentrations low relative to the levels in the liver, i.e. maintained against considerable concentration gradients.[2] In the halothane perfusions the leakage was relatively small considering the size of the dose and, except for threonine and possibly lysine and histidine, tended to be recovered when administration of the anaesthetic was stopped. Trichloroethylene, however, caused in some cases a much greater loss from the cells which was not recovered in the remaining 90 min of perfusion. The greatest degree of leakage was generally of those amino acids normally maintained against the greatest concentration gradients,[2] namely aspartate, glutamate, glycine, alanine, serine and lysine and the loss was small, or these was no measurable loss, of those acids normally maintained against the smallest gradients, namely cystine, arginine, phenylalanine and tyrosine (no figures are available for liver levels of tryptophan). Changes in the perfusate level of histidine do not fit into this pattern, for its concentration fell after administration of trichloroethylene (see Fig. 1), although the normal liver level is considerably higher than the perfusate level (Spruyt[10]). This result indicates a profound fall in the intracellular concentration of histidine.

Turning to the branched chain amino acids, during normal blank perfusions the perfusate (extracellular) concentrations of valine, leucine and isoleucine become greater than their intracellular concentrations[10] after 1–2 hr. Halothane had little or no effect on this continuous outflow, but the reduced output of these acids after tricloroethylene treatment demonstrates that in these perfusions

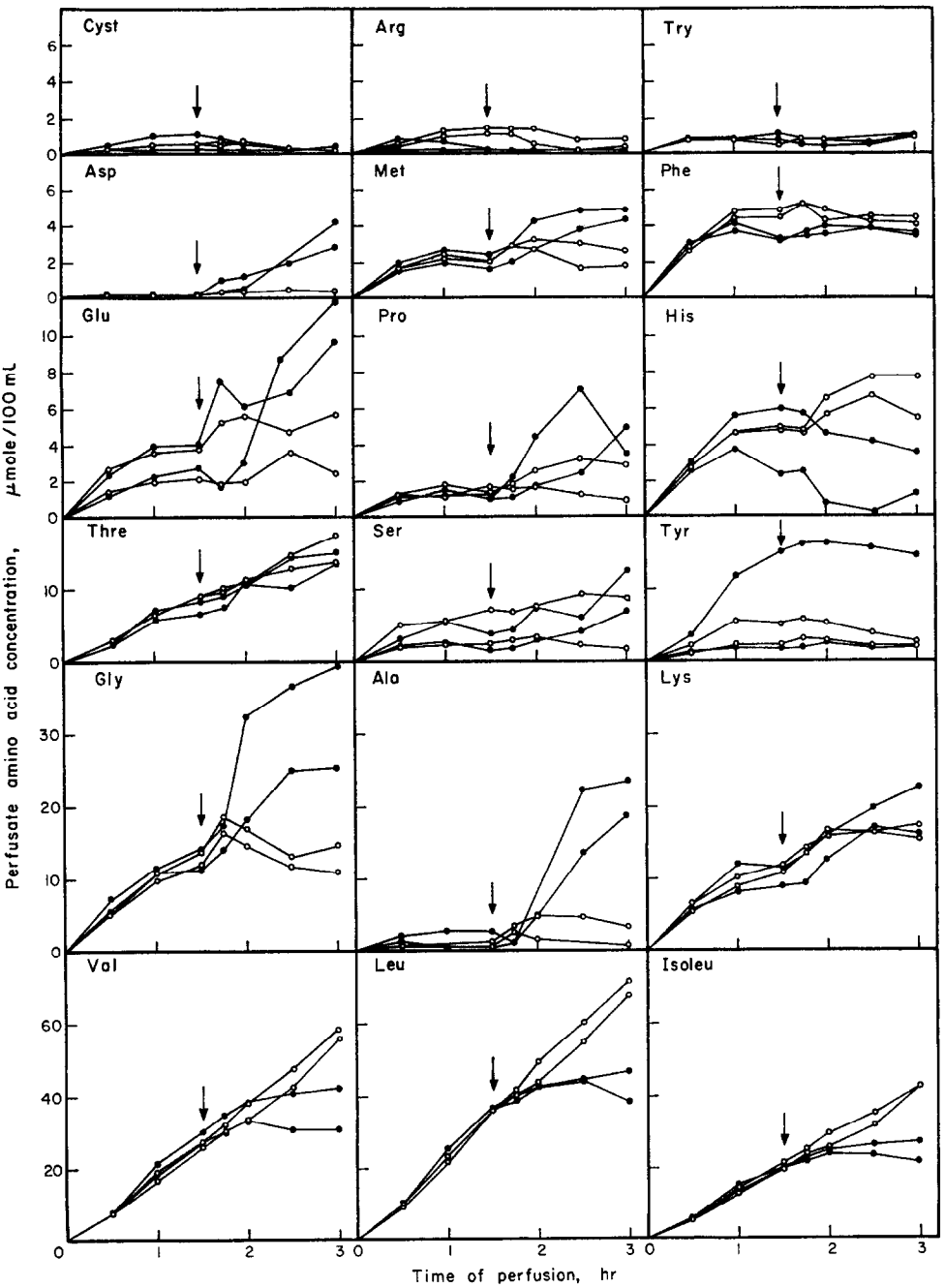

FIG. 1. Effects of halothane and trichloroethylene on the perfusate levels of each of the amino acids. Each anaesthetic was administered to the perfusate during two blank perfusions for 10 min starting at the time indicated by the arrows. The open circles (—○—) indicate perfusate amino acid levels during the halothane perfusions and the closed circles (—●—) indicate the levels during the trichloroethylene perfusions.

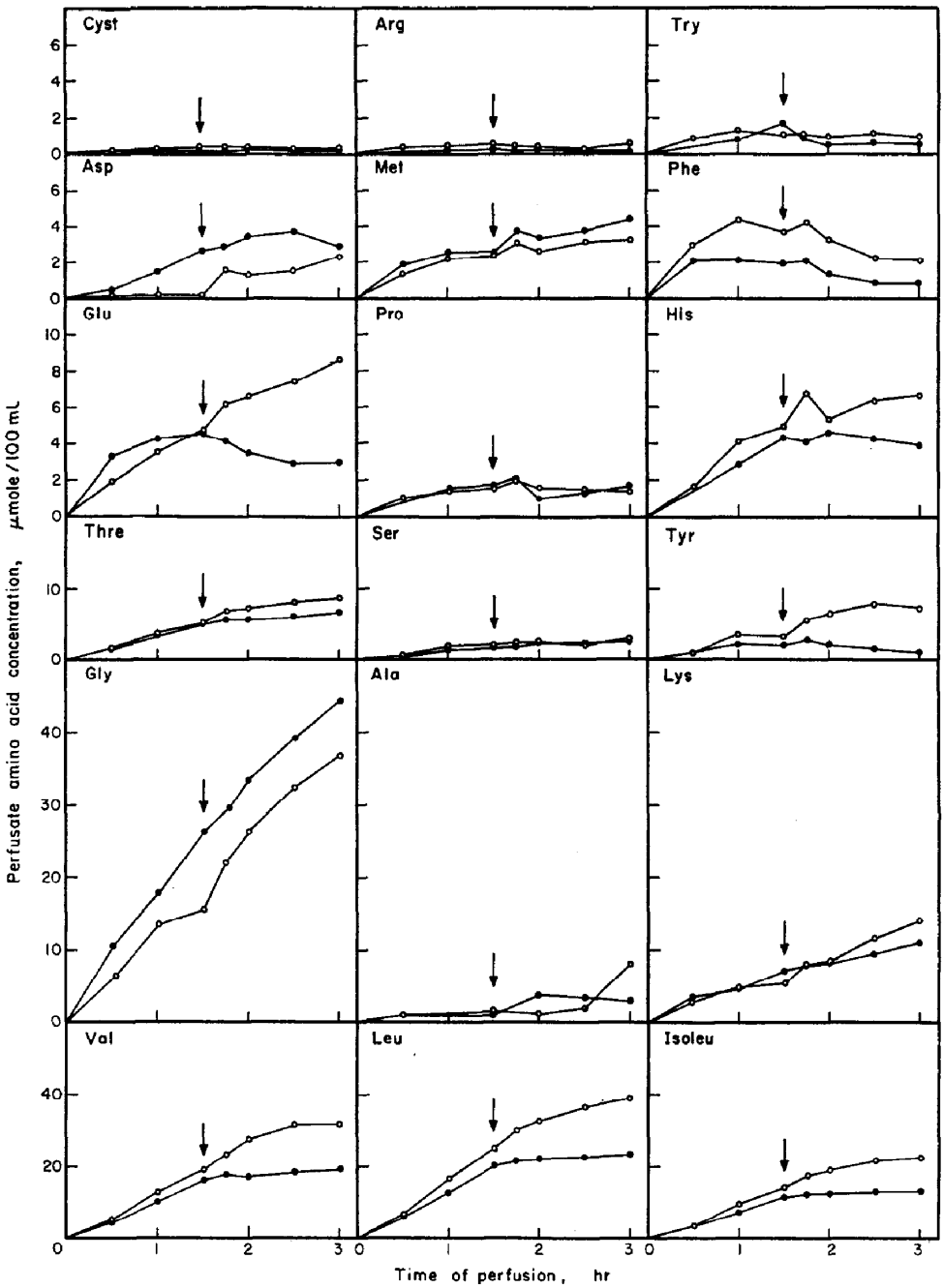

FIG. 2. Effects of pentobarbitone and thiopentone on the perfusate levels of each of the amino acids. Each anaesthetic was added to the perfusate at the time indicated by the arrows. The open circles (—○—) indicate perfusate amino acid levels during the pentobarbitone perfusions and the closed circles (—●—) indicate the levels during the thiopentone perfusions.

the ability of the liver cells to increase the concentration gradients of the branched chain group was diminished. Thus trichloroethylene impaired the ability of the cells to maintain the normal concentration gradients of the main group of amino acids and to increase the gradients of the branched chain acids.

The effects of the barbiturates pentobarbitone and thiopentone on the amino acid pattern of the perfusate were more complex than those of the halogenohydrocarbons described above. The results (Fig. 2) suggest that when pentobarbitone and thiopentone were used for the operation to remove the liver from the rat, amino acid transport was affected during the subsequent perfusion even before addition of the anaesthetic to the perfusate. The rate of output of the branched chain amino acids was reduced, compared with that in both the halothane and trichloroethylene perfusions, and in the thiopentone perfusion there was evidently a considerable leakage of aspartate and glycine before addition of thiopentone to the perfusate (compare results for the first 90 min of perfusion shown in Figs. 1 and 2).

When pentobarbitone was added to the perfusate there was a marked loss from the cells of aspartate, glutamate, glycine, alanine and lysine, some loss of methionine, histidine, threonine and tyrosine, and no loss of cystine, arginine, tryptophan, phenylalanine, proline and serine. In addition, the output of the branched chain acids was inhibited. Thiopentone also caused a marked inhibition of the output of the branched chain group and a considerable leakage of glycine, but there was an actual uptake by the liver of alanine (after an initial loss) and glutamate, and of the amino acids with ring structures, tryptophan, phenylalanine, histidine, tyrosine and probably proline. This suggests either a fall in the intracellular levels of these acids or that thiopentone (which also contains a ring structure), accumulated in the cells,[11–13] inhibited their mediated efflux, resulting in a net inflow. An instance already reported of an effect of a barbiturate on transport processes is the inhibition by Cyclopal sodium of glucose transport into muscle and brain of dogs.[14]

The results given here support the earlier report[3] that massive doses of halothane have relatively little effect on membrane functions of rat liver but that large doses of trichloroethylene cause considerable damage to membrane functions. The results also support the suggestion[3, 5, 15] that the barbiturates have effects on metabolic processes involving amino acids.

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