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AMINO ACID METABOLISM IN THE PERFUSED RAT LIVER

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A perfused organ should provide a useful tool for metabolic studies, especially for investigation of the significance in the metabolism of the whole organ of reactions known to occur in broken cell preparations. Perfusion of rat livers with saline solution through the vena cava has confirmed the importance of pyruvate in transamination reactions (Bristow & Kerly, 1964) but further study of amino acid metabolism in the liver would be facilitated by a preparation which simulated more closely normal physiological conditions. In 1949 Brauer & Pessotti introduced a technique for perfusion of rat livers with homologous blood through the portal vein, and Brauer and his collaborators have attempted to define the basic physiology of this preparation, particularly in regard to its haemodynamics and the excretion of bile. Although in later papers a number of improvements have been described, their technique remains somewhat complex and most subsequent studies have been based on the simpler procedure of Miller, Bly, Watson & Bale (1951). Unfortunately not only do Miller and his co-workers give insufficient information on some important experimental points (e.g. the method of filming the blood in the oxygenator), but they have varied the conditions, such as the composition of the perfusion medium, from one set of experiments to another. Other authors have introduced modifications so that experimental conditions have varied considerably and few studies, even from the same laboratory, are strictly comparable. Therefore before attempting a study of amino acid metabolism in livers perfused with blood it was necessary to develop a standard technique which would allow the liver to be maintained as nearly as possible in a normal physiological condition for several hours and then to investigate changes in amino acid composition of liver, perfusion medium and bile under basal conditions. The results of this investigation are described below.

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METHODS

Perfusion technique

The apparatus (Fig. 1) was essentially that described by Miller *et al.* (1951) but incorporated the following modifications introduced by other workers: a micro-flow pH electrode (Yemm & Cocking, 1955), a magnetic stirrer for the perfusion medium reservoir (Yemm & Cocking, 1955; Cohen & Gordon, 1958) and humidifying dishes within the perfusion cabinet (Cohen & Gordon, 1958). The pump was a Palmer micro-perfusion model, modified so that compression of thick-walled elastic polythene (polyethylene) tubing forced blood up through a pair of flapper-type non-return valves made from 'Perspex' (polymethylmethacrylate) similar to type XT 5120 supplied by Messrs Xlon.

The perfusion medium was pumped at a rate of 30 ml./min into the oxygenation chamber from which half passed to the liver and half through the overflow to the medium reservoir. The oxygenation chamber had an involute at the top so that, as the perfusion medium overflowed, it spread out as a thin film. For most of the experiments described in this report the chamber had straight sides and filming was initiated by filling the chamber with physiological saline solution which drained away as circulation of the medium was started. The filming produced by this method was sufficiently superior to that obtained on a dry chamber to more than off-set the inevitable but relatively minor dilution and loss of perfusion medium which resulted from this procedure. For later experiments (after perfusion 57) the oxygenation chamber has been modified by the introduction of a series of circumferential troughs (as shown in Fig. 1) which allow momentary pooling of the perfusion medium at four points during its descent. This pooling prevents rivulet formation and ensures that the integrity of the blood film, and hence the efficient exchange of respiratory gases, is maintained. After the trough system was introduced it was no longer necessary to fill the chamber with saline solution to obtain satisfactory filming of the perfusion medium.

The filter used between the pH electrode and the pumping mechanism was similar to that described by Miller but the second filter, at the base of the hydrostatic reservoir, consisted of a circular sheet of stainless-steel wire gauze (14 mesh 26 S.W.G.) wrapped round with a double layer of fine nylon stocking (15 denier). The organ chamber was machined from Perspex and had a ledge to support a sheet of perforated polythene on which the cannulated liver was placed. The isolated organ was covered with a very thin sheet of polythene to protect it against drying. The organ chamber and overflow were so adjusted that the pressure in the portal vein was maintained within the physiological range at 13 cm water.

The whole perfusion apparatus was contained in a cabinet thermostatically controlled at 38° by a contact thermometer, the air within the cabinet being circulated by a fan. The medium reservoir, hydrostatic reservoir and portal-vein cannula, but not the rest of the apparatus, were treated with silicone before the start of an experiment by the method suggested by Dacie (1956). After an experiment they were cleaned in boiling KOH (5% in alcohol); the polythene tubing was used for one experiment only and then discarded.

Animals. Albino rats, bred in the Department, were used as blood and liver donors. They were maintained on a standard diet (Parkes, 1946; diet 41B) and fasted 24 hr before use.

Perfusion medium. Blood was obtained immediately before perfusion from 20 to 30 male rats weighing between 200 and 300 g. The animals were lightly anaesthetized with ether and blood (6–8 ml. from each rat) was withdrawn by cardiac puncture into siliconed syringes and pooled in a polythene bottle containing sufficient heparin (Pularin, Evans) and tetracycline hydrochloride (Achromycin, Lederle) to give concentrations of 15 and 20 mg per 100 ml. respectively. In most experiments glucose was also added to the blood, the amounts being shown in the appropriate tables. During the experiments the perfusion medium was thoroughly oxygenated by a stream of moist oxygen (95%) and CO_2 (5%). The pH of the perfusate was maintained nearly constant by the removal of the respiratory gases from the oxygenation chamber with a water pump (see Fig. 1). Haematocrit studies (see below)

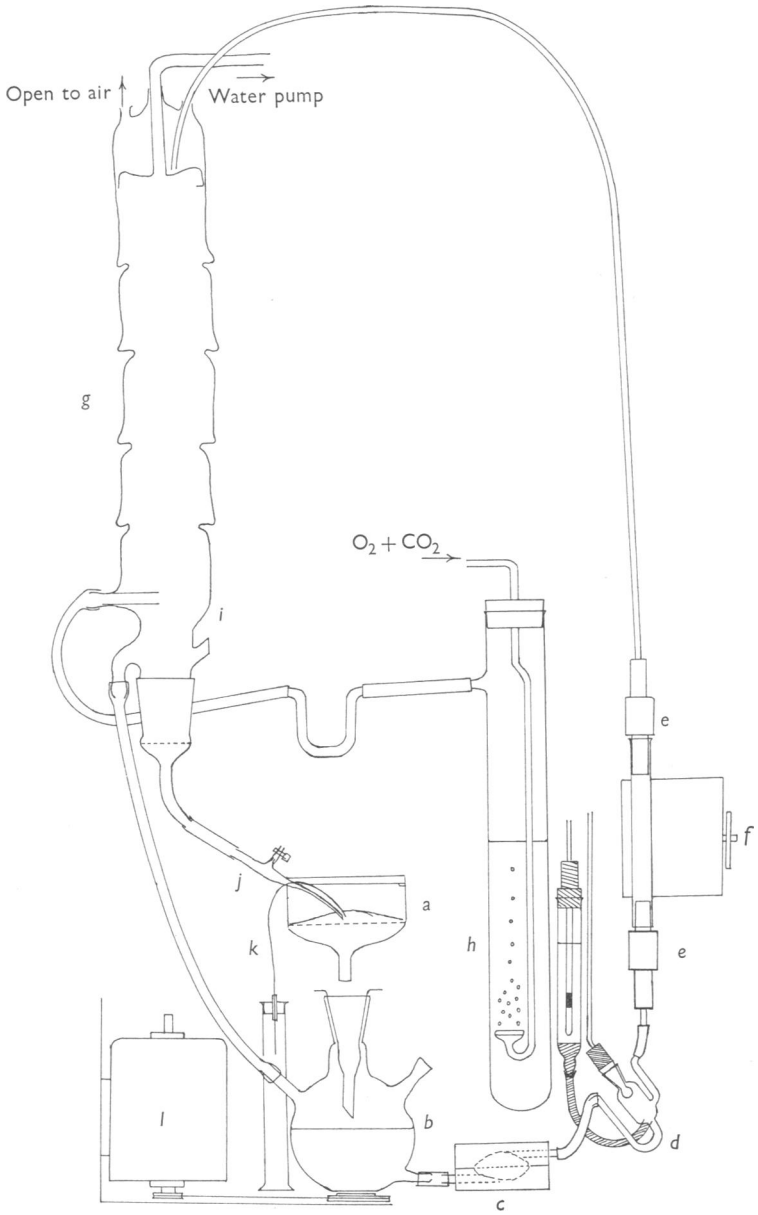

Fig. 1. Diagram of perfusion apparatus. *a*, Organ chamber; *b*, perfusion medium chamber; *c*, filters; *d*, micro-electrodes; *e*, valves; *f*, pump; *g*, oxygenation chamber; *h*, humidifier for $O_2 + CO_2$; *i*, hydrostatic reservoir; *j*, portal vein cannula; *k*, outlet from bile duct cannula; *l*, magnetic stirrer.

showed that some haemoconcentration occurred during perfusion and that this could be prevented by the addition of 0·9 % NaCl solution in amounts equal to or a little greater than the volume of bile produced.

Operative procedure. The method of isolating the liver and transferring it to the perfusion chamber was based on suggestions made by several previous workers. Two fasted rats (one

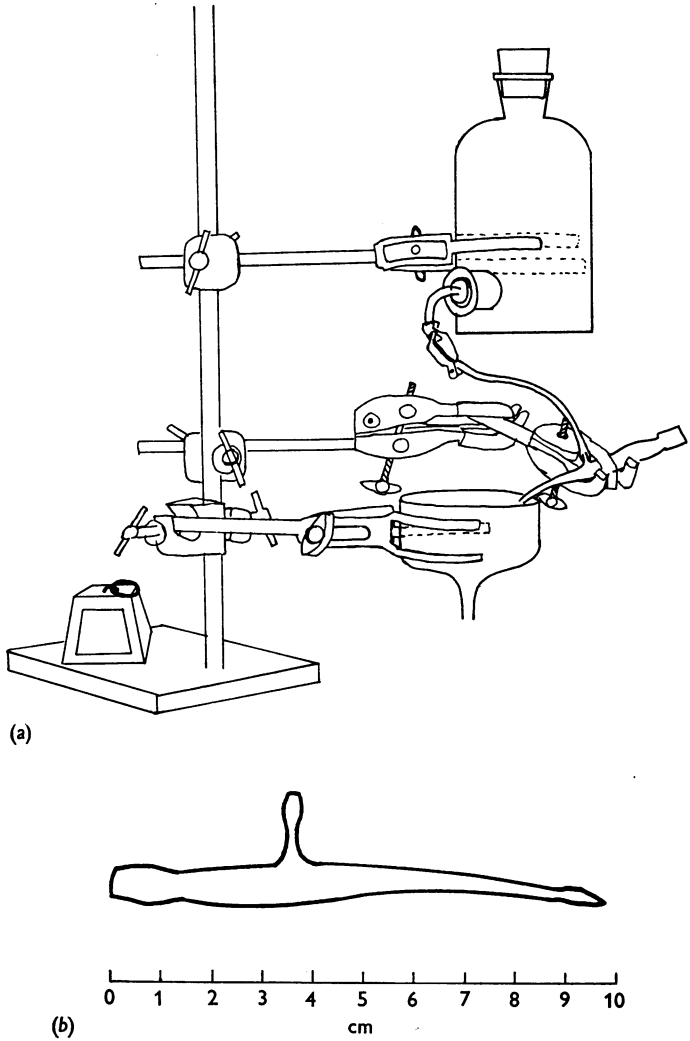

Fig. 2. (*a*) Assembly for liver cannulation: clamps (Gallenkamp's microid) arranged so that the assembly can be transferred and reclamped into the perfusion circuit without disturbance. (*b*) Enlarged, scale drawing of cannula.

to act as a reserve) weighing 270–300 g were anaesthetized by the intraperitoneal injection of 0·2 ml. pentobarbital (Nembutal, Abbots veterinary 60 mg/ml.) The cannula, organ chamber and an aspirator were assembled and held in clamps as shown in Fig. 2. The side

arm of the cannula was connected by rubber tubing to the aspirator, which was then filled with 150 ml. warm saline solution containing 5–10 % rat blood. The large proximal end of the cannula was covered with a polythene cap, all air was forced out of the cannula and the tubing connecting the aspirator and cannula clamped.

To ensure that the perfusate was warm and well oxygenated by the time the liver was introduced into the apparatus, circulation was now started, the hydrostatic and medium reservoirs being connected directly by a piece of polythene tubing which dipped directly into the latter. Blood, prepared as described above, was filtered through nylon into a siliconed measuring cylinder, a sample was retained for analysis and the required volume refiltered through fresh nylon into the perfusion-medium reservoir.

When the depth of anaesthesia was satisfactory, one of the rats was secured on the operating platform, its abdomen swabbed with alcohol and a skin incision made through the length of the bloodless abdominal mid line. The skin was freed from the abdominal wall musculature by blunt dissection, a lateral incision made on each side between the main blood vessels and the flaps of skin turned back. Similar incisions were made in the anterior abdominal musculature itself and a gauze swab, moistened with warm saline solution, placed over the exposed abdominal contents. Now, with precautions against air emboli, 0·1 ml. heparin was injected intrasplenically. The common bile duct was then ligated at its junction with the duodenum and the adhesions joining the liver to its neighbouring organs and structures were carefully severed. The oesophagus and accompanying blood vessels were cut between ligatures and the gastrohepatic ligaments stripped away. With the liver area now largely cleared any remaining perihepatic adhesions were severed, the abdominal vena cava and portal vein freed from their surrounding tissues and the portal vein, its tributaries and the hepatic artery loosely ligated.

With the aid of a lens the common bile duct was cannulated with fine polythene tubing (Portex '00', external diameter 0·628 mm) as closely as possible to its junction with the duodenum. The ligatures on the portal-vein tributaries and hepatic artery were then tightened, the cannula removed from its clamp and the portal vein tied and, with precautions against emboli, cannulated as distal to the liver as possible to avoid obstruction of small branches by the tip of the cannula. Infusion of saline solution containing 5–10 % rat blood was started and the inferior vena cava immediately incised below the liver to allow free outflow of infusate. The 'venous return' was allowed to issue from the open vein, for in a clean preparation there is no need for a vena-cava cannula. Furthermore, the danger of obstructing the hepatic lymph vessels which accompany the vein (Bollman, Cain & Grindlay, 1948; Popper & Schaffner, 1957) is avoided. The liver was dissected free and mounted in the organ chamber, diaphragm surface down, on the platform of perforated polythene sheeting and any adhering fat or diaphragm trimmed away.

The assembly was now transferred to the perfusion cabinet, the clamp holding the organ chamber interlocking with a similar clamp on the stand in the perfusion cabinet. The mouth of the medium reservoir was covered with a piece of thermoplastic film (Parafilm, Gallenkamp) to prevent dilution of the medium by saline solution dripping from the liver; the tubing leading from the hydrostatic reservoir to the medium reservoir clamped with a haemostat and cut about 2 cm below the clamp. The saline-solution infusion from the aspirator was slowed down with a bulldog clamp and the end of the tube leading from the hydrostatic reservoir slipped over the proximal end of the portal-vein cannula (care being taken to avoid the inclusion of any air bubbles). The haemostat was then removed to allow blood to flow through the liver and the saline-solution infusion was completely stopped by a second clamp and the tubing cut between the two clamps. The mouth of the reservoir was uncovered to receive the venous return, which by this time was no longer diluted with saline.

The bile duct cannula was led into a siliconed 10 ml. graduated cylinder containing 2·0 ml. 1 % aqueous picric acid to deproteinize the bile. The organ chamber was secured by the per-

fusion cabinet clamp and the aspirator assembly removed. A piece of very fine polythene film was placed over the liver to prevent drying, the portal vein and bile cannulae were secured to the edge of the organ chamber by Plasticine, the lid of the organ chamber was fitted into place and the fan (temporarily stopped to minimize spattering of blood and saline solution) restarted.

The entire operation usually took from 25 to 30 min and could be carried out by a single operator. The interval between the portal vein ligation and the establishment of the saline solution infusion should not be more than 2–3 min and, with adequate isolation of the portal vein before cannulation, was usually much less. Inspection of the bile flow and venous return at the beginning of each perfusion is most important. Bile should flow spontaneously as regular droplets, one every 5–7 min. A sluggish bile flow can always be corrected by adjustment of the cannula, which often becomes twisted on itself during transfer of the organ chamber. A sluggish venous return, however, is usually indicative of obstruction of circulation to small lobes by the tip of the portal-vein cannula, or some otherwise inadequate preparation; such preparations are unsatisfactory and should be discarded.

Analysis of liver, perfusion medium and bile

Liver. The condition of the liver during perfusion was evaluated by observation of the rate of flow of perfusate and of bile; its appearance at the end of a perfusion was also recorded. In experiments designed to test the validity of the perfusion technique, water content was determined by drying to constant weight (D'Silva & Neil, 1954) and glycogen concentration by the anthrone method (Seifter, Dayton, Novic & Muntwyler, 1950). When individual amino acids were to be determined a small lobe of the isolated liver was ligated at its base and excised just before the liver was placed in the perfusion cabinet. This lobe was blotted and immediately dropped into a known weight of 1 % aqueous picric acid. Perfusion of the liver was started and immediately thereafter the sample of liver was weighed and homogenized. At the end of the perfusion a portion of a large lobe of the liver was excised and treated in a similar manner to the lobe removed at the start of perfusion. The liver samples were homogenized in a Waring Blendor with 20 vol. of 1 % picric acid, the precipitates removed by centrifugation and the supernatants passed through a small coloumn of Dowex 2×8 anion exchange resin to remove excess picric acid. Portions of the eluates were hydrolysed in 6N-HCl for 24 hr at 110° C before determination of amino acids as described below.

Perfusion medium. Determinations of haemoglobin (oxyhaemoglobin method, Dacie, 1956) and packed cell volume (Dacie, 1956) were made on samples of the whole medium. Plasma samples were analysed for haemoglobin (Dacie, 1956), glucose (Mark's (1959) modification of Huggett & Nixon's (1957) method), lactic acid (Barker & Summerson, 1941), protein (Lowry, Rosebrough, Farr & Randall, 1951), amino-N (Saiffer, Gerstenfeld & Harris, (1960), urea-N (Ratner's (1955) modification of Archibald's (1945) method), sodium and potassium (King & Wootton, 1956). For estimation of individual amino acids plasma samples were deproteinized and prepared for analysis as described by Stein & Moore (1954). In a few experiments the amino acid content of plasma after hydrolysis was also determined. Samples of plasma, after deproteinization, were hydrolysed in 6N-HCl for 24 hr at 110°, the hydrolysates purified by the method of Tallan, Moore & Stein (1954) and amino acids determined as described below.

Bile. Quantitative amino acid analysis of bile was carried out on bile collected during perfusion and, for comparison, on bile produced by anaesthetized rats.

Three male rats, similar to those used for the perfusion experiments, were fasted and anaesthetized with pentobarbitone. An incision was made in the abdominal mid line and the bile duct cannulated. The abdominal incisions were then closed with Michele clips, the animals were returned to their cages and light anaesthesia was maintained with booster doses of pentobarbitone. Bile was collected over a 3 hr period in graduated siliconed cylinders containing 1 % aqueous picric acid, as in the perfusion experiments, and then pooled. Samples, freed from

picric acid by passage through a Dowex column as described above, were analysed for amino acids either directly, or after hydrolysis in 6N-HCl for 24 hr at 110°, as described below.

Amino acid analysis. For quantitative determination amino acids were separated on cation exchange columns (Moore & Stein, 1951, 1954). The resin used was analytical grade AG 50 × 5 minus 400 mesh obtained from Bio-Rad Laboratories, Richmond, California. Elution of the amino acids was conducted under a constant pressure of 10 cm mercury and at a rate of 10–12 ml./hr. Buffer was added to the top of the column of resin in such a way that the gradient elution could be initiated, without loss of pressure, in a few seconds. Ninhydrin colour, developed in the eluate fractions, was measured in a Unicam SP 600 spectrophotometer.

There are certain difficulties of interpretation inherent in this technique owing to overlapping of peaks. These were partially resolved by separation of the double peaks by paper chromatography in the propanol–ethanol–pyrophosphate system of Hanes (Hanes, 1961; Hanes, Harris, Moscarello & Tigane, 1961; Matheson, Tigane & Hanes, 1961). Glycine and citrulline are eluted as a single peak but in plasma samples chromatography showed that the peak was composed almost entirely of glycine and all results have been calculated on this assumption. This probably introduced some error as later work, with a more sensitive technique, has shown the presence of citrulline in rat plasma (J. Spruyt, personal communication).

The amides, asparagine and glutamine, are also eluted as a single peak, but again chromatography showed that the amount of asparagine in the plasma samples was negligible. There is a further difficulty in determining these amides since 50–70% of the glutamine in a sample is decomposed during absorption on and elution from the column and further amounts may be destroyed by over-heating during preparation of the sample. All the results for amides are therefore too low and the corresponding values for glutamic acid too high. When no over-heating occurred values for both amides and for glutamic acid should be comparable from one experiment to another; experiments where over-heating did occur, further distorting the values, are noted in the tables.

Before application to the column, sulphur-containing amino acids and glutathione are converted to sulphonates by treatment with sodium sulphite (Moore, Spackman & Stein, 1958). Unfortunately in some experiments an old sample of sulphite was used and these compounds were not reduced so that on elution oxidized glutathione emerged along with α-aminobutyric acid, cystine just after or along with valine, and a mixed disulphide immediately before valine, so that all four peaks were obscured; experiments in which this happened are also noted in the tables. Occasional difficulties (also noted) were experienced with other peaks.

Two *N*-methyl histidines were found in the plasma samples; that first emerging from the column is described as 1-methyl histidine and that following it as 3-methyl histidine using Meister's (1957) nomenclature, although Smith & Birchenough (1960) have pointed out that if the conventional numbering of the imidazole ring were followed the names should be reversed. No values were calculated for tryptophan, which is not quantitatively determined by this technique. Ammonia and urea are included in some tables for comparison although the values do not represent the true concentrations, ammonia being derived largely from destruction of amides and urea being underestimated.

RESULTS

Evaluation of the standard preparation

After twenty-eight perfusions had been carried out to develop the technique, samples of liver and plasma were analysed in a further five experiments to check the viability of the preparation before full amino

acid analysis was undertaken. For these perfusions 100 ml. perfusate was used and 5 ml. samples were removed for analysis, the initial sample at the start of circulation, the second immediately after the liver was introduced (zero time) and the others at intervals as noted in the tables. In two of these experiments no liver was included in the perfusion circuit so that changes in the medium during circulation through the apparatus, but not through a liver, could be assessed. In these experiments the second sample (zero time) was taken 30 min after the start of circulation, approximately at the time a liver would have been introduced. In two experiments (Nos. 32 and 34) 0·9% NaCl solution was added to the medium during perfusion to counteract the haemoconcentrating effect of bile formation. Analysis showed that the glucose level in the perfusion medium fell during circulation before the introduction of the liver; therefore in one experiment (No. 34) 50 mg glucose was added to the perfusate before the start of circulation.

TABLE 1. Comparison of the composition of rat livers before and after perfusion

Samples of liver from ten fasted rats (mean and S.E.) and from three livers at the end of perfusion.

Perfusion no.	Time of perfusion (hr)	Glycogen (g/100 g wet wt)	Water/solid ratio
Fasted rats	—	0·32 ± 0·05	2·78 ± 0·21
31	4	0·65	2·44
32	4	0·32	2·35
34	4½	—	2·40

Changes in the liver. After perfusion for 4–4½ hr there was no obvious sign of deterioration except for small, scattered areas of haemorrhage and, in two cases, incomplete perfusion of the tip of one small lobe. Bile production decreased somewhat (Table 2) during perfusion but the mean hourly volumes compared favourably with those found in anaesthetized rats. Bile production in the intact animal was found to be 0·6 ml./hr over a period of 5 hr, a value similar to that reported by Krayer (1928) for anaesthetized rats weighing 200–300 g, 0·5 ml./hr, and by Friedman, Byers & Michaelis (1950) for unanaesthetized rats, 5·0 ml./100 g rat in 24 hr.

Water content remained within the range observed for livers from fasted rats (Table 1) and there was no obvious sign of oedema. In perfusion No. 32 the glycogen content was maintained at the fasting level, but in No. 31 it was somewhat higher; in the latter experiment the level of glucose in the perfusate rose to an unusually high value (Table 3) and it is possible that the liver donor had not been adequately starved.

Changes in the perfusion medium. In the liverless experiments (Table 2) the decrease in pH was much greater than in the perfusion experiments,

which suggests that the liver may play a role in the maintenance of a steady hydrogen ion concentration. The rate of removal of respiratory gases was, so far as possible, constant from one experiment to another and, in spite of the somewhat crude method of regulating CO_2 concentration, the pH of the medium fell only slightly during perfusion.

TABLE 2. Rate of bile flow and changes in composition of medium (whole blood) during perfusion

100 ml. rat blood containing 20 mg. Achromycin and 15 mg. Pularin circulated through perfusion apparatus, samples withdrawn and 0·9 % NaCl solution added at times shown. For liverless experiments samples at time '0' were withdrawn 30 min after the start of circulation.

Time (hr)	0·9 % NaCl soln. added (ml.)	Bile produced (ml.)	Decrease in pH	Haemoglobin (g/100 ml.)	Cells $\frac{\text{R.B.C. vol.}}{\text{perfusate vol.}} \times 100$
		Perfusion No. 29. No liver			
Initial	—	—	—	13·4	—
0	—	—	—	13·1	37·9
½	—	—	0·04	13·0	—
1½	—	—	0·12	13·0	—
2½	—	—	0·18	12·9	37·6
3½	—	—	0·20	13·1	—
4½	—	—	0·25	13·5	42·0
		Perfusion No. 30. No liver			
Initial	—	—	—	13·9	—
0	—	—	—	13·9	39·4
½	—	—	0·12	13·7	—
1½	—	—	0·25	13·9	—
2½	—	—	0·34	13·9	41·0
3½	—	—	0·39	13·9	—
4½	—	—	0·43	14·3	45·6
		Perfusion No. 31. Liver perfused			
Initial	—	—	—	14·8	41·5
0	—	—	—	14·1	—
1	—	1·0	0·04	13·9	—
2	—	2·0	0·09	13·9	33·3
3	—	2·8	0·10	14·3	—
4	—	3·5	0·13	15·4	40·0
		Perfusion No. 32. Liver perfused			
Initial	—	—	—	16·5	45·0
0	—	—	—	15·4	—
1	1·0	1·0	0	15·4	—
2	1·0	1·8	0	15·0	43·4
3	2·0	2·5	0·04	15·8	—
4	0	3·0	0·05	15·4	40·0
		Perfusion No. 34. Liver perfused (50 mg glucose added to perfusate)			
Initial	—	—	—	—	—
0	—	—	—	16·1	—
½	1·0	0·2*	0	16·0	—
1½	1·0	0·9	0·08	15·3	42·1
2½	1·0	1·7	0·08	15·3	—
3½	0	2·3	0·10	15·7	42·3

* Obstruction to bile drainage corrected after this reading.

Changes in concentration of haemoglobin and of sodium, and in packed cell volume (Tables 2 and 3) indicate changes in water content of the perfusion medium. The 'wet-filming' technique for initiating circulation caused some dilution and this is reflected in the decreased values in all these three parameters between the initial and zero-time samples. In the

TABLE 3. Changes in composition of perfusion medium plasma during circulation

100 ml. rat blood containing 20 mg Achromycin and 15 mg Pularin circulated through perfusion apparatus, samples withdrawn at times shown. For liverless circulations samples at time '0' were removed 30 min after start of circulation.

Time (hr)	Haemoglobin (mg/100 ml.)	Glucose (mg/100 ml.)	Lactic acid (mg/100 ml.)	Protein (g/100 ml.)	Amino-N (mg/100 ml.)	Urea-N (mg/100 ml.)	Sodium (m-equiv/l.)	Potassium (m-equiv./l.)
			Perfusion No. 29. No liver					
Initial	26·4	70·5	101·0	6·4	4·8	18·0	142	2·8
0	22·8	60·0	85·8	6·0	4·7	18·3	—	—
½	26·4	29·0	116·5	6·3	5·6	18·6	131	2·6
1½	43·2	17·5	142·7	6·3	6·2	18·6	131	3·9
2½	67·0	0	183·5	6·0	6·2	18·0	164	4·7
3½	96·5	0	154·0	6·2	7·2	18·2	131	5·4
4½	239·0	—	160·0	—	7·8	18·3	153	6·2
			Perfusion No. 30. No liver					
Initial	13·9	46·3	129·0	5·5	6·3	14·4	153	3·3
0	27·8	16·3	152·0	5·7	7·8	14·4	149	3·6
½	62·5	6·3	201·0	5·8	9·0	14·1	151	3·6
1½	114·7	0	220·0	6·1	9·2	14·7	149	4·8
2½	205·0	0	210·0	6·5	9·6	14·4	151	6·4
3½	240·0	0	170·0	6·0	9·6	15·0	149	8·0
4½	285·0	—	—	6·1	—	—	—	—
			Perfusion No. 31. Liver perfused					
Initial	4·2	91·6	68·5	6·7	6·1	16·2	154	4·6
0	8·9	66·7	109·5	6·0	7·4	16·3	146	5·2
1	14·8	155·0	130·0	6·2	6·6	21·1	162	6·3
2	19·2	158·5	123·0	6·2	7·8	25·0	156	7·5
3	—	173·5	101·0	6·5	9·0	30·0	160	8·8
4	37·9	170·0	82·5	6·6	10·9	33·9	156	10·5
			Perfusion No. 32.* Liver perfused					
Initial	15·8	80·7	44·6	6·1	6·3	16·2	156	5·4
0	30·0	63·0	54·2	5·7	6·0	15·5	150	6·2
1	29·1	75·8	40·4	5·5	5·6	20·7	146	6·7
2	41·3	80·7	34·5	5·8	6·3	24·3	156	8·2
3	48·7	79·0	35·5	5·6	6·9	26·3	154	9·4
4	56·3	78·2	36·5	5·7	7·9	29·3	158	10·5
			Perfusion No. 34.* Liver perfused (50 mg glucose added to perfusate)					
Initial	6·0	103·5	58·5	6·0	5·9	16·6	156	3·8
0	24·8	89·6	44·1	5·5	4·3	18·4	146	5·6
½	64·6	103·5	27·2	5·3	4·9	21·0	151	6·9
1½	94·0	93·2	39·0	5·6	7·1	28·4	151	7·5
2½	—	96·5	—	5·4	—	—	146	8·1
3½	—	86·2	36·3	5·5	9·2	36·6	146	9·5

* 0·9 % NaCl solution added, see Table 2.

liverless experiments little further change occurred until late in the experiment, but in perfusion No. 31 the increase in all three values is evidence that haemoconcentration occurred during perfusion. Therefore in subsequent perfusion experiments 0·9% NaCl solution was added in amounts equal to or a little greater than the volume of bile produced (Table 2). In perfusions Nos. 32 and 34 the haemoglobin and sodium concentration and the packed-cell volume did not change significantly, which suggests that the haemoconcentration observed in perfusion No. 31 was largely, if not entirely, due to extraction of bile.

Changes in perfusion medium plasma. In the liverless experiments the glucose content of the perfusion medium fell rapidly, being completely removed after 1–2 hr (Table 3). In experiment No. 30 the initial level of blood glucose was abnormally low probably because the perfusate had been kept at 37° C instead of at room temperature before the start of circulation so that increased glycolysis had occurred. In both these liverless experiments glycolysis was rapid during circulation and the fall in blood glucose was accompanied by a rise in lactic acid. The blood lactate concentration fell again once glucose had disappeared suggesting that lactate was used by the blood cells. At about this time, when glucose had disappeared, haemolysis increased markedly. In these liverless experiments the small increase in the concentration of protein and urea could be explained on the basis of the haemoconcentration occurring late in the experiments, to which reference has already been made. The increase in amino-N and potassium concentration was definitely larger than could be explained on this basis and was probably due to haemolysis.

In the perfusion experiments haemolysis was much less than that found when the medium circulated without passage through a liver. The blood-glucose level decreased, as in the previous experiments, during the period before the introduction of the liver into the circulation. To counteract this low glucose concentration at the start of perfusion (which was even greater when the reserve rat had to be used as liver donor) in most experiments glucose was added to the medium before the start of circulation; one such experiment (No. 34) is shown in Tables 2 and 3. In all cases, for the first 1–2 hr after the medium started to perfuse the liver the blood-glucose level rose; after that time the level remained more or less steady, except in perfusion No. 31, when it rose to an unusually high level; as has been suggested previously, it is possible that the liver donor in this experiment had not been adequately starved and the liver contained an abnormally large amount of glycogen at the start of perfusion.

In the perfusion experiments the lactic acid concentration in the medium was somewhat lower at the start of perfusion than at the corresponding time in the liverless experiments; this is probably merely a

reflexion of the amount of glycolysis which had taken place; during perfusion the level remained steady or fell slightly.

It has already been pointed out that the 'wet-filming' technique used in these experiments caused a small dilution of the circulating blood and this accounts for the drop in protein concentration between the initial and zero values. The small increase observed in perfusion No. 31 was a reflexion of the haemoconcentration which occurred in this experiment; in perfusions Nos. 32 and 34, where saline solution was added to counteract bile formation, the protein concentration remained steady. Urea concentration increased and the rise in concentration represents a total production of 3–5 mg urea-N/hr. The increases in amino-N and in potassium concentration were similar in both perfusion and in liverless circulation experiments, but as in the former haemolysis was less, part of the increase during perfusion may have been hepatic in origin. Sodium concentration remained nearly constant, in agreement with the finding that the water content of the liver did not change during perfusion.

Changes in amino acid concentration during perfusion

When the reliability of the preparation had been established a further series of livers was perfused and amino acid analyses carried out to determine changes taking place in the perfusion medium, liver and bile. In all these experiments glucose (75 mg/100 ml.) was added to the medium and the volume was increased to 150–200 ml. to allow removal of larger samples (20 ml.) for analysis. After removal of each sample 0·5 ml. NaCl (0·9%) was added to counteract the haemoconcentration due to bile formation. No sample was removed at the start of perfusion when the liver was introduced into the circuit as it was thought advisable to leave a period for the preparation to stabilize. In retrospect it would have been an advantage to have checked the levels at this time as results suggest that metabolic activity proceeded in an orderly manner throughout perfusion and that the changes occurring in the first 30 min were not peculiar to that period. During all experiments the medium plasma was analysed for haemoglobin, lactic acid, amino-N and urea-N (values for experiments Nos. 40, 49, 51 and 61 are shown in Table 5) to ensure that these perfusions could be considered to be 'standard', in particular to check that the increase in medium volume had not altered the behaviour of the preparation.

Plasma amino acids. The initial amino acid content was determined in two experiments and the increase in amino acid concentration on hydrolysis was estimated on one of these samples (Table 4). Apart from the low value of glutamic acid in perfusion No. 40, the values for the individual acids in the two specimens are in good agreement and are similar to those reported by Rogers, Spolter & Harper (1962) and by Miller (1962).

Comparison of the amounts of amino acid before and after hydrolysis of plasma (treated with picric acid to remove protein) shows that no major quantities of conjugated amino acids are present in rat plasma, a finding in agreement with that reported by Stein & Moore (1954) for human

TABLE 4. Amino acids, free and conjugated, in rat plasma, before and after perfusion through liver

170 ml. medium perfused for 2½ hr. Samples deproteinized and prepared for analysis according to the method of Moore & Stein. Portions of samples from perfusions Nos. 61 and 62 hydrolysed in 6N-HCl at 100° for 24 hr before application to the column.

	Amino acid content (μmoles/100 ml.)				
	Initial samples			Samples after perfusion for 2½ hr	
	Perfusion No. 40.	Perfusion No. 62		Perfusion No. 61	
	Before hydrolysis	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis
Leucine	14·6	15·3	16·7	42·9	59·0
Isoleucine	14·1	12·2*	9·8	27·6	27·2
Valine	16·3	20·0	19·2	43·7	45·4
Glycine	25·9	33·0	41·5	16·0	52·2
Alanine	27·0	34·0	37·7	25·9	40·3
α-Aminobutyric acid	1·1	1·2	2·1	1·3	6·6
Serine	18·1	22·0	6·2	5·2	2·0
Threonine	19·9	25·3	14·3	9·1	4·8
Aspartic acid	2·0	2·5	10·5	1·0	29·7
Glutamic acid	5·2	30·3	82·4	17·3	102·0
Aspargine + Glutamine	43·8	35·2	1·5	28·5	0
Arginine	14·0	—	12·0	Trace	6·7
Lysine	22·8	38·0	41·8	12·1	25·6
Ornithine	3·2	4·0	10·3	6·3	—
Cystine + cysteine	1·8	2·2	2·2	4·6	20·6
Glutathione	—	†	†	20·1	0
Methionine	—	—	1·3	—	2·4
Taurine	19·4	20·4	17·3	11·7	15·3
Phenylalanine	6·7	6·8	8·1	5·5	12·7
Tyrosine	9·2	8·7	2·0	3·6	6·0
Histidine	4·9	6·8	8·1	6·2	11·5
Proline	7·9	13·6	17·3	4·0	14·4
N-1-Me-histidine	1·6	2·2	1·5	1·5	1·2
N-3-Me-histidine	0·6	1·0	3·7	Trace	13·6
Total amino acids	280·1	334·7	367·7	294·1	499·2
Ammonia‡	30·9	77·3	—	55·6	—
Urea‡	461·8	404·0	—	582·0	—

* Including a small amount of methionine.

† Any present included with cystine + cysteine.

‡ Values are not quantitative and are included for comparison only.

— Sample lost or otherwise unsatisfactory.

plasma. The level of several amino acids increased after hydrolysis but the increase in total ninhydrin-positive material was only about 10%. There was no change in the single peak due to sulphonated compounds derived from glutathione, cystine and cysteic acid so that it is unlikely that the

plasma contained appreciable amounts of glutathione. Stein & Moore (1954) found that threonine, serine, tyrosine and proline all decompose during acid hydrolysis and, as was to be expected, after hydrolysis the concentration of the first three acids decreased; however, that of proline increased, as did that of ornithine, histidine, 3-methyl histidine, aspartic and glutamic acids (the two latter by larger amounts than could be accounted for by amide destruction), indicating that all these acids are present partly in conjugated form.

When the medium was circulated through the apparatus without perfusion through a liver (Table 5, No. 49) the concentration of all amino acids increased, although that of the amides remained nearly constant. This generalized increase was presumably due to haemolysis, but as the amino acid content of rat erythrocytes has not been reported it is impossible to say whether or not the variation in the amount of increase for different amino acids reflects the amino acid content of the red cells or is due to some other cause. The large rise in ammonia concentration towards the end of perfusion, without a corresponding fall in amide content, may represent a marked change in erythrocyte metabolism at this time.

During perfusion through a liver, changes in concentration of amino acids presented a very different picture (Tables 4 and 5). The concentration of the majority decreased steadily during perfusion, but that of the branched-chain acids leucine, isoleucine and valine increased to a much greater extent than that observed during circulation without passage through a liver. The concentration of glutamic acid and of histidine also increased but only by about the same amount as during circulation without a liver and the changes in concentration of three others, α-aminobutyric acid and the methyl-histidines, were probably not significant, the amounts of these three acids present being very small and not recorded in Table 5.

Conjugation of amino acids after perfusion was assessed by analysis of one sample from perfusion No. 61 before and after hydrolysis (Table 4). There was considerably more conjugation than in plasma before perfusion, the total increase in amino acid concentration after hydrolysis being about 70 % compared with only 10 % before perfusion. Exact comparisons of changes in conjugation of individual amino acids are not possible as determinations before and after perfusion were not made in the same experiment but the appearance of glutathione and the increase in amide concentration recorded during perfusion (Table 5) account at least in part for increases after hydrolysis in glycine, glutamic acid, cysteine and aspartic acid. The concentration of most other amino acids also increased on hydrolysis and this was especially marked for 3-methyl histidine, which in the free state almost disappeared during perfusion; in

TABLE 5. Changes in medium plasma amino acids during perfusion

Rat blood to which had been added 75 mg glucose, 20 mg Achromycin and 15 mg Pularin per 100 ml. circulated through perfusion apparatus, samples withdrawn at times shown (calculated from time of introduction of liver or, for liverless circulation, 30 min after start). Haemoglobin, lactic acid, amino-N and urea-N values were also estimated on most samples and values are included to show that preparations behaved in a standard fashion.

	Perfusion No. 49. No liver 155 ml. medium					Perfusion No. 40. Liver included. 160 ml. medium				Perfusion No. 51. Liver included. 170 ml. medium					Perfusion No. 61. Liver included. 170 ml. medium				
Perfusion time (hr):	0	½	1	1½	2	½	1	2	3	½	1	1½	2	2½	½	1	1½	2	2½
Plasma amino acids (μmoles/100 ml.)																			
Leucine	13·6	14·3	12·8	17·4	18·2	25·0	35·6	45·2	62·3	18·5	23·8	27·1	35·9	44·0	15·5	21·3	25·7	31·9	42·9
Isoleucine	9·7*	10·6	11·7	12·0	16·5*	15·3	25·2*	28·3	34·1	12·5	15·1	17·7	23·3*	30·1*	11·2	13·8	16·4	23·3	27·6
Valine	15·8	18·0	18·1	20·3	24·5	33·6	39·4	63·4	73·6	24·4	31·1	35·0	40·9	45·3	22·3	22·3	26·9	—	43·7
Glycine	34·9	36·1	41·6	40·8	45·5	—	22·4	13·9	13·0	26·6	19·3	17·5	16·2	12·7	28·2	21·4	22·7	22·4	16·0
Alanine	37·2	42·7	49·4	53·5	57·0	12·9	12·6	6·8	8·9	23·9	17·2	18·6	20·3	17·5	30·8	20·3	22·9	28·3	25·9
Serine	17·9	16·4	19·8	19·2	21·1	9·3	8·3	—	8·2	10·3	7·1	6·5	5·2	5·1	14·8	11·5	8·3	7·1	5·2
Threonine	20·6	20·4	23·9	22·9	24·5	14·7	14·8	—	13·9	19·0	14·1	13·2	10·8	9·2	22·2	18·8	14·0	13·4	9·1
Aspartic acid	0·7	0·6	1·0	1·1	—	1·3	0·1	—	0·4	0·4	0·4	0·4	1·0	0·8	1·2	1·1	0·8	0·8	1·0
Glutamic acid	9·5	11·9	12·4	15·4	16·8	9·5	9·8	11·3	9·6	10·6	11·7	13·0	22·5†	16·4	36·2†	13·9	24·5	30·0†	17·3
Asparagine / Glutamine	39·5	38·0	40·1	34·1	38·6	21·4	36·5	—	76·3	27·4	32·0	37·5	36·5†	50·1	19·7†	28·5	24·7	22·2†	28·5
Arginine	12·1	8·3	13·2	11·0	13·7	6·8	4·0	2·0	Trace	—	—	—	—	—	5·0	—	—	—	Trace
Lysine	33·2	28·5	37·5	29·5	41·7	18·9	15·9	10·6	10·7	25·8	17·4	16·7	11·5	10·4	29·5	22·7	18·9	—	12·1
Ornithine	4·0	—	—	—	—	5·1	6·8	5·1	5·6	7·4	7·6	6·1	5·8	—	6·7	5·6	7·7	—	6·3
Glutathione	1·1	1·8	1·5	2·3	3·4	—	—	—	—	4·2‡	4·7‡	9·7‡	10·7‡	12·8‡	—	18·2	20·9	—	20·1
Methionine	2·4*	2·6	3·2	2·7	—	2·5	—	—	—	3·5	2·0	2·3	—	—	1·1	1·2	1·1	—	—
Taurine	18·7	17·5	22·8	23·2	22·8	15·4	18·8	—	13·3	13·0	15·9	13·5	11·6	11·7	25·6	23·2	18·5	14·4	11·7
Phenylalanine	6·9	6·8	7·5	7·6	8·7	6·2	4·8	5·7	4·9	4·7	4·5	4·6	4·5	4·3	7·2	5·4	6·1	7·3	5·5
Tyrosine	9·7	9·2	9·8	9·5	10·6	10·6	12·4	12·8	13·9	6·7	3·9	3·7	2·1	2·0	7·6	5·2	4·4	—	3·6
Histidine	4·0	3·8	4·7	3·7	5·5	3·7	4·3	7·3	7·2	4·3	4·5	4·1	5·4	5·4	5·9	6·3	6·8	—	6·2
Proline	13·6	14·4	14·9	15·9	16·5	—	7·1	—	6·4	9·7	8·4	7·0	4·5	3·5	12·4	6·5	8·1	6·5	4·0
Ammonia§	53·8	59·1	62·9	64·4	100·5	50·0	49·8	38·6	49·9	49·2	39·3	70·7	70·4	68·6	86·1†	67·0	78·5	—	55·6
Plasma haemoglobin, lactic acid, amino-N and urea-N (mg/100 ml.)																			
Haemoglobin	22·0	24·0	40·0	44·0	57·0	—	—	—	—	27·0	36·0	50·0	65·0	74·0	22·0	31·0	36·0	50·0	51·0
Lactic acid	64·6	79·8	86·9	93·9	>100·0	52·7	34·0	24·3	8·1	42·2	27·0	32·4	37·3	38·2	67·3	55·1	45·9	45·7	46·9
Amino-N	6·0	6·4	6·8	6·9	7·1	8·3	8·5	9·4	11·9	5·3	5·3	5·3	5·4	5·8	5·3	5·3	5·1	5·4	5·5
Urea-N	16·1	16·1	16·1	16·1	16·1	21·5	21·5	25·4	28·9	17·5	19·2	20·0	21·7	21·7	18·7	19·9	21·0	22·6	25·2

* Small amounts of methionine included with isoleucine.

— Sample lost or otherwise unsatisfactory.

† Excessive decomposition of glutamine during preparation of sample.

‡ Values too low owing to disulphide formation.

§ Values not quantitative, included for comparison only.

contrast the concentration of the branched-chain amino acids hardly changed after hydrolysis.

Miller (1962) has also investigated the concentration of free (but not conjugated) plasma amino acids during perfusion of rat livers. He found similar changes, that is a general decrease in concentration of most amino acids but an increase in that of the branched-chain amino acids. In most

TABLE 6. Amino acids of rat liver

Picric acid extracts of liver hydrolysed in 6 N-HCl for 24 hr at 110°; amino acids separated by column chromatography according to the method of Moore & Stein.

	μmoles/100 g wet wt.	
	Initial sample (0·446 g liver)	Sample from liver perfused for 2½ hr (0·834 g liver)
Leucine	27·7	32·5
Isoleucine	19·4	15·7
Valine	111·1	69·0
Glycine	1507·1	576·1
Alanine	120·3	152·9
α-Aminobutyric acid	5·0	7·2
Serine	9·3	4·4
Threonine	16·7	9·9
Aspartic acid	74·8	64·0
Glutamic acid	735·7	906·1
Asparagine + Glutamine	4·9	1·7
Arginine	121·3	70·9
Lysine	97·5	33·2
Cystine + cysteine	126·0	26·2
Methionine	1·5	5·6
Taurine	1271·3	702·0
Phenylalanine	11·0	8·9
Tyrosine	—	2·3
Histidine	25·5	41·0
Proline	26·5	20·6
1-Me-histidine	11·7	—
3-Me-histidine	55·3	34·6
Unidentified		
Pre-taurine	59·6	7·3
Pre-aspartic acid (i)	9·7	3·3
(ii)	2·3	1·7
Pre-methionine (i)	4·8	2·4
(ii)	—	2·6
(iii)	—	3·3
Total ninhydrin-positive compounds	4334·7	2734·3

of his experiments one or more amino acids were added to the perfusate and perfusion was continued for 5–6 hr, so that his results are not directly comparable with those recorded in Table 5, but in the one experiment for which he records full amino acid analysis of plasma before and after perfusion (for 6 hr with added glutamine) changes in amino acid concen-

tration are of the same general pattern except for rises in concentration of alanine and of glutamic acid, rises which are probably accounted for by the addition of glutamine to the perfusate. He also found that, whilst most amino acids are oxidized to a greater extent in liver than in peripheral tissue, the reverse is the case for the branched-chain amino acids. He suggests that the general fall in level of amino acids during perfusion is due to oxidation by the liver but that the branched-chain acids are not

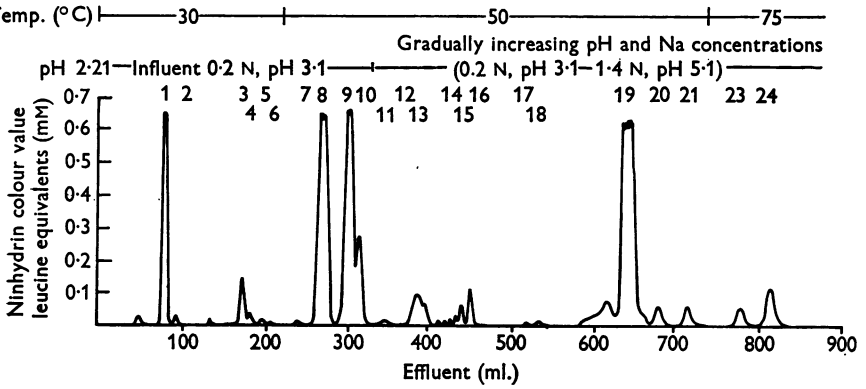

Fig. 3. Liver amino acids. Diagram of ninhydrin-positive compounds separated from a hydrolysate of a protein-free extract of perfused liver. 1, Taurine; 2, urea; 3, aspartic acid; 4, threonine; 5, serine; 6, glutamine–asparagine; 7, proline; 8, glutamic acid; 9, glycine–citrulline; 10, alanine; 11, α-aminobutyric acid; 12, valine; 13, cystine; 14, methionine; 15, isoleucine; 16, leucine; 17, tyrosine; 18, phenylalanine; 19, ammonia; 20, lysine; 21, histidine; 22, 1-methylhistidine 23, 3-methylhistidine; 24, arginine.

removed by oxidation, the rise observed presumably being due to secretion by the liver. Whilst differences in rate of oxidation by the liver may be a factor in controlling amino acid level in the perfusate, the results recorded in Table 4 suggest that differences in amount of conjugation must also be considered.

Liver amino acids. Amino acids were determined in hydrolysed picric acid extracts of liver before and after perfusion (Table 6). All the acids were present in liver at a higher concentration than in plasma and the relative proportions were also quite different. After perfusion the concentration of most acids was decreased, the total amount of amino acid falling by about 35 %. This fall was most marked for glycine and taurine, which were present in unperfused liver in high concentration. The decrease in glycine content is probably associated with bile formation but taurine is not present in large amounts in rat bile. The concentration of a few amino acids, leucine, alanine, α-aminobutyric, glutamic and histidine,

increased slightly after perfusion; the increase in alanine and glutamic acid concentration may perhaps be due to their formation during transamination reactions. A number of unidentified compounds giving a positive ninhydrin reaction were present in liver before perfusion and although,

TABLE 7. Amino acids of rat bile

Normal bile collected from 3 anaesthetized rats and pooled. All samples collected in picric acid, amino acids separated by column chromatography before or after hydrolysis (as indicated) for 24 hr at 110°.

Amino acid content (μmoles/100 ml.)

	Bile from anaesthetized rats		Bile from perfused livers				
			Before hydrolysis			After hydrolysis	
	Before hydrolysis	After hydrolysis	Perfusion No. 37	Perfusion No. 40	Perfusion No. 60	Perfusion No. 61	Perfusion No. 73
Leucine	43·1	84·2	49·1	49·1	31·2	23·3	43·7
Isoleucine	25·8†	38·6	25·0	20·2	19·5	11·4	20·6
Valine	20·9	151·3	42·3	29·5	19·1	13·2	54·3
Glycine	43·5	558·6	68·9	83·3	63·2	267·3	486·9
Alanine	10·7	34·4	22·2	11·6	14·4	19·6	15·0
α-Aminobutyric acid	Masked	6·3	Masked	Masked	6·1	4·0	5·1
Serine	15·0	13·2	13·0	12·3	13·3	—	4·0
Threonine	17·1	25·5	17·8	14·0	20·9	2·5	5·5
Aspartic acid	2·6	60·6	14·5	8·6	10·2	13·0	24·6
Glutamic acid	89·7	>420·0	66·9	91·2	95·0	281·1	508·6
Asparagine + Glutamine	27·1	0·6	45·1	35·1	22·7	—	1·8
Arginine	6·1	18·8	2·2	4·6	—	—	5·4
Lysine	7·3	39·3	6·0	—	6·7	7·8	11·9
Ornithine	Masked	Masked	1·5	Masked	1·2	Masked	Masked
Cystine + cysteine	20·3	—	—	—	—	30·3	24·5
Glutathione	>124·0	0	126·2	35·2*	70·0	0	0
Methionine	Masked†	14·9	4·6	9·6	5·4	1·2	3·1
Taurine	19·7	31·5	37·7	84·2	74·9	37·2	84·9
Phenylalanine	14·8	38·5	10·7	13·3	10·6	9·0	16·4
Tyrosine	9·7	13·6	9·4	11·8	5·7	—	1·2
Histidine	6·9	18·3	8·2	8·6	8·0	5·6	11·3
Proline	11·6	58·0	Masked	11·8	—	15·3	26·8
1-Me-histidine	—	1·4	1·9	1·0	—	—	1·0
3-Me-histidine	—	5·0	1·0	1·0	—	259·0	333·2
Unidentified (total)	101·0	23·8	76·8	74·1	—	2·9	8·8
Total amino acids	485·1	1278·2	651·1	608·3	498·1	1003·9	1698·6
Ammonia‡	83·4	0	47·2	73·2	93·0	—	1000·0
Urea‡	697·4	0	740·0	585·6	650·5	0	0

* Excluding dipeptide.

† Some methionine included with isoleucine.

‡ Values are not quantitative and are included for comparison.

— Sample lost or otherwise unsatisfactory.

like the known amino acids, they decreased in concentration during perfusion additional unknown compounds were produced (Table 6). The elution diagram (Fig. 3) for this analysis is reproduced to indicate the position of these compounds relative to known amino acids.

Bile amino acids. The amino acid content of bile secreted by anaesthetized rats and by perfused rat liver was very similar (Table 7 and Fig. 4). Identification of compounds eluted from the column has been made from their relative positions on the effluent curve, but without supplementary tests identification cannot be made with complete confidence in all cases. No attempt was made to separate glycine and citrulline or asparagine and glutamine, or to identify the several unknown compounds giving a positive

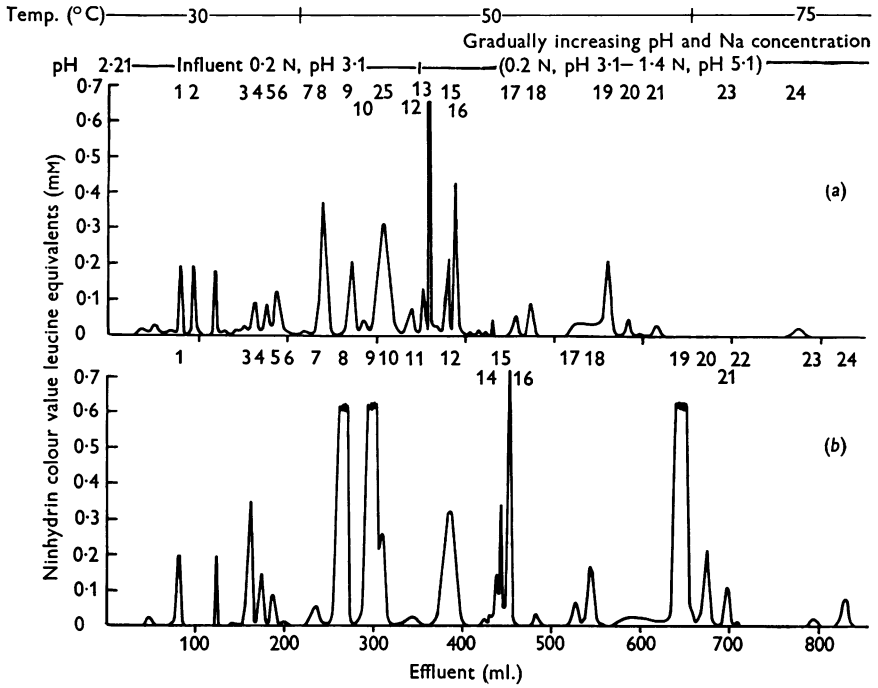

Fig. 4. Amino acids in rat bile. Diagram of ninhydrin-positive compounds separated from (*a*) unhydrolysed and (*b*) hydrolysed samples (2 ml.) of bile from anaesthetized, intact rats. Peaks 1–24 numbered as in Fig. 3; peak 25, oxidized glutathione.

ninhydrin reaction. The three compounds in unhydrolysed bile which are eluted immediately following alanine (Fig. 4*a*) have been identified as glutathione, glutathione–cysteine disulphide and valine respectively; further studies on bile, to be reported later, support this interpretation.

The total amino acid concentration of bile, both from intact rats and from perfused livers, was considerably greater than that of plasma; all the common amino acids were present, in most cases at concentrations greater than those found in plasma, only alanine, arginine, asparagine–glutamine, lysine, serine, and threonine being present at lower concentrations. In bile produced from both sources the concentrations of every

acid-stable amino acid increased on hydrolysis, the increase being larger in bile from intact animals, possibly because the amino acid concentration of the medium fell during perfusion whilst that of blood is maintained constant in the intact animal. Bile is therefore a secretion characterized by an impressive degree of amino acid conjugation. The acids most involved in conjugation are glycine, glutamic acid, aspartic acid, lysine and proline, but not taurine, whose concentration was not much greater in the hydrolysed than in the unhydrolysed samples. (The absence of a cysteic acid or cystine peak in the chromatogram of hydrolysed bile from anaesthetized rats is unexplained as such a compound should have been produced by hydrolysis of the glutathione present in the unhydrolysed sample.) The bulk of the unidentified ninhydrin-positive compounds proved to be acid-labile. One difference between normal and perfusion bile was the large increase in concentration of 3-methyl histidine on hydrolysis of the latter. This increase suggests that the liver is the site of methylation of the imidazole ring and the difference observed between bile secreted by intact rats and by perfused livers merits further investigation.

DISCUSSION

The results of these experiments emphasize that in an investigation of amino acid metabolism in the perfused liver changes in concentration in the liver itself and in the bile secreted, as well as in the plasma, should be considered. Although in many respects the normal condition of the liver and blood can be maintained during perfusion it must be remembered that *in vivo* passage through other tissues is continually modifying the circulating blood; substances may be secreted into or withdrawn from it. Whilst the depletion of amino acids which occurs during perfusion could be controlled by addition, probably most satisfactorily by continuous infusion, the problem of removing those substances which accumulate during perfusion, notably the branched-chain amino acids and urea, is more difficult to solve, but it is one which should be tackled if conditions during perfusion are to simulate closely those found *in vivo*.

SUMMARY

1. The technique for perfusion of rat livers with homologous blood through the portal vein has been modified to allow greater standardization of the preparation.

2. Observation of the state of the livers after perfusion and determination of water and glycogen content of the livers, of rate of flow of perfusate and of bile, of pH, haemoglobin and packed-cell volume of medium and of haemoglobin, glucose, lactic acid, protein, amino-N,

urea-N, sodium and potassium in medium plasma showed that the preparations behaved in a standard manner and that the changes recorded were reproducible from one preparation to another.

3. In the conditions of these perfusions there was little haemolysis and the pH and glucose content of the medium remained nearly constant; there was no formation of glycogen or of protein by the liver; urea was formed at a rate of approximately 6·2 mg urea-N per liver/hr.

4. Full amino acid analysis of medium, of liver at the start of perfusion, and of bile produced, showed that each of these contains all the common amino acids and also, in the case of liver and bile, a number of unidentified compounds which give a positive reaction with ninhydrin. There is little conjugation of amino acids in plasma, but a considerable amount in bile.

5. During perfusion of livers for $2\frac{1}{2}$ hr the concentration of most amino acids fell in both medium plasma and liver. Exceptions were the rise in concentration of the branched-chain acids, leucine, isoleucine and valine in plasma, and of alanine and glutamic acid in liver. The amount of conjugated acids present in plasma increased considerably during perfusion.

6. Bile was secreted at nearly the same rate during perfusion as by anaesthetized rats; its amino acid composition was similar to that of normal bile, except that the total amount of conjugation was less; the amount of 3-methyl histidine present in perfusion bile in conjugated form was very high.

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