Catabolism of Plasma Albumin by the Perfused Rat Liver

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The liver is known to be a major site of plasma-protein synthesis, but there is little information concerning its role in the catabolism of native plasma proteins. The ease with which the isolated perfused liver can be maintained under physiological conditions would appear to make it an ideal system for studying plasma-protein breakdown. However, the ability of the liver to take up particulate material is well known, and Gordon (1957) has recently shown that the perfused rat liver rapidly catabolizes small proportions of altered protein molecules present in preparations of labelled plasma protein. Perfusion experiments, which are necessarily of short duration, can therefore be used to assess the physiological role of the liver in protein breakdown only if the labelled protein used is free from altered molecules.

Rapidly catabolized components can be removed by preliminary injection of the labelled protein solution into a living animal; after the required period of 'screening' the plasma of this animal is used as the labelled protein solution to be tested (McFarlane, 1956). Using a screening period of 48 hr. in the rat, Gordon (1957) found in three experiments that the perfused rat liver catabolized about one-tenth of the homologous albumin broken down in the whole animal. Such data can be regarded as having physiological significance only if it is possible to establish a minimum catabolic rate which is unaffected by more prolonged screening of the labelled albumin. In the present investigation the effect of the length of screening upon the rate of homologous albumin catabolism by the perfused rat liver has been studied. Observations regarding the time taken for the release of diffusible label from [^{131}I]albumin by the perfused liver are also recorded and in addition the capacity of the liver for breaking down native and denatured albumins is compared.

METHODS

Perfusion apparatus. This was basically that described by Miller, Bly, Watson & Bale (1951), but having the following modifications: (1) The dimensions of tubing and glassware have been reduced so that an initial blood volume of 55 ml. allows a sufficient margin for sampling. (2) Haemo-concentration, which readily occurs during the course of a perfusion, is reduced by bubbling the entering oxygen through two gas washers at 37°, by balancing the rates of entry and exit of gas with two flow-meters and also by including at the back of the box two lengths of gauze suspended in beakers of water. (3) A magnetic stirrer has been included in the blood reservoir to facilitate rapid mixing.

Blood donors. These were rats of the hooded strain maintained at the National Institute for Medical Research. Animals were starved overnight and 5–8 ml. of blood was removed by cardiac puncture under ether anaesthesia. At least 3 weeks elapsed before any rat was again used as a blood donor. For each perfusion 0·25 ml. of heparin (1250 i.u.) was added to 55 ml. of blood.

Perfusion technique. Liver donors were hooded male rats which had been starved overnight. The surgical technique described by Miller *et al.* (1951) has been slightly modified. In order to minimize hepatic damage which might arise from products of intestinal infarction the bile duct and portal vein were cannulated without previous ligation of the gastric and duodenal blood vessels. Perfusion of the liver was commenced as soon as the superior vena cava had been cannulated and was followed by dissection of the liver from the animal. By this means the time during which the liver was without a blood supply was reduced from about 6 min. to less than 2 min. The temperature (37–38°) and rate of blood flow through the liver (5–7·5 ml./min.) were allowed to stabilize for $\frac{1}{2}$ hr. before the addition of labelled protein.

Supplements in the form of glucose and amino acids are usually added in liver perfusion experiments (Miller *et al.* 1951; Jensen & Tarver, 1956; Gordon, 1957). However, during a series of 4 hr. liver perfusions in which about 55 ml. of blood was used without supplements the blood-sugar concentration increased by 19–58 mg./100 ml. while the amino acid level rose by 22–67 mg./100 ml. (Tables 2 and 3). Because of this finding no supplements were used in the present experiments.

Preparation of albumin. Albumin fractions were prepared by: (1) zone electrophoresis of rat serum on a column of treated cellulose (Porath, 1954; Campbell & Stone, 1956). The albumin peak was located by measurement of the ultraviolet absorption of 3 ml. volumes of eluate at 280 mμ. The pooled albumin solution was reduced to suitable volume by pressure dialysis at 4° before labelling; (2) chromatography of rat serum on a column of carboxymethylcellulose (Peterson & Sober, 1956). Protein fractions were obtained by gradient elution with two sodium acetate buffers [pH 5·0 (0·05 M), and pH 5·1 (0·40 M) respectively]. The pooled albumin solution was concentrated by pressure dialysis and freeze-dried before labelling. After being labelled with ^{131}I these albumin preparations were mixed with rat serum and examined by paper electrophoresis. Subsequent strip-counting showed that the radioactivity was confined to the albumin band (Fig. 1).

In experiments on denatured protein either bovine serum albumin (Armour and Co. Ltd., Eastbourne, Sussex) or rat albumin was used. The protein after iodination was brought to pH 9·0 by addition of 0·45 m-equiv. of NaOH/g., the final protein concentration being 10% (w/v). It was then denatured by heating at 80° for 5 min. The solution was readjusted to pH 7·0 before addition of the denatured protein to the perfusion circuit.

Iodination. This was usually performed according to the method of McFarlane (1956). In a few instances, however, the following modified method recommended to us by Dr McFarlane was used. The protein solution was adjusted to pH 4·0 by addition of glycine buffer (7·2 g. of glycine dissolved in 96 ml. of 1·47% NaCl and 12 ml. of N-HCl). This acidified protein was mixed with the labelled free-iodine solution and iodination allowed to proceed by raising the pH of the mixture to 8·0 with 0·02N-NaOH. Free iodide was removed by passage of solutions through anion-exchange columns of De-Acidite (Permutit Co. Ltd., Gunnersbury Avenue, London, W. 4). In all instances the mean ratio of iodine bound to protein was less than 1 g.-atom/mole.

Removal of denatured material from the iodinated protein. This was achieved by intravenous or intraperitoneal injection of the labelled protein solution into a living rat, which was bled by cardiac puncture after a period of between 15 min. and 72 hr. This procedure, known as 'screening' (McFarlane, 1956), has been used as a preliminary to a number of perfusions reported below. The plasma containing screened ^{131}I-labelled albumin was dialysed overnight against 0·9% NaCl containing NaI. The final iodinated protein solution contained 0·15–0·97% of the total radioactivity in the supernatant after precipitation of protein with 10% trichloroacetic acid at 20°.

Measurement of radioactivity. Solutions containing ^{131}I were measured by scintillation counting. The standard deviation of counts recorded in this study did not exceed ±3%.

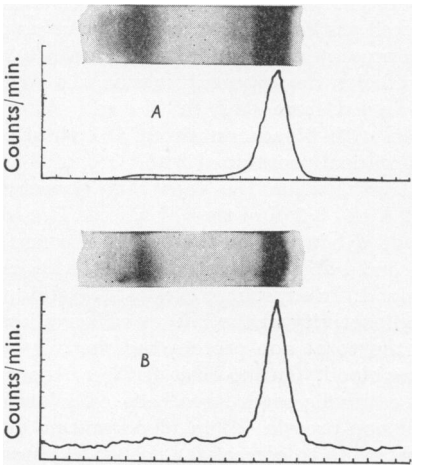

Fig. 1. Radioactivity tracings of ^{131}I-labelled albumins mixed with rat serum and analysed by paper electrophoresis. *A*, Albumin separated by zone electrophoresis; *B*, albumin separated by chromatography (see Methods).

Plasma-protein estimations. These were done by the biuret method of Gornall, Bardawill & David (1949).

Blood-glucose estimations. Blood samples (about 0·2 ml.) were collected into tubes containing 2 mg. of NaF and blood sugar was estimated within 24 hr. according to the method of Somogyi (1945).

Plasma amino acid estimations. These were done by a ninhydrin method (Jacobs, 1956).

Paper electrophoresis. Plasma samples were analysed by paper electrophoresis according to the method of Jencks, Jetton & Durrum (1955) and the proportion of albumin was estimated by the elution of strips stained with bromophenol blue.

Estimation of degree of catabolism. Blood samples were withdrawn 20 min. after addition of the labelled albumin and then at hourly intervals for 4 or 5 hr. The following estimations were made on each sample. (*a*) Radioactivity/ml. of whole blood; (*b*) non-protein radioactivity/ml. of whole blood. Blood (1 ml.) was added to 1 ml. of 0·1% NaI and 2 ml. of 20% trichloroacetic acid was added. The radioactivity of 2 ml. of the supernatant (equivalent to 0·5 ml. of blood) was measured after centrifuging; (*c*) radioactivity/ml. of plasma; (*d*) haematocrit.

The level of non-protein ^{131}I did not increase during the initial 20 min. after the addition of labelled protein to the perfusion circuit (see below). The catabolic rate during subsequent hourly intervals has been calculated by the method illustrated in Table 1. Results are expressed as: (*a*) uncorrected percentage catabolism/hr., which is the increment of non-protein ^{131}I/ml. of blood/hr. expressed as a percentage of the total ^{131}I/ml. of blood. In an experiment with tracer amounts of denatured labelled protein the total radioactivity/ml. of blood fell progressively during perfusion as a result of uptake of protein by the liver as well as adsorption on the walls of the apparatus. In this experiment [rat liver perfusion (R.L.P.) 67, Table 3] the non-protein ^{131}I increment/ml. of blood is expressed as a percentage of the average protein-bound ^{131}I/ml. of blood during the corresponding interval; (*b*) corrected percentage catabolism/hr. The correction factor used was:

$$\frac{\text{Vol. of plasma in perfusion circuit (ml.)} \times \text{protein concn. (mg./ml.)}}{\text{Wt. of rat (g.)} \times 2{\cdot}9},$$

i.e. the percentage catabolism/hr. was corrected to that which would have been observed with a plasma-protein pool size equal to that of the donor rat; (*c*) mass of albumin catabolized/hr. The proportion of albumin in six pooled samples of rat plasma measured by paper electrophoresis was 40±4% of the total protein. During the course of perfusion the plasma-protein concentration frequently increased (Tables 2 and 3), but the albumin percentage as determined by electrophoresis remained constant. The mass of albumin catabolized during each hour was therefore calculated from the uncorrected percentage catabolism × 40% of the plasma-protein mass in the perfusion circuit.

RESULTS

Recovery of ^{131}I from blood. Protein catabolism has been calculated from the proportion of ^{131}I in blood which is non-precipitable by trichloroacetic acid. When Na^{131}I is added to blood and

Table 1. *Example of the method of calculating rates of albumin catabolism by perfused liver*

^{131}I-Labelled rat albumin (75 μc) screened for 48 hr. was added at zero time. Wt. of donor rat, 322 g. (R.L.P. 54).

Sample no.	Time after ^{131}I-albumin	Vol. of plasma in samples (ml.) (1)	Whole blood: Total ^{131}I (μc/ml.)	Whole blood: Non-protein ^{131}I (μc/ml. $\times 10^3$)	Whole blood: Non-protein ^{131}I as % of total	Total plasma ^{131}I (μc/ml.)	Vol. of plasma in perfusion circuit (ml.) (2)
1	20 min.	2·3	1·20	2·77	0·23	1·82	40·8
2	1 hr. 20 min.	2·3	1·19	3·70	0·31	1·80	38·5
3	2 hr. 20 min.	2·3	1·20	4·78	0·40	1·80	36·2
4	3 hr. 20 min.	2·2	1·22	5·80	0·47	1·84	33·9
5	4 hr. 20 min.	—	1·23	6·90	0·56	1·90	31·7

Sample no.	Plasma protein concn. (mg./ml.)	Correction factor (3)	Albumin pool (mg.) (4)	Mean albumin catabolism/hr.: Uncorrected % (5)	Corrected % (6)	Albumin (mg.) (7)
1	74·3	—	—	—	—	—
2	76·6	3·18	1180	0·08	0·25	0·95
3	80·9	3·15	1170	0·09	0·28	1·05
4	86·4	3·14	1170	0·07	0·22	0·82
5	89·3	3·05	1130	0·09	0·27	1·02
			Mean	0·08	0·25	0·96

(1) Vol. of blood sample × (100–95 % haematocrit)/100.

(2) Initial plasma vol. $= \frac{\text{Dose of } ^{131}\text{I } (\mu\text{c})}{\text{Mean plasma } ^{131}\text{I } (\mu\text{c/ml.})}$. Subsequent plasma volumes = (initial vol. − cumulative vol. of plasma in blood samples).

(3) $\frac{\text{Plasma vol. (ml.)} \times \text{plasma-protein concn. (mg./ml.)}}{\text{Wt. of donor rat (g.)} \times 2{\cdot}9}$.

(4) 40 % (plasma vol. × protein concn.).

(5) $\frac{\text{Increment non-protein } ^{131}\text{I/ml. of blood/hr.} \times 100}{\text{Total } ^{131}\text{I/ml. of blood}}$.

(6) Uncorrected percentage catabolism × correction factor.

(7) Uncorrected percentage catabolism × albumin pool (mg.).

precipitated at a final concentration of 10 % trichloroacetic acid in the presence of 0·05 % unlabelled NaI, the radioactivity is quantitatively recovered in the supernatant.

Stability of [^{131}I]*albumin in the absence of a liver.* Radioactivity is not released from undenatured [^{131}I]albumin pumped around the perfusion circuit in the absence of a liver (Gordon, 1957). Since it was thought possible that denatured albumin might be catabolized by leucocytes a similar control experiment was performed in which 10 mg. of heat-denatured bovine serum albumin was added to 58 ml. of blood in the circuit. During 5 hr. the non-protein ^{131}I varied from 1·64 to 1·74 % of the total ^{131}I, but a progressive increase was not observed.

Distribution of iodide in the perfusion circuit. Concentration of iodide to a significant extent in either the liver or bile would invalidate calculations of catabolic rate based upon the level of non-protein ^{131}I in the perfusing blood. Control experiments have been performed in which tracer amounts of ^{131}I (1·04 μc carrier-free ^{131}I) were mixed with rat blood in the perfusion circuit. In one experiment the initial volume of distribution of ^{131}I in the apparatus (calculated by isotope dilution) was 65 ml. This increased to 68 ml. within 20 min. after hepatic cannulation, presumably as a result of the passage of ^{131}I into liver tissue and bile as well as the addition to the perfusion circuit of blood present in the donor liver. During a further 4 hr. perfusion the apparent volume of distribution of ^{131}I varied from 68·2 to 71·5 ml. After 5 hr. perfusion with blood containing ^{131}I the livers in two experiments contained 2 and 3 % of the initial radioactivity, while the total bile samples contained 2·4 and 3·0 % of the ^{131}I added. The concentration of ^{131}I in bile at the end of the perfusions was 1·1 and 1·25 times respectively that present in whole blood. In experiments with [^{131}I]albumin the total radioactivity in the bile was always less than 3 % of the total non-protein radioactivity in the perfusing blood. On the basis of these observations protein catabolic rates have been calculated from levels of non-protein ^{131}I in blood and no attempt has been made to correct for the small percentage of non-protein radioactivity distributed in hepatic tissue and bile.

Effect of hepatic blood flow on rate of albumin catabolism. The rate of hepatic blood flow varied in

different perfusions from 5 to 7·5 ml./min. In one experiment the rate of blood flow through a liver (wt. 10 g.) was artificially reduced from 7 to 2·3 ml./min. after about 2½ hr. perfusion. The rate of catabolism of ^{131}I-labelled rat albumin screened for 48 hr. remained constant throughout, the mean corrected percentage catabolism being 0·23 %/hr. during the first half of the experiment and 0·26 %/hr. after reduction of the blood-perfusion rate.

Catabolism of [^{131}I]*albumin*

Appearance of diffusible ^{131}I. The latent period which intervenes between the addition of labelled protein and the release of non-protein ^{131}I into the perfusing blood was measured with both native rat albumin and heat-denatured bovine serum albumin. In the experiment shown in Fig. 2, 1·9 mg. of ^{131}I-labelled rat albumin previously screened for 48 hr. was added to the circuit 15 min. after equilibration of temperature and perfusion rate. The labelled protein solution contained 0·20 % of non-protein-bound radioactivity. A total of six samples taken within 30 min. after the addition of labelled protein contained 0·18–0·20 % of non-protein ^{131}I. Thereafter the percentage increased at a constant rate over a period of 5 hr. Extrapolation of the linear plot of ^{131}I release indicates that a period of approximately 20 min. intervenes between the addition of [^{131}I]albumin and the release of non-protein-bound label (Fig. 2). A similar latent period was observed in the case of ^{131}I-labelled bovine serum albumin previously denatured by heating at 80° for 5 min. (Fig. 3).

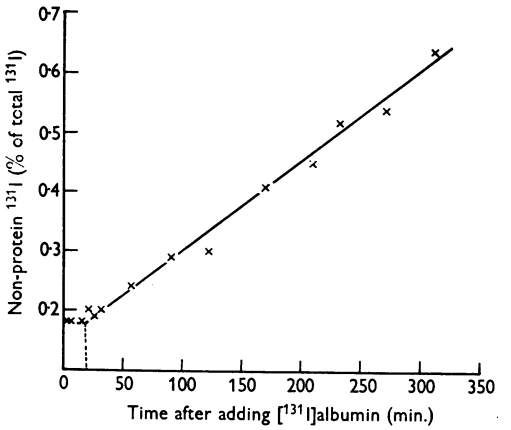

Fig. 2. Experiment showing that a period of 20 min. intervenes between the addition of ^{131}I-labelled albumin and the liberation of non-protein ^{131}I into the perfusion circuit. At zero time 1·9 mg. of ^{131}I-labelled rat albumin previously screened for 48 hr. and containing 0·18 % of non-protein-bound radioactivity was added (R.L.P. 68).

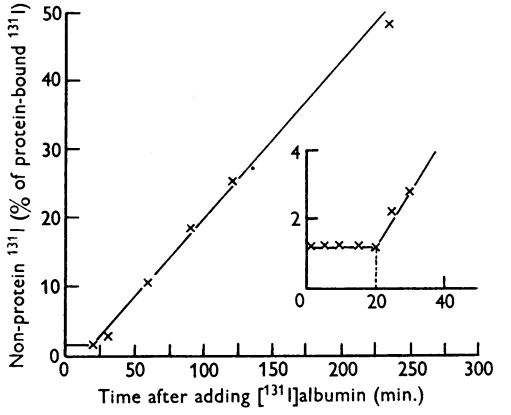

Fig. 3. Experiment showing that a period of 20 min. intervenes between the addition of ^{131}I-labelled, heat-denatured bovine serum albumin and the liberation of non-protein ^{131}I into the perfusion circuit. At zero time 0·9 mg. of heat-denatured bovine serum albumin containing 1·2% of non-protein ^{131}I was added (R.L.P. 67).

Catabolism of ^{131}I*-labelled albumin screened for less than* 24 *hr.* The breakdown rate of rat albumin separated by electrophoresis and screened for 1–23 hr. showed considerable variation (Table 2 and Fig. 4). In three experiments the corrected catabolic rates were between 0·80 and 3·53 %/hr. In two experiments in which chromatographically separated albumin was screened for 1 and 3 hr. the corrected catabolic rates were 1·08 and 0·31 %/hr. respectively.

Catabolism of [^{131}I]*labelled albumin screened for* 48–72 *hr.* The breakdown rate of rat albumin prepared either by electrophoresis or chromatography and screened for 48 hr. was measured in six experiments (Table 2, Fig. 5*a*). The corrected catabolic rates varied from 0·25 to 0·41 %/hr. and the absolute rates of catabolism were 0·96–1·49 mg. of albumin/hr. Labelled albumin prepared by chromatography and screened for 72 hr. (Fig. 5*b*) gave results within the same range, the corrected catabolic rates in two experiments being 0·30 and 0·36 % and the absolute rates of catabolism 0·94 and 1·13 mg. of albumin/hr. respectively.

Catabolism of heat-denatured bovine serum albumin. Catabolism of a tracer dose of bovine serum [^{131}I]albumin proceeded at a linear rate (Fig. 3) of 13·2 % of the labelled protein/hr. In order to compare the relative capacities of the liver for breaking down native and denatured protein two further experiments were performed in which the quantity of denatured albumin added was approximately equal in weight to the pool of rat albumin used in the perfusions. In R.L.P. 69 (Table 3) 640 mg. of denatured bovine serum [^{131}I]albumin in 28 ml. of 0·9 % NaCl was added to 64 ml. of blood in the perfusion circuit. The haematocrit was 25 and the plasma-protein concentration 4·3–4·7 %. Catabolism proceeded at a linear rate of

Table 2. *Details of individual perfusions*

R.L.P. no.	Wt. of donor rat (g.)	Wt. of liver (g.) (1)	Albumin preparation: Fractionation (2)	Albumin preparation: Iodination (3)	Duration of screening	Dose of [131I]albumin: mg.	Dose of [131I]albumin: μc	Protein concn. (g./100 ml.): Initial	Protein concn. (g./100 ml.): Final
48	275	7·3	*P*	*J*	1½ hr. and 15 min. (5)	—	26	6·7	7·6
49	292	—	*P*	*J*	1 hr.	—	39	7·7	7·1
52	345	11·3	*P*	*J*	23 hr.	3·5	59	7·5	9·8
59	267	7·1	*C*	*J*	3 hr.	0·7	29	7·6	8·8
65	280	8·3	*C*	pH	1 hr.	0·2	28	7·1	8·1
50	305	—	*P*	*J*	48	2·9	58	6·9	7·2
53	302	7·9	*P*	*J*	48	8·5	67	7·2	8·9
54	322	9·8	*C*	*J*	48	3·5	75	7·4	8·9
55	337	8·8	*C*	*J*	48	3·0	24	6·9	8·6
56	335	8·2	*C*	pH	48	3·0	69	7·6	7·6
68	310	8·2	*C*	*J*	48	1·9	96	6·7	7·4
60	303	9·0	*C*	*J*	72	1·1	31	8·2	8·8
66	260	6·9	*C*	pH	70	0·2	24	7·0	7·8

R.L.P. no.	Haematocrit: Initial	Haematocrit: Final	Amino acid (mg./100 ml.): Initial	Amino acid (mg./100 ml.): Final	Blood sugar (mg./100 ml.): Initial	Blood sugar (mg./100 ml.): Final	Initial albumin pool (mg.) (4)	Mean albumin catabolism/hr.: % (uncorr.)	Mean albumin catabolism/hr.: % (corr.)	Mean albumin catabolism/hr.: Mg. of albumin
48	35	39	27	77	91	119	870	0·42	1·11	—
49	43	43	—	—	66	124	1180	1·11	3·53	—
52	39	45	29	96	77	116	1168	0·31	0·80	—
59	35	37	—	—	—	—	1130	0·09	0·31	—
65	40	40	—	—	—	—	1110	0·33	1·08	—
50	42	40	—	—	90	110	1060	0·14	0·41	1·49
53	37	38	20	56	81	104	970	0·16	0·38	1·38
54	36	39	54	76	92	111	1180	0·08	0·25	0·96
55	37	40	—	—	116	127	1220	0·13	0·38	1·49
56	36	36	—	—	109	126	1390	0·09	0·28	1·08
68	38	—	—	—	—	—	1270	0·10	0·29	1·03
60	39	37	—	—	—	—	1320	0·10	0·36	1·13
66	41	41	—	—	—	—	1030	0·10	0·30	0·94

(1) Wt. of liver at the end of perfusion.
(2) *P* = albumin prepared by electrophoresis; *C* = albumin prepared by chromatography (see Methods).
(3) *J* = iodination by method of McFarlane (1956); pH = iodination by modified method described in text.
(4) 40% (initial plasma vol. × initial protein concn.).
(5) After screening for 1½ hr. a serum sample was screened for a further 15 min. in a second rat.

Table 3. *Details of experiments in which* ^{131}I*-labelled, heat-denatured albumin was used*

R.L.P. no.	Wt. of donor rat	Wt. of liver	Albumin fraction	Iodination (1)	Dose of [131I]albumin: (mg.)	Dose of [131I]albumin: μc	Protein concn. (g./100 ml.): Initial	Protein concn. (g./100 ml.): Final	Haematocrit: Initial	Haematocrit: Final	Mean albumin catabolism rate: % pool/hr.	Mean albumin catabolism rate: mg./hr.
67	300	—	Rat	*J*	0·9	64	—	—	—	—	13·2	—
69	300	8·4	Bovine serum albumin	pH	640	47·4	4·3	4·7	25	25	0·42	2·3
70	283	8·3	Bovine serum albumin	pH	786	51·4	8·5	9·2	44	44	0·78	5·2

(1) *J* = Iodination by method of McFarlane (1956); pH = iodination by modified method described in text.

0·42% of the labelled protein/hr. which corresponds to the breakdown of 2·3 mg. of denatured bovine serum albumin/hr. In R.L.P. 70 (Table 3) a higher haematocrit and protein concentration was achieved by removing 8 ml. of plasma from 59 ml. of pooled rat blood before the addition of 786 mg. of denatured bovine serum [^{131}I]albumin in 10 ml. of 0·9% NaCl. The haematocrit was 44 and the protein concentration 8·5–9·2%. The catabolic rate declined progressively during this experiment, the average rate being 0·78% of the pool/hr., which corresponds to 5·2 mg. of denatured bovine serum albumin/hr.

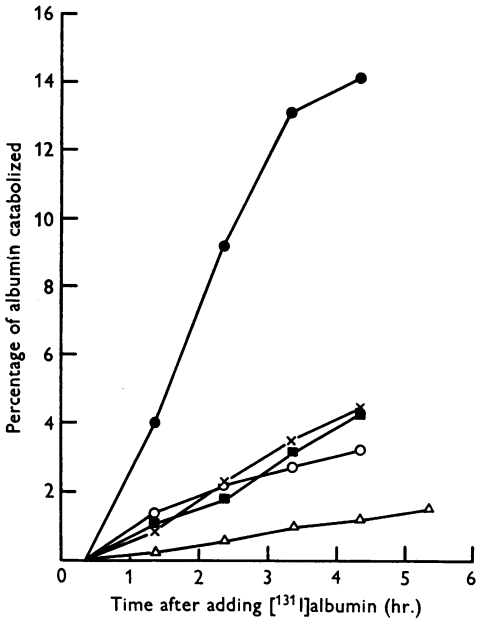

Fig. 4. Rate of catabolism of rat albumin screened for up to 24 hr. The rat-liver perfusion (R.L.P. 48–65) experiments are the same as those in Table 2. The percentage of albumin catabolized is corrected to the pool size of the donor rat. ×, R.L.P. 48; ●, R.L.P. 49; ○, R.L.P. 52; △, R.L.P. 59; ■, R.L.P. 65.

DISCUSSION

In these experiments the release of ^{131}I from labelled albumin is used to calculate rates of albumin catabolism. It is therefore assumed that ^{131}I is liberated from the labelled protein only during reactions involving rupture of peptide bonds. This assumption may be invalid under certain conditions of protein fractionation and labelling. Thus Margen & Tarver (1957), using human serum albumin labelled with ^{35}S and with ^{131}I at a ratio of 3–4 atoms of iodine/mol. of albumin, found that iodine was lost from the protein at a more rapid rate than the sulphur label. If all albumin molecules in these experiments were doubly labelled, and this is not certain, the results indicate the occurrence of deiodination without protein breakdown. On the other hand, the behaviour *in vivo* of [^{131}I]-albumin and -globulin labelled by the method of McFarlane (1956) has been shown in both the rabbit (Cohen, Holloway, Matthews & McFarlane, 1956) and the

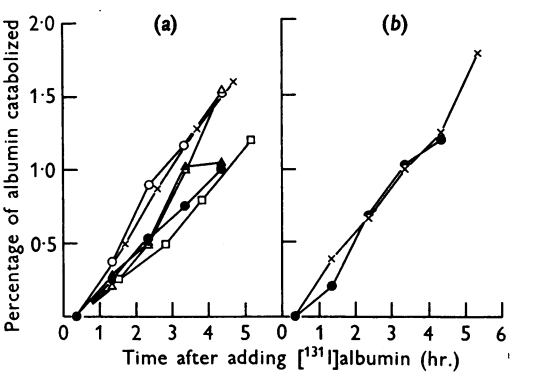

Fig. 5. Rate of catabolism of rat albumin screened for (*a*) 48 hr. and (*b*) 70–72 hr. The rat-liver perfusion experiments (R.L.P. 50–66) are the same as those in Table 2. The percentage of albumin catabolized is corrected to the pool size of the donor rat. (*a*) ×, R.L.P. 50; ○, R.L.P. 53; ●, R.L.P. 54; △, R.L.P. 55; ▲, R.L.P. 56; □, R.L.P. 68. (*b*) ×, R.L.P. 60; ●, R.L.P. 66.

Table 4. *Comparison of catabolic rates of screened rat albumin in living rat and perfused liver*

		Electrophoretic albumin	Chromatographic albumin
Mean catabolic rate *in vivo*	(mg./hr.)	9·6*	8·6†
Mean catabolic rate in perfused liver	(mg./hr.)	1·4	1·1
Mean catabolic rate in perfused liver	(% of total)	14·6	12·8

* Albumin prepared by zone electrophoresis and screened for 100 hr. after labelling (C. Matthews & A. S. McFarlane, personal communication).

† Albumin prepared by chromatography and screened for 48 hr. after labelling (S. Cohen, unpublished observations).

rat (Campbell, Cuthbertson, Matthews & McFarlane 1956) to be almost identical with that of the corresponding proteins labelled with ^{14}C by a biosynthetic procedure. Moreover, the elimination of ^{14}C- and ^{131}I-labelled antibody globulins is identical either in the presence or absence of an immune response (J. H. Humphrey & A. S. McFarlane, unpublished work, cf. McFarlane, 1957). It is concluded from these experiments that ^{131}I-labelled protein prepared by methods used in this investigation does not undergo de-iodination unless sufficient proteolysis has occurred to render the products soluble in 10% trichloroacetic acid.

A second assumption in these perfusion experiments is that the ^{131}I-labelled compounds liberated during catabolism are not reincorporated into newly formed protein. Inability to re-utilize the label has been demonstrated in animals receiving inactive iodide, by feeding large doses of ^{131}I-labelled plasma protein and by subsequent failure to detect a significant amount of the label in plasma protein, as well as by almost complete recovery of the label in the urine within two days (Cohen *et al.* 1956). The fact that iodinated products of protein breakdown are not re-utilized makes the ^{131}I label of unique value for measurements of protein catabolism.

In studies in living animals of plasma-protein metabolism extending over several weeks, the presence of a small proportion of denatured molecules in the labelled protein is of little importance and cannot usually be detected with certainty. In liver-perfusion studies, on the other hand, where only about 0·1% of the injected labelled protein may be broken down per hour a small proportion of altered molecules which are selectively taken up and catabolized will lead to gross overestimation of the overall catabolic rate. Experience with the perfused liver suggests that [^{131}I]albumin solutions frequently contain 1–3% of rapidly catabolized components. 'Screening' of the labelled protein in a living animal is therefore an essential preliminary to liver-perfusion studies. The effect of this screening process is shown in the results recorded above. Where the duration of screening was less than 24 hr. the catabolic rate, expressed as a percentage of the intravascular albumin pool, of the donor rat varied from 0·21 to 3·53%/hr. The results after screening for 48 hr. were far more consistent, and in six experiments the corrected catabolic rates were 0·28–0·41%/hr. All rapidly catabolized components appear to have been removed in these experiments, since catabolic rates were within the same range after a further 24 hr. screening of the dose material. The results obtained after screening for 48 hr. or more are therefore taken as a measure of the catabolic rate of native albumin. The mean breakdown rate in these eight experiments is 0·33% of the intravascular albumin pool of the donor rat/hr., which corresponds to 1·2 mg. of albumin/hr. In Table 4 the rates of catabolism *in vivo* of screened ^{131}I-labelled albumin prepared by two different methods of fractionation are compared with the breakdown rates of similar protein preparations by the perfused liver. It is apparent from these data that the liver is responsible for catabolizing 13–15% of all the albumin broken down in the whole animal.

The observed low rates of catabolism of native albumin may be due to the fact that the perfused livers had partly lost their ability to break down this protein. The numerous functional activities retained by the perfused rat liver have been outlined by Miller, Burke & Haft (1956). Jensen & Tarver (1956) have shown that perfused livers continue to remove colloidal chromic phosphate at a rate equal to that observed *in vivo*, and for 4 hr. or more show no fall in protein synthetic activity. Livers perfused in this Laboratory continue to secrete bile, have been shown to incorporate labelled amino acids into plasma protein, and rapidly take up and catabolize small proportions of altered protein molecules (Gordon, 1957). In addition, the experiments reported above show that the perfused liver is able to catabolize 2–5 times more denatured bovine serum albumin than rat albumin. It is unlikely therefore that the low breakdown rate observed with native albumin results from an impairment of the protein catabolic function of the perfused liver. Since albumin synthesis is known to occur exclusively in the liver it is apparent from the present experiments that the breakdown of albumin *in vivo* must occur mainly in cells which are not involved in the synthesis of this protein.

A latent period of 15–20 min. has been found to intervene between the injection of labelled amino acids and the appearance of labelled protein in the bloodstream (e.g. Green & Anker, 1955). Peters (1957), using chicken-liver slices, has recently shown that only 2–3 min. is required for the incorporation of amino acids into a non-diffusible material bound to cytoplasmic particles and having the electrophoretic and immunological properties of native albumin. This suggests that the lag period observed for the extracellular appearance of newly synthesized plasma protein may be due mainly to the time taken for release of serum albumin from its bound form. The similar interval of 20 min. which elapses before non-protein-bound ^{131}I is released from labelled albumin by the perfused liver may therefore represent predominantly the time required for albumin to enter the cell and become bound to the appropriate enzyme system.

SUMMARY

1. The catabolism of serum albumin labelled with ^{131}I was investigated in the isolated perfused rat liver.

2. A period of about 20 min. intervenes between the introduction of labelled rat albumin into the perfusion circuit and the appearance of non-protein-bound ^{131}I. A similar latent period is observed with heat-denatured bovine serum albumin.

3. Iodinated protein solutions were 'screened' by injection into rats for various periods before use in liver-perfusion studies, in order to remove the small proportion of molecules which are catabolized rapidly by the liver. With screening periods of up to 24 hr. the subsequent rate of catabolism shows wide variation. Labelled albumin separated either by chromatography or electrophoresis and screened for 48 or 72 hr. is broken down during 4 or 5 hr. perfusion at a constant rate which corresponds to about 14% of the total albumin breakdown *in vivo*.

4. The perfused liver is able to break down heat-denatured bovine serum albumin at 2–5 times the rate observed for screened rat albumin.

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REFERENCES

Campbell, P. N. & Stone, N. E. (1956). *Biochem. J.* **62**, 9P.
Campbell, R. M., Cuthbertson, D. P., Matthews, C. & McFarlane, A. S. (1956). *Int. J. App. Radiat. Isotopes*, **1**, 66.
Cohen, S., Holloway, R. C., Matthews, C. & McFarlane, A. S. (1956). *Biochem. J.* **62**, 143.
Gordon, A. H. (1957). *Biochem. J.* **66**, 255.
Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
Green, H. & Anker, H. S. (1955). *J. gen. Physiol.* **38**, 283.
Jacobs, S. (1956). *Analyst*, **81**, 502.
Jencks, W. P., Jetton, M. R. & Durrum, E. L. (1955). *Biochem. J.* **60**, 205.
Jensen, D. & Tarver, H. (1956). *J. gen. Physiol.* **39**, 567.
Margen, S. & Tarver, H. (1957). *Ann. N.Y. Acad. Sci.* **70**, 49.
McFarlane, A. S. (1956). *Biochem. J.* **62**, 135.
McFarlane, A. S. (1957). *Ann. N.Y. Acad. Sci.* **70**, 19.
Miller, L. L., Bly, C. G., Watson, M. L. & Bale, W. F. (1951). *J. exp. Med.* **94**, 431.
Miller, L. L., Burke, W. T. & Haft, D. E. (1956). *Some Aspects of Amino Acid Supplementation*, p. 44. New Brunswick: Rutgers University Press.
Peters, T. (1957). *J. biol. Chem.* **229**, 659.
Peterson, E. A. & Sober, H. A. (1956). *J. Amer. chem. Soc.* **78**, 751.
Porath, J. (1954). *Acta chem. scand.* **8**, 1813.
Somogyi, M. (1945). *J. biol. Chem.* **160**, 61, 69.

Comparative Studies of 'Bile Salts'

11. 3α:6α:12α-TRIHYDROXYCHOLANIC ACID AND RELATED SUBSTANCES*

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There has lately been renewed interest in cholanic acids hydroxylated at C-6. Hyocholic acid from pig bile (Haslewood, 1954*a*) has been shown to be 3α:6α:7α-trihydroxycholanic acid (Haslewood, 1956; Ziegler, 1956*a*, *b*; Hsia *et al.* 1957*a*). Matschiner *et al.* (1957) have isolated from rat bile two compounds which are probably 3α:6β:7α- and 3α:6β:7β-trihydroxycholanic acids (Hsia *et al.* 1957*a*, *b*, 1958*a*; Kagan & Jacques, 1957). From the urine of surgically jaundiced rats which had been given, intragastrically, hyodeoxycholic (3α:6α-dihydroxycholanic) acid there was obtained what is probably 3α:6α:7β-trihydroxycholanic acid (Hsia *et al.* 1958*b*). Other C-6 hydroxylated 5α- and 5β-cholanic acids have been described by Gotô (1955) and Kagan (1957). Hsia *et al.* (1957*b*), Kagan (1957) and Schubert & Damker (1957) have devised methods of making substituted $\Delta^{6:7}$-cholenic acids.

The compound 3α:6α:12α-trihydroxycholanic acid is of special interest to us, first because it is one likely, on biogenetic grounds, to occur in biles and secondly because 3:6:12-trihydroxycholanic acid was stated by Ohta (1939) to have been derived by permanganate oxidation from the 'tetrahydroxy-norsterocholanic acid' first isolated by him from 'Gigi' fish bile. By direct infrared spectroscopic examination of the methyl ester of this latter compound it was concluded (Haslewood & Wootton, 1956) that it could not have the structure attributed to it by Ohta (1939), namely $C_{27}H_{46}O_6$, a 3:6:12:24-tetrahydroxy coprostanic acid; it was

* Part 10: Anderson, Haslewood & Wootton (1957).