that whilst exchange may play only a small part in normal protein synthesis *in vivo*, it may become a dominant route for amino acid incorporation *in vitro* when cellular organization is disrupted or modified.

3. The radioactivity in crude enzymic hydrolysates of yeast or *Chlorella* protein is much more rapidly incorporated into ribonuclease than is the activity of purified hydrolysates. The activating factor has not been identified.

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The Use of the Isolated Perfused Liver to Detect Alterations to Plasma Proteins

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Although the ability of the liver to take up microscopically visible matter is well known, and although this organ has been shown to be a major site of plasma protein synthesis (Miller, Bly, Watson & Bale, 1951), little evidence has yet been obtained concerning its ability to take up and catabolize plasma proteins. An experiment in which Miller, Burke & Haft (1955) used ^{14}C -labelled rat plasma for this purpose is one of the few exceptions (see below). The present study has employed ^{131}I -labelled plasma proteins and the isolated perfused rat liver. This system has been found to be extremely sensitive to very small proportions of altered molecules present in samples of purified albumin and γ -globulin isolated either by electrophoresis or by precipitation with solvents or salts. At intervals during the perfusions, plasma samples have been obtained and treated with trichloroacetic acid. The amounts of ^{131}I then remaining soluble have been taken as representing the successive degrees of proteolysis.

The isolated perfused liver has several advantages for studies of this kind in addition to the primary one that it gives information about a single organ. These include the absence of any significant lymph pool, so that the complication is avoided of the initial period observed *in vivo* during which equilibration with lymph is occurring. This is of special value if it is desired to assess the initial rate of breakdown of a protein. Also, the size of the pool (and/or concentration) of the circulating plasma can readily be varied, and toxic substances which might lead to death in the whole animals can be employed. The limitations of this system include the short periods for which individual experiments can be conducted. This means that if two proteins with very different half-lives are present, the rapid initial breakdown of the one will overshadow the breakdown of the other. However, the rate of catabolism of a protein which is only slowly metabolized can be investigated if the material with short half-life is first removed by preliminary injection of the

Table 1. *Details of individual perfusions*

The pool ratio (column 9) is given by $0.0024 \times [\text{wt. of liver donor (g.)}]/[\text{wt. of plasma protein in circuit (g.)}]$ (Campbell *et al.* 1956). The correction of the percentage of initial dose of [^{131}I]protein catabolized/hr. (column 10) is for the amount of plasma protein in the perfusion circuit and size of liver donor (cf. p. 258). The [^{14}C]lysine (column 14) is uncorrected for dilution of [^{14}C]lysine with lysine originating from breakdown of liver proteins (cf. Jensen & Tarver, 1956). RLP 43 and RLP 12 were on Lister Institute preparations. In RLP 12, the 100 mg. of amino acids used was added 3.25 hr. after the time of the first sample. For RLP 40, extra plasma was added after 2.5 hr. (cf. Fig. 1 for details).

		Initial dose of [¹³¹ I]protein		In perfusion circuit at start			Initial haematocrit (%) (6)	Percentage of initial dose of [¹³¹ I]protein catabolized in 5 hr. (uncorr.) (7)
RLP no.		(mg.) (1)	(μC) (2)	Blood (ml.) (3)	Plasma protein			
					(g.) (4)	(g. %) (5)		
19	Rat-albumin preparation 2	0.53	18.8	89	3.27	3.84	29.0	1.25
40	Rat-albumin preparation 4 (partially denatured)	—	24.5	70	2.62	4.22	29.0	8.43
9	Rat-γ-globulin preparation 1	—	12.6	91	3.32	3.91	28.5	1.56
31	Rat-γ-globulin*	1.6	20.0	75	3.59	4.40	30.0	10.3
43	Human albumin fractionated with ether	45	37.4	70	2.70	3.98	27.2	0.61
45	Human albumin denatured by urea	1.0	4.5	74	2.53	4.31	27.7	1.27
15	Human γ-globulin fractionated with Na ₂ SO ₄	2.9	8.9	84	3.21	3.77	27.0	1.43
12	Human γ-globulin fractionated with ether	—	15.0	131	4.77	4.07	30.0	3.78
21	Human albumin + antibody	1.3	6.9	95	3.98	4.47	30.5	23.0
23	Human albumin + antibody	1.1	2.92	94	4.01	4.26	26.0	24.0
Screened preparations								
34	Rat-albumin preparation 3	—	21.0	71	3.4	4.66	27.3	0.29
38	Rat-albumin preparation 4	4.5	22.3	64	3.02	5.03	28.3	0.26
39	Rat-albumin preparation 4	6.7	28.5	76	3.53	4.76	27.0	0.23
32	Rat-γ-globulin preparation 2	1.0	11.2	54	2.20	3.78	24.3	0.55
36	Rat-γ-globulin preparation 3	1.0	42.0	78	3.45	4.66	29.7	0.55
41	Human-albumin preparation 1	6.5	6.8	73	2.94	4.15	27.0	0.34
42	Human-albumin preparation 1	13.0	6.1	74	3.47	4.75	27.3	0.25

RLP		Wt. of liver donor (g.) (8)	Pool ratio (9)	Percentage of initial dose of [¹³¹ I]protein catabolized/ hr. (corr.) (10)	Percentage of initial dose found in liver after perfusion with saline (11)	Also added at start		Percentage of [¹⁴ C]lysine of initial dose incorporated in plasma protein at 4 hr. (14)
no.	Unscreened preparations					L-Lysine HCl (mg.) (12)	¹⁴ C radio- activity (μC) (13)	
19	Rat-albumin preparation 2	322	0.236	—	—	11.0	5.0	3.2
40	Rat-albumin preparation 4 (partially denatured)	295	0.270 (initial)	6.24	2.8	9.0	5.0	4.2
9	Rat-γ-globulin preparation 1	340	0.250	—	—	13.0	3.5	2.8
31	Rat-γ-globulin*	284	0.208	—	4.75	—	—	—
43	Human albumin fractionated with ether	294	0.261	—	—	—	—	—
45	Human albumin denatured by urea	348	0.330	—	4.74	—	—	—
15	Human γ-globulin fractionated with Na ₂ SO ₄	305	0.228	—	—	11.0	5.0	3.9
12	Human γ-globulin fractionated with ether	285	0.144	—	—	—	—	—
21	Human albumin + antibody	284	0.172	—	53.4†	11.0	5.0	4.7
23	Human albumin + antibody	264	0.160	—	69.7†	11.0	5.0	4.0
Screened preparations								
34	Rat-albumin preparation 3	296	0.208	0.283	0.32	—	—	—
38	Rat-albumin preparation 4	306	0.236	0.217	0.33	11.0	5.0	6.8
39	Rat-albumin preparation 4	259	0.176	0.260	0.39†	11.0	5.0	5.4
32	Rat-γ-globulin preparation 2	331	0.328	0.334	1.5	—	—	—
36	Rat-γ-globulin preparation 3	315	0.219	0.500	0.80	11.0	5.0	6.2
41	Human-albumin preparation 1	300	0.245	0.277	0.46	9.0	5.0	5.7
42	Human-albumin preparation 1	246	0.17	0.296	0.315	9.0	5.0	6.2

* Heated for 2 min. at 65°.

† Not washed out with saline.

‡ Incompletely perfused out with saline.

mixture into a living rat. The plasma of this animal is then taken after a period long enough to ensure the removal of the rapidly degraded material and used as the dose material for the liver perfusion. This procedure, known as 'screening' (McFarlane, 1956), has been used as a preliminary to a number of the perfusions reported below.

In any perfusion system it is necessary to show that the perfused organ is fully functional. For this purpose, in the present experiments, a ^{14}C -labelled amino acid has been added to the blood, and incorporation of the isotope in the plasma proteins has been measured (Miller *et al.* 1951). Ability to synthesize plasma proteins has been considered a sufficient criterion for the functioning of the liver. To eliminate the possibility that liver ^{14}C protein might have escaped into the plasma, an experiment was carried out in which ^{14}C -labelled albumin was specifically isolated by means of an anti-serum.

METHODS

All perfusions were carried out with an apparatus of the type described by Miller *et al.* (1951), simplified by omission of the rubber diaphragm and transfer of the syringe unit to the outside of the box. In order to minimize the volume of blood required, the reservoir was filled with glass beads before use.

Animals. Rats of the hooded strain maintained at the National Institute for Medical Research, Mill Hill, were used for all experiments except RLP 34, which was carried out with a liver and blood of the hooded strain from the Rowett Research Institute, Bucksburn, Aberdeenshire. Liver donors were starved overnight before use. Blood was withdrawn by heart puncture and heparinized (30 i.u./ml.).

Supplements

Glucose. 500 mg. of glucose was added at the start of each perfusion.

Amino acids. A mixture of the same 18 amino acids as given by Miller *et al.* (1951) was used, but in different proportions which were adjusted to agree more nearly with those known to be present in rat plasma (Henderson, Schurr & Elvehjem, 1949). Except where otherwise stated, 100 mg. of the amino acid mixture was added to each perfusion at the same time as the ^{131}I protein.

Preparation of proteins and iodination with ^{131}I

Unless otherwise stated, all proteins were prepared by electrophoresis on treated cellulose columns according to Porath (1954). After concentration by pressure dialysis, they were examined by electrophoresis on paper and then iodinated with ^{131}I by the method of McFarlane (1956). The human γ -globulin and albumin used in RLP 12, 43 and 45 were made by the Medical Research Council Blood Products Unit at the Lister Institute, Elstree, Herts., by the ether-fractionation method of Kekwick & MacKay (1954).

The human albumin used for the urea-denaturation experiment, RLP 45, was from the same batch as that used for RLP 43. Denaturation with 8M-urea and subsequent removal of the urea by dialysis and regeneration of the

albumin by heating to 41° for 30 min. at pH 5.25 were carried out as described by Neurath, Cooper & Erickson (1942); they showed that horse-serum albumin regenerated in this way has a molecular weight similar to that of native albumin, but differs from it in solubility and electrophoretic mobility.

The weights of iodinated plasma proteins used have been small (Table 1). Therefore, to guard against losses due to protein adsorption on glassware, the solutions have been mixed with an excess of uniodinated rat serum. This was usually carried out immediately before dialysis (RLP 9, 19, 21, 23); however, the human γ -globulin used for RLP 12 and 15 was mixed with rat serum soon after iodination and used several days later. In RLP 31, the γ -globulin was not mixed with serum until after the heating procedure.

Screening. Solutions of ^{131}I proteins were injected intraperitoneally into rats, volumes up to 3 ml. being used. After 48 hr. the animal was bled as extensively as possible by cardiac puncture. Sometimes a second cardiac bleeding was made possible by injecting 10 ml. of warm 0.9% (w/v) NaCl into the inferior vena cava immediately after the first bleeding.

Estimation of the degree of catabolism. The percentages of ^{131}I proteins catabolized at intervals during each perfusion were estimated by removal of blood samples, which were promptly centrifuged in haematocrit tubes and then kept at 2° . At the end of each perfusion the plasmas were separated and 1 ml. of each was stirred with 3 ml. of 10% (w/v) trichloroacetic acid. Carrier NaI (approx. 10 mg.) was added before to this precipitation, and when ^{14}C lysine incorporation was being measured this amino acid (approx. 5 mg.) was also added as carrier. The supernatants thus formed were removed at the centrifuge without delay and estimated for radioactivity. For measurements of ^{14}C the precipitates were handled as described by Miller *et al.* (1951).

Measurements of radioactivity. ^{14}C Protein samples were oxidized to CO_2 and estimated as described by Bradley, Holloway & McFarlane (1954). The error on the final samples was $\pm 3\%$. Solutions containing ^{131}I were estimated in a scintillation counter (cf. Cohen, Holloway, Matthews & McFarlane, 1956). Errors were greatest in the estimations of non-protein-bound ^{131}I . Counting of trichloroacetic acid supernatants was usually continued long enough to give $\pm 5\%$ accuracy on the final sample of each series.

Plasma-protein estimations. These were done on 0.1 ml. samples of the plasma by the biuret method of Gornall, Bardawill & David (1949).

RESULTS

Sources of error

In perfusions in which low rates of catabolism occurred, possible inaccuracies in estimating the proportion of the radioactivity not due to protein became specially important. Thus concentration due to evaporation during the perfusion must lead to too high apparent rates of catabolism. Despite the use of a gas wash-bottle incorporating a sintered plate for the inflow of oxygen, some concentration occurred. Corrections based on the observed

changes in radioactivity/ml. of plasma have therefore been applied. For perfusions 32-45 a second gas washer was introduced, with the result that volume changes due to evaporation were greatly reduced. Since changes in volume due to evaporation can, under certain conditions, lead to significant errors and also because some of the rates of catabolism reported below are small, some examples of the apparent effect of volume changes on rates of catabolism are given in Table 2.

In perfusions 9-21, appropriate volumes of Ringer solution-water (1:1, v/v) were added to compensate for evaporation. However, since this procedure required complicated and possibly inaccurate corrections for dilution, as well as for concentration due to evaporation, it was abandoned, and, in perfusions 32-45 where measurements of the catabolism of screened proteins were undertaken, only corrections for evaporation and for sampling (see below) were required.

As shown below (p. 259), the initial plasma volumes have been calculated in two ways. Comparison shows differences of up to 15% for the results obtained by the two methods. This is thought to be largely due to error in reading the haematocrit tubes, which were graduated only at intervals of 0.2 ml. For this reason the values for the initial plasma volumes have been derived from the degree of dilution of the ^{131}I added initially.

As shown in Table 2, errors are larger the greater the initial non-protein radioactivity of the dose. In the present work, errors due to this cause have been kept low by dialysing the ^{131}I protein solutions, before use, for 20 hr. against 2 l. of 0.9% NaCl at 2° with rocking of the containing vessel. In this way, at worst, the level of non-protein radioactivity present initially has doubled during the experimental period. The effect of an increase of this magnitude is shown in Table 2. In most of the experiments much larger increases of non-protein radioactivity took place. More prolonged dialysis would presumably have reduced still further the levels of non-protein radioactivity of the dose solutions. Since, however, the aim in most of this

work was to examine the behaviour of proteins unmodified except by the addition of the ^{131}I label, more prolonged dialysis, carrying with it a greater risk of some slight degree of denaturation, was thought undesirable.

Another type of error would arise if ^{131}I iodide or iodinated protein were to become adsorbed on any of the surfaces of the perfusion circuit. Control (i) (see below), in which ^{131}I iodide was used, was therefore carried out. Measurement of total blood radioactivity at intervals during certain perfusions excluded loss of ^{131}I protein.

It may be noticed that the larger the amount of protein in the perfusion circuit the larger has been the factor by which the observed degree of catabolism has been multiplied to give the final figures for the screened proteins. Since errors must thereby be proportionately increased, it is evidently desirable to work with the minimum volume of blood that will still allow circulation and a sufficient margin for sampling.

Corrections applied to observed values

(a) *For volume of blood used and size of liver.* Since rather different volumes of blood have been used in the various experiments, and also because the observed degree of proteolysis must depend to some extent on the size of the liver being perfused, some of the results have been multiplied by a factor $(0.0024 \times \text{liver donor weight})/(\text{plasma protein weight in perfusion circuit})$, called 'the pool ratio' (Campbell, Cuthbertson, Matthews & McFarlane, 1956). The main purpose of this correction is to allow comparison of the results of one experiment with another. It is also necessary for the attempt made below to calculate the proportion of albumin catabolized in the liver *in vivo* (cf. Discussion and Table 5). However, such calculations are valid only if the rate of catabolism has been shown to be exponential over the period of the experiment, and the absolute amount of protein broken down to be constant despite different pool sizes. That this may be true for screened proteins is suggested both by the results shown in Figs. 4 and 5 and by the experiment (Fig. 1) in which the pool size was increased during the perfusion. On the other hand, with some of the uncreened dose materials the rate of catabolism fell markedly during the perfusion, so that except for RLP 40 such corrections have not been made. Any attempt to deduce a 'rate of catabolism' which is valid for a longer period than the 5 hr. used for these experiments involves the assumption of a continuing constant rate of catabolism. This can be expected only if the homogeneity of the dose material is absolute. The aim of the screening experiments has been to achieve this, but it is recognized that further work is required to show how nearly homogeneity has, in fact, been attained.

Table 2. *Size of errors resulting from (undetected) concentration of blood during perfusion*

The non-protein radioactivity resulting from 10% concentration is expressed as a percentage of that appearing when no concentration has taken place (no concn. = 100%).

Initial non-protein radioactivity (arbitrary units)	Non-protein radioactivity appearing during the perfusion (arbitrary units)	Non-protein radioactivity resulting from 10% concn.
10	100	112
10	10	122
10	1	222

Table 3. *Example of corrections for changes in volume of perfusion circuit due to sampling*

After removal of each sample the observed value of the radioactivity of trichloroacetic acid supernatant has been multiplied by a factor equal to (total vol. of blood remaining in the perfusion circuit)/(initial vol. of blood in the perfusion circuit). The figures in column (3) were obtained by multiplying those in column (2) by $\frac{4}{3}$.

Period	Vol. of blood in circuit during each period (ml.) (1)	Increase in counts/3 ml. of trichloroacetic acid supernatant/min. (2)	Increase in counts/ml. of plasma appropriate to each period (3)	Increase in counts/ml. of plasma corr. for pool size appropriate to each period (4)	Cumulative increase (5)
1	103	137	183	178	178
2	98	148	197	182	360
3	95	313	417	373	733
4	92	393	524	455	1188
5	89	349	465	390	1578

Fig. 1. Catabolism of rat albumin (RLP 40). This preparation of [131 I]albumin had become partially denatured on standing. At 2.5 hr. after the initial sample was taken, 8.9 ml. of rat plasma that had previously been concentrated to contain 1.6 g. of plasma protein was added. Initial pool ratio, 0.270; final pool ratio, 0.168. ○, Values obtained by use of the true pool ratios appropriate to each period; ×, initial pool ratio used for calculation instead of that actually existing. Both sets of figures have been corrected for sampling. The perfusion rate fell from 7 to 5 ml./min. on addition of the extra plasma, and then rose again to 6 ml./min.

(b) *For sampling and evaporation.* See below and Table 3.

Methods of calculation

To obtain rates of catabolism knowledge is required of the volume of blood initially present in the perfusion circuit. Since some loss of blood must take place during the setting up, blood volumes used in the present calculations (Table 1) have been derived either from the volumes left in the circuit at the end of each experiment with appropriate

corrections for sampling and evaporation (perfusions 9–21) or from the blood or plasma radioactivity/ml. Since values obtained by the latter method are believed to be more accurate than the corrected blood volumes derived from the final blood-volume measurements, they have been used for the calculations of perfusions 23–45 given in Table 1. The percentages of protein catabolized/hr. have been calculated as shown in the following example, in which the figures from RLP 36 (Table 1) have been used:

(1) The volume of blood in circulation at the end of the perfusion is estimated as follows: Volume of blood pumped out (62 ml.) + [(Radioactivity of washings from perfusion circuit + liver saline perfusate)/(Radioactivity/ml. of final blood) (5.62/0.416 = 13.5 ml.)] = 75.5 ml. (total vol.).

(2) From the final blood volume and haematocrit (31.4%) the volume of red cells in final blood is calculated to be 23.7 ml. To this, the volume of red cells of all samples (6.6 ml.) is added and from the initial haematocrit the initial plasma and blood volumes are obtained (72 and 102 ml. respectively).

(3) The volume of plasma initially in the perfusion circuit is estimated by dividing the initial dose of [131 I]protein by the radioactivity/ml. of the plasma. (0.5 ml. of plasma was diluted to 10.5 ml., and 3 ml. of this solution was used for counting. Therefore, plasma vol.

$$= \frac{42.2 \times 0.5 \times 3 \times 7.2 \times 10^5}{58400 \times 10.5} = 74.2 \text{ ml. and blood volume} = 106 \text{ ml.}$$

(4) A correction is applied for sampling as shown in Table 3, using the blood volume obtained in (3) above.

(5) Cumulative increases are multiplied by the appropriate factor to correct for any volume change due to evaporation, i.e. final volume/initial volume = 1.0 as estimated by change in total radioactivity/ml. of plasma samples.

(6) To obtain the percentage of the dose catabolized during 5 hr. the corrected non-protein radioactivity formed/ml. of blood or plasma is expressed as a percentage of the initial dose/ml.

$$= \frac{1578 \times 106}{42.2 \times 7.2 \times 10^5} = 0.55.$$

(7) The resulting percentage (expressed/hr.) is divided by the pool ratio, i.e. (weight of protein assumed to have been present in the plasma of the donor rat [2.4 mg./g. body weight])/(weight of plasma protein initially present in the perfusion circuit) = $(0.55/0.219 \times 5) = 0.50\%$.

Control experiments

(i) *Recovery of [^{131}I]iodide.* Since the amount of protein catabolized has been estimated from the proportion of ^{131}I rendered non-precipitable by trichloroacetic acid, a control experiment was carried out to ascertain recovery of [^{131}I]iodide added to rat blood. The same conditions were used as in the liver perfusions except for the absence of the liver. After addition of 2.87 μC in 0.7 mg. of carrier NaI to 104 ml. of rat blood, radioactivities in the whole blood, whole plasma and plasma trichloroacetic acid supernatants were measured at hourly intervals for 5 hr. Recovery in plasma and in the plasma trichloroacetic acid supernatants was the same, namely 71% of the added dose. Variation between samples was small, being $\pm 3.8\%$ of the dose for the plasma and $\pm 3.1\%$ for the plasma trichloroacetic acid supernatants. No trend in these figures could be detected. Correction for plasma volumes on the assumption of equal concentration of iodide inside and outside the red cells gave a total recovery of 102%. Recovery in the whole blood measured directly was 97% of the dose. In this series of measurements the value at 10 min. was low and has been excluded from the average.

(ii) *Stability of [^{131}I]albumin in absence of a liver.* In another control experiment again with no liver present, 10 μC of iodinated rat albumin (approximately 0.3 mg. of albumin) was added to 113 ml. of rat blood and circulated for 3 hr. At this time a further 10 μC of the same solution was added and the experiment was continued for 3 hr. Measurements of the radioactivity in samples taken at hourly intervals showed 2.1% to be non-precipitable by trichloroacetic acid. After the second addition this figure remained almost the same (2.2%).

(iii) *Absence of exchange of [^{14}C]lysine with circulating antibody γ -globulin and isolation by electrophoresis and precipitation with an antiserum of ^{14}C -labelled rat albumin.* The liver of a rat weighing 263 g. was perfused with 116 ml. of blood, 58 ml. of which was obtained from rats which 6 weeks previously had received two intramuscular injections of human albumin each of 5 mg. in 0.5 ml. of Freund's adjuvant mixture (Freund & McDermott, 1942). An interval of a week was allowed between the injections. The antibody level in the plasmas at the time of use lay between 1.0 and 1.6 mg./ml. L-Lysine (11 mg.) was included in 100 mg. of the amino acid mixture, and 500 mg. of glucose plus Ringer's solution to a total volume of 138 ml. were used. Half an hour after starting the perfusion,

10 μC (0.5 mg.) L- ^{14}C]lysine was added and samples were taken after 10 min., 2 hr. 40 min. and 5 hr. 10 min. No radioactivity could be detected in the first sample, whereas 2.35 and 5.0% of the dose appeared in the proteins of the subsequent samples. After addition of human albumin at optimum proportions to another sample of the final plasma and standing for 2 days at 0° , the antigen-antibody complex precipitate was centrifuged off. After being washed three times with saline at 0° , followed by washes with 1:1 (v/v) ethanol-water, ethanol and ether, the precipitate was dried and estimated for ^{14}C . The specific activity was found to be less than 2% of that of the protein of the whole plasma, which was precipitated for this purpose by trichloroacetic acid and washed as described above. Since no precautions for the removal of complement were taken, this level of radioactivity in the antibody precipitate may be considered as being due to coprecipitation with traces of the radioactive proteins present in the plasma. Exchange between free [^{14}C]lysine and antibody lysine, and thus presumably that of the other plasma proteins also, if it occurs at all cannot be at a level which needs to be taken into account for the present experiments.

To investigate further the nature of the radioactive proteins that had appeared during the perfusion, 12 ml. of the final plasma was subjected to electrophoresis in a starch slab. Veronal buffer (0.1 M) at pH 8.6 and 3.0 v/cm. for 24 hr. were used. Elution of those parts of the starch expected to contain the albumin and the γ -globulin gave 160 and 18 mg. of protein respectively. Approximately 1 mg. of this albumin plus 30 mg. of total serum proteins from a rabbit hyperimmunized against rat albumin (kindly made available by Dr P. N. Campbell) in a volume of 4 ml. of 0.9% (w/v) NaCl was kept at 37° for 1 hr. The precipitate which formed was removed and washed as described above, except that only a single wash with saline was done; 8.0 mg. of protein was obtained. Both this sample and the protein obtained by trichloroacetic acid precipitation of a sample of the eluate from which it came were estimated for ^{14}C . The radioactivity of the eluate protein (albumin fraction) was 720 disintegrations/min./mg. of C, whereas that of the antigen-antibody complex was 100 disintegrations/min./mg. of C. These figures indicate that 81% of the radioactivity present in the eluate was precipitated by the anti-albumin serum. Only the 0.52% of the total radioactivity recovered from the starch appeared in the γ -globulin fraction, which is in accord with the finding of Miller & Bale (1954) that no more than traces of γ -globulin are synthesized in the rat's liver.

(iv) *Effect of protein-pool size.* Assuming that there is no special selection of [^{131}I]protein molecules for catabolism, it is to be expected that the rate of

Table 4. *Distribution of radioactivity between liver and blood after 5 hr. perfusion as a percentage of initial radioactivity*

	In liver	In blood			
		Non-precipitable by 8% trichloroacetic acid		Particulate or associated with red cells	Sol. protein
		Uncorr.	Corr. for pool size		
Human albumin + antibody (RLP 23)*	69.5	16.3	—	9.9	4.1
Rat albumin, partially denatured (RLP 40)	2.8	3.71	—	—	—
Rat γ -globulin, heated 2 min. at 65° (RLP 31)	4.75	9.44	—	—	—
Rat albumin, screened (average of perfusions RLP 34, 38 and 39)	0.35	2.68	1.30	—	—
Rat γ -globulin, screened (average of perfusions RLP 32 and 36)	1.15	2.78	1.95	—	—
Human albumin, screened (average of perfusions RLP 41 and 42)	0.39	2.96	1.43	—	—

* Not finally perfused with saline. Perfusion time 4 hr. 10 min.

liberation of ^{131}I from protein will be proportional to the concentration of [^{131}I]protein in the protein passing through the liver. In order to check this assumption, in RLP 40, 1.6 g. of rat plasma was added 2.5 hr. after the start. Fig. 1 shows the rates of catabolism thus found, plotted with and without correction for the change in pool size. The straight line obtained after proportional correction for increased pool size confirms the validity of the assumption.

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The results for these experiments are presented in Tables 1 and 4. The latter shows the observed distribution of radioactivity between liver and blood.

DISCUSSION

Very different proportions of the variously treated plasma proteins were broken down during the 5 hr. perfusion period (Table 1). For convenience the results may be divided into three groups: (1) the screened proteins, 0.23-0.55% of which were catabolized; (2) the various preparations isolated by the usual methods (cf. p. 257 for details) and not specially treated. These, of which 0.61-3.8% was catabolized, will be referred to as the 'normal' proteins; (3) those proteins which have been partially or completely denatured or caused to aggregate and were catabolized to the extent of 10.3-20.4%. The single exception to this scheme is the urea-denatured, and then regenerated, human albumin used in RLP 45, of which only 1.27% was catabolized. The much lower figures obtained for the screened proteins as compared with those referred to above as 'normal' suggests that the screening process removed a proportion of protein molecules which, because they had been altered in some way, would otherwise have been preferentially taken up by the perfused liver and catabolized. Since, with screened rat albumin, only 0.25% of the protein initially added is cata-

Fig. 2. Catabolism of human serum albumin plus antibody (RLP 21). The liver donor used had been immunized against human serum albumin. The proportion of the blood from rats similarly treated was 18%. During 9 min. after the addition of the human serum albumin the perfusion rate fell steadily to one-quarter of its initial value (approximately 7 ml./min.). It then rose again, finally attaining a rate of approximately 10 ml./min. The figures for percentage catabolized have been corrected for sampling; since 16 ml. of Ringer's solution was added as replacement for the blood samples when each was taken, correction has also been made for this dilution.

bolized during the 5 hr. period, amounts of the order of 3% of the protein under examination can readily be detected, if present in such form as to be largely taken up by the liver during this period.

The question of the origin of the small proportion of altered molecules in the 'normal' proteins is of some importance but cannot be usefully discussed without further investigation. Understanding of

the relative importance of the rate of uptake of proteins into the liver cells and their subsequent rate of catabolism and the significance of prior denaturation or aggregation for the rates of the two processes, or both, also requires much further work. Yet certain facts indicate that the rate of uptake is of over-riding importance as a factor controlling catabolism, at least where altered proteins are concerned. Thus when human [^{131}I]albumin was allowed to react with antibody during a perfusion (RLP 21 and RLP 23 and Fig. 2) nearly the whole of the labelled protein was removed from the blood during the next 4 hr. 10 min. and a very high rate of catabolism took place (Table 4). Evidently under these experimental conditions the liver can rapidly catabolize a native heterologous protein.

Since the ability of the liver to catabolize denatured plasma proteins has not previously been studied, comparisons can most usefully be made with the relative rates of breakdown of native and denatured plasma proteins when these are treated with enzymes *in vitro*. Under these conditions Bernheim, Neurath & Erickson (1942), using pancreatin as a source of trypsin, have shown that denatured horse plasma albumin is broken down rather less than twice as fast as is the native protein, and that this rate is unchanged after regeneration (cf. Methods). With pseudoglobulin, on the other hand, a much greater increase in proteolytic rate took place after denaturation. The rates of breakdown of protein molecules within cells must of course be governed by more complex factors than those operating in the experiments reported by Bernheim *et al.* (1942). Yet in the present work it has been observed that albumin before and after urea denaturation (RLP 43 and RLP 45) showed rather similar differences to those observed in the *in vitro* system of Bernheim *et al.* (1942). Approximately 10% of

the preparation of rat γ -globulin which had been heated to 65° for 2 min. (RLP 31, Fig. 3) was broken down in the course of 5 hr. No doubt the relative lability of this protein towards enzymes as observed by Bernheim *et al.* (1942) for horse pseudoglobulin, and some degree of aggregation, contributed to this result.

Screened proteins. In order to investigate the behaviour of proteins unmodified except by the introduction of the label, a number of perfusions were carried out on screened proteins. Details of this procedure are given above. Using the same strain of rats, McFarlane & Matthews (personal communication) have shown that, 48 hr. after injection, 42–57% of rat albumin and 58–66% of rat γ -globulin remain unexcreted. It therefore seems reasonable to assume that at this time nearly the whole of any substance with a catabolic rate as fast as that of a partially denatured protein will have disappeared. The catabolic rates obtained for these screened proteins are shown in Table 1. Evidently the screening process has reduced both catabolic rates to less than 50% of their previous values, with γ -globulin still being broken down slightly faster than albumin (Figs. 4, 5). Comparison of these rates with those observed *in vivo* suggests that *in vivo* only a small proportion of each protein is broken down in the liver. An attempt to estimate this proportion quantitatively may be made for rat albumin as shown in Table 5.

A similar calculation for γ -globulin may be made, but is of less interest because of the lack of any accurate estimate of the proportion of γ -globulin in rat serum. Accurate estimates of the rate of catabolism of human albumin in rats are not available, but McFarlane (1956) has shown in the rabbit that before the onset of immunity this protein is broken

Fig. 3. Catabolism of rat γ -globulin previously heated for 2 min. at 65° (RLP 31). The figures for percentage catabolized have been corrected only for sampling. The rate of perfusion was approximately 7 ml./min.

Fig. 4. Catabolism of rat albumin after screening: Δ , RLP 34; $\square$, RLP 38; $\circ$, RLP 39. The same preparation of [^{131}I]albumin was used for RLP 38 and 39. The figures for percentage of protein catabolized have been corrected for pool size and sampling, and in RLP 38 for 4% concentration due to evaporation. This last figure is based on the change in total radioactivity/ml. of the samples. The rates of perfusion were approximately 8 ml./min. for all three perfusions.

Table 5. Comparison of rate of catabolism of rat plasma albumin in the perfusion circuit and in vivo

The figures are those from RLP 34 (Table 1).

Total plasma protein in perfusion circuit	3.4 g.
Wt. assumed to be albumin (50%)	1.7 g.
Albumin catabolized	{ 0.059%/hr. 1.0 mg./hr.
Wt. of liver donor (rat)	296 g.
Plasma albumin of donor, assuming 2.4 mg. of plasma protein/g. body wt. (Campbell <i>et al.</i> 1956), and 50% of this as albumin	0.355 g.
Rate of catabolism of albumin <i>in vivo</i> (Campbell <i>et al.</i> 1956)	{ 2.5%/hr. 8.9 mg./hr.
Rate of catabolism by the perfused liver (as % of rate of catabolism <i>in vivo</i>) is therefore	11.2

Fig. 5. Catabolism of rat γ -globulin after screening (RLP 36). The figures for percentage of protein catabolized have been corrected for pool size, sampling and 2% concentration due to evaporation. This last figure is based on the change in total radioactivity/ml. of the samples. The rate of perfusion was approximately 8 ml./min.

down only slightly faster than is the homologous albumin. As shown in Table 1, the catabolic rates for screened heterologous and homologous albumins observed with the perfused rat liver are closely similar. Apparently the proteolytic enzymes of the liver do not distinguish the small differences between these molecules.

An alternative explanation for the observed very low rates of catabolism of native plasma proteins might be forthcoming if the livers used for these perfusions had partly lost, under the conditions of the experiments, their original ability to break down protein. It is difficult wholly to exclude this possibility, but two circumstances appear to reduce the likelihood of such an explanation. First, there is the ability of the livers to incorporate L-[^{14}C]lysine into plasma proteins in the same perfusions in which extremely low rates of catabolism were observed. The control experiments reported above, in which albumin containing ^{14}C is characterized by means of an antiserum, are here relevant. The second circumstance is the marked ability of these livers to catabolize partially denatured protein or proteins present as antigen-antibody complexes. Thus in the perfusion in which human albumin was allowed to react with an antiserum, 30.5% remained in the

blood after 4 hr. 10 min., and 53% of this was no longer in the form of protein. If then the perfused livers are fully functional, it seems probable that *in vivo* also only a small proportion of the total breakdown of plasma proteins is brought about by the liver. If this is so, the finding of Miller *et al.* (1955) that ^{14}C -labelled plasma proteins are broken down as fast by the perfused liver as in the whole animal will require reinterpretation. At the moment it is possible only to note that the rate of catabolism reported by Miller *et al.* (1955) is very much faster than anything observed in the present experiments, at least when screened proteins have been used.

As shown in Table 4, some proportionality exists between the amounts of the various proteins broken down during 5 hr. and the amounts of each (as measured by ^{131}I) which were found in the liver at the end of the perfusion. However, in perfusion 23, where the antigen-antibody complex was present presumably as larger aggregates, the liver was able to remove much more protein than could immediately be catabolized. Only very small proportions of screened proteins (0.35% of the dose for rat albumin) were found in the livers after perfusion with 30 ml. of saline at the end of the experiment. Possibly only very small amounts of these proteins are able to penetrate any of the liver cells except those of the reticuloendothelial system. On the other hand, previous work by means of fluorescent antibodies and bovine serum albumin (Coons, Ledue & Kaplan, 1951) has shown that this protein can be revealed within the nuclei of mouse hepatic cells. If these experiments were repeated with screened proteins it might be possible to demonstrate whether the increased breakdown characteristic of partially denatured or particulate proteins is due only to increased catabolism in the Kupfer cells, or whether all the cells of the organ play some part. However, the contrast between the slow rate of breakdown of soluble native albumin and the rapid degradation of this protein when it occurs as part of an antigen-antibody complex emphasize the great reserves of proteolytic capacity in the normal liver.

SUMMARY

1. The behaviour of serum albumin and γ -globulin labelled with ^{131}I has been investigated in the isolated perfused rat liver.

2. Such a liver has been found capable of preferentially removing and then breaking down very small proportions of altered protein molecules present in preparations of plasma proteins isolated by the usual methods. The proportions of such altered molecules actually present have been found to vary widely according, at least in part, to the methods used for the preparation of each protein.

3. Very rapid breakdown occurred when human albumin was presented to the liver as part of an antigen-antibody complex.

4. On the other hand, very slow liberation of ^{131}I was found if the [^{131}I]proteins were first injected into rats and then, 48 hr. later, the plasma of these animals was used as the dose material for the liver perfusions. No difference could be detected between the behaviour of rat and human albumins which had been thus pretreated.

5. The observed rate of catabolism of rat albumin if pretreated as above was found to be very low. This suggests that in the rat the liver is responsible for not more than about 10 % of the total breakdown of serum albumin which occurs *in vivo*.

6. The proportions of these proteins which were retained in the liver after 5 hr. perfusion and after finally perfusing out with saline have been measured.

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Glutamic Dehydrogenase in the Developing Chick Embryo

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The processes of morphogenesis and differentiation in embryos may be expressed on the molecular level as an increasing complexity of arrangement of proteins in the embryonic cell. This arrangement may be accompanied by development or loss of certain enzymic activities during differentiation until the complement of enzymes characteristic of adult tissue is attained. Boell (1955) has reviewed the considerable amount of evidence that enzyme development is linked with functional differentiation during development.

The ontogenic sequence of carbohydrate and protein as primary energy sources during develop-

ment of the chick embryo (Needham, 1931) may be accompanied by the appearance of enzymes concerned with the conversion of carbohydrate into protein precursors. Glutamic dehydrogenase (GDH) plays an interesting role in amino acid metabolism, by providing a possible pathway for the production of glutamic acid from carbohydrate sources by the fixation of ammonia, or as an 'L-amino acid oxidase' in conjunction with the transaminases (Braunstein & Bychkov, 1939; Braunstein & Azarkh, 1945; Meister, 1955).

GDH activity has been measured in blastoderms, embryos and their extra-embryonic tissues during