- Fischer, P. & Goutier-Pirotte, M. (1954). *Arch. int. Physiol.* **62**, 76.
- Hatch, M. D. & Turner, J. F. (1960). *Biochem. J.* **76**, 556.
- Henneman, D. H., Altschule, M. D. & Gonez, R. M. (1954). In *Glutathione*, p. 299. Ed. by Colowick, S. P. *et al.* New York: Academic Press Inc.
- Ingram, V. M. (1955). *Biochem. J.* **59**, 653.
- Kolthoff, J. M., Stricks, W. & Morren, L. (1954). *Analyt. Chem.* **26**, 366.
- Narahara, H. T. & Williams, R. H. (1959). *J. biol. Chem.* **234**, 71.
- Nickerson, W. J. & Falcone, G. (1956). *Science*, **124**, 318.
- Pihl, A. & Eldjarn, L. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, **13**, 43.
- Pihl, A., Eldjarn, L. & Bremer, J. (1957). *J. biol. Chem.* **227**, 339.
- Pranker, T. A. J. (1961). *The Red Cell*, Oxford: Blackwell Scientific Publications.
- Racker, E. (1954). In *Glutathione*, p. 165. Ed. by Colowick, S. P. *et al.* New York: Academic Press Inc.
- Racker, E. (1955). *J. biol. Chem.* **217**, 867.
- White, A. & Beach, E. F. (1938). *J. biol. Chem.* **122**, 219.

Biochem. J. (1962) **82**, 197

Catabolism of γ -Globulin by the Isolated Perfused Rat Liver

By S. COHEN,* A. H. GORDON AND CHRISTINE MATTHEWS†
National Institute for Medical Research, Mill Hill, London, N.W. 7

(Received 31 July 1961)

Experiments with ^{14}C -labelled amino acids have shown that plasma protein with the electrophoretic mobility of γ -globulin is not synthesized by the normal rat liver (Miller & Bale, 1954). In the present investigation the catabolism of γ -globulin by the isolated, perfused rat liver has been investigated and directly compared with that of albumin. In these experiments, γ -globulin prepared by chromatography, labelled with radioactive iodine and screened in a living rat for 2 days before use, was catabolized at a rate equivalent to about 30 % of that observed in the whole animal. On the other hand albumin, which is known to be synthesized exclusively in the liver (Miller, Bly, Watson & Bale, 1951; Gordon & Humphrey, 1960), is broken down at a significantly slower rate equivalent to less than 15 % of the total catabolism of this protein *in vivo* (Cohen & Gordon, 1958). These experiments indicate that to a large extent the catabolic sites of both albumin and γ -globulin differ from their respective sites of synthesis.

In the present investigation, as in previous studies, it was observed that the concentration of plasma protein in the perfusing blood usually increased progressively during the course of the experiment, despite the fact that haemoconcentration did not occur. This phenomenon appears to be relevant to an assessment of the functional state of the perfused liver. The amount of protein discharged into the perfusing blood has therefore been measured, and plasma samples have been assayed

for the presence of solubilized liver protein; this part of the investigation has been the subject of a brief preliminary report (Gordon, 1961).

METHODS

Perfusion apparatus and technique. This was basically the same as that used by Miller *et al.* (1951) but having the modifications described by Cohen & Gordon (1958). Haemoconcentration, which readily occurs during the course of a perfusion, was not completely controlled by measures previously adopted (Cohen & Gordon, 1958); for this reason 0.9 % NaCl equivalent to 1.5 times the volume of bile produced was added to the blood reservoir at intervals of about 1 hr.

Liver donors were hooded male rats that had been starved overnight. The perfusion technique used in these experiments was that described by Cohen & Gordon (1958).

Preparation of blood for perfusion. Blood donors were rats of the hooded strain maintained at the National Institute for Medical Research. For perfusions 122–128 (Table 1) 20–30 mg. of heparin (2000–3000 i.u.) was added to 65 ml. of rat blood. In the remaining experiments, the erythrocytes obtained from similar samples of fresh heparinized blood after removal of the plasma and leucocyte layer were washed in 2 vol. of 0.9 % NaCl and suspended in an appropriate volume of serum which had been prepared on the previous day by the following methods: (i) In perfusions 131 and 133, plasma containing 0.167 vol. of 3.8 % (w/v) sodium citrate was clotted by addition of CaCl_2 (0.015 m-equiv./ml. of plasma). (ii) In perfusions 136, 137 and 138, plasma separated by centrifuging of blood in plastic tubes was subsequently allowed to clot. Serum was stored overnight at 2° and mixed with the washed erythrocytes immediately before the experiment.

Preparation of protein fractions. γ -Globulin was prepared from a sample of pooled rat serum by chromatography on diethylaminoethylcellulose (Peterson & Sober, 1956). The

* Present address: Department of Immunology, St Mary's Hospital Medical School, London, W. 2.

† Present address: Radiotherapeutic Research Unit, Hammersmith Hospital, London, W. 12.

buffer used was 5 mM-phosphate, pH 7.1, with a gradient to 0.3M-NaH₂PO₄. γ -Globulin which was eluted in the first chromatographic peak was concentrated by pressure dialysis and stored at 2° after Seitz filtration. This protein solution contained no rapidly sedimenting material when examined in the ultracentrifuge and appeared as a single peak with a slightly asymmetrical leading edge and $S_{20,w}$ 7.07. After being labelled with ¹³¹I the γ -globulin was mixed with rat serum and examined by paper electrophoresis. Subsequent strip counting showed that the radioactivity was confined to the γ -globulin band. Albumin was prepared by ion-exchange chromatography according to the procedure described by Cohen & Gordon (1958).

Iodination. Solutions containing 10 mg. of γ -globulin were labelled with carrier-free ¹³¹I or ¹²⁵I. Radioactive iodine solutions were free from reducing agent and condensed into 0.02N-NaOH. Iodine monochloride was used as the inactive carrier solution according to the method of McFarlane (1958). Free iodide was removed from the labelled protein solutions by passage through anion-exchange columns of De-Acidite (The Permutit Co. Ltd., London, W. 4); 40–60% of the initial radioactivity was bound to protein. The mean ratio of iodine bound to protein (mol.wt. 150 000) was 1–1.5 g.atom/mole.

Removal of denatured material from the iodinated protein. This was achieved by intraperitoneal injection of the labelled-protein solution into a living rat, which was bled by cardiac puncture after 48 hr. The plasma containing the screened ¹³¹I-labelled γ -globulin was dialysed overnight against 0.9% NaCl containing NaI. The final iodinated protein solutions contained 0.04–0.23% of the total radioactivity in the supernatant after precipitation with 10% (w/v) trichloroacetic acid.

Measurement of radioactivity. Solutions containing both ¹³¹I and ¹²⁵I were measured by scintillation counting with a pulse-height analyser having a 9v channel width. Measurements of ¹³¹I were made with the channel in the region of the 0.36 Mev γ -ray peak and ¹²⁵I was measured in the region of the 0.035 Mev peak. In the samples which contained both isotopes ¹²⁵I measurements were corrected for ¹³¹I counts (equivalent to 20% of the count rate observed in the ¹³¹I channel). ¹³¹I measurements, on the other hand, did not require correction for ¹²⁵I as the latter isotope gave a negligible counting rate in this channel. The s.d. of counts recorded in this study did not exceed $\pm 3\%$.

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

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

Erythrocyte-sedimentation rate. This was measured on heparinized blood samples in Wintrobe tubes which were read after 1 hr.

Detection of liver proteins in plasma samples. A rabbit was injected with homogenate of rat liver suspended in complete Freund's adjuvant. The rabbit serum was adsorbed with rat plasma and tested against plasma samples obtained at the end of the perfusion period by the gel-precipitation method of Ouchterlony (1953). The densities of the precipitation lines were noted after 3 days at 2°.

An attempt was made to assess semiquantitatively the minimum amount of solubilized liver protein present in the

final plasma from the rat of Expt. 133. A 0.9% NaCl extract of rat liver was prepared by the method of Goranov, Todorov, Skatschokova, Hlebarova & Kuzmanova (1959). The liver extract (0.68 mg./ml.) was arranged in a gel alternatively with appropriate dilutions of the final plasma. The plasma dilution that gave lines of similar density and position to those obtained with the liver extract was assessed.

Measurement of alterations in the mass of plasma protein. The initial plasma-protein mass (A) was calculated by isotope dilution from the expression, $A = BD/C$, where B is ¹³¹I (μ C) added to the perfusion circuit, C ¹³¹I (μ C/ml.) in the first plasma sample, and D protein concentration (mg./ml.) of the first plasma sample.

The cumulative loss of protein (E) was estimated from the volume and protein concentration of each plasma sample.

The expected final plasma-protein mass (F) = $A - E$.

The actual mass of plasma protein (G) in the circuit at the end of the perfusion period was calculated by isotope dilution from

$$G = \frac{\text{Final protein-bound } ^{131}\text{I } (\mu\text{C})}{\text{Protein sp.act. } (\mu\text{C/mg.}) \text{ of final sample}} = \frac{H}{I}$$

where H = initial ¹³¹I – (cumulative ¹³¹I samples + ¹³¹I in bile + total diffusible ¹³¹I in blood at the end of perfusion) and

$$I = \frac{\text{Final protein-bound } ^{131}\text{I } (\mu\text{C/ml.})}{\text{Final protein concn. (mg./ml.)}}$$

The amount of protein entering the plasma during the course of the perfusion is $G - F$.

Estimation of degree of catabolism. Blood samples (2.5–3.0 ml.) were withdrawn at the intervals shown in Table 2. 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. (e) Plasma-protein concentration.

The catabolic rate was calculated from increments in non-protein ¹³¹I by using the method set out in detail by Cohen & Gordon (1958). Results are expressed as: (a) Uncorrected catabolism/hr., i.e. increase in non-protein-bound radioactivity per unit time as a percentage of the injected dose. (b) Corrected percentage catabolism/hr. The correction factor used was

$$\frac{\gamma\text{-globulin in perfusion circuit (mg.)}}{\text{Wt. of donor rat} \times 0.405},$$

i.e. the percentage catabolism/hr. was corrected to that which would have been observed with a γ -globulin pool size equal to that of the donor rat. Paper electrophoresis of pooled rat plasma showed that γ -globulin was 14% of the total protein. The initial γ -globulin in the perfusion circuit was calculated from volume of plasma in perfusion circuit (ml.) $\times$ 14% protein concentration (mg./ml.); subsequent values for γ -globulin = initial value – cumulative loss of γ -globulin in plasma samples. (c) Mass of γ -globulin catabolized/hr. was calculated from the uncorrected percentage catabolism $\times$ γ -globulin mass present in perfusion circuit.

Estimation of fractional catabolic rates, liver pool sizes and other rate constants for albumin and γ -globulin. In two experiments the breakdown rates of ^{131}I -labelled albumin and ^{125}I -labelled γ -globulin were estimated simultaneously. At the end of each of these experiments the liver was perfused with 30 ml. of 0.9% NaCl through the portal vein. Part of the liver was then homogenized in 0.9% NaCl containing NaI and protein was precipitated with 20% trichloroacetic acid. ^{131}I and ^{125}I were estimated in the supernatant fluid and precipitate, and the proportions of the injected radioactivity remaining in the liver in protein-bound and diffusible form were calculated for albumin and γ -globulin.

Pools of protein-bound and diffusible radioactivity in blood and liver were assumed to be connected as shown in Fig. 1. Protein pools, but not breakdown-product pools, were assumed to be in equilibrium and fractional exchange rates of breakdown products were regarded as remaining constant. M_i = mass of protein in the i th pool, K_{ij} = fraction of protein in the i th pool passing into the j th pool per unit time, X_i = fraction of the injected dose in the i th pool at time t .

Increments of diffusible radioactivity were plotted as shown in Fig. 4; the slopes of these curves were calculated from terminal linear portions which, on extrapolation, cut the abscissa at times referred to below as intercepts.

Five ratios were obtained from the experimental results:

R_s = ratio of albumin slope to γ -globulin slope,

R_i = ratio of albumin intercept to γ -globulin intercept,

R_l = ratio of liver albumin activity to liver γ -globulin activity,

R_b = ratio of liver albumin diffusible activity to liver γ -globulin diffusible activity,

R = ratio of circulating albumin to circulating γ -globulin (determined by paper electrophoresis = 3).

It can be shown (see Appendix) that:

(a) The ratio of albumin to γ -globulin catabolic rates expressed as fractions of circulating albumin and γ -globulin can be calculated from

$$\frac{K_{23} M_2 (\text{alb.})}{M_1} \bigg/ \frac{K_{23} M_2 (\text{glob.})}{M_1} = R_s.$$

(b) The ratio of liver albumin to liver γ -globulin mass can be calculated from

$$\frac{M_2 (\text{alb.})}{M_2 (\text{glob.})} = R_l R.$$

(c) The ratio of albumin to γ -globulin catabolic rates expressed as fractions of liver albumin and γ -globulin can be calculated from

$$\frac{K_{23} (\text{alb.})}{K_{23} (\text{glob.})} = \frac{R_s}{R_l}.$$

(d) The ratio of the fractional rates at which breakdown products leave the liver (expressed as fractions of liver breakdown-product pools) can be calculated from:

$$\frac{K_{34} (\text{alb.})}{K_{34} (\text{glob.})} = \frac{R_s}{R_b}.$$

(e) The ratio of fractional rates at which albumin and γ -globulin enter the liver (minimum values expressed as fractions of circulating-blood pools) can be calculated from:

$$\frac{K_{12} (\text{alb.})}{K_{12} (\text{glob.})} > \frac{R_l}{R_i} \text{ if } \frac{R_s}{R_b} < 1.$$

(f) The ratio of fractional rates at which albumin and γ -globulin leave the liver (expressed as fractions of liver protein pools) can be calculated from

$$\frac{K_{21} (\text{alb.})}{K_{21} (\text{glob.})} > \frac{1}{R_i} \text{ if } \frac{R_s}{R_b} < 1 \text{ and } \frac{R_l}{R_i R_s} > 1$$

$$\text{or } \frac{K_{21} (\text{alb.})}{K_{21} (\text{glob.})} < \frac{1}{R_i} \text{ if } \frac{R_s}{R_b} < 1 \text{ and } \frac{R_l}{R_i R_s} < 1.$$

RESULTS

Experiments with ^{131}I -labelled γ -globulin

Increase in plasma-protein mass during perfusion. Haematocrit readings (Table 1) indicate that haemoconcentration did not occur during the course of these perfusions. Despite this, plasma-protein concentration increased in seven out of ten experiments and in five of these the increment was greater than 10 mg./ml. (Table 1). Calculations based on isotope dilution showed that the mass of plasma protein increased in all perfusions by an amount which varied between 30 and 380 mg. Part of this increment appeared to be liver protein since the final plasma sample from eight perfusions tested against an adsorbed rat-liver antiserum all gave precipitation lines (Table 1) and these varied in number and intensity (Gordon, 1961). In experiment 133 calculations based on isotope dilution indicated that 260 mg. of protein was added to the plasma during perfusion; a semi-quantitative immunological technique (see under Methods) gave a value of more than 2 mg. of liver protein/ml. of plasma at the end of the perfusion. The final volume of plasma was 25 ml. so that at least 50 mg. of liver protein had entered the blood during 5 hr. of perfusion in this experiment. In addition, the haemoglobin concentration in the

Fig. 1. Assumed interconnexions between blood and liver pools.

Table 1. Details of individual perfusions

Blood for perfusion consisted of: A, heparinized blood; B, washed erythrocytes mixed with serum prepared by method (i); C, washed erythrocytes mixed with serum prepared by method (ii), see text. (1) Calculated by isotope dilution (see text). (2) Immunological test (see text). (3) 14% (initial plasma vol. $\times$ initial protein concn.). (4) Mean values after second hour of perfusion.														
Expt. no.	Blood prep.	Wt. of donor rat (g.)	Duration of perfusion (hr.)	Haematocrit		Protein concn. (mg./100 ml.)		Increase in plasma-protein mass (mg.)	(2) Liver-protein test	(3) Initial γ -globulin pool (mg.)	(4) Mean γ -globulin catabolism/hr.			
				Initial	Final	Initial	Final				Uncorrected (%)	Corrected (%)	mg. of γ -globulin	
122	A	225	4	38	38	65	79	40	+	395	0.09	0.34	0.31	
123	A	230	5	42	42	78	91	140	+	508	0.18	0.81	0.76	
124	A	275	5	43	39	70	70	180	+	384	0.15	0.40	0.44	
127	A	260	6	42	39	70	81	380	+	399	0.12	0.35	0.36	
128	A	250	6	37	36	66	74	30	+	382	0.13	0.44	0.49	
131	B	280	4	36	37	58	69	210	+	360	0.20	0.44	0.59	
133	B	290	5	43	41	61	84	260	+	266	0.28	0.53	0.63	
136	C	300	4	35	34	69	69	50	.	343	0.15	0.35	0.43	
137	C	320	5	25	25	86	86	40	.	606	0.11	0.38	0.39	
138	C	315	5	34	35	76	80	290	+	606	0.10	0.40	0.48	
										Mean	0.15	0.44	0.49	
										Range	0.09-0.28	0.34-0.81	0.31-0.76	

Blood for perfusion consisted of: A, heparinized blood; B, washed erythrocytes mixed with serum prepared by method (i); C, washed erythrocytes mixed with serum prepared by method (ii), see text. (1) Calculated by isotope dilution (see text). (2) Immunological test (see text). (3) 14% (initial plasma vol. $\times$ initial protein concn.). (4) Mean values after second hour of perfusion.

final sample was 3 mg./ml. greater than in the initial sample, indicating that 75 mg. of haemoglobin had been released into the perfusing plasma.

Variation of anti-coagulants. When heparinized blood was used it was noticed that the erythrocyte-sedimentation rate increased progressively during the course of the perfusion and on microscopical examination this was found to be associated with clumping of erythrocytes. When a mixture of serum and washed erythrocytes was used for perfusion, the sedimentation rate remained unchanged throughout the experiment and erythrocytes showed no tendency to clump. This modification of technique did not, however, produce any obvious change in the breakdown rate of labelled γ -globulin or in the quantity of protein released into the circulation during perfusion (Table 1).

Appearance of diffusible ^{131}I . In two experiments (137 and 138) six blood samples were taken during the initial 2 hr. of perfusion. Non-protein ^{131}I did not appear in significant amounts during the first 20 min.; thereafter the rate of release of diffusible

Fig. 2. Release of non-protein-bound ^{131}I into the perfusing blood after the addition of ^{131}I -labelled γ -globulin. At zero time the labelled protein contained 0.18% of non-protein-bound ^{131}I in Expt. 138 (O) and 0.16% in Expt. 137 (●).

¹³¹I increased progressively for 2 hr. and was approximately linear during the remainder of the experiments (Fig. 2). The mean values for the ten perfusions listed in Table 2 show the same latent period, followed by a progressively increasing rate of ¹³¹I release and finally a linear rate between the second and fifth hours of perfusion (Fig. 3).

Rate of γ-globulin catabolism. The breakdown rate of ¹³¹I-labelled γ-globulin has been measured in ten experiments; the values shown in Table 1 are mean rates observed after the second hour of perfusion. Corrected catabolic rates varied from 0.34 to 0.81 %/hr. (mean 0.44 %/hr.) and absolute rates of catabolism were 0.31–0.76 mg./hr. (mean 0.49 mg./hr.).

A comparison of the corrected catabolic rates obtained for screened albumin in eight experiments (Cohen & Gordon, 1958) and for γ-globulin in ten experiments is shown in Table 3. The average corrected catabolic rate for γ-globulin is 0.444 %/hr. and for albumin 0.306 %/hr. A *t* test between these means is not entirely valid because the variability of the γ-globulin group is greater than that of the albumin group; however, the probability of getting a value for *t* of 2.776 by chance is about 1 in 20 to 1 in 50.

Experiments with ¹³¹I-labelled albumin and ¹²⁵I-labelled γ-globulin

The catabolic rates of ¹³¹I-labelled albumin and ¹²⁵I-labelled γ-globulin, which had been screened together for 48 hr. in a living rat, were directly

Fig. 3. Average figures for non-protein ¹³¹I observed in ten perfusions with ¹³¹I-labelled γ-globulin (Table 2). Increments of non-protein-bound ¹³¹I are corrected on the basis of the intravascular γ-globulin-pool size of the liver donor.

Table 2. Increase in diffusible ¹³¹I during liver perfusion

Increments of diffusible ¹³¹I (% total ¹³¹I) are corrected on basis of the intravascular γ-globulin mass of the liver donor (see text).

Expt. no.	Time after adding ¹³¹ I γ-globulin (min.)										
	5	20	60	80	120	140	180	200	240	260	300
122	—	0.19	—	0.19	—	0.43	—	0.66	—	1.10	—
123	—	0.23	—	0.59	—	1.14	—	1.90	—	2.73	—
124	—	0.20	—	0.40	—	0.81	—	1.14	—	1.64	—
127	0.22	—	0.26	—	0.47	—	0.86	—	1.14	—	1.43
128	0.20	—	0.42	—	0.78	—	1.28	—	1.80	—	2.17
131	0.20	—	0.30	—	0.54	—	0.94	—	1.46	—	1.85
133	0.16	—	0.31	—	0.66	—	1.27	—	1.91	—	2.25
136	—	0.25	—	0.41	—	0.77	—	1.09	—	1.47	—
137	0.16	0.17	0.31	—	0.56	—	0.87	—	1.39	—	—
138	0.18	0.18	0.31	0.40	—	0.85	—	1.20	—	1.61	—
Mean	0.19	0.19	0.31	0.40	0.60	0.80	1.04	1.20	1.54	1.71	1.93

Table 3. Catabolic rates (corrected to the pool size of the liver donor) for albumin (Cohen & Gordon, 1958) and γ-globulin

	No. of expts.	Duration of screening (hr.)	Mean catabolic rate (%/hr.)	S.D.	$t = 2.776$
¹³¹ I-labelled albumin	8	48–72	0.306	0.062	
¹³¹ I-labelled γ-globulin	10	48	0.444	0.141	

compared in two liver-perfusion experiments by using heparinized blood. The rate of release of non-protein radioactivity was initially faster for albumin; after the second hour, however, the release of radioactivity from γ -globulin (0.45 and 0.34 % intravascular pool of the liver donor/hr.) was considerably faster than that of albumin (0.25 and 0.18 %/hr.) in both experiments (Fig. 4).

The catabolic rate of albumin, expressed as a fraction of the circulating pool, is therefore about half that observed for γ -globulin. However, the ratio of albumin to γ -globulin is lower in the liver

Fig. 4. Catabolism of ^{131}I -labelled albumin (O) and ^{125}I -labelled γ -globulin (●) by the perfused rat liver. Labelled proteins were screened for 48 hr. in a living rat before use. The level of non-protein radioactivity is corrected on the basis of the pool size of the liver donor (see text). At zero time the blood level of diffusible radioactivity (% total radioactivity) was 0.20% for ^{131}I and 0.08% for ^{125}I in Expt. 162, and 0.07% for ^{131}I and 0.04% for ^{125}I in Expt. 163.

Table 4. Catabolic rates, liver pool sizes and other ratios and rate constants for albumin and γ -globulin

All values are expressed as ratios of albumin to γ -globulin (see Methods for symbols used). The ratio of albumin to γ -globulin in the circulating blood is 3:1.

Expt. no.	R_0	R_i	R_t	Catabolic rate (% of circulating pool) ($K_{22}M_2/M_1$)	Liver protein pool (M_2)	Catabolic rate (% of liver pool) (K_{22})	Transfer rate of breakdown products (K_{24})	Transfer rate of circulating protein (K_{12})	Transfer rate of liver protein (K_{21})
162	0.54	0.42	0.68	0.54	1.26	1.28	0.79	> 3.1	> 7.4
163	0.52	0.45	0.72	0.52	1.35	1.16	0.73	> 2.5	> 5.5

(1.3:1) than in the circulation (3:1), so that catabolic rates of the two proteins are similar when calculated as fractions of the liver pools (Table 4). The rate of exchange between circulating and liver albumin is considerably greater than the estimated exchange rate for γ -globulin; the observed difference in the intercepts for the two proteins is due mainly to a difference in K_{21} (Table 4).

DISCUSSION

The release of ^{131}I from labelled γ -globulin has been used to calculate rates of catabolism in these experiments. This procedure can be regarded as valid only if deiodination is always indicative of proteolysis and if ^{131}I -labelled compounds liberated during catabolism are not reincorporated into newly formed protein. The evidence indicating that these requirements are fulfilled by the ^{131}I -labelled proteins used in these investigations has been presented by Cohen & Gordon (1958). Results would be invalidated by the presence in the labelled proteins of denatured material, which would be rapidly catabolized by the perfused liver (Gordon, 1957). With ^{131}I -labelled albumin, denatured molecules were effectively removed from the initial labelled preparation by screening in a living animal for 48 hr. (Cohen & Gordon, 1958). In the present investigation it has been assumed that a similar period of screening is adequate for ^{131}I -labelled γ -globulin; the initial slow rise in the breakdown rate of these preparations suggests that this assumption is valid.

Some departure from a physiological state must be expected during the perfusion of an isolated organ. Many studies testify to the remarkable viability *in vitro* of the rat liver and the numerous functional activities retained after several hours of perfusion have been outlined by Miller, Burke & Haft (1956) and by Ostashever, Gray & Graff (1960). Despite this, however, the results reported above indicate that appreciable amounts of solubilized liver protein enter the circulating blood during the course of perfusion. In ten experiments the estimated amount of protein discharged into the blood varied from 30 to 380 mg. (mean 160 mg.). An increase in plasma-protein mass is to be expected as a result of plasma-protein synthesis (about 100 mg./5 hr.) and also from haemoglobin released by erythrocyte damage (about 75 mg./5 hr. in these experiments). In addition, however, the eight final plasma samples tested by an immunological technique all showed the presence of solubilized liver protein, as described in a brief report (Gordon, 1961). The amount of this protein was not reduced by preventing the progressive erythrocyte clumping which occurred when heparinized blood was used. In one experiment (133) a

semiquantitative technique showed that at least 50 mg. of liver protein entered the perfusing blood in 5 hr. Liver protein cannot be demonstrated with the same techniques in the plasma of normal rats, but appears within 6 hr. after injecting 1 mg. of *Escherichia coli* endotoxin (Gordon, 1961); its appearance during the course of perfusion may be due to some degree of hepatocellular damage. On the other hand, decrease in protein content of the liver is not necessarily associated with any irreversible loss of function, since rats when transferred to a non-protein diet are known to lose up to a quarter of their liver protein within 2 days (Kosterlitz, 1947). To what extent loss of protein from the liver may interfere with the protein catabolic function that has been studied here is unknown. Livers perfused under the same conditions can certainly synthesize albumin at a rate equivalent to that observed in the intact animal (Gordon & Humphrey, 1960).

A latent period of 15–20 min. has been found to intervene between the injection of ^{131}I -labelled albumin and the appearance of non-protein-bound ^{131}I in the perfusion circuit; thereafter, the release of radioactivity proceeds at a constant rate during 4 or 5 hr. of perfusion (Cohen & Gordon, 1958). The rate of release of radioactivity from ^{131}I -labelled γ -globulin increased progressively and did not become constant until after the second hour (Fig. 2); this slow rise for γ -globulin must be attributed to the fact that the rate of exchange of this protein is slower than that of albumin when expressed as a fraction of the liver-protein pool. The overall rate of transfer of γ -globulin across capillary walls is appreciably slower than that of albumin in the living rat (S. Cohen, unpublished data). This difference is unlikely to be manifest in the liver, however, since hepatic sinusoids have been shown to contain large fenestrations, which allow free passage of plasma across the capillary walls (Bennett, Luft & Hampton, 1959); the passage of γ -globulin into the liver must therefore be relatively retarded at the cell surface. The possibility that the slow rise in catabolic rate is due to a relatively slow re-entry into the circulation of the products of γ -globulin breakdown appears to be excluded by the present data (Table 4).

After the second hour of perfusion the mean breakdown rate was equivalent to 0.44% of the intravascular γ -globulin pool of the liver donor per hour. On the basis of previous experiments (Cohen & Gordon, 1958) it seemed likely that the fractional breakdown rate (% of donor pool/hr.) observed in the perfused liver was significantly greater for γ -globulin (mean 0.44%/hr.) than for albumin (mean 0.31%/hr.). This difference has been confirmed in double-labelling experiments in which the two proteins were studied simultaneously. It has

also been shown that catabolic rates for albumin and γ -globulin are similar when expressed as fractions of the liver protein pools. This suggests that catabolic mechanisms may be similar for the two proteins and that the absolute mass broken down per unit time depends upon the amount of protein present in the liver pool.

The mean catabolic rate *in vivo* of ^{131}I -labelled γ -globulin prepared by the same methods as were used in the present study is 37% of the intravascular pool/day and the average rate for albumin is 69%/day (Cohen, 1957). Thus the liver catabolizes about 30% of all the γ -globulin broken down in the whole animal, and less than 15% of the total albumin (Cohen & Gordon, 1958).

SUMMARY

1. The catabolism of γ -globulin prepared by chromatography, labelled with ^{131}I and screened for 48 hr. in a living animal was investigated in the isolated perfused rat liver.

2. In ten perfusions the mean catabolic rate of γ -globulin (0.44% of the intravascular γ -globulin pool of the liver donor/hr.) was equivalent to about 30% of the total γ -globulin breakdown *in vivo*.

3. In two perfusions ^{125}I -labelled γ -globulin and ^{131}I -labelled albumin were studied simultaneously. The rate of entry of γ -globulin into the liver was slower and its breakdown rate (% of intravascular pool) more rapid than that of albumin. The ratio of albumin to γ -globulin was lower in the liver (1.3:1) than in the circulation (3:1) so that breakdown rates for the two proteins were similar when expressed as fractions of the liver pools.

4. Protein released into the perfusing blood (mean = 160 mg. of protein/4–5 hr.) consists in part of solubilized liver protein; this suggests that some hepatocellular damage occurs during the course of perfusion.

We are grateful to Dr P. A. Charlwood, Dr J. H. Humphrey and Dr A. S. McFarlane for much helpful discussion and thank Dr R. H. Pain, who performed the ultra-centrifugal analysis. The skilled technical assistance of Mr L. Assemakis and Miss Dorothy Parr is gratefully acknowledged.

APPENDIX

Pools are assumed to be connected as shown in Fig. 1.

The assumptions made are given in the Methods section.

M_i = mass of protein or degradation product in i th pool.

K_{ij} = fraction of protein in i th pool passing into j th pool per unit time.

X_i = fraction of injected dose in i th pool at time t .

$K_{12}M_1 = (K_{21} + K_{23})M_2$.

The equations of Rescigno (1956) can be applied to the left-hand half of the system, i.e. pools 1 and 2 and a 'sink' 3. This gives the following equation:

$$X_2 = \frac{K_{12}}{(b_2 - b_1)} (\exp[-b_1 t] - \exp[-b_2 t]).$$

Where $b_1 b_2 = K_{12} K_{23}$

and $b_1 + b_2 = K_{12} + K_{21} + K_{23}$.

For the right-hand half of the system (i.e. pools 3 and 4):

$$\frac{dX_3}{dt} = K_{23}X_2 + K_{43}X_4 - K_{34}X_3$$

and $\frac{dX_4}{dt} = K_{34}X_3 - K_{43}X_4$.

When $t = 0$, $X_3 = 0$, $X_4 = 0$, $\frac{dX_3}{dt} = 0$

and $\frac{dX_4}{dt} = 0$.

Solving for X_3 and X_4 , the following equations are obtained:

$$X_3 = K_{12}K_{23} \left(\frac{(K_{43} - b_1)(1 - \exp[-b_1 t])}{b_1(b_2 - b_1)(K_{34} + K_{43} - b_1)} + \frac{(K_{43} - b_2)(1 - \exp[-b_2 t])}{b_2(b_1 - b_2)(K_{34} + K_{43} - b_2)} - \frac{K_{34}(1 - \exp[-(K_{34} + K_{43})t])}{(K_{34} + K_{43})(K_{34} + K_{43} - b_1)(K_{34} + K_{43} - b_2)} \right)$$

$$X_4 = K_{12}K_{23}K_{34} \left(\frac{(1 - \exp[-b_1 t])}{b_1(b_2 - b_1)(K_{34} + K_{43} - b_1)} + \frac{(1 - \exp[-b_2 t])}{b_2(b_1 - b_2)(K_{34} + K_{43} - b_2)} + \frac{(1 - \exp[-(K_{34} + K_{43})t])}{(K_{34} + K_{43})(K_{34} + K_{43} - b_1)(K_{34} + K_{43} - b_2)} \right)$$

Approximations

(1) $K_{12} \ll K_{21} + K_{23}$

$$b_1 = \frac{1}{2}(K_{12} + K_{21} + K_{23})$$

$$- \sqrt{[(K_{12} + K_{21} + K_{23})^2 - 4K_{12}K_{23}]}]$$

$$\therefore b_1 \simeq 0, \quad \therefore 1 - \exp[-b_1 t] \simeq b_1 t,$$

$$\therefore b_2 \simeq K_{21} + K_{23}, \quad \therefore b_1 \simeq \frac{K_{12}K_{23}}{(K_{21} + K_{23})}.$$

(2) $K_{43} \ll K_{34}$.

(3) When $t > 2\frac{1}{2}$ hr.,

$$X_4 = St - St_0$$

where S = slope of linear part of curve of X_4 against time, and t_0 = intercept on time axis.

Therefore when $t > 2\frac{1}{2}$ hr., each $1 - \exp[-bt]$ term in equation for X_4 is equal to 1, bt or 0. At least one $1 - \exp[-bt]$ term is equal to 1 and at least one $1 - \exp[-bt]$ term = bt . Therefore if $b_2 \sim K_{34}$, both $1 - \exp[-b_2t]$ and $1 - \exp[-K_{34}t]$ are equal to 1. The only alternative is for $b_2 \gg K_{34}$ or $K_{34} \gg b_2$. If $b_2 \gg K_{34}$ the intercept is negative, and if $K_{34} \gg b_2$ the slope is zero, and so neither of these alternatives is possible.

$$\therefore X_4 \simeq \frac{K_{12}K_{23}}{K_{21}+K_{23}}t - \frac{K_{12}K_{23}}{(K_{21}+K_{23})} \left(\frac{1}{K_{34}} + \frac{1}{K_{21}+K_{23}} \right)$$

$$\therefore S = \frac{K_{12}K_{23}}{K_{21}+K_{23}} = \frac{K_{23}M_2}{M_1} \quad (1)$$

$$\text{and} \quad t_0 = \frac{1}{K_{34}} + \frac{1}{K_{21}+K_{23}} \quad (2)$$

$$\text{Similarly,} \quad X_3 \simeq \frac{K_{23}M_2}{K_{34}M_1} \quad (3)$$

$$\text{and} \quad X_2 = \frac{M_2}{M_1} \quad (4)$$

The equations (a)–(f) given in the Methods section are readily obtained from equations (1)–(4).

Equations (1)–(4) may be solved to obtain values for the four unknowns, K_{12} , K_{23} , K_{21} , K_{34} (and hence M_2/M_1) in terms of S , t_0 , X_3 and X_2 . However, these results have not been used as it was considered that the absolute experimental results

were not sufficiently accurate. Only ratios of values of S , t_0 , X_3 and X_2 for albumin to the same values for γ -globulin have been used.

REFERENCES

- Bennett, H. S., Luft, J. H. & Hampton, J. C. (1959). *Amer. J. Physiol.* **196**, 381.
 Cohen, S. (1957). *S. Afr. J. med. Sci.* **23**, 245.
 Cohen, S. & Gordon, A. H. (1958). *Biochem. J.* **70**, 544.
 Goranov, I., Todorov, Y., Skatshokova, X., Hlebarova, M. & Kuzmanova, P. (1959). *Nature, Lond.*, **184**, B.A. 64.
 Gordon, A. H. (1957). *Biochem. J.* **66**, 255.
 Gordon, A. H. (1961). *Nature, Lond.*, **189**, 727.
 Gordon, A. H. & Humphrey, J. H. (1960). *Biochem. J.* **75**, 240.
 Gornall, A. C., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
 Jencks, W. P., Jetton, M. R. & Durrum, E. L. (1955). *Biochem. J.* **60**, 205.
 Kosterlitz, H. W. (1947). *J. Physiol.* **106**, 194.
 McFarlane, A. S. (1958). *Nature, Lond.*, **182**, 53.
 Miller, L. L. & Bale, W. F. (1954). *J. exp. Med.* **99**, 125.
 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). *12th Conf. Protein Metabolism, Rutgers University*, p. 44.
 Ostashever, A. S., Gray, I. & Graff, S. (1960). *Amer. J. Physiol.* **199**, 395.
 Ouchterlony, O. (1953). *Acta path. microbiol. scand.* **32**, 231.
 Peterson, E. A. & Sober, H. A. (1956). *J. Amer. chem. Soc.* **78**, 751.
 Rescigno, A. (1956). *Biochim. biophys. Acta*, **21**, 111.

Biochem. J. (1962) **82**, 205

The Dependence of the Respiration of Brain Cortex on Active Cation Transport

By R. WHITTAM

Department of Biochemistry, University of Oxford

(Received 17 July 1961)

The widespread dependence upon respiration of the maintenance of concentration gradients of sodium and potassium between cells and plasma has been established during the past decade, the notable exception being mammalian erythrocytes, which depend on glycolysis. The work of Turner, Eggleston & Krebs (1950) and of Krebs, Eggleston & Turner (1951), for example, showed that brain-cortex slices lose potassium when respiration is inhibited with respiratory poisons and, further, that the exchange of potassium between cells and medium is at the high rate of about 4 m-equiv./kg.

wet wt./min., representing the passive outward leakage which is made good by an active influx. The loss of potassium caused by 2,4-dinitrophenol suggested that active transport did not depend directly upon respiration as such but upon a supply of adenosine triphosphate, the utilization of which is inhibited by this compound.

More recent experiments with other cells have shown unequivocally that adenosine triphosphate facilitates active movements of Na^+ and K^+ ions. Thus the efflux of sodium from squid giant axons is inhibited by cyanide, yet the injection into poisoned