Plasma Protein Catabolism by the Perfused Rat Liver

THE EFFECT OF ALTERATION OF ALBUMIN CONCENTRATION AND DIETARY PROTEIN DEPLETION

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1. The isolated perfused rat liver was used to study degradation rates of plasma albumin, transferrin and fibrinogen. 2. Constant fractional rates were observed for all three proteins even when the albumin concentration was drastically increased by the addition of large amounts to the perfusate pool. 3. Livers taken from rats deprived of dietary protein for 14–18 days showed greatly diminished fractional catabolic rates for albumin when perfused with blood from similarly deprived animals. 4. These rates could be restored to near-normal values by adding albumin or by perfusing with blood from normally fed rats. 5. These findings are consistent with the theory of pinocytosis as a step in the degradation of plasma proteins by hepatic parenchymal cells.

Perfusion of isolated rat livers has indicated that this organ cannot account for more than 10–15% of the albumin catabolized by the whole animal *in vivo* (Cohen & Gordon, 1958; Gordon, 1962). Despite many investigations, no other organ or tissue capable of any higher rate of catabolism, on a weight-for-weight basis, has been found, so the use of the liver perfusion system as a model for studying albumin breakdown seems justified. Because during perfusion the rate of catabolism of labelled albumin, as measured by release of iodide, remains fairly constant for 6–8 h, the system seems especially valuable for assessment of short-term effects of acutely induced changes, an assessment that is complicated in the intact animal by problems of iodide excretion and exchange.

In the present study liver perfusion was used to examine the relationship between the rate of albumin catabolism and its plasma concentration, and to clarify the adaptive fall in catabolism that accompanies low-protein feeding *in vivo*. In addition, the immediate effect on transferrin and fibrinogen breakdown of abruptly raising the concentration of albumin in the perfusing fluid is considered in the light of a possible endocytic mechanism for catabolism of all three proteins.

MATERIALS AND METHODS

Perfusion apparatus and technique. These were essentially the same as described by Cohen & Gordon (1958) and Gordon (1962).

Animals. Blood donors were rats of the hooded strain

maintained at the National Institute for Medical Research on a normal diet or, in some experiments, a non-protein diet for 14–18 days. (The non-protein diet for rats was kindly supplied by Dr C. R. C. Heard, London School of Hygiene and Tropical Medicine, London W.C.1., U.K., and consisted of: maize starch, 54 g; potato starch, 10 g; glucose, 15 g; arachis oil, 15 g; salt mixture, 5 g; B-vitamin mixture, 1 g. It was fed *ad libitum*).

Liver donors were rats of the same stock fed on similar diets. Neither group of rats was starved.

Preparations of proteins for iodination. Albumin was obtained from rat plasma by treatment with $(\text{NH}_4)_2\text{SO}_4$ (at 50% saturation), acidification of the supernatant and preparative polyacrylamide-gel electrophoresis (Brownstone, 1969).

Transferrin was isolated on DEAE-Sephadex by the method of Gordon & Louis (1963) except that preliminary separation was by passage through Sephadex G-150 instead of by electrophoresis.

Fibrinogen was prepared by precipitation with $(\text{NH}_4)_2\text{SO}_4$ as described by Regoezi (1970). The purity of the protein preparations was checked by disc gel electrophoresis (Gordon, 1969).

Iodination of proteins for catabolic rate measurements. This was performed by the ICl method of McFarlane (1958), with ^{131}I or ^{125}I to give 0.5–1.0 g-atom of I/mol of albumin or transferrin; for fibrinogen rather less ICl was used to give not more than 0.5 g-atom/mol. After iodination non-protein iodide was removed by passage through an ion-exchange resin. For fibrinogen this was first washed with 5 mM-trisodium citrate.

Iodinated albumin and transferrin were screened biologically (McFarlane, 1956) by intraperitoneal injection into rats and bleeding 48 h later by cardiac puncture. The plasma thus obtained was dialysed overnight against NaCl–NaI to remove all but traces of non-protein radioactivity (0.1–0.2% of the total radioactivity). Fibrinogen

was screened for only 18 h after intravenous injection, and the blood was collected into potassium oxalate solution and passed through a citrate-saline exchange resin to remove non-protein iodide. Silicon tubes were used throughout for this protein and 1 g of ϵ -aminohecanoic acid was added to the perfusion system to minimize fibrinolysis.

Preparation of bulk albumin. For Expts. 4-6 large amounts of fibrinogen-free albumin had to be added to the blood. This was prepared as described above except that electrophoresis was omitted. For Expts. 7 and 8 transferrin-free albumin was prepared from the supernatant after treatment of rat plasma with $(\text{NH}_4)_2\text{SO}_4$ by the method described above. In both cases disc electrophoresis confirmed freedom from contamination.

Measurement of catabolic rates. Plasma containing labelled proteins was added about 10-15 min after perfusion of the liver had begun. When ^{131}I was used as the protein label, 10-15 μCi was added; for ^{125}I , 15-40 μCi . Samples were withdrawn at 30-60 min intervals for measurement of radioactivity on (i) whole plasma and (ii) a protein-free supernatant. The latter was prepared from 1.0 ml of plasma to which was added 1.0 ml of 0.33 M-NaI and 2.0 ml of 20% (w/v) trichloroacetic acid; after centrifugation the supernatant was passed through a glass tube containing a small pledget of cotton wool to remove minute solid particles. Since non-protein radioactivity usually constituted less than 1% of the total, any contamination by protein-bound material could lead to serious errors. Radioactivity was measured on 0.1 ml samples of whole plasma and 2 ml samples of the non-protein supernatant (equivalent to 0.5 ml of the original plasma). Depending on the amount of radioactive material used, the volume of perfusing fluid and the rate of catabolism, the latter gave a value of 100-1000 c.p.m. Samples were counted for two 20 min periods in a Packard Autogamma spectrometer with channel discrimination to separate the contributions of the two isotopes. The fractional catabolic rate was derived from the iodide released per hour, expressed as a percentage of the mean total protein-bound radioactivity during that hour; with appropriate adjustments for volumes of distribution and amounts removed, the absolute rate (mg of albumin degraded/h) could be determined.

Determination of albumin concentration. This was carried out in duplicate on selected samples from each perfusion by the method of Fernandez, Sobel & Goldenberg (1966). The haematocrit was measured at the beginning and end of each experiment for calculation of albumin pool masses.

Design of experiments. In three separate experiments (Expts. 1-3) normal rat livers were perfused with normal rat blood in the presence of ^{131}I -labelled albumin until a steady rate of breakdown could be shown (usually $3\frac{1}{2}$ h). A concentrated solution of unlabelled albumin was then added in an amount almost sufficient to double the plasma albumin concentration. The continuing catabolism of albumin was measured for a further 3 h.

The catabolism of labelled fibrinogen (Expts. 4-6) and transferrin (Expts. 7 and 8) was measured under the same conditions before and after addition of a similar amount of unlabelled albumin free of contamination by these two proteins.

In another set of experiments the rate of albumin

breakdown was studied with livers taken from rats fed on non-protein diet for 14-18 days; these were perfused with blood taken from normal rats or from rats similarly deprived of protein (Expts. 9-13). In Expts. 12 and 13, in which both the liver and the perfusing blood had been taken from protein-deprived animals, unlabelled albumin was added after $3\frac{1}{2}$ h to study the effect of an abrupt increase in its concentration.

RESULTS

Fig. 1 shows albumin breakdown in Expts. 1-3 before and after the addition of unlabelled albumin. In all cases the fractional rate of catabolism was unaffected by the albumin load; because of the augmented pool, this meant a substantial increase in the absolute amount degraded per hour (Table 1).

The effect of an additional albumin load on fibrinogen and transferrin catabolism (Expts. 4-8) is shown in Fig. 2. The fractional rate of breakdown remained constant for transferrin and showed no significant deviation from normal for fibrinogen. Since the added albumin was not contaminated by either of these proteins, their concentrations should not have changed, so the absolute amounts degraded per hour must also have remained constant.

Also shown in Table 1 are albumin catabolic rates measured with liver from protein-deprived rats in the presence of normal blood (Expts. 9 and 10) and blood from protein-deprived animals (Expts. 11, 12 and 13). When normal blood was used the fractional rate was just below the range found with normal livers, but it was abnormally low when blood from protein-deprived rats was used; in one case (Expt. 11) it was immeasurably low, and in the others it became elevated when albumin was added to the perfusate pool (Expts. 12 and 13).

Fig. 1. Catabolism of ^{131}I -labelled albumin by perfused rat liver. Increase in non-protein-bound iodide/protein-bound iodide ratio with time, before and after the addition of an unlabelled albumin load (arrow). O, Expt. 1; □, Expt. 2; Δ, Expt. 3 (see Table 1).

Table 1. *Catabolism of albumin by perfused livers of rats fed on normal and protein-free diets*

Albumin catabolic rates are expressed as (non-protein-bound iodide/protein-bound iodide) %/h and as mg of albumin degraded/h, all perfusions being continued for at least 3½ h. (a) Control period; (b) after addition of unlabelled albumin load. Abbreviations: NL, normal liver; NB, normal blood; OPL, liver from protein-deprived rat; OPB, blood from protein-deprived rat.

		Concn. of plasma albumin (mg/ml)	Albumin catabolic rate	
			(%/h)	(mg/h)
NL + NB	Controls (n = 12)	23.8	Mean 0.068 Range 0.045–0.115	0.74 0.53–1.28
	Expt. 1 (a)	21.8	0.115	1.28
	(b)	38.9	0.117	2.46
	Expt. 2 (a)	22.6	0.072	0.67
	(b)	42.0	0.075	0.97
	Expt. 3 (a)	24.6	0.062	0.78
	(b)	37.6	0.064	1.11
	Expt. 9	22.2	0.040	0.40
OPL + NB	Expt. 10	20.9	0.041	0.35
OPL + OPB	Expt. 11	18.9	No measurable catabolism	
	Expt. 12 (a)	18.7	0.013	0.10
	(b)	21.6	0.043	0.34
	Expt. 13 (a)	20.7	0.032	0.27
	(b)	39.5	0.068	1.05

Fig. 2. Catabolism of ^{131}I -labelled fibrinogen (O, Δ and $\square$) and ^{125}I -labelled transferrin ($\bullet$ and $\blacktriangle$) by perfused rat liver. Increase in non-protein-bound iodide/protein-bound iodide ratio with time, before and after the addition of an unlabelled albumin load (arrow).

DISCUSSION

These experiments confirm the usefulness of the closed perfusion circuit for studying plasma protein catabolism by the isolated rat liver, and serve to elucidate some of the problems concerning mechanisms of breakdown. In theory increased catabolism may result from (i) an increase in concentration of a protein with maintenance of a constant fractional breakdown rate (this would be consistent with the commonly held view of first-order kinetics for plasma albumin degradation; McFarlane, 1964), and (ii) an increase in fractional catabolic rate with or without a change in concentration. Because the

concentration of only one protein has been changed in these experiments, it is possible to distinguish between these mechanisms.

In all cases, except that in which liver from protein-deprived rats was used (see below), addition of albumin to the perfusate pool failed to alter the fractional rate of catabolism of this protein. By maintaining a constant rate the liver was able to degrade larger absolute amounts of albumin. This state of affairs has also been shown to exist *in vivo* (Friedberg, 1963; Rothschild, Oratz, Evans & Schreiber, 1964; Andersen & Rossing, 1967; Hoffenberg, 1970), thus providing the body with a powerful and prompt means of eliminating a protein overload and of maintaining plasma protein concentrations within a relatively narrow normal range.

The precise mechanism by which plasma proteins are degraded remains controversial, although evidence is accruing to favour engulfment by a process of pinocytosis, then transfer in small vesicles to secondary lysosomes. This sequence has been shown for the removal of formaldehyde-treated ^{131}I -labelled albumin by hepatic cells (Mego & McQueen, 1965, 1967; Mego, Bertini & McQueen, 1967; Bertini, Mego & McQueen, 1967), and degradation of denatured albumin molecules by lysosomal proteolytic enzymes has also been demonstrated by Coffey & de Duve (1968). Bocci, Masti, Pacini & Viti (1968) showed that polymorphonuclear leucocytes were capable of catabolizing denatured proteins but not screened albumin molecules. Indeed the fate of undenatured protein molecules is less certain (Mego & McQueen, 1965;

Bocci *et al.* 1968), although Freeman, Gordon & Humphrey (1958) adduced evidence for their breakdown by hepatic parenchymal cells after blockade of reticuloendothelial activity by carbon particles. Gordon & Jacques (1966) considered that this catabolism took place in lysosome-containing liver fractions.

The observed constant fractional rate of albumin catabolism may be explained in terms of liquid endocytosis, which, as Jacques (1969) has observed, introduces into the cell all the substances present in the external milieu in proportion to their concentration. However, possible selective adsorption of individual plasma proteins at the cell membrane before engulfment must be kept in mind. Evidently non-proportionality of catabolic rates with plasma concentrations does not exclude pinocytosis, and a constant catabolic rate as observed in these experiments does not prove its existence, but the occurrence of this process seems more likely. Further, since altered albumin concentrations did not affect the fractional catabolic rates of either transferrin or fibrinogen, any process involving competition at sites of uptake or catabolism is rendered improbable. The evidence from the present experiments with livers from normal rats suggests that there might be a continuous uptake of plasma by pinocytosis at a fixed rate.

The only exception to this fixed fractional breakdown rate for albumin was found in experiments with livers taken from protein-deprived rats. These appeared to catabolize considerably less albumin when perfused with blood of lower albumin concentration taken from similarly deprived animals. Addition of enough albumin to raise this concentration by a few mg/ml restored the catabolizing capacity of the liver to near-normal values (Expt. 12), whereas an approximate two fold increase in the concentration led to a parallel rise in the fractional catabolic rate (Expt. 13). Under conditions of protein loss or deprivation *in vivo*, abnormally low fractional catabolic rates for albumin have been found (Matthews, 1961; Freeman & Gordon, 1964; Hoffenberg, Black & Brock, 1966), and it is likely that extreme conditions of depletion are associated with diminished efficiency of albumin degradation. It is possible that a 'threshold' of albumin concentration exists, below which catabolism of this protein becomes extremely low or even ceases. This could be explained by slowing down of the rate of pinocytosis, by decreasing proteolytic enzyme activity within the lysosomes, or by diminution in the number of lysosomes available for catabolism. The prompt return to near-normal catabolic rates when albumin is added or normal blood is used in the

perfusion is noteworthy but fails to distinguish between these possibilities.

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REFERENCES

- Andersen, S. B. & Rossing, N. (1967). *Scand. J. clin. Lab. Invest.* **20**, 183.
- Bertini, F., Mego, J. L. & McQueen, J. D. (1967). *J. cell. Physiol.* **70**, 105.
- Bocci, V., Masti, L., Pacini, A. & Viti, A. (1968). *Exptl Cell Res.* **52**, 129.
- Brownstone, A. D. (1969). *Analyt. Biochem.* **27**, 25.
- Coffey, J. W. & de Duve, C. (1968). *J. biol. Chem.* **243**, 3255.
- Cohen, S. & Gordon, A. H. (1958). *Biochem. J.* **70**, 544.
- Fernandez, A., Sobel, C. & Goldenberg, H. (1966). *Clin. Chem.* **12**, 194.
- Freeman, T. & Gordon, A. H. (1964). *Clin. Sci.* **26**, 17.
- Freeman, T., Gordon, A. H. & Humphrey, J. H. (1958). *Br. J. exp. Path.* **39**, 459.
- Friedberg, W. (1963). *Am. J. Physiol.* **204**, 501.
- Gordon, A. H. (1962). *Biochem. J.* **82**, 531.
- Gordon, A. H. (1969). In *Laboratory Techniques in Biochemistry and Molecular Biology*, vol. 1, p. 50. Ed. by Work, T. S. & Work, E. Amsterdam: North-Holland Publishing Co.
- Gordon, A. H. & Jacques, P. (1966). *Proc. Conf. Labelled Proteins in Tracer Studies, Pisa*, p. 127. Ed. by Donato, L., Milhaud, G. & Sircis, J. Brussels: Euratom.
- Gordon, A. H. & Louis, L. N. (1963). *Biochem. J.* **88**, 409.
- Hoffenberg, R. (1970). In *Plasma Protein Metabolism: Regulation of Synthesis, Distribution and Degradation*. Ed. by Rothschild, M. A. & Waldmann, T. A. New York: Academic Press Inc. (in the Press).
- Hoffenberg, R., Black, E. & Brock, J. F. (1966). *J. clin. Invest.* **45**, 143.
- Jacques, P. J. (1969). In *Lysosomes in Biology and Pathology*, vol. 2, p. 395. Ed. by Dingle, J. T. & Fell, H. B. Amsterdam: North-Holland Publishing Co.
- McFarlane, A. S. (1956). *Biochem. J.* **62**, 135.
- McFarlane, A. S. (1958). *Nature, Lond.*, **182**, 53.
- McFarlane, A. S. (1964). In *Mammalian Protein Metabolism*, vol. 1, p. 298. Ed. by Munro, H. N. & Allison, J. B. London: Academic Press (Inc.) Ltd.
- Matthews, C. M. E. (1961). *J. clin. Invest.* **40**, 603.
- Mego, J. L., Bertini, F. & McQueen, J. D. (1967). *J. Cell Biol.* **32**, 699.
- Mego, J. L. & McQueen, J. D. (1965). *Biochim. biophys. Acta*, **100**, 136.
- Mego, J. L. & McQueen, J. D. (1967). *J. cell. Physiol.* **70**, 115.
- Regoecezi, E. (1970). *Clin. Sci.* **38**, 1.
- Rothschild, M. A., Oratz, M., Evans, C. & Schreiber, S. S. (1964). *J. clin. Invest.* **43**, 1874.