drug. It thus seems probable that the amount of pyruvate produced by the parasite is greater than the capacity of the host to metabolize or excrete it.

We wish to thank Mr L. Hills and Mr K. Gammage for invaluable assistance in the maintenance of these strains and the handling of the experimental animals.

REFERENCES

Arnstein, H. R. V. & Keglević, D. (1956). *Biochem. J.* **62**, 199.
Brand, T. von (1951). In *Biochemistry and Physiology of Protozoa*, vol. 1. Ed. by Lwoff, A. New York: Academic Press.
Chen, G. (1948). *J. infect. Dis.* **82**, 226.
Christophers, S. R. & Fulton, J. D. (1938). *Ann. trop. Med. Parasit.* **32**, 43.
El Hawary, M. F. S. & Thompson, R. H. S. (1954). *Biochem. J.* **58**, 519.
Feller, D. D., Strisower, E. H. & Chaikoff, I. L. (1950). *J. biol. Chem.* **187**, 571.
Friedemann, T. E. & Haugen, G. E. (1943). *J. biol. Chem.* **147**, 415.
Fulton, J. D. & Grant, P. T. (1955). *Exp. Parasit.* **4**, 377.
Fulton, J. D. & Grant, P. T. (1956). *Biochem. J.* **63**, 274.
Fulton, J. D. & Stevens, T. S. (1945). *Biochem. J.* **39**, 317.
Gidez, L. I. & Karnovsky, M. L. (1954). *J. biol. Chem.* **206**, 229.
Gilvarg, C. & Bloch, K. (1951). *J. biol. Chem.* **193**, 339.
Grant, P. T. & Fulton, J. D. (1956). *Biochem. J.* **63**, 8P.
Harvey, S. C. (1949). *J. biol. Chem.* **179**, 435.
Hatcher, W. H. & Mueller, W. H. (1929). *Trans. Roy. Soc. Can.* (Sect. 3), **23**, 35.
Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
Krebs, H. A., Smyth, D. H. & Evans, E. A. (1940). *Biochem. J.* **34**, 1041.
Marks, P. A. & Horecker, B. L. (1956). *J. biol. Chem.* **218**, 327.
Meyerhof, O. & Junowicz-Kocholaty, R. (1943). *J. biol. Chem.* **149**, 71.
Moulder, J. W. (1948). *J. infect. Dis.* **83**, 33.
Phares, E. F. & Long, M. U. (1955). *J. Amer. chem. Soc.* **77**, 2556.
Popják, G. (1950). *Biochem. J.* **46**, 560.
Reeves, R. E. (1941). *J. Amer. chem. Soc.* **63**, 1476.
Rittenberg, D. & Foster, G. L. (1940). *J. biol. Chem.* **133**, 737.
Ryley, J. F. (1956). *Biochem. J.* **62**, 215.
Schambye, P., Wood, H. G. & Popják, G. (1954). *J. biol. Chem.* **206**, 875.
Somogyi, M. (1952). *J. biol. Chem.* **195**, 19.
Swim, H. E. & Krampitz, L. O. (1954). *J. Bact.* **67**, 419.
Tobie, E. J., Brand, T. von & Mehlman, B. (1950). *J. Parasit.* **36**, 48.
Topper, Y. J. & Hastings, A. B. (1949). *J. biol. Chem.* **179**, 1255.
van Niel, C. B., Thomas, J. O., Ruben, S. & Kamen, M. D. (1942). *Proc. nat. Acad. Sci., Wash.*, **28**, 157.
Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.
Voegtlin, C. & Smith, H. W. (1920). *J. Pharmacol.* **15**, 453.
Williamson, J. (1953). *Exp. Parasit.* **2**, 348.
Wood, H. G. (1946). *Physiol. Rev.* **26**, 198.
Wood, H. G. (1955). *Physiol. Rev.* **35**, 841.
Yorke, W. & Murgatroyd, F. (1930). *Ann. trop. Med. Parasit.* **24**, 449.

On the Determination of the Time Required for Biosynthesis of a Single Protein Molecule: Experiments with Rat Pancreatic Ribonuclease

By VALDA M. CRADDOCK AND C. E. DALGLIESH
Postgraduate Medical School, Ducane Road, London, W. 12

(*Received* 29 *October* 1956)

It has been pointed out by one of us (Dalgliesh, 1953) that the time required for biosynthesis of a single molecule of protein must be known before conclusions about the mechanism of protein biosynthesis can be drawn from certain kinetic data, or from the occurrence of unequal labelling in proteins (references summarized by Steinberg, Vaughan & Anfinsen, 1956). If the time required for biosynthesis of a single molecule of protein is other than short, there should be a measurable delay between the addition of a labelled amino acid to a system actively synthesizing protein and the first appearance of free labelled protein in that system. There are in the literature several examples of the occurrence of such delays, e.g. in the synthesis of serum albumin *in vitro* (Peters, 1953), or whole serum proteins *in vivo* (Green & Anker, 1955), of γ-globulins *in vivo* (Askonas, Humphrey & Porter, 1956), of Bence–Jones protein *in vivo* (Putnam, Meyer & Miyake, 1956) and of the proteins of the pancreatic juice *in vivo* (Junqueira, Hirsch & Rothschild, 1955). The time before appearance of the labelled protein in these cases must include other factors, e.g. diffusion and mixing, besides the time of biosynthesis. To determine the true time of biosynthesis of a protein molecule, it seemed desirable to investigate synthesis of a single molecular species in as simple a system as possible. For

an initial experimental approach we chose for investigation rat pancreatic ribonuclease. Ribonuclease is a fairly stable protein, readily isolated in the pure state. It is a component of pancreatic-juice proteins, which exhibit a time-lag in incorporation (Junqueira *et al.* 1955), and it can exhibit differences in specific activity of amino acid residues in different parts of the protein chain (Vaughan & Anfinsen, 1954) which might also be due to an appreciable time being required for biosynthesis of a single molecule (Dalgliesh, 1953). Our results have not allowed the time of biosynthesis of the protein to be deduced, but suggest the possible existence of differences between the modes of amino acid incorporation *in vivo* and *in vitro* and indicate the desirability of reassessing the relative roles of *de novo* synthesis and exchange.

MATERIALS AND METHODS

Animals. Adult female white rats were used. Each animal was killed by a blow on the head, and the pancreas removed as rapidly as possible.

^{14}C-*Labelled amino acids.* These were prepared biosynthetically (Dalgliesh & Dutton, 1956). Labelled protein from the food yeast *Torulopsis utilis* or the green alga *Chlorella vulgaris* was hydrolysed enzymically, and the aromatic amino acids were removed (for other experiments) by starch chromatography (Moore & Stein, 1949). The total non-aromatic fraction was used in the present experiments.

Paper chromatography. Chromatography was carried out by the descending technique on Whatman no. 1 paper. The organic layer of a freshly prepared butanol–acetic acid–water mixture (4:1:5, by vol.; Partridge, 1946) was used as solvent.

Radioautography. Radioautography of paper chromatograms was carried out on Ilford X-ray film, with exposures varying from 15 hr. to 9 weeks as appropriate.

Estimation of amino acids. Estimations were by the ninhydrin procedure of Cocking & Yemm (1954).

Estimation of total protein. The modified Folin method of Lowry, Rosebrough, Farr & Randall (1951) was used.

Estimation of ribonuclease. (*a*) In eluates from chromatographic columns a modification of the method of Kunitz (1940) was used, 0·3 ml. of eluate being incubated with nucleic acid for 20 min. at pH 5·0; unhydrolysed nucleic acid was precipitated and removed by filtration, and the soluble nucleotides in the filtrate were determined by their absorption at 260 mμ.

(*b*) The ribonuclease content of pancreas slices was determined by the procedure mentioned above after preliminary purification by $(NH_4)_2SO_4$ precipitation (described below) and adjustment to pH 5·0.

(*c*) Purified ribonuclease was determined by its absorption at 278 mμ. This was found to be linearly related to concentration up to 3 mg./ml.

Estimation of radioactivity. 'Infinite thinness' counting of samples evaporated on 1 cm.^{2} nickel planchets was used, with a thin mica end-window Geiger counter. Samples of an appropriate concentration giving no appreciable self-absorption were applied as drops at several different points on the planchet (to ensure greater spread if the protein became deposited at the perimeter of the dried spots). Background determinations were made on the planchets before applying the samples, and appropriate corrections to the count made for background and coincidence. Counting was continued sufficiently long to give a standard error of the count of $\pm 3\%$, or better.

Procedure in incorporation experiments. Pancreases from groups of ten rats were chopped with a tissue chopper (McIlwain & Buddle, 1953), and the slices, 0·38 mm. thick, were placed in 15 ml. of Krebs–Ringer–bicarbonate solution (Umbreit, Burris & Stauffer, 1949) containing casein hydrolysate (0·045%, w/v; 0·67 mg.) and approximately 20 μc of ^{14}C-labelled mixed aliphatic amino acids (in about 1 mg., the exact amount depending on the source). The slices were gassed for 2 min. with $O_2 + CO_2$ (95:5), and incubated in closed flasks at 37°, with constant shaking, for periods of up to 2 hr. The reaction flask was then plunged into an ice–water mixture, 2N-H_2SO_4 added to bring the final concentration to 0·25N, and the tissue kept at 0° through the following crude fractionation of pancreas protein.

The pancreas slices were weighed and ground in the incubation medium with sand, and the mixture was filtered through glass wool; the residue was washed with 0·25N-H_2SO_4, and the washings were added to the filtrate. The extract was brought to 0·6 saturation with $(NH_4)_2SO_4$, and the protein precipitate filtered off after standing at 0° for 1 hr.; trichloroacetic acid was added to the filtrate to give a concentration of 10% (w/v) and the mixture left overnight. The protein precipitate containing the ribonuclease was centrifuged, washed twice with 10% (w/v) trichloroacetic acid and twice with 95% (v/v) ethanol, and dried in a desiccator. Casein hydrolysate (15 mg.) was added to the dry protein to facilitate subsequent location of the amino acid peak on chromatography; the mixture was dissolved in phosphate buffer (1 ml.; see below) and run on a chromatography column.

Chromatographic purification of rat ribonuclease. The method used was similar to that developed by Hirs, Moore & Stein (1953) for purifying the ox enzyme by use of the ion-exchange resin Amberlite IRC-50. Species differences between ox and rat ribonucleases (Craddock & Dalgliesh, 1956*a*) made it necessary to use a lower pH for purifying rat ribonuclease than ox ribonuclease. Sodium phosphate buffer (0·2M), pH 6·25, was used, with a column 0·9 cm. × 30 cm. The fractions were analysed for ribonuclease, total protein and amino acids as described above.

The ribonuclease-containing fractions were pooled, brought to 10% (w/v) with respect to trichloroacetic acid and allowed to stand overnight. The precipitate was washed twice with 10% (w/v) trichloroacetic acid and twice with 95% ethanol, and dried in a desiccator.

RESULTS

It was hoped that use of a small animal, such as the rat, might lead to simpler and more economical requirements for incubation experiments than would be involved in working with the more usually investigated dog or ox pancreas. We intended to facilitate isolation of labelled rat ribonuclease by dilution with unlabelled ox ribonuclease, which is conveniently prepared in quantity. This approach

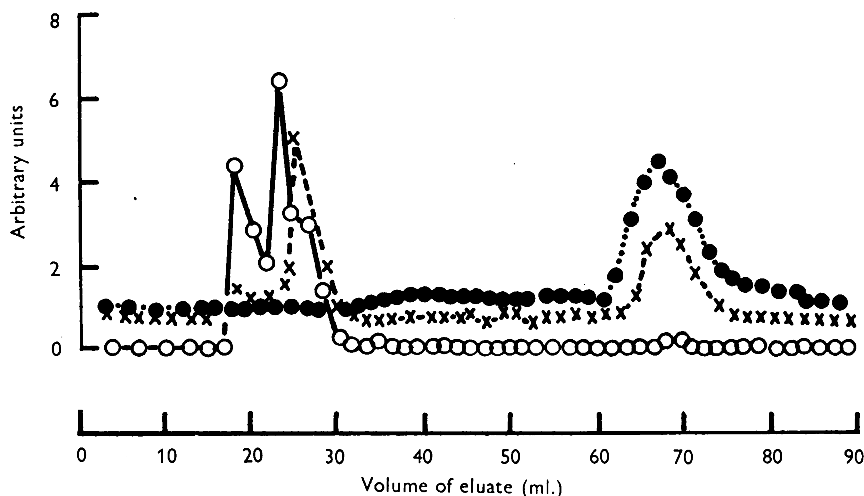

Fig. 1. Chromatographic purification of rat pancreatic ribonuclease on an Amberlite IRC-50 column, 0·2M-phosphate buffer, pH 6·25, being used as eluent. ●, Ribonuclease activity; ○, ninhydrin colour; ×, total protein.

had to be rejected owing to the marked species difference between the rat and beef enzymes (Craddock & Dalgliesh, 1956*a*) resulting in considerable differences in chromatographic behaviour. Conditions were, however, readily found under which rat pancreatic ribonuclease could be separated by ion-exchange chromatography from other proteins in the pancreas extracts and from amino acids. A typical separation is shown in Fig. 1. The efficiency of the separation of amino acids and ribonuclease was investigated by incubating ribonuclease with labelled amino acids under the conditions of the incorporation experiments, except that the pancreas slices were omitted. The ribonuclease after reisolation showed no detectable radioactivity. It was calculated that incorporation into, or adsorption by, the protein of 0·003 % of the added amino acid radioactivity would have been detectable. This is well below the lowest value obtained in the incorporation experiments.

On aerobic incubation of rat-pancreas slices a steady rate of oxygen uptake was observed over a 2 hr. period, and this rate was unaffected by additions of glucose (up to 0·2 %) or casein hydrolysate (up to 0·045 %). On addition of labelled amino acids, radioactivity appeared in the ribonuclease subsequently isolated. That this was true metabolic incorporation was shown by the fact that no radioactivity was incorporated under anaerobic conditions. There was no detectable change in the total amount of ribonuclease during incubation. All incorporation experiments were carried out in the presence of unlabelled casein hydrolysate, to ensure the presence in the incubation medium of all amino acids essential for ribonuclease biosynthesis. Casein hydrolysate does not contain tryptophan,

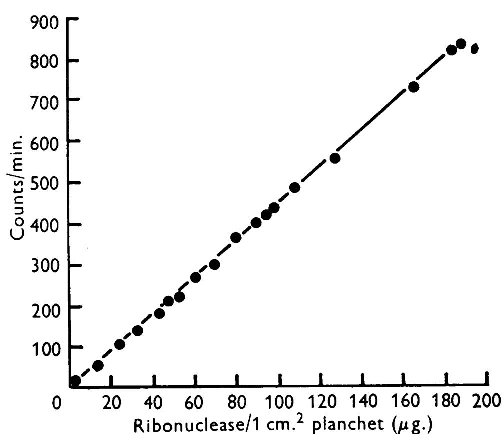

Fig. 2. Determination of protein-bound radioactivity of rat pancreatic ribonuclease by 'infinite thinness' counting.

but tryptophan is known not to be a component of ox ribonuclease (Tristram, 1950). In view of the species differences between rat and ox ribonucleases (Craddock & Dalgliesh, 1956*a*) we applied to the rat enzyme the reaction of Dickman & Crockett (1956) for the detection of bound tryptophan. No tryptophan could be detected.

The concentration of protein in the purified ribonuclease fraction could be accurately determined by its ultraviolet absorption. The radioactivity of the small amounts of protein obtained was found to be conveniently measurable by 'infinite thinness' counting. A large preparation of labelled ribonuclease was prepared batchwise from fifty rats and the self-absorption examined when counted under these conditions. From Fig. 2 it can be seen that the

count rate and concentration are linearly related up to 200 μg./1 cm.^{2} planchet, showing that there is negligible self-absorption in this range. Fig. 2 also gives an indication of the precision of the method.

The incorporation of labelled aliphatic amino acids derived from yeast protein is shown in Fig. 3. It will be seen that the amount of radioactivity incorporated varies approximately linearly with time, but there is no evidence of any marked delay before the first appearance of protein-bound radioactivity, as was found *in vivo* by Junqueira *et al.* (1955) for the total proteins of pancreatic juice, of which ribonuclease is one of the components (Rothschild & Junqueira, 1956). An obvious possible explanation of this apparent discrepancy was that incorporation in our experiments had occurred by exchange rather than by *de novo* synthesis. If true synthesis had occurred the specific activities of the individual amino acids of the ribonuclease should be in the same ratio as the specific activities of the amino acids in the incubation medium. The small scale of our experiments only allowed us to investigate this point qualitatively. One-dimensional chromatograms were run of the incubation medium and a hydrolysate (20 hr. at 105° in a sealed tube with 6N-HCl) of the corresponding preparation of ribonuclease, and

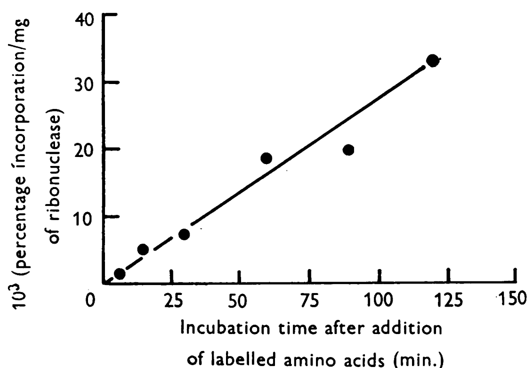

Fig. 3. Incorporation of the unpurified aliphatic fraction of an enzymic hydrolysate of yeast protein into the ribonuclease of rat-pancreas slices.

radioautographs of the chromatograms prepared and compared. In one such experiment chromatograms of measured fractions of the medium and of the hydrolysate, containing 220 and 12 μmc, were exposed for 2 and 28 days respectively. The distribution of activities in the two showed no obvious differences. This is compatible with *de novo* synthesis, but does not exclude random exchange (see Discussion). It was calculated that the amount of synthesis indicated by the amount of radioactivity incorporated was within the experimental error of the determinations of total ribonuclease activity in the incubation mixture.

An incorporation curve similar to that in Fig. 3 was obtained with the aliphatic amino acids from a hydrolysate of *Chlorella* protein. In both yeast and *Chlorella* amino acid preparations, however, paper chromatography and radioautography showed the presence, on or close to the origin of the chromatograms, of an appreciable amount of radioactivity; this was associated with a brown colour but was apparently ninhydrin-negative. Protein hydrolysates from *Chlorella* protein were therefore purified by paper chromatography. The impurities on the origin were cut off and the amino acids on the remainder of the sheets eluted and concentrated. Absence of ninhydrin-negative contaminants was confirmed by chromatography and autoradiography. To our surprise the purified preparations were incorporated much more slowly than the original unpurified preparations (cf. Craddock & Dalgliesh, 1956*b*) (Table 1). The presence of unlabelled casein hydrolysate in the incubation medium ensured that this low rate of incorporation of the purified preparations was not due to absence of an essential amino acid. The contaminating material near the origin of the preparative chromatograms was therefore eluted, and was found to give a much higher rate of incorporation than the original preparations (Table 1). Since the labelled amino acid mixture used in the control experiments (incubation either without pancreas slices or without oxygen) had not been purified in this manner, the material on the origin contained a substance or substances markedly stimulating incorporation of

Table 1. *Incorporation of different preparations of labelled amino acids into the ribonuclease of rat-pancreas slices*

Slices were incubated aerobically under the conditions described in the text. Incorporation is expressed as $10^3 \times$ (percentage incorporation/mg. of ribonuclease).

Amino acid preparation	Incorporation	
	After 30 min.	After 120 min.
Unpurified aliphatic fraction from yeast protein	8·1	33·3
Unpurified aliphatic fraction from *Chlorella* protein	4·4	17·6, 16·3
Purified amino acids from *Chlorella* protein	1·1	3·3
Slow-running material from *Chlorella* protein	—	45·7
Purified amino acids from *Chlorella* protein after further passage through a starch column	—	3·2

amino acids. Chromatography and radioautography of the eluate of the contaminating material showed the presence of some ninhydrin-positive radioactive material, but in amount very small compared with the ninhydrin-negative labelled material. It therefore seemed probable that the ninhydrin-negative material was itself incorporated, and that it represented amino acid derivatives more readily available for protein synthesis than were free amino acids. The method of preparation of the labelled protein (Dalgliesh & Dutton, 1956), involving extraction with cold and hot trichloroacetic acid, made it unlikely that the protein was contaminated by nucleic acid or nucleotides. Consideration of the various steps in the preparation of the protein hydrolysates suggested that a stage at which amino acid derivatives might be formed was during the chromatography on starch or during subsequent storage of the concentrated eluates from the starch columns, when reaction between amino acids and carbohydrate might have occurred. Such reaction would be in accord with the brown colour, low R_F value, and apparent ninhydrin-negativity of the stimulating factor; moreover, carbohydrate derivatives of amino acids are known to stimulate biosynthesis of reticulocyte protein (Borsook, Abrams & Lowy, 1955). However, when a sample of the purified amino acid preparation was passed through a starch column and stored, it showed no increase in degree of incorporation (Table 1). The stimulating factor has not been further identified, and in particular a peptide nature for it has not been excluded.

DISCUSSION

We have found that under the conditions of our investigations the curve for the incorporation of amino acid radioactivity into ribonuclease intercepts the time axis at or near the origin (Fig. 3). If the premises outlined in the introduction are correct, this would indicate that biosynthesis of a single molecule of ribonuclease is a rapid process (for a fuller discussion see Dalgliesh, 1957). On the other hand, the incorporation curve obtained *in vitro* is quite different from that obtained *in vivo* by Junqueira *et al.* (1955), who found a marked time lag before radioactivity appeared in pancreas proteins. This suggests, as a possible alternative explanation, that the mode of incorporation differs in the two cases. An incorporation curve of the type we have found could obviously occur if there were exchange of free amino acids in the medium with the corresponding amino acids in the protein chain. If participation of a template in protein biosynthesis is assumed, a plausible mechanism is for amino acids to be laid down on the template surface, say by loose attachment of activated derivatives or by formation of template–amino acid bonds, followed by combination of the amino acid residues and a 'peeling off' of the peptide chain so formed. It is implicit in the template hypothesis that any region of the protein corresponds to an appropriate region of the template, and it is not unreasonable to expect that in the normal course of cellular events a region of a protein chain might collide with the appropriate region of the corresponding template. It may well be that when such an event occurs the protein can become temporarily reattached to the template over a small part of its length, with labilization of the residues in this region and possible exchange with similar residues in the medium. Exchange would thus be a limiting form of the same process that brings about *de novo* synthesis. Under normal circumstances exchange might well make a negligible contribution to the net incorporation of amino acids into protein. But if for any reason normal synthesis were inhibited, collisions between protein and template might continue to give rise to incorporation by exchange. Such incorporation might occur for any residue for which corresponding free amino acids were available, and the protein might thus attain specific activities of its individual residues similar to those of the corresponding free amino acids of the cell. In short, the disruption of cellular organization associated with *in vitro* preparations may result in a normal mechanism behaving in an abnormal manner, so that an incorporation of amino acids which *in vivo* proceeds largely by *de novo* synthesis, proceeds *in vitro* largely by exchange. The labelled proteins formed in the two cases would be indistinguishable by the techniques described in this paper, and more refined techniques will be necessary before the time required for biosynthesis of a single molecule of protein can be determined by the approach we have used. It may well be significant that most of the cases (already cited) in which marked delays have been observed before appearance of radioactivity in protein, have occurred with *in vivo* systems, and it would seem highly desirable that the kinetics of protein synthesis should as far as possible be studied in such systems under conditions in which net synthesis, as well as incorporation, can be demonstrated.

SUMMARY

1. Activity–time curves have been determined for the incorporation of radioactive aliphatic amino acids into the ribonuclease of rat-pancreas slices. In contrast with results obtained by other workers *in vivo*, no appreciable delay was observed before the appearance of radioactivity in protein.

2. The results are discussed in relation to various time factors in the biosynthesis of protein. It is suggested that exchange may be a limiting form of a template mechanism of protein synthesis, and

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.

We are most grateful to the Medical Research Council for support.

REFERENCES

Askonas, B. A., Humphrey, J. H. & Porter, R. R. (1956). *Biochem. J.* **63**, 412.

Borsook, H., Abrams, A. & Lowy, P. H. (1955). *J. biol. Chem.* **215**, 111.

Cocking, E. C. & Yemm, E. W. (1954). *Biochem. J.* **58**, xii.

Craddock, V. M. & Dalgliesh, C. E. (1956*a*). *Bull. Soc. chim. Belg.* **65**, 76.

Craddock, V. M. & Dalgliesh, C. E. (1956*b*). *Biochem. J.* **64**, 39P.

Dalgliesh, C. E. (1953). *Nature, Lond.*, **171**, 1027.

Dalgliesh, C. E. (1957). *Science*, **125**, 271.

Dalgliesh, C. E. & Dutton, R. W. (1956). *J. chem. Soc.* p. 3784.

Dickman, S. R. & Crockett, A. L. (1956). *J. biol. Chem.* **220**, 957.

Green, H. & Anker, H. S. (1955). *J. gen. Physiol.* **38**, 283.

Hirs, C. H. W., Moore, S. & Stein, W. H. (1953). *J. biol. Chem.* **200**, 493.

Junqueira, L. C. U., Hirsch, G. C. & Rothschild, H. A. (1955). *Biochem. J.* **61**, 275.

Kunitz, M. (1940). *J. gen. Physiol.* **24**, 15.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.

McIlwain, H. & Buddle, H. L. (1953). *Biochem. J.* **53**, 412.

Moore, S. & Stein, W. H. (1949). *J. biol. Chem.* **178**, 53.

Partridge, S. M. (1946). *Nature, Lond.*, **158**, 270.

Peters, T. (1953). *J. biol. Chem.* **200**, 461.

Putnam, F. W., Meyer, F. & Miyake, A. (1956). *J. biol. Chem.* **221**, 517.

Rothschild, H. A. & Junqueira, L. C. U. (1956). *Nature, Lond.*, **178**, 258.

Steinberg, D., Vaughan, M. & Anfinsen, C. B. (1956). *Science*, **124**, 389.

Tristram, G. R. (1950). *Advanc. Protein Chem.* **5**, 83.

Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949). *Manometric Techniques and Tissue Metabolism*, 2nd ed. p. 119. Minneapolis: Burgess Publ. Co.

Vaughan, M. & Anfinsen, C. B. (1954). *J. biol. Chem.* **211**, 367.

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

By A. H. GORDON

National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W. 7

(*Received* 30 *October* 1956)

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