Utilization of Glucose for the Synthesis of Protein in Chicken and Rat Liver and Rat Hepatoma *in vitro*

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Zamecnik, Loftfield, Stephenson & Steele (1951) showed that when slices of rat hepatoma were incubated with uniformly labelled [^{14}C]glucose the radioactivity of the tissue protein was 10–15 times that of normal liver tissue taken from the same animal and treated in a similar manner. Zamecnik Frantz, Loftfield & Stephenson (1948) had previously used [^{14}C]alanine in similar experiments in which they showed that there was a greater incorporation of radioactivity into the hepatoma than into the liver slices, although the differences were not as great as those found with [^{14}C]glucose. The high growth rate of malignant tissue as compared with its tissue of origin, and the possibility that tumour tissue may be able to utilize glucose more effectively for the synthesis of protein than normal tissue, are good reasons for the further investigation of glucose utilization for the synthesis of protein.

Rabinovitz, Olson & Greenberg (1954) have shown that an exchange reaction can take place *in vitro* between preformed protein and the amino acid pool. Thus under these conditions the incorporation of radioactive amino acid into protein does not necessarily imply that complete protein synthesis has occurred. It was desirable, therefore, to study the transfer of radioactivity from [^{14}C]-glucose to a protein, the synthesis of which could be followed. Peters & Anfinsen (1950) have described such a system in which the amount of serum albumin formed by chick-liver slices is estimated by an immunological method. However, it seemed improbable that any extensive synthesis of albumin would take place in rat hepatoma, so that it was decided first to compare the incorporation of ^{14}C into both the serum albumin and a soluble protein fraction after incubation of chick-liver slices with [^{14}C]alanine and [^{14}C]glucose. Alanine was chosen as the amino acid, since it is known to be formed from glucose. It would then be possible to determine the ratio of the specific radioactivity of alanine in the albumin, of which there had been a net synthesis during incubation, and in the soluble protein fraction, after incubation of liver slices with [^{14}C]alanine and [^{14}C]glucose respectively. If the ratio of specific radioactivities were found to be the same with the two substrates then it would seem probable that the transfer of ^{14}C from [^{14}C]glucose to the soluble liver-protein fraction is a reflexion of the utilization of glucose for protein synthesis. The experiments reported show that this is in fact the case.

Zamecnik *et al.* (1951) showed that the amount of glycogen present in hepatoma slices was very small compared with that present in liver slices. Thus if [^{14}C]glucose is added to liver slices the dilution of [^{14}C]glucose through the breakdown of glycogen is much greater in the liver than in the hepatoma slices. Zamecnik *et al.* (1951) overcame this difficulty by starving the animals over a prolonged period (6 days), after which the amount of glycogen in the liver was negligible. However, Wyshak & Chaikoff (1953) have shown that the metabolism of glucose in the liver is greatly reduced in rats which have been starved for 3 days or more. Thus it seemed undesirable to starve the rats in the present experiments. The difficulty arising from the dilution of [^{14}C]glucose was, therefore, overcome by measuring the specific radioactivity of the glucose during the course of the experiment. It is shown that when the specific radioactivity of the glucose present in the medium in which the hepatoma and liver slices are incubated is approximately the same, the radioactivity of the tissue protein from the hepatoma is very much higher than that from the liver slices.

MATERIALS AND METHODS

Animals. Chickens were of the Brown Leghorn strain, bred at the A.R.C. Poultry Centre, Edinburgh.

Rats were Wistar albinos highly inbred at this Institute. They were fed on rat-cube diet (No. 86, Rowett Institute, Aberdeen, Scotland).

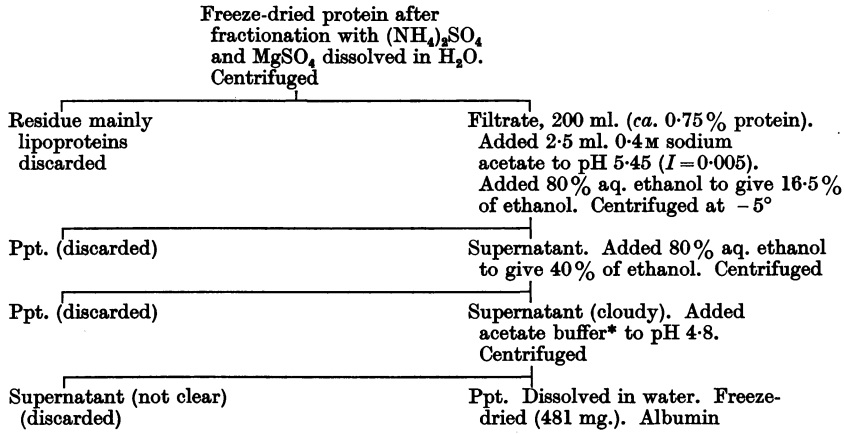
Production of hepatomas. Male rats (160–180 g.) were kept on a diet containing 0·06% *p*-dimethylaminoazobenzene. The diet, which was fed *ad libitum*, was prepared as described by Kline, Miller, Rusch & Baumann (1946). The casein (Lactic; unextracted) and the salt mixture (DL6) were obtained from Glaxo Laboratories, Greenford, Middlesex. The maize oil was obtained from the British Oil and Cake Mills, Albion Wharf, Erith, London, S.E. Vitamins A and D were supplied in the form of Adexolin (Glaxo Laboratories) at the rate of 1 drop/kg. of diet. After 10 weeks on the diet the animals were subjected to laparotomy and placed on a normal diet. They were maintained

on the normal diet for at least 2 weeks before being used. After this treatment most of the animals had liver tumours. Some of the livers were affected in only one lobe, whereas others were widely infiltrated with tumour. When the metabolism of 'normal' and hepatoma tissue was to be compared, the livers of several rats were used. The 'normal' liver slices were obtained from the unaffected lobes of livers, which contained only small amounts of tumour tissue.

Radioactive substances. Uniformly labelled D-[^{14}C]-glucose (100 μC $\equiv$ 1·64 mg.), L-[^{14}C]alanine (100 μC $\equiv$ 5·2 mg.) and [^{14}C]glycine (100 μC $\equiv$ 8·6 mg.) were supplied by the Radiochemical Centre, Amersham.

thus prepared. This was examined in a Tiselius electrophoresis apparatus (A. Hilger and Co., London) in veronal and acetate buffer (I = 0·1) at a concentration of 1–2% protein.

Preparation of antisera against chick albumin. An alum-precipitate of the chick albumin (389 mg.) was prepared by the method of Proom (1943). The precipitate was suspended in 25 ml. M/15, pH 6·7, phosphate buffer containing 1:10 000 Thiomersalate (British Drug Houses Ltd.) and homogenized in a glass homogenizer. Rabbits were then given nine intravenous injections of the suspension by the ear vein over 3 weeks as follows: 2 of 0·2 ml., 2 of 0·4 ml., 5 of 0·8 ml. Ten days after the last injection the rabbits



* Buffer 5 ml. 10 N acetic acid, 2·5 ml. 4 M sodium acetate, 10·5 ml. of ethanol. Water to 25 ml.

Fig. 1. Purification of chick serum albumin by ethanol fractionation.

Purification of chick serum albumin. Although Peters & Anfinsen (1950) described the use of antiserum for estimating the amount of albumin formed by chick-liver slices, they did not give any adequate details for the preparation of chick albumin. A brief description of the method used is therefore given.

The chickens were starved overnight and then killed by decapitation. Blood was collected and allowed to clot, and the serum separated by centrifugation. The serum was treated with $MgSO_4$ according to the method of Popják & McCarthy (1946). After removal of the precipitate by filtration the $MgSO_4$ was removed from the filtrate by dialysis and the solution was treated with 0·5 saturated $(NH_4)_2SO_4$ (31 g./100 ml.). After removal of the precipitate the filtrate was saturated with $(NH_4)_2SO_4$, the precipitate collected by filtration, dissolved in water and dialysed against distilled water until free of $(NH_4)_2SO_4$. Examination of this solution by filter-paper electrophoresis in veronal buffer, pH 8·6, I = 0·05 (Flynn & de Mayo, 1951), showed it to contain only traces of globulin in addition to albumin. The purification of albumin was then completed with ethanol by a modification of Method 6, of Cohn *et al.* (1946). Details of the method used are given in Fig. 1. The fractionation described applies to the serum obtained from three hens (each about 2 kg. body weight) which gave 70 ml. of serum in all. The composition of the various fractions was followed throughout the procedure by filter-paper electrophoresis. Altogether 1·03 g. of albumin were

were killed and serum was collected. The serum was fractionated by the addition of 2 vol. of 27% (w/v) Na_2SO_4 dissolved in sodium phosphate buffer, pH 7·8 (I = 0·1), to produce a final concentration of 18% (w/v). The precipitated globulins were redissolved in water and reprecipitated at 16% (w/v) Na_2SO_4, dialysed and freeze-dried. The amount of antibody in the rabbit serum was estimated and found to average 10 mg./ml. of serum. A test for the homogeneity of the antiserum was carried out according to Oudin (1948). This showed that in addition to the main component three components are present in very small amounts.

Incubation procedure for tissue slices. Slices were cut and suspended in a bicarbonate medium as described by Peters & Anfinsen (1950). After a preliminary washing of the slices in the medium (150 ml. of medium for 6 g. of slices) at room temperature, to reduce the amount of albumin present, they were drained on filter paper and divided into the appropriate number of batches. Each weighed batch of slices was incubated in 10 ml. of the same medium, which contained any additions of radioactive glucose or amino acid required, at 37° in a gas phase of $O_2 + CO_2$ (95:5).

In the experiments with chicks, the liver slices were obtained from animals 6–12 weeks old and about 2 g. of slices were used in each incubation flask. In the experiments involving rat liver and hepatoma only about 1 g. of tissue was used in each flask.

Microdetermination of serum albumin in liver and hepatoma slices. The method used is based on that of Peters & Anfinsen (1950). Preliminary tests showed that in order to obtain a satisfactory calibration curve over the range 5–60 μg. of albumin, it was necessary to use 3 mg. of freeze-dried antiserum. The procedure as applied to the estimation of albumin in liver and hepatoma slices was as follows.

After incubation of the slices at 37° the flasks are cooled on ice. The slices and medium are then homogenized in a Potter-type glass homogenizer and the tissue suspensions are centrifuged at 2° for 20 min. at 15 000 *g*. The supernatant, which is not clear, is diluted with an equal vol. of 0·9% NaCl containing 1:10 000 Thiomersalate and frozen overnight. This procedure appears to cause denaturation of the lipoproteins, for the supernatant is usually completely clear after a second centrifugation. For the accurate estimation of albumin it is essential that this be so.

For each estimation 0·3 ml. of a 1% solution of freeze-dried antiserum in 0·9% NaCl, which has been completely cleared by centrifuging, is used. Sufficient of the supernatant from the tissue homogenates is used to give 10–60 μg. albumin. For comparison tubes containing similar quantities of albumin are set up as standards. The volume of each tube is made up to 1·5 ml. with 0·9% NaCl, and tubes are incubated at 37° for 40 min. and then set aside overnight at 2°. The precipitate is spun down at 2°, washed twice with 0·9% NaCl at 2° and then dissolved in 1·5 ml. 0·1 M-Na_2CO_3. After complete solution of the protein precipitate has been ensured the tubes are again centrifuged to spin down any insoluble material. The optical density of the clear solutions is read at 280 mμ. in a spectrophotometer with 0·5 cm. quartz cells. Determinations were made at two different concentrations on each tissue supernatant and the results were averaged.

Large-scale precipitation of albumins with antisera. When sufficient albumin was required for determination of radioactivity, an excess of antiserum was added to the extract containing the albumin. After incubation the precipitated albumin was centrifuged and the supernatant tested to ensure excess of antiserum. The precipitated albumin was then washed with 0·9% NaCl at 2° and then with ethanol, ethanol–ether–$CHCl_3$ (2:2:1, v/v) and finally acetone.

Measurement of radioactivity

Protein. Trichloroacetic acid (TCA) precipitates of the proteins were treated as described by Zamecnik *et al.* (1951). The dried protein was then plated either on 1 cm.^{2} polythene disks or on 0·3 cm.^{2} Perspex disks and counted at infinite thickness as described previously (Campbell & Work, 1952).

Specific activity of alanine in tissue proteins. After hydrolysis of the protein (5–10 mg.) in a sealed tube with 5 N-HCl for 18 hr. at 110°, alanine was isolated by paper chromatography by using first phenol–water (4:1) and then pyridine–water (4:1), as described by Steinberg & Anfinsen (1952). Whatman no. 3 paper was used for chromatography. The paper was pretreated by washing first with N-Na_2CO_3, then with distilled water, 2 N-acetic acid and finally distilled water. Ordinary descending chromatographic procedures were used for this washing, each solvent being run for 24 hr. With paper thus treated the R_F of alanine when phenol was used as solvent was consistently less than that of the marker alanine. It was, therefore, necessary to determine the position of the alanine on these chromatograms by using absorption under ultraviolet light. The purity of the alanine obtained by these methods was checked using butanol–acetic acid–water (4:1:5) (Partridge, 1948) as solvent for a third chromatogram with Whatman no. 4 paper. The alanine obtained was dissolved in 10% aq. *iso*propanol and samples were placed on clean 2 cm.^{2} polythene disks. A 0·05% solution of the non-ionic detergent BRIJ 35 (Honeywill and Stein Ltd., 21 St James's Square, London) was added to each disk to effect a complete spread of the alanine solution. The disks were then dried in a vacuum desiccator over P_2O_5. The radioactivity of the disks was determined by means of a Geiger end-window counter. The amount of alanine on each disk was determined by reaction with ninhydrin, according to the method of Cocking & Yemm (1954). The reaction was carried out in 1 in.-diameter boiling tubes capped with funnels, in an ammonia-free room. The tubes were heated for 20 min., cooled for 5 min. and diluted with 5 ml. of 60% ethanol. The colour was read in a spectrophotometer at 570 mμ. in 1 cm. cells. The procedure gave consistently lower blanks than that described by Moore & Stein (1948) and was found to be convenient. A satisfactory standard curve for amounts of alanine between 5 and 45 μg. was obtained.

Specific activity of glucose in tissue homogenates. Samples of the supernatant from homogenates of liver and hepatoma slices were quickly freeze-dried and then thoroughly dried in a vacuum desiccator over P_2O_5. The residue was extracted with 5 ml. of dry pyridine at 100° for 10 min. as described by Malpress & Morrison (1949). The pyridine was removed from the extracts under reduced pressure and the residues dissolved in 1 ml. of water. The aqueous extract was chromatographed overnight on Whatman no. 4 paper which had been washed with acetic acid and ammonia as recommended by Isherwood & Hanes (1953). Butanol–acetic acid–water (4:1:5) (Partridge, 1948) was used as the solvent. The position of the radioactive glucose on the chromatogram was determined by autoradiography. The glucose was eluted from the paper with water and its radioactivity determined as described for alanine. The amount of glucose on each disk was determined by the method of Hagedorn & Jensen (1923*a*, *b*). Although this procedure did not provide completely pure glucose, since there was some contamination with phosphorus-containing substances, its extent was slight and did not differ appreciably for tumour and liver tissue.

RESULTS

Chick serum albumin

Apart from the work of Peters & Anfinsen (1950) there seems to be no description in the literature of the purification of chick serum albumin. Before the method described was used, the methanol-precipitation method of Pillemer & Hutchinson (1945), which works well for the preparation of human serum albumin, was tried without success. In Fig. 2 are shown the electrophoretic diagrams obtained when chick serum albumin (1%) was examined in veronal buffer at pH 8·53 and in acetate buffer at pH 4·50. It will be observed that under these conditions there is no evidence of a

second component. However, the result of the Oudin procedure (see Methods section) showed that the albumin contained at least three minor components.

Synthesis of serum albumin in chick-liver slices

Fig. 3 shows the results obtained when a standard solution of chick serum albumin was assayed by the antiserum method previously described. Since small differences in the incubation and centrifuging procedures were liable to affect the results it was found more satisfactory to run standard tubes in each assay. The main source of difficulty was the presence of fat in the tissue extracts. High-speed centrifuging was the most effective method of clearing extracts of fatty suspensions, and this was greatly facilitated by the denaturation of lipoproteins which takes place on freezing the extracts. The results obtained with the method as described were usually accurate to within ± 10%, although, for no apparent reason, isolated results were sometimes obtained which were well outside these limits.

Altogether six experiments were carried out in which chick-liver slices were incubated in bicarbonate medium for different times and the amount of albumin in the medium plus slices was measured. In many of the experiments small quantities of radioactive amino acids or glucose were added to the slices before incubation and in these cases the radioactivity of the protein in the supernatant, which was precipitated by TCA, has been determined. The results of five such experiments are shown in Table 1. One other experiment has been omitted because the fatty nature of the liver made accurate estimation of the amount of albumin in the tissue extracts impossible.

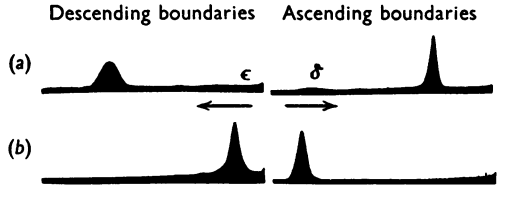


Fig. 2. Electrophoretic patterns of purified serum albumin. Each arrow indicates the position of the boundary at the start of the run and the direction of movement. Potential gradient, 4v/cm. $I=0{\cdot}1$, 1% protein. (*a*) Sodium veronal buffer, pH 8·53. Duration of run, 4 hr. 38 min. (*b*) Sodium acetate buffer, pH 4·50. Duration of run, 3 hr. 36 min.

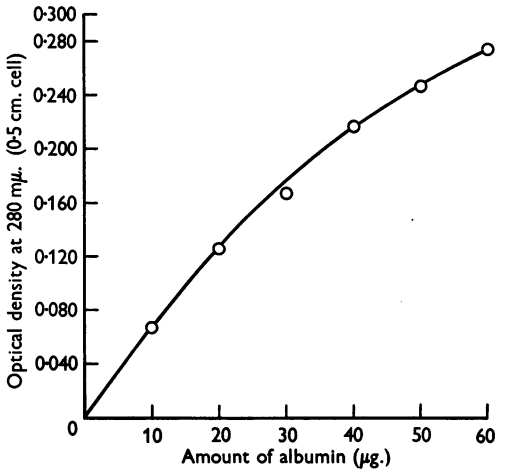


Fig. 3. Standard curve for estimation of chick serum albumin.

Incorporation of radioactivity into various liver-protein fractions

In order to determine which liver-protein fractions incorporated the most radioactivity during the incubation of slices with uniformly labelled [^{14}C]glucose, the radioactivity of different protein fractions was determined. The results are shown in Table 2. The albumin, which was precipitated with antiserum, contained approximately ten times its weight of non-radioactive protein, since in the antibody–antigen precipitate the ratio of the two proteins is about 10:1. Thus the radioactivity of the albumin in the liver was very much higher than that of any of the other protein fractions tested.

Table 1. *Rate of synthesis of albumin in chick-liver slices*

Results are given in mg. albumin/g. wet tissue; percentage increase is shown in brackets. Radioactivity is expressed as counts/min./sq.cm. infinite thickness.

Expt. no.	Additions	0 hr. Albumin	0 hr. Counts	2 hr. Albumin	2 hr. Counts	4 hr. Albumin	4 hr. Counts
I	None	0·78	—	1·28 (64)	—	1·44 (85)	—
II	2 μc [^{14}C]glycine	1·66	0	1·98 (19)	118	2·40 (44)	321
III	2 μc [^{14}C]glucose	1·18	1	1·89 (60)	211	2·68 (127)	385
IV	20 μc [^{14}C]glucose*	1·36	—	1·74 (28)	—	2·02 (49)	2000
VI	5 μc [^{14}C]alanine*	0·78	—	1·01 (30)	—	1·72 (133)	1120

* Present only in the flask incubated for 4 hr.

Table 2. *Radioactivity of various protein fractions after incubation of chick-liver slices with [^{14}C]glucose for 2 and 4 hr.*

Radioactivity is expressed in counts/min./0·3 cm.^{2} infinite thickness.

Protein preparation	Count 2 hr.	Count 4 hr.
Homogenate of slices + medium; TCA ppt.	51	89
Supernatant of above; TCA ppt.	—	122
Supernatant of above; 0·5 saturated $(NH_4)_2SO_4$	79	—
Supernatant of above; saturated $(NH_4)_2SO_4$	70	—
Supernatant of above; albumin pptd. with antiserum	—	102

Estimation of specific radioactivity of alanine at infinite thinness

Fig. 4 shows the results obtained when the radioactivity of a standard solution of [^{14}C]alanine was estimated on 2 cm.^{2} polythene disks as described previously. The results show that over the range 10–70 μg. of alanine the amount of self-absorption is insignificant. Radioactive measurements on alanine isolated from protein were made on amounts of alanine which fell between these limits.

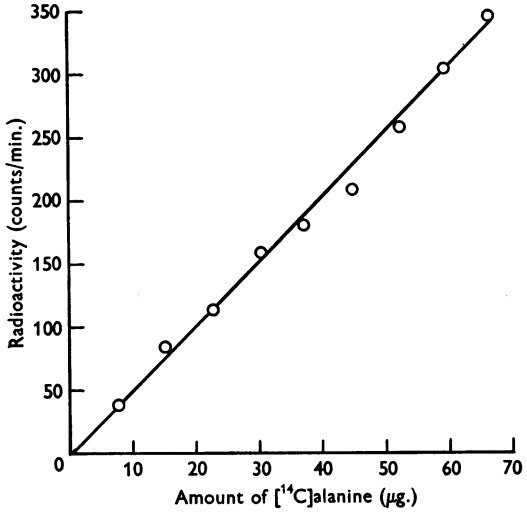


Fig. 4. Self-absorption curve for determination of specific radioactivity of [^{14}C]alanine.

As was previously explained (Methods section) the alanine isolated from protein hydrolysates by paper-partition chromatography was first assayed for radioactivity on polythene disks and then the amount of alanine on each disk was determined by interaction with ninhydrin. One of the dangers of such a method is that in eluting the alanine from the chromatogram some ninhydrin-reacting substance other than alanine may be eluted, even though the chromatography paper has undergone a careful washing procedure. On the basis that any such impurity in the [^{14}C]alanine would not be radioactive, the following method for checking the amount of such contamination of the alanine has been devised: the radioactivity of the alanine obtained from liver protein was determined on samples containing various amounts of alanine. The relationship between the amount of substance which reacts with ninhydrin and the radioactivity of the samples is shown in Fig. 5. The position where the graph cuts the base line must represent the amount of ninhydrin-reacting substance,

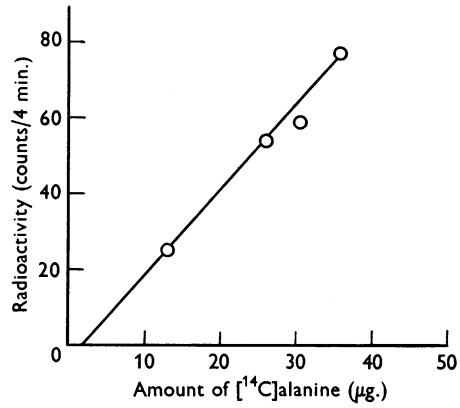


Fig. 5. Relation between amount of ninhydrin-reacting substance (expressed as alanine) and radioactivity of eluent from chromatogram.

which is not radioactive. It will be seen that the amount of such impurity is small compared with that of alanine. This method was considered more satisfactory than one involving the elution of control strips of the paper chromatogram. In triplicate determinations of the specific radioactivity of alanine the results obtained agreed to within ± 8 %.

After chick-liver slices had been incubated with [^{14}C]glucose (Exp. IV, Table 1) for 4 hr. the slices and medium were homogenized and the suspension was centrifuged. One part of the supernatant

obtained was treated with antiserum and another with 10% TCA. Alanine was isolated from each of the precipitates and its specific radioactivity determined. A determination was made on similar precipitates obtained after incubation of chick-liver slices with [^{14}C]alanine (Exp. VI, Table 1). The results of these experiments are given in Table 3. As was previously observed the alanine obtained from the antiserum-precipitated albumin is diluted with the alanine present in the non-radioactive antiserum, but the alanine in each case will have been diluted to the same extent.

Determination of specific radioactivity of [^{14}C]*-glucose during incubation of normal liver slices and hepatoma slices and correlation with the radioactivity of the tissue protein*

The method used for measuring the radioactivity of the [^{14}C]glucose isolated from tissue extracts was similar to that used for [^{14}C]alanine. Since similar quantities of glucose and alanine were estimated, it has been assumed that the self-absorption of glucose in the radioactive assay is negligible, as was found for alanine. Since the Hagedorn & Jensen method used for the estimation of glucose is not as sensitive as the ninhydrin method used for the estimation of alanine, the accuracy of the assay for glucose is less than that for alanine. The accuracy of the determination of the specific activity of glucose is about ±15%, which is sufficient for the present purpose of the experiments.

The results are shown (Table 4) of two experiments in which the specific radioactivity of the glucose in the medium and slices, and the radioactivity of the soluble protein obtained by precipitation with TCA, were estimated. It will be observed that the protein obtained from the tissue slices at time 0 hr. had negligible radioactivity. It was consistently found that the procedure adopted for the treatment of the tissue-protein precipitates ensured the removal of both free amino acids and glucose from the protein precipitates.

The amount of protein precipitated by the addition of TCA to the supernatant of the homogenates obtained from each flask was weighed, and was found to be closely similar for the hepatoma and liver tissue. Thus the amount of [^{14}C]glucose/g. tissue protein was the same for hepatoma and liver slices.

Preliminary experiments indicated that although the amount of glucose present in the liver slices during the incubation period was sufficient to enable the radioactivity of the glucose to be measured, this was not so in hepatoma slices. This was no doubt due to the small quantity of glycogen known to be present in hepatoma tissue compared with that in liver (Zamecnik *et al.* 1951). For this reason it was necessary to add inactive glucose to the hepatoma slices before the start of the incubation. The results shown in Table 4 suggest that in liver slices there is a rapid breakdown of glycogen leading to a lowering of the specific radioactivity of the glucose.

Table 3. *Specific radioactivity of alanine isolated from albumin and TCA-precipitated protein after incubation of chick-liver slices with* [^{14}C]*glucose and* [^{14}C]*alanine*

Radioactivity is expressed as counts/4 min./μM alanine.

	Incubation with	
	[^{14}C]glucose	[^{14}C]alanine
Albumin	185	980
TCA ppt.	183	848

DISCUSSION

Although Zamecnik *et al.* (1951) showed that under *in vitro* conditions hepatoma tissue utilized glucose more effectively for the synthesis of protein than did liver tissue, this result could not be confirmed *in vivo*. These authors were able to show that after feeding [^{14}C]glucose to rats the specific activity of the non-protein fraction of the hepatoma was less than that of the liver, whereas the radioactivity of the tissue proteins was not significantly different. This suggested a circulatory impairment of the hepatoma tissue, when compared with the normal liver tissue. They were later able to confirm this

Table 4. *Relationship between radioactivity of* [^{14}C]*glucose and tissue protein during incubation of rat-liver and hepatoma slices*

Results are expressed as counts/min./mg. glucose and counts/min./0·3 cm.^{2} protein at infinite thickness. Each incubation flask contained approx. 1 g. of tissue slices together with 0·033 mg. of [^{14}C]glucose in Expt. 1 and 0·013 mg. of [^{14}C]glucose in Expt. II. In addition, 10 mg. of inactive glucose was added to each flask containing the hepatoma slices.

		Liver			Hepatoma		
		0 hr.	2 hr.	4 hr.	0 hr.	2 hr.	4 hr.
Expt. I	Glucose	42 350	12 350	10 350	26 800	—	17 400
	Protein	2·5	19	33	3	—	327
Expt. II	Glucose	12 005	4 605	3 560	7 688	4 144	1 545
	Protein	0	9	21	0	61	105

after injection of [^{14}C]alanine. However, as Gaitonde & Richter (1955) have recently observed in brain tissue, a failure to incorporate an injected radioactive amino acid into a tissue protein does not imply a low rate of synthesis of the proteins in that tissue; a knowledge of the radioactivity of the free amino acid is necessary before any deductions may be drawn.

The purpose of the experiments described was to compare the utilization of glucose for the synthesis of protein in hepatoma and normal rat liver. However, it was not possible to follow the synthesis of a specific protein in the hepatoma tissue, and a mixture of the soluble proteins had to be used. It was desirable therefore that a comparison should be made between the transfer of ^{14}C from [^{14}C]glucose into a specific protein, of which there was a net synthesis in liver tissue, with the transfer of ^{14}C to a mixture of soluble liver proteins. Preliminary experiments (Table 2) indicated that such a mixture of soluble protein contained more radioactivity after incubation of liver slices with [^{14}C]-glucose than did the whole liver protein.

The experiments on chick-liver slices (Table 1), in which the amount of albumin was estimated during the course of incubation, leave little doubt that a net synthesis of albumin does take place under these conditions and thus confirms the results of Peters & Anfinsen (1950). The incorporation of [^{14}C]alanine into the albumin in Experiment VI suggests that the increase in albumin is real and that it is not merely being released from the tissue during the incubation period. However, the possibility of an exchange between the amino acid and the preformed protein, as mentioned in the Introduction, cannot be excluded.

The specific radioactivity of alanine in the albumin obtained after incubation of chick-liver slices with [^{14}C]glucose (Expt. IV, Table 1) under conditions of net albumin synthesis is compared with that in the soluble protein fraction in Table 3. Since the alanine in the albumin will have been diluted by that in the non-radioactive antiserum used in the precipitation of the albumin, the alanine in the albumin has a much higher specific radioactivity than that in the soluble protein fraction. In the utilization of glucose for protein synthesis the glucose must be metabolized to amino acids, which in turn are converted into protein. Since little is known about the distribution of the intracellular enzymes in these two reactions it was necessary to determine the specific radioactivity of an amino acid, known to be formed from glucose, in the two protein preparations after incubation with the radioactive amino acid. The amino acid chosen was alanine. The results of the specific radioactivity determinations are also shown in Table 3. It will be observed that within the experimental error of the methods used the ratio of the specific activities of alanine in the two protein preparations is the same in the two experiments. This demonstrated that alanine formed from glucose is relatively as available for the synthesis of albumin and the mixture of soluble proteins as is alanine added to the incubation medium.

The results of the experiments on rat-liver hepatoma may now be considered in the light of the results obtained with chick liver. Since the rat-liver and hepatoma slices were incubated with [^{14}C]glucose under the same conditions as the chick-liver slices we may assume that incorporation of radioactivity into the soluble protein fraction is a reflexion of the synthesis of protein. In the experiments for which the results are shown in Table 4, the radioactivity of the hepatoma protein is in each case higher than that of the corresponding liver protein. In the first experiment, the specific activity of the [^{14}C]glucose was greater in the hepatoma than in the liver, but the difference does not account for the tenfold difference in activity of the proteins. In the second experiment the activity of the glucose was always less in the hepatoma than in the liver, yet the hepatoma protein was five times as active as the liver protein. It is clear, therefore, that glucose is more effectively used for the synthesis of protein in hepatoma than normal liver tissue.

Three possible explanations of this result may be considered: First, hepatoma protein might contain a preponderance of those amino acids that are derived from glucose, compared with liver protein. Zamecnik, Frantz & Stephenson (1949) and Zamecnik & Frantz (1949) carried out an amino-acid assay of liver and hepatoma protein, but found no such differences. Secondly, it is possible that in hepatoma tissue there are mechanisms for synthesizing amino acids from glucose such as do not occur in liver tissue. Zamecnik *et al.* (1951) have examined this possibility, but proline was the only amino acid which was radioactive in the hepatoma protein and was not radioactive in the liver protein after incubation of the hepatoma and liver slices with [^{14}C]glucose. This could hardly account for the differences in activity of the proteins. Thus it must be concluded that the better utilization of glucose by hepatoma tissue for the synthesis of protein under *in vitro* conditions is due to a more rapid incorporation of amino acids into the hepatoma protein.

SUMMARY

1. The preparation of chick serum albumin is described. The preparation was homogeneous at pH 8·53 and 4·50 in the Tiselius electrophoresis

apparatus. However, an immunological method showed the presence of three additional minor components.

2. An antiserum, prepared by injection of chick serum albumin into rabbits, was used to demonstrate the net synthesis of serum albumin by chick-liver slices. The results of Peters & Anfinsen (1950) were thus confirmed.

3. The incorporation of radioactivity into serum albumin and a soluble protein fraction was studied after incubation of chick-liver slices with [^{14}C]glucose and [^{14}C]alanine respectively. The specific radioactivity of the alanine in each protein fraction was determined.

4. The incorporation of radioactivity into a soluble protein fraction of rat hepatoma and rat liver was studied after incubation of the tissue slices with [^{14}C]glucose. The specific radioactivity of the glucose during the incubation period was followed. It was thus shown that under these conditions there is a better utilization of glucose by hepatoma tissue for the synthesis of protein than there is by liver tissue.

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REFERENCES

Campbell, P. N. & Work, T. S. (1952). *Biochem. J.* **52**, 217.
Cocking, E. C. & Yemm, E. W. (1954). *Biochem. J.* **58**, xii.
Cohn, E. J., Strong, L. E., Hughes, W. L. jun., Mulford, D. J., Ashworth, J. N., Melin, M. & Taylor, H. L. (1946). *J. Amer. chem. Soc.* **68**, 459.
Flynn, F. V. & de Mayo, P. (1951). *Lancet*, **2**, 235.
Gaitonde, M. K. & Richter, D. (1955). *Biochem. J.* **59**, 690.
Hagedorn, J. C. & Jensen, B. N. (1923*a*). *Biochem. Z.* **135**, 46.
Hagedorn, J. C. & Jensen, B. N. (1923*b*). *Biochem. Z.* **137**, 92.
Isherwood, F. A. & Hanes, C. S. (1953). *Biochem. J.* **55**, 824.
Kline, B. E., Miller, J. A., Rusch, H. P. & Baumann, C. A. (1946). *Cancer Res.* **6**, 1.
Malpress, F. H. & Morrison, A. B. (1949). *Nature, Lond.*, **164**, 963.
Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
Oudin, J. (1948). *Ann. Inst. Pasteur*, **75**, 30, 109.
Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
Peters, T. jun. & Anfinsen, C. B. (1950). *J. biol. Chem.* **186**, 805.
Pillemer, L. & Hutchinson, M. C. (1945). *J. biol. Chem.* **158**, 299.
Popják, G. & McCarthy, E. F. (1946). *Biochem. J.* **40**, 789.
Proom, H. (1943). *J. Path. Bact.* **55**, 419.
Rabinovitz, M., Olson, M. E. & Greenberg, D. M. (1954). *J. biol. Chem.* **210**, 837.
Steinberg, D. & Anfinsen, C. B. (1952). *J. biol. Chem.* **199**, 25.
Wyshak, G. H. & Chaikoff, I. L. (1953). *J. biol. Chem.* **200**, 851.
Zamecnik, P. C. & Frantz, I. D. jun. (1949). *Cold Spr. Harb. Symp. quant. Biol.* **14**, 199.
Zamecnik, P. C., Frantz, I. D. jun., Loftfield, R. B. & Stephenson, M. L. (1948). *J. biol. Chem.* **175**, 299.
Zamecnik, P. C., Frantz, I. D. jun. & Stephenson, M. L. (1949). *Cancer Res.* **9**, 612.
Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L. & Steele, J. M. (1951). *Cancer Res.* **11**, 592.

The Proteins of *Hevea brasiliensis* Latex

1. PROTEIN CONSTITUENTS OF FRESH LATEX SERUM

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Analytical studies (Altman, 1946) have shown that fresh unammoniated *Hevea* latex contains about 1% of protein, part of which is adsorbed at the surface of the rubber particles while the remainder is distributed between the aqueous serum phase of the latex and the lutoid bodies first described by Homans & van Gils (1948). Although it is generally recognized that the presence of protein in fresh latex is an important factor controlling its colloidal stability, further information on the concentration and properties of the individual protein components, and their distribution between the above three phases, is necessary for a better understanding of the colloidal behaviour of latex.