the transformation of dehydro*epi*androsterone into androst-5-ene-3β:16α:17β-triol.

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The Role of Serum Albumin as a Precursor of the Soluble Tissue Proteins and the Serum Globulins of the Rat Bearing Liver Tumours

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In a previous paper the evidence for supposing that one body protein can be converted into another without complete degradation to amino acids was reviewed (Askonas, Campbell, Godin & Work, 1955). In particular, it was shown that peptides for synthesis of milk protein are not supplied by partial hydrolysis of plasma protein (see also Godin & Work, 1956). Since this work was completed, Babson & Winnick (1954) have suggested that implanted tumours in the rat may derive amino acids for the synthesis of tissue proteins by the partial breakdown of plasma protein. It was decided therefore to study the role of plasma protein for the synthesis of tissue protein in an autologous tumour.

For this purpose a rat bearing liver tumours induced by feeding 4-dimethylaminoazobenzene was used. The approach was to inject two amino acids which were not interconvertible; one of the amino acids was injected in the free state and the other bound in the form of serum albumin. By a comparison of the radioactivity of each amino acid in the tissue proteins it was hoped to compare the incorporation into the tissues of a free amino acid and a protein-bound amino acid under identical conditions and to determine whether liver tumour differs from liver in this respect.

The rat bearing liver tumours was injected with a solution containing free [^{14}C]alanine and serum albumin labelled with [^{14}C]lysine. After 1·5 hr. the rat was killed and the radioactivity of alanine and lysine in the soluble tissue proteins was determined. For the purposes of comparison not only was liver and liver tumour studied but also kidney and spleen. In order to determine the effect on these radioactivity determinations of the presence of unchanged radioactive serum albumin, the amount of the latter in the tissue extracts was determined immunochemically. The influence of variations in

the pool sizes of lysine and alanine in the tissues was allowed for by determining the radioactivity of these amino acids occurring free in the tissues.

Recently Maurer & Müller (1955) concluded that serum albumin is converted into serum globulin in the rat without first being completely broken down to free amino acids. In an attempt to confirm this observation the serum proteins from the rat which had been injected with [^{14}C]lysine-labelled albumin and [^{14}C]alanine were fractionated by zone electrophoresis and the radioactivity of the lysine and alanine in the fractions was determined.

Preliminary results of some aspects of this work have been reported (Campbell, Jones & Stone, 1956).

MATERIALS AND METHODS

Animals and production of liver tumours. Tumours were induced in rats by feeding them on a diet containing 0·06% of 4-dimethylaminoazobenzene as described by Campbell (1955).

Radioactive substances. Uniformly labelled L-[^{14}C]alanine (100 μc/5·2 mg.) and L-[^{14}C]lysine (100 μc/2·5 mg.) were supplied by the Radiochemical Centre, Amersham.

[^{14}C]*Lysine-labelled serum albumin.* This was prepared from the serum of a rat which had received an intraperitoneal injection of 50 μc (1·25 mg.) of uniformly labelled L-[^{14}C]lysine as described by Campbell & Stone (1957).

Injection of [^{14}C]*alanine and* [^{14}C]*lysine-labelled albumin into a rat bearing liver tumours.* After laparotomy, a male rat (310 g. body wt.) was chosen which had several large discrete liver tumours, but which had some unaffected liver. The rat had been on a normal diet for 3 weeks after the diet containing 4-dimethylaminoazobenzene. The purified [^{14}C]lysine-labelled albumin (0·4 μc/150 mg.) and L-[^{14}C]alanine (20 μc, approx. 1 mg.) were dissolved in 3 ml. of saline and injected into the femoral vein of the rat under light ether anaesthesia. The animal was left without food, but with water, for 1·5 hr. and then anaesthetized with ether. Blood (5 ml.) was removed by heart puncture and the liver was perfused with 40 ml. of warm saline. The liver (containing liver tumour), kidneys and spleen were then removed and cooled on ice. After separation of the tumour and liver, samples of each were taken for histological examination.

Preparation of soluble fractions from tissues. The tissues were homogenized in a Potter-type all-glass homogenizer in cold saline, at 4500 rev./min. for 2 min. The soluble fraction was obtained by centrifuging as previously described (Campbell & Halliday, 1957).

Isolation of tissue proteins. Samples of the soluble fraction from the tissues were treated with an equal volume of 10% (w/v) trichloroacetic acid (TCA) and the precipitates treated to remove non-protein substances as described by Zamecnik, Loftfield, Stephenson & Steele (1951). In this procedure the protein precipitates are washed twice with 5% TCA, heated for 20 min. at 90° with 5% TCA, and washed with warm ethanol, warm ethanol–ether–chloroform (2:2:1) and finally with warm acetone. In view of the results of Korner & Debro (1956), who showed that after precipitation of rat-serum albumin with TCA some of the protein dissolved in ethanol, the loss of albumin when the tissue proteins were treated according to Zamecnik *et al.* was determined. Rat-serum albumin (2·4 mg.) labelled with [^{14}C]lysine was dissolved in saline (4 ml.) and the solution was centrifuged to remove traces of insoluble substances. This solution (1 ml.) was added to an extract (1 ml.) of rat liver prepared as described in the last section. Protein was then precipitated by the addition of an equal volume of 10% TCA. In one case (*a*) the precipitate was treated by the procedure of Zamecnik *et al.*, and in the other (*b*) the precipitate was washed with ethanol. Approx. 8 mg. of dry protein was obtained in each. The radioactivity of protein prepared by method (*a*) was 32 counts/min. at infinite thickness, whereas the corresponding count for that prepared by method (*b*) was 22. Thus, as expected from the results of Korner & Debro, there was a loss of approx. 30% of the albumin when the cold TCA precipitate was washed with ethanol.

The results obtained by the procedure of Zamecnik *et al.* were then compared with those of a procedure involving coagulation of protein by heat. The experiment was similar to that already described, except that rather less radioactive albumin was used. The proteins were treated either as described by Zamecnik *et al.* (method *a*) or the protein was coagulated by boiling the solution for 10 min., an equal volume of acetone being added to the cooled mixture, and centrifuging and finally washing with acetone. When the radioactivity of the proteins was determined on duplicate samples, that treated by method (*a*) gave 22 and 25 counts/min. and that treated by coagulation 23 and 26 counts/min. It is therefore concluded that no significant amount of albumin is lost when tissue proteins are treated by the procedure of Zamecnik *et al.* The results suggest that whereas some albumin is soluble in organic solvents when it is precipitated with cold 5% TCA this albumin is no longer soluble when it has been heated at 90° in 5% TCA.

Isolation of amino acids from tissue proteins. Alanine and lysine were isolated from hydrolysates of approximately 50 mg. of the tissue proteins on columns of Deacidite FF (6% cross-linked, particle size 50 μ; 0·9 cm. diam., 30 cm. long; The Permutit Co. Ltd., London) (Campbell, 1956) and columns of Zeo-Karb 225 (0·9 cm. diam., 100 cm. long) as described by Campbell & Halliday (1957).

Isolation of free amino acids from tissues. After removal of the protein in the tissue extracts by the addition of TCA, alanine and lysine were isolated from the supernatants by ion exchange and paper chromatography as previously described (Campbell & Halliday, 1957).

Fractionation of serum proteins. Serum obtained from the sample of blood was fractionated by the addition of an equal volume of saturated $(NH_4)_2SO_4$. The precipitate was removed by centrifuging, and dissolved in water, dialysed and freeze-dried. The supernatant was dialysed and freeze-dried. Each fraction was then further purified by zone electrophoresis with partially acetylated cellulose in columns 1·5 cm. diam., 40 cm. long, as described by Campbell & Stone (1957). The fractions obtained were freeze-dried after removal of the buffer by dialysis against distilled water.

Estimation of serum albumin in tissue extracts and serum-protein fractions. An antiserum obtained by injecting rabbits with a purified preparation of rat-serum albumin was used. Substances which interfere with the estimation were first removed from the tissue extracts by adsorption on to a precipitate produced by the addition of chick-serum albumin and chick-albumin antiserum. The method has been previously described in detail (Campbell & Stone, 1957).

Estimation of blood content of tissue extracts. The amount of blood in the supernatant from homogenates of various tissue extracts was determined by the method of Holzer, Sedlmayr & Kiese (1956).

Immunoelectrophoresis. This was carried out on fractions of rat-serum protein according to the method of Grabar & Williams (1955).

Measurement of radioactivity

Tissue proteins and freeze-dried serum proteins. These samples were plated on 0·28 cm.² Perspex disks and counted at infinite thickness in a Geiger–Müller counter with a mica end-window.

Amino acids from hydrolysates of tissue proteins. If 3 mg. or more of alanine or lysine was obtained from a protein hydrolysate the sample was converted into its dinitrophenyl (DNP) derivative and was then counted at infinite thickness on 0·28 cm.² Perspex disks. The details of this procedure have been previously described (Campbell & Halliday, 1957).

If less than 3 mg. of amino acid was obtained then the amount of amino acid was determined by reaction with ninhydrin according to the method of Cocking & Yemm (1954). The radioactive amino acid was then diluted with sufficient inactive amino acid to provide at least 20 mg. of DNP-amino acid after treatment of the mixture with dinitrofluorobenzene. The activity of this derivative was determined by counting at infinite thickness on 1 cm.² polythene disks.

Amino acids from hydrolysates of serum proteins. The amount of alanine and lysine obtained from these hydrolysates was so small that it was necessary to dilute the samples before conversion into their DNP derivatives as described in the last section. The DNP-amino acids were then counted at infinite thickness on 1 cm.² polythene disks. However, sometimes the activity was too low for accurate counting and in these cases 10 mg. samples of the DNP derivatives were subjected to combustion and the radioactivity of the CO_2 was determined by gas counting (Salmony & Whitehead, 1954). An oxidizing mixture (Van Slyke & Folch, 1940) was used for the combustion, and the CO_2 passed through a solid CO_2-ethanol trap to remove water. The radioactivity was expressed as counts/min./mg. of free amino acid. The actual count obtained from the combustion of 10 mg. of DNP-amino acid was approx. 15 times that obtained by counting at infinite thickness on a 1 cm.² disk, for which approx. 20 mg. was required.

Free amino acids from tissue extracts. The activity of these amino acids was determined by counting samples at infinite thinness on 2 cm.² polythene disks as previously described (Campbell, 1955).

RESULTS

Histological examination of the liver from the rat which was injected showed that the tissue was substantially free from malignant cells. The liver tumour consisted mainly of cholangioma cells with some hepatoma, but did not contain normal liver cells.

Radioactivity of the free amino acids and protein-bound amino acids in the tissues

In Table 1 are shown the results of these determinations. The ratio of the free (F) to protein-bound (P) alanine and lysine in each tissue is also given. The differences between the F:P ratios for alanine and for lysine in liver, liver tumour and spleen are not significantly different but the F:P ratio for kidney lysine is significantly lower than that of kidney alanine.

Size of pools of free amino acids in the tissues

Owing to the small amount of free amino acid in the tissue extracts care was taken to ensure that the methods used in the isolation were essentially quantitative. It has already been shown that the ion-exchange fractionation used is quantitative for alanine and lysine (Hirs, Moore & Stein, 1954), and the losses on the paper chromatography of alanine were very small. Thus the amount of free amino acid isolated provides an approximate measure of the size of the pools of lysine and alanine in the various tissues. Since it has been shown that the amount of protein precipitated from the supernatant fraction of tissue homogenates with TCA is reproducible (Campbell, 1955), the concentration of free amino acid may be related either to the total amount of protein in the tissue extract or to the wet weight of the tissue. The results shown in Table 2 indicate that the relative concentration of lysine in liver,

Table 1. *Radioactivity of amino acids in the supernatant fraction of homogenates of rat tissues 1·5 hr. after injection of* [¹⁴C]*alanine and* [¹⁴C]*lysine-labelled serum albumin*

The radioactivity of the amino acids isolated from the protein precipitated with trichloroacetic acid is compared with that of the free amino acids in the extract. Radioactivities have been corrected to counts/min./cm.² at infinite thickness. F:P ratios are expressed as ±S.E.M. derived from the coefficient of variation of the radioactivity determinations of the protein and free amino acids.

	Lysine			Alanine		
	Protein amino acid (P)	Free amino acid (F)	Ratio, F:P	Protein amino acid (P)	Free amino acid (F)	Ratio, F:P
Liver	46	730	15·8±2·6	387	5851	15·1±2·1
Liver tumour	123	853	6·9±1·3	1201	7058	5·9±0·95
Spleen	114	363	3·2±0·43	706	2855	4·0±0·33
Kidney	194	275	1·4±0·13	1297	2802	2·2±0·15

Table 2. *Concentration of alanine and lysine in various tissues*

Results are expressed both as mg. of amino acid isolated/g. of protein precipitated from the supernatant of the tissue homogenate with trichloroacetic acid and as μg./g. of the tissue (wet wt.) from which the extract was prepared.

	Amount of free amino acid			
	Lysine monohydrochloride		Alanine	
Tissue	mg./g. of sol. protein	μg./g. wet wt.	mg./g. of sol. protein	μg./g. wet wt.
Liver	2·3	94	1·2	50
Liver tumour	3·1	162	2·4	122
Spleen	2·9	256	1·5	130
Kidney	6·0	320	3·2	173

Table 3. *Estimation of amount of blood in the tissues of the rat*

Results are expressed as percentage of blood in the wet tissue.

Tissue	Amount of blood (%)
Liver, perfused	1·1
Liver, non-perfused	3·0
Liver tumour (non-perfused)	1·3
Kidney	1·2

Table 4. *Serum albumin in the supernatant fraction of tissue homogenates*

	Concn. of albumin in extracts (μg./ml.)		
Tissue	Total	Due to blood	Albumin as % of total protein
Liver (perfused)	211	103	1·4
Liver tumour	840	58	9·9
Spleen	420	32*	4·1
Kidney	388	38	5·9

* Calculated on the assumption that the spleen contains 1% of whole blood.

liver tumour and kidney is similar irrespective of the method of comparison. The same is also true for alanine. However, this is not so for spleen. Whereas the lysine content of this tissue is 50% of that of kidney based on soluble protein, it is 80% based on tissue weight. Similarly, the alanine content of spleen is 50% of that of kidney based on soluble protein, but 76% based on tissue weight. The discrepancy for spleen may be connected with the change in the size and composition of this organ which accompanies the development of liver tumours in rats (Åkerfeldt, 1954).

Amount of blood in the tissue extracts

In order to account for the amount of albumin in the tissue extracts due to the presence of blood it was necessary to estimate the amount of blood in the extracts of various tissues. The method used for the estimation of blood depends on the determination of the amount of haemoglobin present in the extract, so that it is essential to haemolyse all the blood. Since the extracts obtained from the rat which had received radioactive amino acids were made in 0·9% NaCl the blood estimations were made on extracts prepared from tissues of a similar rat with 0·125% NaCl. The results of these estimations expressed as percentage of blood in the wet tissue are shown in Table 3. Since the liver of the rat which received the radioactive amino acids was perfused with saline, the results for a perfused and non-perfused liver are compared. The amount of blood in spleen could not be estimated by such methods since the amount of haemoglobin would not be expected to be a true reflexion of the amount of blood present in this tissue.

Amount of serum albumin in tissue extracts

It has previously been shown (Campbell & Stone, 1957) that in order to obtain reproducible results it is necessary to 'clear' tissue extracts with an antibody–antigen precipitate before estimating their albumin content. The chick albumin–antiserum reaction has been used for this purpose. In the present experiments it was necessary to show that the results obtained with such 'cleared' extracts represented the true level of rat-serum albumin in the tissue extracts. This was achieved by adding to samples of each of the four tissue extracts amounts of serum albumin that corresponded to approx. 50 and 100% of the amount of albumin contained in the extracts. A full recovery of the added albumin within the experimental error of the method was obtained.

It is therefore concluded that the albumin estimations on the tissue extracts represent the absolute amount of serum albumin in the extracts. Duplicate estimations were within 10% of the mean.

The amount of albumin in haemolysed rat blood was found to be 25·4 mg./ml. It was therefore possible to calculate the amount of albumin in the extracts which was due to the presence of blood.

Table 5. *Fractionation of serum proteins obtained from a rat bearing a liver tumour, 90 min. after injection of* [^{14}C]*alanine and* [^{14}C]*lysine-labelled albumin*

For details of the nature of the fractions see Figs. 1 and 2. Radioactivities are expressed as counts/min./cm.^{2} at infinite thickness. The amount of albumin in the fractions was determined by means of an antiserum.

Fraction	Nature	Amount (mg.)	Radioactivity	Albumin (%)
A	Albumin + α-globulin	42	91	—
A′	Purified albumin	31	125	76
B	β-Globulin	14	21	3·5
C	γ-Globulin	14	22	3·0
D	Albumin + unknown	9	51	—
E	Albumin + α-globulin	22	77	20

The results are shown in Table 4, together with the amount of albumin in the extracts expressed as a percentage of the total protein precipitated with TCA. The results were corrected to allow for differences in the amount of N in the albumin preparations used as a standard and in the TCA protein precipitates. It will be seen that, except for the liver extract, which has a very low albumin content, the contribution of the blood to the total albumin in the tissue extracts is very small. The low level in liver may be associated with the fact that it was the only organ to be effectively perfused. It must be concluded that large amounts of serum albumin are 'stored' in the tissues. This has been shown with rabbit skin by Humphrey, Neuberger & Perkins (1956).

Fractionation of serum proteins

The sample of serum protein (116 mg.) obtained from the rat bearing liver tumours was first fractionated with $(NH_4)_2SO_4$. The two fractions obtained were further purified by zone electrophoresis. Figs. 1 and 2 show the results obtained and the way in which the fractions were subdivided. The yields of the freeze-dried protein fractions are shown in Table 5. Fraction A was further purified on another zone-electrophoresis column to remove small amounts of a second component. The conditions were exactly the same as those used in the first run, the fractions containing the peak of the protein being collected. After dialysis and freeze-drying, this fraction was designated A′. It will be seen from Table 5 that this procedure raised the radioactivity of the fraction from 91 to 125 counts/min.

The nature of fraction E, which was the fastest-running component in the fraction precipitated with 0·5 saturated $(NH_4)_2SO_4$, was further investigated by immuno-electrophoresis. As was previously shown (Campbell & Stone, 1957), a small amount of α-globulin in the preparation of rat-serum albumin may be detected by this method. The components of fraction E which reacted with the antiserum proved to be identical with those present in the purified albumin.

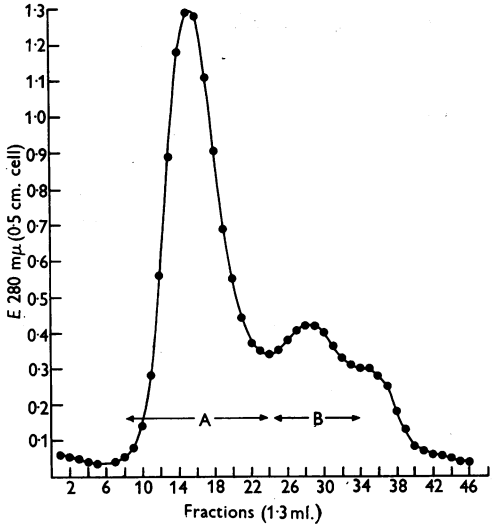

Fig. 1. Zone electrophoresis of rat-serum proteins obtained from whole serum after removal of the precipitate formed with 0·5 saturated $(NH_4)_2SO_4$. Protein (55 mg.) was added to the column (46 cm. × 1·5 cm.) packed with cellulose. A current of 10 mA (500 v) was applied for 24 hr. Borate–phosphate buffer (see Materials and Methods) was used.

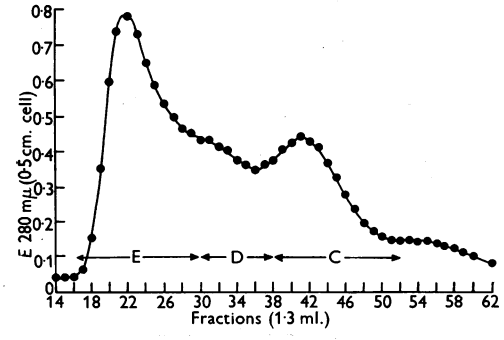

Fig. 2. Zone electrophoresis of rat-serum proteins obtained from whole serum by precipitation with 0·5 saturated $(NH_4)_2SO_4$. Protein (45 mg.) was run under the same conditions as described in Fig. 2.

The identity of the protein fractions was studied by electrophoresis on paper in veronal buffer, pH 8·6 (Flynn & de Mayo, 1951). An indication of the major protein constituents of the various fractions is given in Table 5. The serum proteins from rats bearing liver tumours differ from those of normal rats. A more detailed study of this aspect of the work will be published later.

The results of the determination of the amount of albumin in the protein fractions is also given in Table 5. The high radioactivity of fractions D and E compared with B and C is no doubt due to the presence of albumin in these fractions.

Radioactivity of alanine and lysine from serum-protein fractions

The results of these determinations are shown in Table 6. As expected, the radioactivity of the lysine from the albumin fraction greatly exceeded that from the fractions containing β- and γ-globulin. In view of this, even a small amount of albumin in these fractions has a great effect on the radioactivity of the lysine derived from them. The lysine values for fractions B and C are shown in the table before and after allowing for the presence of lysine due to albumin contamination.

DISCUSSION

Yuile, Lamson, Miller & Whipple (1951) were the first to suggest that plasma protein could be converted into tissue protein without first being degraded to free amino acids. They suggested that the plasma protein was broken down intracellularly, and that since the amount of free ^{14}C-labelled amino acid reaching the circulation was very small the degradation did not reach the amino acid level. That injected plasma proteins are able to enter a wide variety of cells without prior degradation was shown by Coons, Leduc & Kaplan (1951) using fluorescent antibody. However, Loftfield & Harris (1956) have shown that a considerable proportion of the amino acids required for tissue-protein synthesis is supplied by the degradation of protein intracellularly, and that the amino acid released does not equilibrate with the circulating amino acids. Thus the results of Yuile *et al.* (1951) may be explained without postulating the interconversion of proteins at a polypeptide level.

Babson & Winnick (1954) injected rats bearing an implanted Walker carcinoma with [^{14}C]leucine-labelled plasma protein and determined the radioactivity of the protein from the tumour, kidney and liver after 2 hr. As a control they injected a similar group of rats with free [^{14}C]leucine. They observed that, compared with the kidney and liver, the tumour incorporated radioactivity from the plasma protein more effectively. Busch & Greene (1955) have obtained very similar results with rats implanted with the Walker carcinoma and with the Jensen sarcoma. These authors discuss the possibility that the results are due to the presence of unchanged radioactive plasma protein in the tumour protein but consider this an unlikely explanation.

Babson & Winnick (1954) also carried out experiments in which they studied the effect on the radioactivity of the tissue proteins of injecting large amounts of inactive leucine with the [^{14}C]leucine-labelled plasma protein. They showed that whereas in the control experiments, in which free [^{14}C]-leucine was injected, the injection of inactive leucine reduced the radioactivity of the tissue proteins, there was no such reduction when the labelled plasma protein was injected. They concluded that the Walker carcinoma and liver and kidney utilized plasma protein without extensive release of leucine in the free form. However, in view of the results of Loftfield & Harris (1956) already referred to, further experiments seem to be necessary before such a conclusion could be accepted.

Campbell & Halliday (1957) have suggested that after the injection of a radioactive amino acid into an animal the ratio of the radioactivity of the free amino acid (F) within a given tissue to that of the same amino acid in the tissue proteins (P) is a measure of the rate of synthesis of the proteins in the tissue. The lower the ratio the greater the rate of protein synthesis, provided that F > P. They further postulated that if the proteins concerned are synthesized from free amino acids, i.e. the entry of each species of amino acid into the protein molecule occurs at the same rate, then the radioactivity ratio F:P should be the same, irrespective of the nature of the amino acid, for a particular tissue at a given time after injection. However, this is only so if the time–radioactivity curves of the different amino acids are similar. If there is a delay in the peak of the time–activity curve for one amino acid compared with another, for example, in the experiments cited where lysine was injected and alanine was formed from glucose, then the F:P

Table 6. *Radioactivity of alanine and lysine obtained from fractions of rat-serum protein*

The rat had received an injection of [^{14}C]alanine and [^{14}C]lysine-labelled albumin 1·5 hr. previously. For designation of fractions see Table 5. Radioactivity is expressed as counts/min./mg. of free amino acid. The effect of the presence of albumin in fractions B and C is shown by the corrected values for lysine in the fractions.

Fraction	Lysine monohydrochloride (uncorrected)	Lysine monohydrochloride (corrected)	Alanine
A′ (albumin)	4692	—	1168
B (β-globulin)	400	244	1496
C (γ-globulin)	335	200	1800

ratio of the one amino acid (alanine) will be higher than that of the other (lysine). A similar situation arises in the present experiments where alanine was injected and lysine was derived through the slow breakdown of albumin.

Before discussing the significance of the results obtained from the determination of the F:P ratio for alanine and lysine in the various tissues as shown in Table 1, the effect on these ratios of the presence of unchanged radioactive serum albumin must be considered.

The importance of the effect of the presence of albumin in the tissues for the present studies arises from the much greater radioactivity of the lysine in the circulating albumin than in the tissue proteins. Thus the radioactivity of the lysine in the albumin obtained from the serum at the time of death was 2325 counts/min. at infinite thickness, which is far in excess of that of the lysine from the various tissue proteins (see Table 1). A similar situation does not arise for alanine. As may be seen from Table 4, the amount of serum albumin in the soluble tissue proteins varies considerably for the different tissues. Three reasons for the presence of serum albumin in the tissues must be considered: first there is albumin due to the presence of blood, secondly that due to the synthesis of serum albumin in the tissue and thirdly that due to 'storage'.

Owing to the small amount of serum albumin in the tissue extracts and the difficulty of obtaining it in a pure state, it was not possible to estimate the radioactivity of the lysine in the albumin present in such extracts. However, the radioactivity of the lysine of the albumin present in the circulating plasma was determined. Hence the effect of the presence of the 'blood' albumin in the tissue extracts on the radioactivity of the protein lysine may be calculated. The results of these calculations, showing their effect on the lysine F:P ratios for the four different tissues, are shown in Table 7. In each the effect is to raise the ratio; this is especially noticeable with liver.

Since the radioactivity of alanine and lysine in the serum albumin in the tissues arising as a result of protein synthesis is likely to be of the same order as that of the mixed tissue proteins, albumin originating in this way need not be considered. It is much more difficult to evaluate the influence of the albumin 'stored' in the tissues. The results of Humphrey *et al.* (1956) on the albumin present in rabbit's skin suggest that such albumin is metabolically relatively inactive. If this is so then 'stored' albumin will have little effect on the lysine radioactivity. The greater the equilibration of such stored albumin with the blood the greater would be its contribution to the radioactivity of the tissue lysine, and hence the corrected F:P ratio would show a corresponding rise.

Table 7. *Effect of blood in the tissue extracts on the radioactivity of the protein lysine*

Results are expressed as in Table 1.

Tissue	Whole protein	Corrected for presence of blood	Corrected F:P ratio
Liver	46	30	24±4·0
Liver tumour	123	107	8·0±1·5
Spleen	114	101	3·6±0·5
Kidney	194	181	1·5±0·13

Table 8. *Comparison of the radioactivity of the free lysine in the tissues 1·5 hr. after injection of [^{14}C]lysine-labelled serum albumin and 1 hr. after injection of [^{14}C]lysine into each rat*

Radioactivity is expressed as counts/min. cm.^{2} at infinite thickness.

Tissue	Radioactivity of lysine monohydrochloride: Labelled protein injected	Free amino acid injected	Amino acid / Protein
Liver	730	5 978	8·2
Liver tumour	853	10 300	12·1
Kidney	275	2 900	10·6

The general effect therefore of the serum albumin in the tissues is to increase the radioactivity of the tissue protein lysine so that the F:P ratios for lysine in Table 1 are too low. Thus the F:P ratios for lysine will tend to be greater than those for alanine. As has been explained, this is to be expected if the peak of the time–activity curve of free [^{14}C]lysine is delayed relative to that of [^{14}C]alanine. If, on the other hand, [^{14}C]lysine were to be derived from the labelled albumin in 'peptide' units, then the effect would be to raise the radioactivity of the lysine in the tissue proteins and so lower the lysine F:P ratios. Owing to the very high radioactivity of the lysine in the albumin compared with that in the tissue, any significant incorporation of lysine derived from the partial degradation of the labelled albumin would have a marked effect on the lysine F:P ratios. Although no firm conclusion can be drawn from these experiments there is no evidence to suggest that partial degradation products of serum albumin are an important source of precursors for the synthesis of tissue proteins. Furthermore, there is no evidence that liver tumour differs from liver in this respect.

The possibility that amino acids derived from serum albumin are preferentially utilized by some tissues for the synthesis of proteins, as is suggested by the results of Busch & Greene (1955), must now be examined. Any such effect would be expected to be reflected in the relative radioactivity of the free amino acids in the tissues. In Table 8 the radio-

activity of lysine in liver, liver tumour and kidney is compared after injection of [^{14}C]lysine-labelled albumin with similar results obtained after injection of free [^{14}C]lysine (Campbell & Halliday, 1957). Although conclusions drawn from such a comparison must be treated with reserve, since the experiments were conducted in different animals and the animals were killed after slightly different times, the results do not indicate any marked preferential utilization of albumin by one tissue over another. However, further experiments are necessary before this possibility can be excluded. It is not possible to draw any valid conclusions from a comparison of the radioactivity of free alanine and lysine in the different tissues (Table 1), for the amount of these amino acids in the tissues varies (Table 2), and alanine is metabolically much more active than lysine.

It is now necessary to discuss the results of the determination of the radioactivity of the lysine and alanine present in the serum proteins. Simpson & Velick (1954) and Heimberg & Velick (1954) have shown that the three rabbit muscle enzymes, phosphorylase, aldolase and glyceraldehyde-3-phosphate dehydrogenase, are synthesized from the same amino acid pool. If this result is capable of general application then plasma proteins may be expected to be synthesized from the same amino acid pool as the soluble proteins in the tissues. In this case, if plasma proteins, like tissue proteins, are synthesized from free amino acids, the ratio of the radioactivity of alanine and lysine after the injection of [^{14}C]lysine and [^{14}C]alanine would be expected to be the same as that of the soluble proteins of the tissue(s) in which the plasma proteins were synthesized.

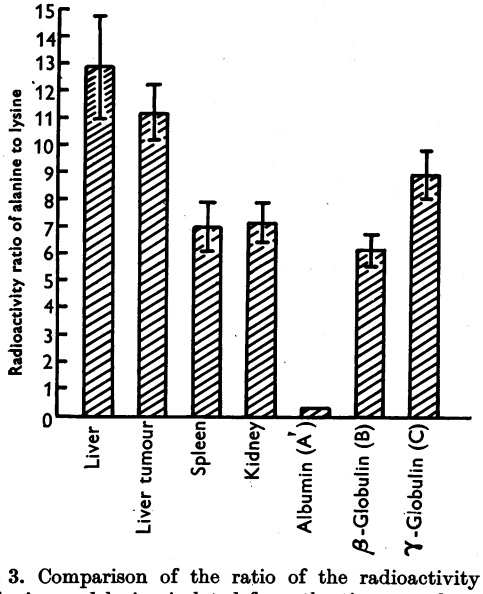

Fig. 3. Comparison of the ratio of the radioactivity of alanine and lysine isolated from the tissues and serum proteins of a rat 1·5 hr. after injection of [^{14}C]alanine and [^{14}C]lysine-labelled albumin. The results are expressed as ±S.E.M. derived from the coefficient of variation of the radioactivity determinations on each amino acid. Allowance has been made for the effect of albumin in the tissue extracts due to the presence of blood, and for albumin in the globulin fractions.

Whereas the synthesis of serum albumin appears to be confined to the liver (Miller, Bly, Watson & Bale, 1951), the synthesis of the other serum proteins takes place in a variety of tissues including the liver (Miller & Bale, 1954; Miller, Bly & Bale, 1954; Askonas & White, 1956). It is probable that the tissues studied in the present experiments (liver, kidney and spleen) are representative of the tissues in which the serum globulins are synthesized. Fig. 3 shows a comparison of the radioactivity of the alanine and lysine isolated from the tissue extracts and the serum-protein fractions. Allowance has been made for the effect of albumin due to the presence of blood in the tissue extracts and for serum albumin in the globulin fractions. Although the ratio for the β-globulin fraction is a little lower than that for the tissue proteins, the difference is not significant. The ratio for the γ-globulin fraction is well within the range for the tissue proteins. As may be seen from the very low ratio for albumin, any extensive conversion of albumin into globulin through partial degradation products would have been reflected in a marked lowering of the globulin ratios.

Maurer & Müller (1955) injected a rat with serum albumin labelled with ^{35}S in the sulphur amino acids. The rats were bled 4 and 25 days after this and the plasma proteins fractionated by paper electrophoresis. They calculated that the radioactivity of the globulins greatly exceeded that to be expected if the radioactive amino acids derived from the serum albumin had equilibrated with the circulating amino acids before being incorporated into the globulins. They therefore postulated that serum albumin was converted into globulin without complete degradation. As has been shown, this reasoning is based on an unwarranted assumption regarding the degree of equilibration of amino acids, and the results of the present experiments do not support the view that serum albumin can be converted into globulin at other than an amino acid level.

SUMMARY

1. A rat bearing liver tumours induced by feeding 4-dimethylaminoazobenzene was intravenously injected with a solution containing [^{14}C]alanine and [^{14}C]lysine-labelled rat-serum albumin. The rat was killed 90 min. after the injection.

2. The liver, liver tumour, kidney and spleen were homogenized and soluble extracts of the tissues were obtained. Samples of the extracts were treated with trichloroacetic acid and the radioactivity of the lysine and alanine in hydrolysates of the precipitated proteins was determined. The radioactivity of the alanine and lysine occurring free in the supernatants of the trichloroacetic acid precipitates was also determined.

3. The amount of serum albumin in the tissue extracts was determined by means of an antiserum. From estimations of the amount of blood in the extracts it could be shown that the total amount of albumin in the extracts greatly exceeded that due to the presence of blood.

4. The plasma proteins obtained from the rat at death were fractionated by zone electrophoresis. Fractions containing albumin plus α-globulin, β-globulin and γ-globulin were obtained. The radioactivity of the alanine and lysine in hydrolysates of each fraction was determined.

5. From the ratio of the radioactivity of the free amino acids in the tissues to that of the amino acids in the tissue proteins it is concluded that there is no evidence that serum albumin is utilized for the synthesis of soluble tissue proteins without first being broken down to free amino acids. In this respect liver tumour did not appear to differ from the other tissues studied.

6. No evidence could be found for the interconversion of serum albumin and serum globulin at other than an amino acid level.

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The Isolation and Properties of a Fragment of Bovine-Serum Albumin which Retains the Ability to Combine with Rabbit Antiserum

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The structural basis of the biological activity of proteins is a problem which is arousing growing interest as knowledge of protein structure increases. One approach (Porter, 1953) is to hydrolyse a protein under conditions in which the biological activity survives and then isolate the fragment responsible for activity, thus reducing the structural work necessary to pinpoint the features essential for activity. Difficulty has always been met in isolating the active fragment, but retention of activity after partial hydrolysis has been demonstrated in a sufficient number of cases to establish that the whole protein molecule is not necessarily essential for activity.