The Isolation of 16 α -Hydroxydehydroepiandrosterone (3 β :16 α -Dihydroxyandrost-5-en-17-one) from the Urine of Normal Men

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The possibility that certain types of urinary steroid conjugates might be selectively hydrolysed by boiling the urine at a neutral pH was first suggested by the work of Speirs, Wragg, Bonner & Homburger (1951), in which it was shown that chloroform-extractable material, active in the mouse-eosinophil test of Speirs & Meyer (1951), could be liberated in urines by this method from patients treated with adrenocorticotrophic hormone. Further attention was drawn to this possibility by the demonstration of Teich, Rogers, Lieberman, Engel & Davies (1953) that sodium dehydroepiandrosterone (3 β -hydroxyandrost-5-en-17-one) sulphate can be hydrolysed in neutral aqueous solution with the production of *i*-androstanolone (6 β -hydroxy-3:5-cycloandrostan-17-one) by continuous extraction with benzene at an elevated temperature (see also Dingemans, Huis in't Veld & Hartogh-Katz, 1952).

In following up this possibility the present authors found (unpublished observations) that the amounts of formaldehydogenic substances extracted by chloroform from urines of normal men could be increased two- to three-fold by boiling the urines at pH 7 for 30 min. before extraction. The original object of the present work was the isolation and identification of the formaldehydogenic substances liberated in this way. This object has not been achieved, but the work has led to the isolation and characterization of a C₁₉-steroid, not hitherto known to occur naturally, which has been shown to be a metabolic product of dehydroepiandrosterone.

RESULTS

Paper-chromatographic investigation of chloroform-extractable reducing substances formed in urine by boiling

Detection of substances X and Y. The possibility was considered at the outset that the chloroform-extractable formaldehydogenic substances liberated by boiling of neutral urine might be C₂₁ α -ketolic 'reducing' steroids. Accordingly, it was decided to examine chloroform extracts of boiled urine by paper-chromatographic methods using

'blue tetrazolium' [2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride] for the detection of reducing substances.

Samples of urine from normal men, a few of which were pre-extracted with chloroform, were boiled for 60 min. and extracted with chloroform. In some of the earlier experiments the urine was adjusted to pH 7.0 before boiling, but in later ones this was not done. The extracts were purified by the procedures described in detail below, and then chromatographed on paper.

The chromatograms of the extracts of the boiled urines, whether pre-extracted with chloroform or not, invariably showed the presence of two blue-tetrazolium-reducing spots, that with the higher R_F (substance X) being more intense than the second spot (substance Y). The R_F values for these substances in the solvent systems used compared with the R_F value of standard compound 17 α :21-dihydroxypregn-4-ene-3:20-dione (11-deoxycortisol) are shown in the table. The presence of these two reducing substances was in no instance detected on the chromatograms of extracts obtained from unboiled urines.

Conversion of X into substance Xa by treatment with acid: preliminary observations on the natures of X and Xa

Extracts of boiled urine were separated into non-ketonic and ketonic fractions by reaction with Girard's reagent T at room temperature in methanolic acetic acid solution. Paper chromatograms on the non-ketonic fractions showed no blue-tetrazolium-reducing spots, while those on the ketonic fractions showed a single spot with an R_F of about 0.28 in system B₂ (Bush, 1952) which was considerably more intense than the spot due to substance Y in the unfractionated extracts. These findings

Table 1

Substance	R_F value in system B ₂ of Bush (1952)	R_F value in modified system B ₂ of Bush (1952)
X	0.48	0.70
Y	0.28	0.57
11-Deoxycortisol	0.37	0.66

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suggested the possibility that, under the acid conditions of the Girard reaction, X had become transformed into another substance (Xa) which resembled Y in its behaviour on chromatography. Proof that this was the case was obtained when it was found that X, after purification by paper chromatography, became transformed into Xa (R_f 0.29 in system B₂) by allowing it to stand for 60 min. at room temperature in 0.1N-H₂SO₄ in 10% (v/v) methanol in water. Since the R_f of chromatographically purified Y was unaffected by this treatment it seems probable that Xa and Y may be identical, but no rigid proof that this is so has been obtained.

The liberation of X from a chloroform-insoluble precursor in urine by boiling and the transformation by acid treatment of X into Xa (with higher R_f) appeared to be strikingly analogous to the hydrolysis of sodium dehydroepiandrosterone sulphate by boiling in neutral aqueous solution with the formation of *i*-androstanolone and the transformation of the latter into dehydroepiandrosterone by acid (Teich *et al.* 1953). Accordingly, the working hypothesis was adopted that Xa might be a steroid of the 5-en-3 β -ol class, that X might be the corresponding 6 β -hydroxy-3:5-*cyclo* compound, and that the parent substance present in urine might be the 3-sulphate of Xa. Support for this hypothesis was provided by the finding that chromatographically purified Xa, but not X, was precipitated by digitonin. Furthermore, Xa gave a positive reaction in the Pettenkofer test as modified by Munson, Jones, McCall & Gallagher (1948).

Isolation and identification of Xa

At this stage of the work it was still assumed that Xa, because of its blue-tetrazolium-reducing power, must be a C₂₁ steroid with an α -ketolic side chain. On the basis of the evidence outlined in the preceding section and in view of the chromatographic behaviour of the compound it was considered that it might be a monohydroxy or a monooxo substitution product of 3 β :21-dihydroxypregn-5-en-20-one. However, owing to the difficulty of obtaining reference samples of more than a few steroids of this class for comparison by paper chromatography it was decided that there was no satisfactory alternative to isolation and characterization of Xa by orthodox methods.

Concentrates rich in X were obtained from chloroform extracts of boiled urine from normal men by conventional solvent-partition procedures followed by adsorption chromatography on magnesium trisilicate-Celite columns. Ketonic fractions obtained from such concentrates by means of the Girard reaction carried out at room temperature were then subjected to partition chromatography on Celite

columns. From the fractions so obtained which were found to contain Xa by paper chromatography crystalline material in a yield of 5–15 mg./100 l. of urine was obtained.

The crystalline substance, when applied to paper and sprayed with blue tetrazolium, was found to have only about one-tenth of the reducing power of 11-deoxycortisol, and since elementary analyses indicated the probable formula C₁₉H₂₈O₃ it was thought at first that the substance might be a non-reducing C₁₉ steroid containing about 10% of Xa as impurity. However, when it was found that the reducing power remained undiminished after repeated recrystallization, that the substance yielded a diacetate which showed reducing power after recrystallization, and that oxidation with periodic acid yielded no significant amount of formaldehyde, it was concluded that the isolated substance was identical with Xa and must be a weakly reducing C₁₉ steroid with an α -ketol group in rings C or D. In this connexion it should be pointed out that when the blue-tetrazolium test was carried out in solution according to Mader & Buck (1952) Xa was found to have 42% of the reducing power of 11-deoxycortisol.

The most likely possibilities were that Xa was either a 16-hydroxy-17-oxo steroid or a 17-hydroxy-16-oxo steroid. Of these two possibilities the second seemed to be excluded by reason of the fact that Xa had $[\alpha]_D + 12^\circ$ (ethanol), since all 17-hydroxy-16-oxo steroids so far examined have been found to be laevorotatory. Support for the view that Xa was a 16-hydroxy-17-oxo steroid rather than a 17-hydroxy-16-oxo steroid was obtained when it was found that whereas 16-oxo-oestradiol-17 β (3:17 β -dihydroxyoestra-1:3:5-trien-16-one) had the same reducing power as 11-deoxycortisol in the blue-tetrazolium test of Mader & Buck (1952) 16 α -hydroxyandrost-4-ene-3:17-dione (Fried, Thoma, Perlman, Herz & Borman, 1955) had a considerably weaker reducing power.

All the available evidence therefore indicated that Xa was probably either 3 β :16 α -dihydroxyandrost-5-en-17-one or 3 β :16 β -dihydroxyandrost-5-en-17-one. Evidence for the presence of a double bond was obtained by reduction in acetic acid solution with hydrogen and a palladium-black catalyst when Xa took up 0.97 molar equivalent of hydrogen. When a platinum oxide catalyst was used the hydrogen uptake was 1.94 molar equivalents.

Since catalytic hydrogenation of 17-oxo steroids gives predominantly the corresponding 17 β -hydroxy compounds (cf. Ruzicka, Goldberg & Meyer, 1935; Sarett, 1947) it was assumed that the fully reduced product yielded by Xa would be either 5 α -androstane-3 β :16 α :17 β -triol or 5 α -androstane-3 β :16 β :17 β -triol. However the possibility could not be ignored that reduction of a 17-oxo

group in Xa might give, in addition, an appreciable amount of the corresponding 17α -hydroxy isomer in either case. Accordingly, the product was treated with an acetone solution of hydrogen chloride to convert the $3\beta:16\alpha:17\alpha$ -triol or $3\beta:16\beta:17\beta$ -triol, which might be present as minor or major components respectively of the reduction product, into the acetone derivative. Fractionation of the reaction product by leaching with benzene showed that the major component was benzene-insoluble and was therefore assumed to be material which had not undergone formation of the acetone derivative. This material yielded an acetate, m.p. $172-174.5^\circ$, and the melting point was not depressed after admixture with authentic 5α -androstane- $3\beta:16\alpha:17\beta$ -triol triacetate. Furthermore, the infrared spectra of the acetate of the Xa reduction product and 5α -androstane- $3\beta:16\alpha:17\beta$ -triol triacetate were identical. Since the possibility of Xa containing a 16 -oxo group had been excluded it follows that the identification of the main component of the Xa reduction product as 5α -androstane- $3\beta:16\alpha:17\beta$ -triol provides proof that Xa is $3\beta:16\alpha$ -dihydroxyandrost-5-en-17-one.

Urinary excretion of X and Y after the administration of dehydroepiandrosterone to a human subject

The subject was a woman, aged 45 years, with carcinoma of the breast who had been treated by bilateral ovariectomy and bilateral adrenalectomy. She was being maintained on 25 mg. of cortisone acetate/day and occasional doses of deoxycorticosterone trimethylacetate.

Complete 24 hr. urine specimens were collected during a 2-day control period. Dehydroepiandrosterone (100 mg.) was then administered by intramuscular injection, and the urine collections continued for 4 further days. Portions of each urine specimen were boiled and extracted as described under Experimental, and the extracts examined by paper chromatography for the presence of substances X and Y.

Neither X nor Y was detected in the extracts of the control urines, but both were found to be present in those of the urines collected on the day of the injection and on the following day. On the subsequent 2 days neither X nor Y could be detected in the extracts. In view of these findings there can be no doubt that both X and Y were produced as metabolic products of the administered dehydroepiandrosterone.

As far as could be judged by visual inspection of the intensities of the spots on the chromatograms it is considered that about 2% of the administered dehydroepiandrosterone was excreted in the urine as X and Y.

EXPERIMENTAL

Methods

Preparation of urine extracts for paper chromatography. Each 24 hr. urine specimen was heated to boiling for 1 hr., cooled and then extracted twice with 2 vol. of CHCl_3 . In the earlier experiments the pH of each urine specimen was adjusted to pH 7.0 before boiling, but in all subsequent ones the urine was boiled without pH adjustment. In a few experiments the urine specimen was pre-extracted with 2 vol. of CHCl_3 before boiling.

The combined CHCl_3 extracts were washed twice with 0.1 vol. of 0.1N-NaOH and twice with 0.1 vol. of water, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue thus obtained was dissolved in 14 ml. of methanol, and the solution diluted with 6 ml. of water and extracted once with 20 ml. of hexane. The hexane extract was back-extracted with 20 ml. of 70% (v/v) aqueous methanol, and the combined aqueous methanol phases were evaporated under reduced pressure to about 10 ml. After dilution to about 30 ml. with water the aqueous residue was extracted once with 50 ml. of ether, and the extract dried over anhydrous Na_2SO_4 and evaporated to dryness.

Paper-chromatographic methods. The solvent systems used were system B_2 (Bush, 1952) and a modification of the Bush (1952) system B_2 (benzene-methanol-water; 10:7:3, by vol.). Amounts of the extracts, each corresponding to one-quarter of a 24 hr. urine specimen, were applied to Whatman no. 42 paper, and, after equilibration in the tanks overnight at $18 \pm 2^\circ$, the chromatograms were developed for 6-8 hr. The papers were sprayed with a 0.066% solution of blue tetrazolium in 3.3% (w/v) NaOH.

Digitonin precipitation. This was carried out as described by Butt, Henly & Morris (1948), with the modification that the reaction mixture was heated under reflux for 30 min. and the solvent then removed under reduced pressure before the ether extraction was carried out.

Miscellaneous. Melting points were determined on a microscope hot-stage and are uncorrected for emergent stem.

Samples for C and H analysis and for optical-rotation measurements were dried to constant weight at 80° *in vacuo* over P_2O_5 .

The solvents and Celite (Celite 535, Johns-Manville Co. Ltd., London) used were purified by the methods employed by Watson & Marrian (1956). Magnesium trisilicate (British Drug Houses Ltd.) was activated by drying overnight at 110° . Celite columns for partition chromatography were prepared as described by Bauld (1955).

Isolation of Xa from urine

In a typical experiment urine from normal men was collected in batches of about 20 l. without preservative. Each batch was boiled for 1 hr. without previous adjustment of pH, and extracted four times with one-tenth volumes of CHCl_3 . The subsequent treatment of the extracts was as described above under *Preparation of urine extracts for paper chromatography*, the appropriate volume corrections being made for the solvent-partition procedures. The residues obtained were stored dry at -20° until 250 l. of urine had been processed.

The combined residues were chromatographed on a column (30 cm. $\times$ 2.5 cm.) containing 60 g. of a mixture of

equal parts by weight of magnesium trisilicate and Celite. The column was prepared in light petroleum (boiling range 80–100°), and was eluted with 800 ml. of a mixture of light petroleum and ethanol, the ethanol concentration being increased in a step-wise discontinuous gradient from 0 to 5% (v/v). Successive 40 ml. fractions of eluate were collected, evaporated to dryness, and one two-hundredth of each residue was chromatographed on paper. Those fractions found to contain X were combined, yielding 550 mg. of gummy residue.

The residue was dissolved in 55 ml. of a mixture of equal volumes of acetic acid and 90% (v/v) methanol in water, and 5.5 g. of Girard's reagent T was added. After the solution had stood overnight at room temperature 10 vol. of ice-cold water and 3 vol. of 2.5 N-NaOH were added, and the non-ketonic fraction was removed by extraction six times with equal volumes of CHCl_3 . The aqueous phase was acidified to pH 1 with H_2SO_4 and extracted during 2 hr. six times with equal volumes of CHCl_3 . The combined extracts containing the ketonic fraction were washed with 0.1 N-NaOH solution and water, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure, yielding a residue of 160 mg.

The ketonic fraction was chromatographed on a column of 60 g. of Celite, with the partition-solvent system methanol-water (80%, v/v)-benzene. Successive 40 ml. fractions of eluate were evaporated to dryness and examined for the presence of Xa by paper chromatography. Those found to contain Xa were combined, yielding 50 mg. of a slightly pigmented semicrystalline solid.

After crystallization three times at -20° from methanol 25 mg. of a white crystalline product, m.p. $177-181^\circ$ (slight yellowing) and $[\alpha]_D^{25} +12^\circ$ (c, 0.478% in ethanol), was obtained (Found: C, 75.1; H, 8.9. Calc. for $\text{C}_{19}\text{H}_{28}\text{O}_3$: C, 75.0; H, 8.9%).

In the blue-tetrazolium reaction of Mader & Buck (1952) the product showed 42% of the reducing power of 11-deoxycortisol, and in the modified Pettenkofer reaction of Munson *et al.* (1948) it gave a blue colour having 65% of the intensity of that given by an equal weight of dehydroepiandrosterone. On dissolving 100 μg . of Xa in conc. H_2SO_4 (4 ml.) an immediate mauve colour with green fluorescence was produced, and after standing at 24° for 2 hr. the solution showed absorption maxima at the following wavelengths, the $E_{1\text{cm}}^{1\%}$ values being given in parentheses: 271 $\text{m}\mu$ (196), 378 $\text{m}\mu$ (315), 400 $\text{m}\mu$ (309), 560 $\text{m}\mu$ (245) and minima at 250 $\text{m}\mu$ (175), 315 $\text{m}\mu$ (163), 390 $\text{m}\mu$ (304), 458 $\text{m}\mu$ (186).

Acetylation of Xa with acetic anhydride and pyridine at room temperature overnight yielded a product which, after crystallization three times from methanol, had m.p. $166-168^\circ$ and $[\alpha]_D^{25} +20^\circ$ (c, 0.494% in ethanol) (Found: C, 70.9; H, 8.5. Calc. for $\text{C}_{23}\text{H}_{32}\text{O}_5$: C, 71.1; H, 8.3%). Subsequently it was found that this m.p. was not depressed after admixture of the acetate with authentic 3β :16 α -dihydroxy-androst-5-en-17-one diacetate, m.p. $164-167^\circ$ (Cooley, Ellis, Hartley & Petrow, 1955).

Characterization of Xa

Hydrogenation with palladium-black catalyst. Xa (10.021 mg.) in 4 ml. of acetic acid was shaken with 1 mg. of Pd black in an atmosphere of H_2 in a Towers microhydrogenation apparatus. After 1 hr. the uptake of H_2 stopped, when 0.97 molar equivalent had been used. The product was recovered by adding an excess of CHCl_3 to the

reaction mixture, washing with Na_2CO_3 solution and water, drying over anhydrous Na_2SO_4 and evaporating to dryness.

Hydrogenation with platinum oxide catalyst. Xa (9.854 mg.) in 4 ml. of acetic acid was hydrogenated, 1 mg. of Adams platinum oxide catalyst being used. The uptake of H_2 stopped after 1 hr., when 1.94 molar equivalents had been used. The product was recovered from the reaction mixture as described above.

Identification of the main component of the reduction product of Xa as 5 α -androstane-3 β :16 α :17 β -triol. The partially reduced product from the Pd-black hydrogenation was rehydrogenated by using platinum oxide as catalyst, and the two specimens of fully reduced Xa were combined. This material (16.7 mg.) was treated with 3 ml. of anhydrous acetone saturated with HCl, and the solution allowed to stand at room temperature for 30 min. Ice-cold water (10 vol.) was then added and the insoluble precipitate filtered off, washed thoroughly with water, and dried *in vacuo* over CaCl_2 . The product was then repeatedly leached with small volumes of benzene until there was no further decrease in weight.

The benzene-insoluble fraction (9.0 mg.) was acetylated with acetic anhydride and anhydrous pyridine at room temperature, and the product crystallized twice from aqueous methanol. It had m.p. $172-174.5^\circ$, and when mixed with authentic 5 α -androstane-3 β :16 α :17 β -triol triacetate (m.p. $172-174^\circ$) the m.p. was unchanged.

Preparation of authentic androstane-3 β :16 α :17 β -triol

Androst-5-ene-3 β :16 α :17 β -triol. 3 β :17 β -Dihydroxyandrost-5-en-16-one was prepared from dehydroepiandrosterone by the method of Stodola, Kendall & McKenzie (1941), and reduced with Na amalgam in ethanol as described by Huffman & Lott (1949) for the corresponding 3 β -methoxy derivative. The product (210 mg.) was subjected to a Girard reaction at room temperature and the non-ketonic fraction (105 mg.) dissolved in 20 ml. of anhydrous acetone. To the solution was added 3 ml. of acetone saturated with HCl, and after the mixture had been swirled continuously for 15 min. the acetone was removed at room temperature under a stream of air. The product was leached with benzene until no further decrease in weight occurred, to remove the acetone derivative of androst-5-ene-3 β :16 β :17 β -triol. The benzene-insoluble fraction (39 mg.) was crystallized twice from ethanol to give androst-5-ene-3 β :16 α :17 β -triol, m.p. $266-270^\circ$ (triacetate, m.p. $187-189^\circ$).

Androstane-3 β :16 α :17 β -triol. The androstenetriol was hydrogenated in acetic acid solution with a platinum oxide catalyst. The product was crystallized from methanol when it had m.p. $251-253^\circ$ and $[\alpha]_D^{25} 0.0^\circ$ (c, 0.495% in ethanol). Marrian & Butler (1944) give m.p. $257.5-259^\circ$ and $[\alpha]_D^{25} 0.0^\circ$ (in methanol). Acetylation yielded the triacetate, which was crystallized from aqueous methanol and had m.p. $172-174^\circ$. Marrian & Butler (1944) give m.p. $174-176^\circ$.

DISCUSSION

The most interesting aspect of the isolation of 16 α -hydroxydehydroepiandrosterone from urine is the probability that this compound may be the intermediate in the metabolic 'hydration' of dehydroepiandrosterone to androst-5-ene-3 β :16 α :17 β -triol.

Fig. 1. Comparison of the hypothetical scheme proposed for the metabolism in man of dehydroepiandrosterone and oestrone to androst-5-ene-3β:16α:17β-triol and oestriol respectively. ↓, Experimentally established reaction; ↓, hypothetical reaction.

The last-named compound, first isolated from the urine of a boy with an adrenal tumour by Hirschmann (1943) and subsequently isolated from the urine of normal men and women by Marrian & Butler (1944), has been shown to be a metabolic product of administered dehydroepiandrosterone in man (Mason & Kepler, 1947), and can also be formed from dehydroepiandrosterone *in vitro* on incubation with rabbit-liver slices (Schneider & Mason, 1948). The metabolic reduction of 16α-hydroxydehydroepiandrosterone to androst-5-ene-3β:16α:17β-triol has not yet been demonstrated, but the analogous reduction of 16α-hydroxyoestrone to oestriol under *in vivo* conditions in man is now known to occur (Brown & Marrian, 1957).

It is of interest to compare the hypothetical scheme for the metabolic formation of androst-5-ene-3β:16α:17β-triol from dehydroepiandrosterone proposed here with that put forward by Marrian, Watson & Panattoni (1957), Marrian, Loke, Watson & Panattoni (1957) and Brown & Marrian (1957) for the formation of oestriol from oestrone (see Fig. 1). In neither case has complete proof for the correctness of the proposed scheme been obtained, but it can now hardly be doubted that the two triols arise by similar metabolic pathways.

SUMMARY

1. It has been shown by paper chromatography that when urine from normal males is boiled for 1 hr. at neutral pH and extracted with chloroform these extracts contain two 'blue-tetrazolium'-reducing compounds X and Y which are not present in extracts of unboiled urine.

2. By treatment with acid at room temperature X was converted into a blue-tetrazolium-reducing compound Xa, which had a higher R_F than X and the same R_F as Y.

3. Xa has been isolated from extracts of urine and characterized as the hitherto undescribed steroid 16α-hydroxydehydroepiandrosterone.

4. By analogy with 6β-hydroxy-3:5-cycloandrostan-17-one which arises from the hydrolysis in neutral solution of dehydroepiandrosterone sulphate and which is transformed by acid into dehydroepiandrosterone it is considered that X is 6β:16α-dihydroxy-3:5-cycloandrostan-17-one formed from the 3β-sulphate of 16α-hydroxydehydroepiandrosterone.

5. 16α-Hydroxydehydroepiandrosterone was shown to be a metabolite of dehydroepiandrosterone, and it is suggested that it is an intermediate in

the transformation of dehydroepiandrosterone into androst-5-ene-3 β :16 α :17 β -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