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The Uptake of ^{35}S into Rat Tissues after Injection of [^{35}S]Methionine

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Labelled amino acids are readily taken up into the proteins of the liver, intestine and most other organs, when administered *in vivo*: but they are taken up to only a very limited extent by the brain (Tarver & Morse, 1948; Greenberg & Winnick, 1948). The earlier workers concluded from this that the brain proteins are relatively inert, resembling in this respect the connective-tissue proteins such as collagen. It is now recognized that the free passage of amino acids from the blood stream into the brain tissue is prevented by the blood–brain barrier: under these conditions the rate of incorporation of a labelled amino acid is not a satisfactory index of the rate of protein formation in the tissue, and the tracer experiments have not really shown that the proteins of the brain are metabolically inert (Friedberg & Greenberg, 1947; Tarver & Morse, 1948).

It would appear that if the slow uptake of a labelled amino acid is due to the inertness of the tissue proteins, the specific radioactivity in the acid-soluble fraction of the tissue should be relatively high, depending on that in the plasma: but if the slow uptake is due to the low penetration of the amino acid into the tissue, then the specific radioactivity in the acid-soluble fraction should be correspondingly low. By comparing the specific radioactivities of a labelled amino acid in the acid-soluble and protein fractions under suitable conditions, it should therefore be possible to obtain evidence of the true rate of incorporation of the amino acid into the tissue proteins considered as a whole.

Methionine labelled with ^{35}S was used in the present investigation, and the naturally occurring L isomer, prepared biosynthetically from yeast, was used in preference to the DL mixture used in previous isotopic experiments. Rat brain and liver tissues were separated into three main fractions containing (*a*) proteins, (*b*) lipids and (*c*) acid-soluble constituents. The specific radioactivity of the sulphur in these fractions at different times after injecting L-[^{35}S]methionine was determined, and ratios giving a measure of the incorporation into the protein and lipid fractions were obtained.

EXPERIMENTAL

[^{35}S]*Methionine.* The L isomer was prepared from yeast grown in a medium containing $^{35}SO_4^{2-}$ (0·5 mc/l.) by the method of Williams & Dawson (1952). The conditions were similar to those described except that the culture medium contained 6 g. $(NH_4)_2HPO_4$/l. and it was carefully adjusted to pH 5·1 using bromocresol green. The incubation was continued for 48 hr. With these modifications the yield was increased to 4·5–5·4 g. crude yeast proteins/l. medium. The yield of methionine was improved somewhat by increasing the number of butanol extractions and by carefully removing all traces of free HCl under reduced pressure before

crystallizing from the pyridine–ethanol mixture. The method worked well in practice, and 3–4 l. medium yielded 100–160 mg. pure L-[^{35}S]methionine giving $0{\cdot}72 \times 10^5$ – $1{\cdot}2 \times 10^5$ counts/min./mg. when measured under a mica-windowed counter. The purity was confirmed by paper chromatography and by a correct analysis for N and S.

The DL-[^{35}S]methionine was obtained from the Radiochemical Centre, Amersham.

Experimental animals. The Wistar albino rats (49–105 g.) were from a pure strain obtained from Messrs A. Tuck and Son, The Mousery, Rayleigh. In a part of the work the rats used were of a large albino strain of the grey Norwegian rat (59–82 g.), designated 'Albino I', that had been bred for several years in this laboratory. The animals received an intraperitoneal injection of 1–3 mg. L-[^{35}S]methionine, giving $9{\cdot}2 \times 10^4$ counts/min., in 0·15 to 0·22 ml. 0·9% NaCl solution. During the experimental period they were given free access to water. They were killed by decapitation and the tissue samples were rapidly removed.

Isolation of tissue fractions. The tissue sample (about 1 g.) was weighed quickly on a torsion balance and disintegrated for 3–5 min. in a centrifuge tube containing 5–8 ml. 10% (w/v) trichloroacetic acid (TCA). The blades of the blender were washed with 1–2 ml. 10% TCA and the tube was stoppered and left at room temp. overnight. After centrifuging, the supernatant extract was filtered. The residue was washed twice with 5 ml. 10% TCA and 3 times with 5 ml. distilled water. The combined extract and washings represented the acid-soluble fraction.

The residue was extracted with 5 ml. acetone, centrifuged, and the supernatant solution filtered. The residue from this was shaken with 10 ml. 50% (v/v) chloroform–ethanol and left overnight at room temp. in a stoppered centrifuge tube. The extract, which was separated by centrifuging and filtration, contained most of the lipid sulphur, but a further small amount was extracted by refluxing the residue twice with 10–15 ml. of the same solvent for 2 hr. on a water bath. The combined acetone and chloroform–ethanol extracts formed the total lipid fraction: the solid residue was taken as the protein fraction.

Oxidation of organic sulphur to sulphate. The weighed tissue sample (0·05–0·5 g.) was placed in a boiling tube containing a few glass beads, and 5 ml. of oxidizing reagent (60% (w/w) A.R. $HClO_4$–conc. HNO_3 (A.R.), 1:3, v/v) were added. The tissue was brought into solution by heating gently with a microburner. Explosions were rare, but precautions were taken throughout in view of the risk. When a clear solution was obtained, a piece of electrolytic copper wire (50–70 mg.) was added, and, after it had dissolved, the tube was loosely closed with a glass stopper and placed in an air-bath at 100–150°. After 30 min. the temp. of the air-bath was raised to 230–250° and the heating was continued for 3 hr. The tube was then cooled and 5 ml. of an aqua regia reagent (1 vol. conc. HCl to 3 vol. of the 3:1 HNO_3–$HClO_4$ acid mixture) were added. Heating in the air-bath at 230–250° was continued for another 3 hr., after which the excess acid was gently boiled off. The dark residue was then heated strongly on the microburner for 1–2 min. and the acid fumes were driven off. The tube was cooled and the residue was dissolved by warming with 2 ml. N-HCl.

In the oxidations carried out by the method of Pirie (1932) a reagent consisting of 3 vol. conc. HNO_3 saturated with copper nitrate and 1 vol. 60% perchloric acid was used, but otherwise the conditions were similar to those described. Oxidations by the Carius method were carried out under the conditions described by Young, Edson & McCarter (1949).

Precipitation of benzidine sulphate. The solution containing the sulphate in N-HCl was concentrated to about 1 ml. by boiling off some of the acid. The solution was made just acid to congo red with N-NaOH, filtered (Whatman no. 42 paper), and washed through with 2–3 ml. distilled water. The benzidine sulphate was precipitated by adding 2 ml. 95% ethanol and 2 ml. benzidine hydrochloride reagent (prepared by dissolving 5 g. benzidine hydrochloride in 40 ml. N-HCl and bringing to 250 ml. with 50% (v/v) aqueous ethanol; the solution was brought to the boil, cooled, filtered and stored in a dark place). The precipitated benzidine sulphate was stirred and allowed to stand 30–60 min. before collection. When the amount of S exceeded about 0·4 mg. in 5–8 ml. solution, the precipitation was rapid and quantitative. With quantities of the order of 50–100 μg. S, the volume of the filtrate was reduced to about 1 ml. before precipitation and 1 ml. benzidine reagent and 1 ml. ethanol were used. With less than 50 μg. S, a known amount of standard Na_2SO_4 solution was added to bring the total amount to about 50 μg. S and a suitable correction was made. It was confirmed in control experiments that the phosphate present in the animal tissues examined did not interfere with the quantitative precipitation of benzidine sulphate under the conditions used.

Collection of benzidine sulphate. It was necessary for assaying the radioactivity that the benzidine sulphate should be collected in as uniform and reproducible a manner as possible. The apparatus used was similar to that described by Pinajian & Cross (1951). It consisted of a sintered glass filter funnel of 2·8 cm. internal diam. and porosity no. 1, with four glass hooks round the upper rim. A thick filter paper (Whatman no. 3) was placed on the sintered disk, wetted with water, and then a thin filter paper (Whatman no. 42, diam. 2·8 cm.) was placed on top. The filter papers were held firmly in position by inserting a glass inner tube of 1·75 cm. internal diam. ground flat at the lower end and fitted with four glass hooks to which springs were attached.

The benzidine sulphate was transferred to the inner tube of the filter funnel, washed down with 1 ml. water saturated with benzidine sulphate, and then washed with 1–2 ml. 95% ethanol so that it formed an even layer in the centre of the filter paper. The benzidine sulphate should have a shiny appearance when dry: if it appeared discoloured at this stage it was redissolved in dil. NaOH, filtered and reprecipitated. After washing the ppt. with a further small volume of 95% ethanol, the glass inner tube was removed and the edges of the filter paper that had been below the ground edge of the tube were washed with a little water and ethanol to remove any residual traces of the reagent, taking great care not to disturb the ppt. The upper filter paper was carefully removed by means of forceps and kept flat while drying on the mounting table of the Geiger counter stand.

Assay of ^{35}S. A Geiger counting tube with a thin end-window (1·7 mg. mica/sq.cm.) was used, and a special stand was constructed to ensure that the samples were brought to exactly the same distance from the window every time. The stand was designed to fit inside the lead castle, immediately below the window of the counter tube. It consisted of a heavy brass plate which could be moved up and down by means of a screw. The filter paper bearing the sample was held flat on the surface of this plate by a second brass plate having a circular hole (1·9 cm. diam.), slightly greater than

that of the space occupied by the benzidine sulphate ppt. After placing the stand in the lead castle, the sample was raised until the brass plate holding it made firm contact with the circular rim of the aperture immediately below the mica window of the counter tube.

The observed rate of counting was corrected for background (10–11 counts/min.), circuit dead time, counter sensitivity and for radioactive decay. A correction for self-absorption was made by multiplying the observed activity of any sample by a correction factor for the thickness of the sample, as described by Henriques, Kistiakowsky, Margnetti & Schneider (1946). The correction factor was obtained from a chart made by plotting the observed radioactivity of standard samples of ^{35}S against mg. benzidine sulphate collected within a fixed area of 2·4 sq.cm. The samples were generally counted at relatively small thicknesses (below 2 mg. benzidine sulphate/sq.cm.) so that the absorption correction was kept small. The measurements of radioactivity were based on at least 1000 counts, which gave a probable error in the counting rate of 2·7% for samples giving not less than 50 counts/min.

Determination of total S. When large amounts of tissue were available, the benzidine sulphate prepared from a portion was collected in a weighed sintered glass crucible (porosity no. 3 or 4), washed with ethanol and dried at 100° to constant weight.

For small amounts of tissue containing less than 1 mg. of S, microtitration with standard alkali was preferred (Fiske, 1921). The filter paper containing the benzidine sulphate used for the assay of radioactivity was suspended in 10 ml. distilled water and the warm solution titrated against 0·01 N-NaOH using phenol red. The titration was continued at the boiling point until a sharp end point was obtained. In a series of nine consecutive estimations on tissue samples containing 0·9–1·2 mg. total S, the mean recovery was 99·5% (±3·7 S.D.) of the value obtained by gravimetric determination. The method was applied successfully to small tissue samples containing as little as 0·05–0·1 mg. S.

RESULTS

The sulphur content of rat tissues

For the purpose of the present investigation it was necessary to know the total sulphur content of different rat tissues. Of the various methods used by previous investigators (Tarver & Schmidt, 1939; Boursnell, Francis & Wormall, 1946), the acid-digestion method of Pirie (1932) is the simplest and most convenient: but preliminary experiments by this method gave values for the sulphur content of brain tissue that were 5–10% lower than those reported by Young *et al.* (1949), who used the Carius bomb method. The Pirie method gave a recovery of sulphur as sulphate that was approximately 100% with cystine, but often only 90–95% with methionine. This agrees with Folch & Lees (1951), who found that methionine resisted oxidation with nitric acid, but was completely oxidized by aqua regia. Young *et al.* (1949) also found that the Pirie method gave low values for certain sulphur compounds.

In the present investigation, the modified Pirie (1932) method using a mixture of perchloric acid with aqua regia gave a recovery of sulphur from methionine of 99·1% ± 1·4 S.D. A series of eight determinations on human brain tissue (parietal cortex) gave a mean sulphur content of 1·58 ± 0·04 S.E., which agreed closely with the value 1·59 ± 0·01 S.E. obtained on the same sample of tissue by the Carius bomb procedure. Values obtained by this method for the sulphur content of Wistar albino rat (150–165 g.) tissues showed good agreement with values previously reported by Young *et al.* (1949) (Table 1).

Most of the sulphur of the tissues is present in the proteins as combined methionine and cysteine; but the lipid fraction contains a variable quantity of sulphatides, and the acid-soluble fraction contains taurine, glutathione, free methionine, cysteine and inorganic sulphate as well as other sulphur compounds present in smaller amounts. The total S content of the three fractions in the liver and brain tissue of six Wistar albino rats (80–150 g.) is given in Table 2. It may be seen that in the brain about 80% of the sulphur is in the proteins, 5% in the lipids and 15% in the acid-soluble fraction. Similar determinations of the sulphur content of the three fractions in the brain and liver of eleven rats of the Albino I strain gave values that agreed closely with these figures, except that the sulphur content of the brain acid-soluble fraction was 14% higher than with the Wistar albino rats. It was noted that the sulphur content of this fraction varied to some

Table 1. *The total sulphur content of some rat tissues, determined by different methods*

Results are expressed as mg. S/g. fresh tissue ± S.E.

Tissue	Carius bomb method*	HNO_3–$HClO_4$ digestion	Aqua regia–$HClO_4$ digestion
Whole brain	1·69 ± 0·09	1·61 ± 0·05	1·73 ± 0·05
Liver	2·55 ± 0·16	2·20 ± 0·21	2·40 ± 0·11
Kidney	2·47 ± 0·11	2·42 ± 0·08	2·58 ± 0·14
Lung	2·10 ± 0·09	2·01 ± 0·08	2·08 ± 0·12
Spleen	2·20 ± 0·18	2·25 ± 0·16	2·56 ± 0·16
Heart	2·89 ± 0·17	2·82 ± 0·08	3·00 ± 0·08
Leg muscle	2·47 ± 0·09	2·56 ± 0·06	2·70 ± 0·13

* The values are taken from Young *et al.* (1949).

Table 2. *The sulphur content of protein, lipid and acid-soluble fractions of Wistar albino rat tissues*

Results are expressed as mg. S/g. fresh tissue ± S.D.

Tissue fraction	Sulphur content: Liver	Sulphur content: Brain
Protein	2·00 ± 0·14	1·36 ± 0·02
Lipid	0·15 ± 0·04	0·11 ± 0·02
Acid-soluble	0·38 ± 0·06	0·29 ± 0·08
Total	2·53	1·76
Whole tissue (total S)	2·40 ± 0·11	1·73 ± 0·05

extent with the age of the animal: this is shown in Table 3 for groups of Albino I rats of different weights. A variation in the sulphur content of this fraction with age has been previously reported by May (1948).

The uptake of ^{35}S into different tissues from DL- *and* L-*methionine*

Of eight tissues examined after intraperitoneal injection of DL-[^{35}S]methionine, kidney showed the highest uptake of the isotope and brain and muscle the lowest (Table 4). The pattern of incorporation was similar to that previously reported for ^{35}S-labelled DL-methionine administered by the intravenous route (Friedberg, Tarver & Greenberg, 1948). To enable comparisons to be made between experiments with animals of different weights and with different doses of ^{35}S, the radioactivity of the tissues is given in terms of the relative specific activity, which is defined as

$$\frac{\text{specific radioactivity (counts/mg. S) of tissue}}{\text{dose administered (counts/mg. body weight)}}.$$

A similar unit was used by Tarver & Morse (1948).

After intraperitoneal injection of L-[^{35}S]methionine, the uptake of isotope was relatively lower in the kidney and higher in the liver than with the DL mixture: brain and muscle again gave the lowest uptake. This was observed both with Wistar albino rats and with a series of rats of the Albino I strain. The relative specific activity in liver tissue was initially nearly 10 times that in brain and muscle, but it gradually fell to a lower value that was maintained for up to 24 hr. after injection: the relative specific activity in liver tissue at 6 hr. was 639, at 8 hr. was 475 and at 24 hr. was 460. A high initial specific activity was to be expected in the liver, since methionine given by intraperitoneal injection would be transported first to the liver via the portal blood vessels before entering the general circulation.

In brain tissue the relative specific activity quickly rose to 132, and there was relatively little change within 24 hr.: the relative specific activity at 6 hr. was 120, and at 24 hr. 132. It appears that the amount of ^{35}S entering the brain tissue was quickly balanced by the amount leaving the tissue, so that a state approaching an isotopic equilibrium was attained.

Table 3. *The variation with age in the sulphur content of the acid-soluble fraction of the rat brain*

Albino I rats were used. Results are expressed as mg. S/g. fresh tissue ± S.D.

Rat weight (g.)	No. of animals	S content
20–30	9	0·44 ± 0·07
30–50	8	0·37 ± 0·05
50–100	7	0·33 ± 0·04
100–200	6	0·29 ± 0·01

The distribution of ^{35}S in the lipid, protein and acid-soluble fractions

Liver tissue. Measurements of the specific radioactivity in the three fractions of liver tissue after the injection of L-[^{35}S]methionine showed a high initial concentration of ^{35}S in the acid-soluble fraction (Table 5). The specific activity of this fraction decreased rapidly as the ^{35}S became distributed throughout the other tissues of the body and was partly eliminated by excretion. The specific activity of the liver protein fraction showed less change. At 3 hr. the ratio of the specific activities in these two fractions

$$\frac{\text{specific activity of protein S}}{\text{specific activity of acid-soluble S}}$$

had risen to 0·14 and after 5 hr. to 0·31.

The specific activity of the liver lipid fractions was unexpectedly high and of the same order as that of the protein fractions, to which they ran

Table 4. *The relative specific activities in different tissues of Wistar albino rats at different times after the intraperitoneal injection of* DL- *and* L-[^{35}S]*methionine*

Rat no. 18 received 0·56 mg. DL-methionine giving 128×10^5 counts/min.; rat no. 17 received 0·47 mg. DL-methionine giving 102×10^5 counts/min.; rats no. 87–90 received 3 mg. L-methionine giving $9{\cdot}2 \times 10^4$ counts/min. The methionine was dissolved in 0·15–0·25 ml. 0·9% NaCl.

Rat no.	Body wt. (g.)	Time after injection (hr.)	Relative specific activity (counts/min./mg. S/counts/min./mg. body wt. administered)							
			Brain	Liver	Kidney	Lung	Spleen	Heart	Muscle	Blood
			DL-[^{35}S]Methionine							
18	49	3	128	646	1050	210	294	165	95	251
17	56	24	158	542	756	241	361	183	91	286
			L-[^{35}S]Methionine							
87	78	0·5	132	1208	384	224	274	178	134	—
88	84	1	185	1592	556	305	—	176	130	—
89	95	3	167	1438	712	364	415	218	97	—
90	105	5	168	796	656	357	369	206	108	—

closely parallel. It was found experimentally that although the lipid fraction contains sulphatides, etc., the actual sulphur content of these compounds was small in comparison with that of the protein part of the lipoproteins extracted by lipid solvents and so included in the lipid fraction. If the radioactivity of the lipid fraction was due mainly to the protein residues of the lipoproteins, that might explain the high specific activity of this fraction.

Brain tissue. The specific radioactivity of the acid-soluble fraction of the brain tissue was consistently low, while the specific activity of the brain protein fraction increased until it exceeded that in the acid-soluble fraction. Assuming that the observed radioactivity was due mainly to [^{35}S]-methionine, it appeared that any methionine that entered the acid-soluble phase of the brain tissue was quickly incorporated into the proteins: the relations were those to be expected if protein formation was actively taking place in the tissue. The ratio of the specific activity of protein S/acid-soluble S was 0·89 at 3 hr. and 1·03 at 5 hr. after the injection.

The specific activity of the brain lipid fraction was extremely low and, in view of the low sulphur content of this fraction (110 μg. lipid S/g. fresh brain tissue) and the low counting rate, the figures in Table 5 for this fraction are given with considerable reserve.

The incorporation of ^{35}S into tissue fractions of Albino I rats

The specific activities of the protein, lipid and acid-soluble fractions of a group of Albino I rats were determined, as before, at different times after injection of L-[^{35}S]methionine. The results for liver and brain tissue were similar in general to those obtained with the Wistar albino rats and confirmed the previous findings: but the specific activity of the brain acid-soluble fraction was lower throughout (Table 6). This might be due in part to a higher content of inactive sulphur compounds in the acid-soluble fraction, since the mean total S content of the acid-soluble fraction was 14% higher than in the Wistar albino rats. The specific activity ratios increased more rapidly and remained at a higher level than in the Wistar albino rats. In the liver tissue the specific activity of the lipid fraction was again high and similar in magnitude to that of the protein fraction. The ratios of specific activity of protein S/acid-soluble S for liver tissue were 0·14 at 0·5 hr., 0·21 at 2 hr., 0·34 at 4 hr., 0·63 at 8 hr. and 1·1 at 24 hr. The individual specific activity figures of the different fractions showed greater variation in the Albino I than in the Wistar albino rats, but the specific activity ratios were again consistent in that the values for different times fell on a smooth curve. The specific activity ratios are largely inde-

Table 5. *The specific activities of lipid, protein and acid-soluble fractions of Wistar albino rat tissues at different times after injection of* L-[^{35}S]*methionine*

Each rat received 3 mg. L-methionine, giving 9·2 × 10^4 counts/min., in 0·15 ml. 0·9% NaCl by intraperitoneal injection.

Rat no.	Body wt. (g.)	Time after injection (hr.)	Specific activity (counts/min./mg. S)			Specific activity ratios	
			Acid-soluble S	Lipid S	Protein S	Lipid S/ acid-soluble S	Protein S/ acid-soluble S
			Liver				
87	78	0·5	6090	834	607	0·14	0·10
88	84	1	8660	901	790	0·10	0·09
89	95	3	5170	578	755	0·11	0·14
90	105	5	1870	454	573	0·24	0·31
			Brain				
87	78	0·5	477	20	80	0·04	0·17
88	84	1	465	64	149	0·14	0·32
89	95	3	184	56	163	0·31	0·89
90	105	5	149	62	153	0·42	1·03

Table 6. *The specific activities of lipid, protein and acid-soluble fractions of the brain of Albino I rats at different times after intraperitoneal injection of* L-[^{35}S]*methionine*

The solution (0·22 ml. 0·9% NaCl) injected contained 1·1 mg. L-methionine giving 9·2 × 10^4 counts/min.

Rat no.	Body wt. (g.)	Time after injection (hr.)	Specific activity (counts/min./mg. S)			Specific activity ratios	
			Acid-soluble S	Lipid S	Protein S	Lipid S/ acid-soluble S	Protein S/ acid-soluble S
18	59	0·5	283	47	127	0·16	0·45
20	69	2	83	28	114	0·33	1·4
21	82	4	151	59	278	0·39	1·8
23	82	8	78	27	140	0·34	1·8
24	80	24	117	19	172	0·16	1·5

pendent of variable factors such as the rate of penetration of the isotope into the tissue and the dose administered: they would appear to be more satisfactory than the individual specific activity figures as an index of the incorporation of isotope into the lipid and protein fractions.

DISCUSSION

The uptake of ^{35}S from [^{35}S]methionine into rat tissues was studied by Friedberg *et al.* (1948) using the DL amino acid. The D isomer is not used directly for protein synthesis in animal tissues: its presence appeared undesirable in isotopic experiments both for that reason and also in view of the report of Simpson & Tarver (1950) that in liver slices it interferes with the incorporation of the L isomer into proteins.

The present study of the rate of uptake of ^{35}S into the rat tissues, using the L isomer of [^{35}S]methionine, showed that there was a rapid appearance of ^{35}S in the liver and other tissues shortly after injection, while the concentration in brain and muscle tissue remained relatively small. The order of radioactivity of the different tissues was similar to that observed by Friedberg *et al.* (1948) with the DL mixture, except that the radioactivity was relatively lower in the kidney and higher in the liver tissue. It was thought at first that this difference might be due to the fact that the L-methionine was given by intraperitoneal injection, whereas the DL mixture had been administered by intravenous injection: but the difference was still observed when both were given by the same route (Table 4). The relatively high concentration of the isotope in the kidney after adminstering the DL mixture may be attributed to the activity of the kidney in withdrawing the unnatural D isomer from the circulation: the kidney is known to contain a D-amino acid oxidase and is active in excreting D-amino acids (Camien, Malin & Dunn, 1951). Apart from the differences observed in the liver and kidney, these experiments gave no indication that the DL mixture behaves differently from the L isomer in the distribution pattern of radioactivity in different tissues.

Gaitonde & Richter (1955) have shown that L-[^{35}S]methionine can be isolated from the brain proteins after administration *in vivo*, and they have obtained evidence that the activity of the tissue is due mainly to [^{35}S]methionine, with a small proportion of [^{35}S]cysteine, combined in the proteins by peptide bonds. It is therefore reasonable to assume that under the conditions of these experiments the incorporation of ^{35}S into the brain proteins is attributable to protein formation in the brain. The present study of the specific activity of the different tissue fractions shows that, while the total ^{35}S content of brain tissue is small, the radioactivity of the brain proteins is high in comparison with that of the acid-soluble fraction. It would appear that any [^{35}S]methionine that gets through the blood–brain barrier into the brain is rapidly incorporated into the brain proteins; the evidence therefore suggests that the metabolic activity of the brain proteins must be relatively high.

The incorporation of ^{35}S from L-[^{35}S]methionine into the proteins and lipid fractions of the tissue followed a pattern that was essentially similar in rats of Wistar albino and Albino I strains, but certain differences were noted in the ratios of the specific activities of various tissue fractions, due possibly to differences in enzymic activity and other factors, in animals of different genetic strains. Large differences in the rate of incorporation of [^{35}S]methionine into liver tissue in rats of different genetic strains have been previously reported by Rutman, Dempster & Tarver (1949). It would appear that in work on protein metabolism involving comparisons between different animals, the animals used should be of uniform genetic strain.

SUMMARY

1. The specific radioactivity of rat tissues was measured at different times after the intraperitoneal injection of L-[^{35}S]methionine into rats. The pattern of uptake of ^{35}S in order of decreasing radioactivity was: liver, kidney, spleen, lung, heart, brain and muscle.

2. The pattern of uptake of ^{35}S from DL-[^{35}S]-methionine differed from that of the L isomer for liver and kidney, but was similar for other tissues.

3. The specific activities of lipid, protein and acid-soluble fractions of brain and liver tissue were measured. There was a relatively rapid uptake of ^{35}S from the acid-soluble into the protein fraction of the brain.

4. The rate of uptake of ^{35}S into the tissues depends on constitutional factors that were found to vary in rats of two different genetic strains, but with animals of uniform strain consistent results were obtained.

5. Values are given for the total sulphur content of rat tissues, and for the sulphur content of the protein, lipid and acid-soluble fractions of liver and brain.

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The Specific Polysaccharides of some Gram-negative Bacteria

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In their work on the specific capsular polysaccharides of the Pneumococci, Goebel and Avery and their collaborators (see Goebel & Avery, 1931; Goebel, 1939) showed the kind of chemical relationship which existed between some of the materials and which accounted for their serological cross-reactivity. A number of specific polysaccharides elaborated by organisms in the Gram-negative group have been examined chemically in spite of the difficulty in obtaining adequate amounts of starting material and of identifying sugars by isolation from complex mixtures in polysaccharide hydrolysates. The development of chromatographic methods of analysis has much simplified the task of identification and much smaller quantities of material are required.

From the specific degraded polysaccharide of the 'Smooth' form of *Shigella dysenteriae*, Morgan (1936, 1938) isolated derivatives of *N*-acetylglucosamine, galactose and rhamnose; Jesiatis & Goebel (1952) found that the *Shigella sonnei* specific lipopolysaccharide contained acetylglucosamine, galactose, glucose and a heptose; Goebel, Binkley & Perlman (1945) isolated specific polysaccharides from some strains of *Shigella flexneri* and demonstrated the presence of acetylglucosamine, glucose and rhamnose. These three Shigella species thus appeared to contain acetylglucosamine and the three possible combinations of two from the other three hexose sugars, galactose, glucose and rhamnose. Aldoheptose sugars have recently been found, not only in *Sh. sonnei* products but also in a specific polysaccharide material obtained from *Sh. flexneri* (Slein & Schnell, 1953) and in the specific protein–polysaccharide complex of *Sh. dysenteriae* (Davies & Morgan, 1954). None of these heptoses has yet been identified by isolation of the sugar or of a characteristic derivative. Although some family relationship is revealed by the qualitative composition of the Shigella polysaccharides, a group of materials which do not generally cross-react serologically (Weil, 1947), the situation has been somewhat complicated by the discovery of a 'phage-resistant mutant of *Sh. sonnei* whose specific lipopolysaccharide appears to contain one unidentified hexosamine and no other sugar residue (Goebel & Jesiatis, 1952).

In the Salmonella group, galactose, glucose and mannose were identified as components of the specific degraded polysaccharides of *Salm. typhosum*, (Freeman, 1942) and *Salm. typhimurium* (Freeman, 1943). These three sugars were also found in the specific polysaccharide of *Salm. abortus equi* by Lüderitz & Westphal (1952*a*) with the addition of hexosamine, rhamnose and abequose.

In two strains of *Escherichia coli* examined by Lüderitz & Westphal (1952*b*), and belonging to different '0' groups, acetylhexosamine, galactose, glucose and rhamnose were present in the polysaccharides; one material also contained xylose whereas the other contained mannose.

Single strains of organisms from other taxonomic groups have yielded polysaccharides composed of yet other groups of sugars, e.g. galactose and mannose in *Haemophilus pertussis* (Akiya, Takahashi, Kuriyama & Ogawa, 1951), hexosamine, glucose and methylpentose in *Serratia marcescens* (Hartwell, Shear, Adams & Perrault, 1943), acetylhexosamine, galactose and mannose in *Proteus vulgaris* (Bendich & Chargaff, 1946), glucose and glucuronic acid in *Klebsiella pneumoniae* (Goebel & Avery, 1927), glucose, galactose, arabinose and glucuronic acid in *Vibrio* spp. (Linton, 1940) and glucosamine, glucose, xylose and rhamnose in *Trichomonas foetus* (Feinberg & Morgan, 1952).