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Hydrocarbons in Pyrethrum Cuticle Wax

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During the period 1928-37, there were many contributions to this *Journal* on the cuticle waxes, as found on the petals of flowers, the protective coatings of fruit and on the leaves of vegetables. For references see Chibnall, Piper, Pollard, Williams & Sahai (1934), Chibnall & Piper (1934), and also Kreger (1948).

In general these cuticle waxes are found to consist of long-chain carboxylic acids of even number, and their esters; even-numbered primary alcohols; and odd-numbered secondary alcohols, ketones and paraffin hydrocarbons. The proportions vary from plant to plant, and the carbon numbers range from C_{24} to C_{36} . These findings caused considerable speculation about the sequence of formation of these materials in the plants, and the subject is still not clarified.

Good methods are available for characterizing pure samples. For hydrocarbons one may mention the transition-point data and the X-ray data of Piper *et al.* (1931), and Piper, Chibnall & Williams (1934). But the real difficulty in this work is the separation of members of a homologous series of these materials; this limits the applicability of the analytical procedures.

The purpose of this work was to examine the hydrocarbon portion of pyrethrum wax, and to attempt to accomplish some resolution of the mixtures found therein.

EXPERIMENTAL

All melting points are corrected.

Materials

Two samples of the crude pyrethrum wax were supplied by the U.S. Industrial Chemicals Corp., these being factory samples from different periods of operation. The crude wax is precipitated by chilling the hexane extract of the dried pyrethrum flowers. These two samples, A, and B, were found to contain hydrocarbon portions of 46 and 17% respectively, as measured by recovery from the light petroleum eluate, of a chromatogram on activated alumina. It is with these hydrocarbon portions of the wax that this communication is principally concerned.

Sample A, once-crystallized from acetone melted at $61.5-64.5^\circ$. The average molecular weight of this sample was 370 ± 15 , as determined by the micro-Rast procedure. Carbon and hydrogen analyses (C 82.08, 82.16; H 13.99, 14.07%) accounted for 96.1% of the same sample. The infrared curves confirmed the presence of oxygen by the carbonyl

band at 5.63μ ., by the alcohol band at 3.05μ ., and by the alcohol or ether band at 9.42μ . By analysis the oxygen content could not be greater than 3.9%. Thus for this molecular weight range there is on the average not more than one oxygen atom per molecule.

Crystallization and chromatography on alumina

The expected hydrocarbon portions were separated from the oxygenated and other material by elution with light petroleum (b.p. $30-60^\circ$), from activated alumina chromatographic columns. Alorco grade F-20 alumina, reactivated for 16 hr. at 205° was used, in the ratio of at least 30 g. alumina/g. of sample. Elution with a given solvent was continued until substantially no more product was obtained. For brevity the results are listed in Table 1, as light petroleum, ether, and methanol eluates. A graded series of benzene-ether eluates moved little of the material until the solvent blend was over 90% ether.

Referring to Table 1, sample A1 gave a negative Davidson test for oxygen (Davidson, 1940), while the sample A2 gave a positive test. By infrared analysis the 5.63μ . carbonyl peak was seen to be present in sample A2 and absent in sample A1. Sample A1 was analysed by the manometric Unterzäucher method (Holowchak & Wear, 1951) giving oxygen values of 0.040, 0.057, 0.056 and 0.040% (average 0.048%). The uncertainty in this analysis at this level is estimated to be 35%. Assuming that a probable impurity would be a ketone, e.g. $C_{25}H_{48}O$ (3.8% oxygen), it appears that the above-described chromatographic conditions give a fairly good separation between paraffins and oxygenated compounds in the C_{30} range. There should not be more than 1.5% of C_{30} ketone or other oxygenated compounds of this molecular-weight range in the light petroleum eluate. Carbon and hydrogen values were C 85.18, 85.19; H 14.60, 14.85%.

Sample B was handled differently in order to attempt to detect the highest molecular weight species of paraffins

Table 1. *Alumina chromatography of crude pyrethrum wax samples*

Fraction no.	Eluent	Weight (%) of charge recovered			
		A	B1	B3	B*
1	Light petroleum (b.p. $30-60^\circ$)	45.5	33.4	12.9	17
2	Ether	22.0	32.1	—	—
3	Methanol	2.1	1.8	—	—
4	Unrecovered	30.4	32.7	—	—

B* Calculated, see text p. 685.

Table 2. *Chromatography of 1.0 g. household paraffin wax on Norit A carbon black*

Fraction no.	Vol. ethyl acetate in effluent (ml.)	Wt. of cut (g.)	M.p. of crystals as discharged ($^\circ$)	M.p., once crystallized from 95% ethanol* ($^\circ$)
1	<30	0.239	43.5-49.5	50.5-52.0
2	30	0.237	49.5-52.0	54.0-56.0
3	100	0.098	49.0-53.5	55.5-57.0
4	145	0.050	48.5-54.5	54.5-56.5
5	168	0.079	50.5-54.5	57.5-59.0
Amount left on column		0.297		
		1.000 g.		

* Of the 0.709 g. recovered from the column, 0.146 g. remained in solution on recrystallization.

present: the sample (2.0 g.) was refluxed in methanol (1 l.), and the solution was allowed to cool to room temperature to crystallize in part. By combining several such operations the following fractions were obtained: B1, recrystallized from methanol, 45.1%; B2, recovered from methanol solution, 16.9%; B3, harder yellow wax which did not dissolve in methanol, 38.0%. Samples B1 and B3, by alumina chromatography, gave the results shown in Table 1. By a separate chromatogram on the composite sample B (sample B*, Table 1), it was found to have a 'paraffin' content of 17%. It was expected that sample B3 might contain paraffins of higher molecular weight, and this was borne out by the carbon-black chromatography.

Urea complex formation

Sample A was treated with urea, following a procedure of Fetterly (1952) to effect a separation between normal and iso paraffins: sample A (0.3725 g., ca. 0.001 mole), urea (1.8330 g., ca. 0.03 mole), isooctane (13 ml.) and methanol (2 ml.) were mixed. Within 2 hr. a curdy precipitate of hydrocarbon-urea complex appeared; it was filtered on a sintered glass funnel, and washed with isooctane. The hydrocarbons recovered from the complex melted sharply at $61.5-61.7^\circ$. The weight of the residue recovered from the isooctane was 0.0068 g. This experiment provides some evidence that the hydrocarbons are probably normal paraffins.

Partial resolution of paraffin fractions by chromatography on carbon

It seemed that the most promising hope of resolving homologous series of hydrocarbons would be by means of chromatography on a suitably activated carbon. In this hydrocarbon field the following studies appeared to give the most promising leads: (1) Claeson has accomplished a quantitative gas phase resolution of C_1-C_7 hydrocarbons on a $ZnCl_2$ -activated carbon, using a dilute atmosphere of ethyl acetate in nitrogen as the displacing agent (Tiselius, 1940, 1941, 1946). (2) Sesquiterpenes have been isolated by Sörm and co-workers, using $ZnCl_2$ -activated carbon, with benzyl alcohol as a displacing agent (Sörm, 1949; Herout, Ružička, Vraný & Sörm, 1950; Herout, 1950). With the exception of Sörm's work on the sesquiterpenes, we have found nothing published in the molecular weight range of the paraffin hydrocarbons of our samples. In favour of such a resolving experiment is the fact that only the odd-numbered paraffins were expected in the mixture.

Household paraffin wax. As a preliminary experiment, a sample of ordinary household paraffin wax, m.p. $48-50^\circ$

(approximate carbon number, C_{22} - C_{28}), was chromatographed on a column of Norit A, using ethyl acetate as displacing or developing agent. The 19 cm.-long column, compacted by evacuation at the bottom of the column, contained 6 g. of Norit A, and the ratio of carbon to wax was 6:1. The column was water-jacketed and maintained at 64-68° to keep the sample melted and to hasten filtration. After placing the wax on a plug of glass wool above the carbon black, it was melted by heat from the jacket, and then ethyl acetate was introduced under a pressure of 20-35 cm. Hg. The results of this experiment, run over a period of 17 hr. at about 10 ml./hr. effluent rate, are listed in Table 2. From this it appears that 71% of the household paraffin wax can be removed from Norit A, and that some resolution of the material will occur in the process.

Pyrethrum wax. This process was now applied to a hydrocarbon fraction obtained by alumina chromatography from the pyrethrum wax (sample A1, Table 1). The Norit A column was 18.5 cm. long by 2.7 cm. diameter, and held 34.9 g. of the carbon black. The sample weighed 1.2439 g., and the ratio of carbon black to wax sample was 28:1. Operating pressure was about 20 cm. Hg. Other details are given in Table 3. In this table and also in Table 4, the displacing agents are described as 'elutents'. (This is open to question, since there is some doubt as to what type of chromatography is being employed.)

No product was obtained by elution with ethyl acetate. The reason for this is not clear. Increased adsorption of higher-molecular-weight species probably would not be a complete explanation in this case. Similarly, isopropyl acetate and ethyl acetoacetate were ineffective. However, benzyl alcohol was effective, and the degree of resolution obtained is indicated by the average carbon numbers of the fractions. These values are calculated from X-ray data, details of which are given in a later section. Elution with phenol at 93-97° did not remove any significant additional amount of wax. It will be noted from Table 3 that only 64.6% of the sample was recovered. Such low recoveries are typical in this work, and indicate how strongly these

materials are adsorbed on certain carbons. They also suggest that species of higher molecular weight than those reported here may be present in the original wax.

In a search for paraffin hydrocarbons of higher molecular weight, sample B 3-1 (Table 1) was examined in a similar way. Details are given in Table 4. Nearly all of the run was made with a mixture of 66% benzyl alcohol and 34% ethyl acetate: 68% of the sample was recovered by this elution process, but another 16% was extracted from the carbon black by refluxing several times with boiling benzene. 16% appears to have been retained on the carbon black.

It is well known that a homologous series of hydrocarbons forms an ideal solution. The estimation of the average carbon number by measuring the X-ray 'd' spacings corresponding to the 'c' dimensions of the average unit cell, assumes that a true weight-average unit cell is seen by the X-ray beam. However, there is some possibility that distribution is weighted in favour of the species of higher molecular weight, since they are higher melting, and may be expected to orient themselves more completely than would the lower members of the series. In order to check on this possibility, we were fortunate to be able to obtain four mass spectrometer analyses, which are shown in Tables 3 and 4. In three cases out of four the agreement between the two methods is surprisingly good.

Perhaps the most interesting result of all is the molecular-weight-distribution data afforded by the mass spectrometer analyses. Table 5 indicates the degree of resolution of the series of hydrocarbons by this type of chromatography. The data also show that all of the even-numbered paraffins are present in small amounts, but some of them, e.g. C_{28} and C_{30} , are present in some cuts to the extent of 7 and 5.5% respectively.

String bean wax. In order to find out if even-numbered paraffins were present in other natural cuticle waxes, a sample of New York State Tendergreen string bean (*Phaseolus aureus*) wax hydrocarbons was examined in a similar manner. This sample C1 (m.p. 63°) was obtained in the same way as sample A1, by elution with light petroleum from

Table 3. *Chromatography of hydrocarbon fraction A1 of pyrethrum wax on Norit A carbon black*

Fraction no.	Temp. (°)	Eluent used and vol. (ml.)	Time interval (hr.)	Approx. effluent rate (ml./hr.)	Percentage charge removed	M.p. of fractions (°)	'c' unit cell dimension (Å)	Average carbon no.	
								X-ray	Mass spectrometer
	43-50	Ethyl acetate (710)	7.4	96	—	—	—	—	—
	58-78	Ethyl acetate (600)	6.5	93	—	—	—	—	—
	73-78	isoPropyl acetate (410)	5	82	—	—	—	—	—
	65-92	Ethyl acetoacetate (461)	10	45	—	—	—	—	—
37	93-96	Benzyl alcohol (232)	9	26	12.8	58.5-60.5	75.99	28.37	28.33
38	93-96		—	26	14.9	62.0-62.5	76.06	28.40	—
39	93-96		—	26	11.0	62.7-63.3	76.09	28.42	—
40	93-96		—	26	7.17	63.4-64.2	76.95	28.75	—
41	93-96		—	26	7.03	64.4-64.6	78.08	29.22	—
42	93-96		—	26	3.75	64.3-64.5 64.7-65.5 64.6-65.4 64.5-65.3 64.3-65.2	78.84	29.53*	29.60*
43-44	93-96		—	26	4.23				
45	93-96		—	26	1.02				
46	93-96		—	26	0.85				
47	93-96		—	26	1.23		—	—	—
48	93-96		—	26	0.59		—	—	—
49	93-97	Phenol (110)	2.5	—	—	—	—	—	—
Percentage of sample recovered				64.6					

* X-ray and mass spectrometer analyses run on composites of fractions 42-47. This material showed no C:O band in the infrared.

alumina. This sample also contains even-numbered paraffins. The results are summarized in Table 5.

X-ray data and densities. The unit cell dimensions were measured on a Phillips diffractometer-type X-ray unit, using $1/12^\circ$ divergence slit, 1° receiving slit; 50000 v and 19 ma.; 2 sec. time constant; Ni-filtered Cu radiation; chart speed of $1/2^\circ$ per min. (2 θ). Readings were the average of 'ascending' and 'descending' values. Calculations were made assuming $\lambda = 1.5418 \text{ \AA}$. At least five and sometimes eight orders of reflexion were observed, and the 'c' values were read from the plot of these readings at a point corresponding to $n=5$.

As observed by other workers, sample preparation influences the degree of orientation of the paraffin chains (e.g. Clark & Smith, 1931). These samples were prepared by placing about 3-4 mg. of the wax on to the 1×1 in. ground-glass end of a microscope slide. The samples were heated to

110° in an air bath, held there for 1 hr., and allowed to cool to room temperature over about 2 hr. The samples on these pieces of microscope slide were placed directly in the diffractometer. The correlation between average carbon number and the 'c' dimension of the unit cell (assumed to be in the orthorhombic form) was made from a plot of thirteen literature values over the range of C_{19} - C_{35} . These values adjusted for current values of physical constants, are chiefly those of Müller & Saville (1925), Müller (1932), Piper *et al.* (1931) and MacArthur (1944). Most of these values have been summarized by Robertson (1953). The slope of this straight line plot is $2.5125 \text{ \AA}$ per carbon number (corresponding to $1.2563 \text{ \AA}$ per carbon number per individual molecule). A sample D1 of purified dotriacontane, $C_{32}H_{66}$, m.p. $69.5-69.7^\circ$, which is at least 99% pure by mass spectrometer analysis agrees closely with this correlation plot.

Table 4. *Chromatography of hydrocarbon fraction B3-1 of pyrethrum wax on Norit A carbon black*

1.8940 g. of wax sample were run on the same column as for sample A1 (Table 3), on 34.9 g. of Norit A, giving a ratio of carbon to wax sample of 18.4:1. Operating pressure was also about 20 cm. Hg.

Fraction no.	Temp. ($^\circ$)	Eluent used and vol. (ml.)	Time interval (hr.)	Approx. rate (ml./hr.)	Percentage of charge removed	'c' unit cell dimension ($\text{\AA}$)	Average carbon no.	
							X-ray	Mass spectrometer
1	62-72	Ethyl acetate (340)	4	85	3.27	—	—	—
2	70	34% Benzyl alcohol plus 66% ethyl acetate (70)	13.5*	46	3.60	76.16	28.42	—
3	71	66% Benzyl alcohol plus 34% ethyl acetate, 18	—	34	5.39	77.50	28.96	28.76
4	71	15	—	27	7.34	77.55	28.97	—
5	71	8	—	14	5.62	78.17	29.23	—
6	70.5	10	—	30	6.20	78.46	29.36	—
7	69.5	10	—	17	5.77	78.59	29.40	—
8	68	15	—	26	5.75	78.84	29.52	—
9	68	25	—	32	8.90	79.58	29.80	—
10	74	25	—	34	5.10	79.56	29.79	—
11	75	25	—	28	4.06	79.84	29.92	—
12	72	25	—	19	2.72	81.32	30.52	—
13	71	45	—	—	0.95	—	—	—
14	70	100% benzyl alcohol (80)	—	16	3.30	83.06	31.00	30.31†
15	—	—	—	—	16.20†	—	—	—
Residue	—	—	—	—	15.83‡	—	—	—
					100.00%			
D1 (control)							31.97	

* 13.5 hr. to collect fractions 2-14 inclusive.

† Free from C:O band in infrared.

‡ Recovered by repeated extraction of the carbon black with boiling benzene.

§ Unaccounted for, and left on column.

|| A purified synthetic sample which is at least 99% dotriacontane by mass-spectrometer analysis.

Table 5. *Molecular-weight distributions of hydrocarbon fractions obtained from some natural waxes*

Sample* no.														Average carbon no.	Source
	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{36}		
A1 37-47	—	—	1.7	30.9	7.2	53.1	2.9	4.2	—	—	—	—	—	28.33	*From pyrethrum flowers: first four samples are fractions from Tables 3 and 4
A1 42-47	—	—	0.6	2.2	2.0	59.5	5.5	26.5	2.0	1.6	0.1	—	—	29.60	
B3 1-3	0.5	0.9	0.4	6.6	3.9	85.9	0.9	0.9	—	—	—	—	—	28.76	
B3 1-14	—	0.5	0.7	1.6	1.7	47.2	4.3	25.0	2.4	6.9	2.0	5.3	2.4	30.31	String beans (New York State Tendergreen; <i>Phaseolus aureus</i>)
C1	—	—	1.9	3.0	2.8	15.9	3.2	60.5	2.1	10.6	—	—	—	30.56	

* The sum of the numbers on a horizontal line total 100% by weight of the fraction which was submitted to mass-spectrometer analysis. C_{24} represents $C_{24}H_{50}$, etc. The procedures and equipment used are similar to those described by O'Neal & Wier (1951).

Table 6. *Density values of normal paraffins computed from X-ray data**

	Computed from X-ray data	Measured at 20°†	Difference
C ₁₈ H ₃₈	—	0.910	—
C ₁₉ H ₄₀	0.916	—	—
C ₂₀ H ₄₂	0.920	0.913	+0.007
C ₂₁ H ₄₄	0.923	—	—
C ₂₂ H ₄₆	0.925	0.916	+0.009
C ₂₃ H ₄₈	0.929	—	—
C ₂₄ H ₅₀	0.931	0.939	-0.008
C ₂₅ H ₅₂	0.934	—	—
C ₂₆ H ₅₄	0.936	0.921	+0.015
C ₂₇ H ₅₆	0.939	—	—
C ₂₈ H ₅₈	0.940	0.927	-0.013
C ₂₉ H ₆₀	0.942	(0.843)	(+0.099)
C ₃₀ H ₆₂	0.944	0.943	+0.001
C ₃₁ H ₆₄	0.945	—	—
C ₃₂ H ₆₆	0.947	0.944	+0.003
C ₃₃ H ₆₈	0.949	—	—
C ₃₄ H ₇₀	0.950	0.946	+0.004
C ₃₅ H ₇₂	0.951	—	—

* Assuming that all of the X-ray data (by various workers) were obtained at a temperature near 20°.

† Seyer *et al.* (1944).

As an attempt to check on the reliability of this plot, densities (ρ) for the C₁₉–C₃₅ paraffins were computed by the relation $\rho = 1.66020 \times 4 \times \text{mol. wt.} / a \times b \times c$. Values for the a and b dimensions were taken as 7.46 and 4.98 Å respectively. The calculated values are given in Table 6, and compared with measured density values of Seyer, Patterson & Keays (1944). Their values are read from their graph at 20°. In the case of C₃₃H₆₆ a value of $\rho = 0.9472$ is computed, as compared to $d_4^{20} = 0.9439$, measured by Seyer & Morris (1939). This latter number is probably one of the most reliable literature values. There are some anomalous results, particularly in the odd series (cf. C₂₉H₆₀), as discussed by Seyer *et al.* (1944).

RESULTS

The hydrocarbon portions of the pyrethrum wax samples examined varied between 45 and 17% of the samples, and the evidence indicates that they are normal paraffins. All members of the series between C₂₄ and C₃₆ have been found. This is in contrast to earlier reports that only odd-numbered normal paraffins are found in natural plant waxes. Up to 7.2% of normal C₂₈H₅₈ was found in one fraction. Analogous results were also found in wax obtained from string beans. If the hydrocarbons are formed from carboxylic acids, as has been postulated, these findings would require at least the temporary existence of odd-numbered carboxylic acids in the plants.

Some chromatographic resolution of these materials has been accomplished by elution from Norit A, with benzyl alcohol, at 70–90°. Under these conditions 65–68% of the samples have been recovered from the columns. Therefore it is possible that higher species than C₃₆H₇₄ may exist in these waxes. In other work, yet to be published, elutions

from Norit A at temperatures up to 230° have yielded additional amounts of product. The results and degree of resolution are best observed in Table 5. Molecular-weight distributions obtained by mass spectrometer analysis are also given.

In addition, densities of the normal paraffins in the range of C₁₉–C₃₅ are computed from X-ray data taken from the literature, and recorded.

DISCUSSION

The finding of even-numbered paraffins is of interest in connexion with the theory of their formation in the living plants. Although the situation is not fully clarified, one possible cycle is that proposed by Chibnall & Piper (1934), wherein odd-numbered paraffins result from β -oxidation of even-numbered acids, followed by decarboxylation and reduction of the odd ketones. However, the presence of even-numbered hydrocarbons would appear to require the existence at least temporarily of some odd carboxylic acids. One means of explaining this begins with unsaturated acids of higher molecular weight. Chibnall & Piper (1934) show such an example, beginning with linolenic acid.

If there should exist in plants a cycle analogous to the coenzyme A (CoA) synthesis route postulated for animal tissues, then both odd and even paraffins could be explained (Lynen, 1953; Lynen & Ochoa, 1953; Stadtman, 1954). These authors state that two molecules of acetyl-CoA condense to yield one molecule of acetoacetyl-CoA. Eight such repetitions of the complete four-stage cycle account for the formation of stearic acid. It is possible that the odd-numbered fatty acids necessary to explain our results could come from slight variations of the CoA cycle:

(a) One molecule of acetyl-CoA and one of propionyl-CoA condense to form β -oxovaleryl-CoA. Thereafter the cycle is continued by additions of only acetyl-CoA. It would seem that this route is less probable than the regular acetyl-CoA cycle, since it is the even-numbered acids which are well known.

(b) If attack at the α -carbon should be a possibility, then at the termination of a normal CoA series, a molecule of propionyl-CoA could react in either sense to form either of two β -keto acids. Both of these on decarboxylation would yield an even-numbered straight-chain ethyl ketone, and ultimately on reduction a paraffin of even-numbered carbon chain. One of these alternatives is shown in Scheme I.

One additional mechanism is proposed below to account for both the odd and even paraffins, beginning only with the known even-numbered fatty acids. In this case alternate α -oxidation and β -oxidation are used to account for formation of a

minor proportion of odd-numbered acids. These latter would be intermediates in the formation of even paraffins as shown in Scheme II: (R represents an even number of carbon atoms). The fact that the ratio of odd to even paraffins is found to be so high experimentally, suggests that the β process proceeds faster than the α process (see Table 5).

Examination of the oxygenated fractions found with the paraffins in the cuticle waxes may give some evidence to support one or other of these theories and is being undertaken.

SUMMARY

1. Some resolution has been accomplished in a homologous series of paraffin hydrocarbons, by chromatography on Norit A carbon black. The degree of resolution is expressed in molecular-weight-distribution data.

2. Contrary to prior reports, all of the normal paraffins (instead of odd-numbered ones only) have been found in pyrethrum wax, and also in a sample of string bean wax. In the former case all members of the series from C_{24} to C_{36} have been found, but higher members might be present.

3. A mechanism is proposed to account for the presence of the even-numbered paraffins.

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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 con-

ditions, 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 $^{35}\text{SO}_4^{2-}$ (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. $(\text{NH}_4)_2\text{HPO}_4/\text{l.}$ 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