REFERENCES

- Bullough, W. S. (1946). *Philos. Trans. B*, **231**, 453.
 Crabtree, C. (1941). *Endocrinology*, **29**, 197.
 Eschenbrenner, A. B. (1944). *J. nat. Cancer Inst.* **5**, 251.
 Eschenbrenner, A. B. & Miller, E. (1945). *Science*, **102**, 302.
 Fishman, W. H. (1947). *J. biol. Chem.* **159**, 7.
 Fishman, W. H. & Fishman, L. W. (1944). *J. biol. Chem.* **152**, 487.
 Friedenwald, J. S. & Becker, B. (1948). *J. cell. comp. Physiol.* **31**, 303.
 Kerr, L. M. H., Graham, A. F. & Levvy, G. A. (1948). *Biochem. J.* **42**, 191.
 Kerr, L. M. H. & Levvy, G. A. (1948). *Nature, Lond.*, **162**, 219.
 Levvy, G. A., Kerr, L. M. H. & Campbell, J. G. (1948). *Biochem. J.* **42**, 462.
 Mills, G. T. (1947). *Nature, Lond.*, **160**, 638.
 Mills, G. T. (1948). *Biochem. J.* **43**, 125.
 Pincus, G. & Martin, D. W. (1940). *Endocrinology*, **27**, 838.
 Roberts, S. & Szego, C. M. (1947). *Endocrinology*, **40**, 73.
 Segaloff, A. (1946). *Endocrinology*, **38**, 212.
 Talbot, N. (1939). *Endocrinology*, **25**, 601.

Concentration of Lipids in the Brain of Infants and Adults

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METHODS

Histologically it has been shown that the fibre tracts of the brain are not fully myelinated at birth, but that myelination is completed later, coincident with the functional development of the central nervous system. Most of the medullated fibres of the nervous system are in the white matter, whereas most of the bodies of the nerve cells are in the grey matter. In order to investigate, therefore, the lipid components of the myelin sheath, the lipid distribution in the grey matter and white matter of the brain of the newborn infant has been compared with that of the adult brain.

The important lipids of the central nervous system are cerebroside, cholesterol and the phospholipins, lecithin, sphingomyelin and kephalin. In a previous report (Johnson, McNabb & Rossiter, 1948*a*) it was shown that white matter of brain is distinguished from grey matter by a greater concentration of cerebroside, free cholesterol and sphingomyelin. It was suggested that these lipids, rather than lecithin and kephalin, formed the lipid components of 'myelin'. Additional evidence was obtained for this view when it was found that medullated peripheral nerve was relatively rich in cerebroside, cholesterol and sphingomyelin, thus resembling the white matter of brain rather than grey matter (Johnson, McNabb & Rossiter, 1948*b*). It has now been shown that white matter of adult brain, in which myelination is complete, differs from 'white matter' of newborn infant brain in that it contains a higher concentration of these same lipids (cerebroside, cholesterol and sphingomyelin).

The brain was removed from each of five infants and five adults as soon as possible after death. The infants, whose ages ranged from 7 months' gestation (premature) to full term, died at birth or shortly afterwards. Samples of both grey matter and 'white' matter were taken from the cerebral hemispheres. The grey matter consisted of a thin shaving from the surface of the cerebrum. As there was practically no visible white matter in the infant, the 'white' sample was taken from the positions where white matter was known to occur in the adult brain.

A sample of tissue (1-3 g.) was rapidly weighed and repeatedly extracted with 50 ml. portions of a 1:1 ethanol-ether mixture as described previously (Johnson *et al.* 1948*a*). Additional samples of both grey matter and white matter were taken for the determination of the wet-weight to dry-weight ratio. A portion of the fresh tissue was added to a tared covered crucible, the crucible and tissue weighed, dried in an oven at 105° for 24 hr., cooled in a desiccator and reweighed. From these figures, the water content of the tissue was calculated. The concentrations of cerebroside, free cholesterol, total cholesterol, total phospholipin, mono-aminophospholipin and lecithin were determined in samples of the extract as previously described (Johnson *et al.* 1948*a*), and from these figures were calculated the concentrations of ester cholesterol, sphingomyelin and kephalin.

RESULTS

The figures for both white and grey matter of adult brain (Table 1) were similar to those obtained previously, when two human brains only were analyzed (Johnson *et al.* 1948*a*), and were considerably greater than those for infant brain, chiefly

Table 1. *Concentration of lipids in grey and white matter of infant and adult brain, expressed as percentage of fresh tissue weight*(Five infant and five adult brains examined. Results given as mean $\pm$ S.E.M.)

	Grey matter			White matter		
	Infant (%)	Adult (%)	Adult/infant ratio	Infant (%)	Adult (%)	Adult/infant ratio
Cerebroside	0.52 $\pm$ 0.06	0.87 $\pm$ 0.24	1.67 : 1	0.58 $\pm$ 0.01	4.78 $\pm$ 0.19	8.25 : 1
Total cholesterol	0.49 $\pm$ 0.06	0.99 $\pm$ 0.02	2.02 : 1	0.67 $\pm$ 0.07	4.22 $\pm$ 0.17	6.30 : 1
Free cholesterol	0.48 $\pm$ 0.07	0.97 $\pm$ 0.05	1.98 : 1	0.64 $\pm$ 0.07	4.15 $\pm$ 0.18	6.50 : 1
Ester cholesterol	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00	—	0.03 $\pm$ 0.03	0.07 $\pm$ 0.07	—
Total phospholipin	1.89 $\pm$ 0.27	3.36 $\pm$ 0.05	1.78 : 1	2.09 $\pm$ 0.19	7.03 $\pm$ 0.29	3.36 : 1
Monoaminophospholipin	1.77 $\pm$ 0.26	2.83 $\pm$ 0.08	1.60 : 1	1.96 $\pm$ 0.20	5.03 $\pm$ 0.37	2.57 : 1
Lecithin	0.75 $\pm$ 0.08	0.99 $\pm$ 0.09	1.32 : 1	0.85 $\pm$ 0.07	1.37 $\pm$ 0.12	1.61 : 1
Sphingomyelin	0.12 $\pm$ 0.05	0.53 $\pm$ 0.04	4.42 : 1	0.13 $\pm$ 0.04	2.00 $\pm$ 0.18	15.40 : 1
Kephalin	1.02 $\pm$ 0.18	1.85 $\pm$ 0.04	1.81 : 1	1.11 $\pm$ 0.13	3.66 $\pm$ 0.27	3.30 : 1

because the water content of adult brain was much less than that of infant brain.

Nevertheless, from Table 1, it can be seen that the ratio of the concentration of each individual lipid in the grey matter of the adult to the concentration of the same lipid in the infant was usually less than 2 and never greater than 4.42. For white matter the ratio for cerebroside, cholesterol (almost all of which was in the free form) and sphingomyelin was much greater, suggesting that it was a greater concentration of these lipids that distinguished the white matter of the adult brain from that of the infant.

The results of Table 1 appear somewhat different after allowance has been made for the water content of the tissues. Table 2 shows that the water content of infant brain was much higher than that of adult brain, and also that the value for grey matter of infant brain was not significantly different from that for white matter. The water content of the adult brain was much less, and it was found that grey matter had more than twice the water content of white matter. Thus, as the brain developed and myelin was deposited, the whole organ lost water, but relatively more was lost from the white matter than from the grey matter.

In Table 3 the values for the lipid components of both grey and white matter of infant and adult brain

are given in terms of dry weight. It can now be seen how strikingly similar was the distribution of lipids in the grey matter of the infant brain to that in the adult. For grey matter, with the exception of sphingomyelin with a ratio of 2.33, the adult to infant

Table 2. *Water content of grey and white matter of infant and adult brain*(Five infant and five adult brains examined. Results given as mean $\pm$ S.E.M.)

	Grey matter (mg./mg. dry tissue)	White matter (mg./mg. dry tissue)
Infant	9.74 $\pm$ 0.69	9.82 $\pm$ 0.72
Adult	5.34 $\pm$ 0.10	2.40 $\pm$ 0.06

ratio for each individual lipid was between 0.80 and 1.23. For white matter, however, the picture was quite different. The ratio for cerebroside and free cholesterol was 2.10 or more, and for sphingomyelin it was 4.87. Thus the white matter of adult brain had a higher concentration of cerebroside, free cholesterol and sphingomyelin than had the white matter of infant brain. The concentrations of total phospholipin in the white matter of the adult brain and infant brain did not differ significantly. There was no difference in the concentration of kephalin, and the greater concentration of sphingomyelin in the white

Table 3. *Concentration of lipids in grey and white matter of infant and adult brain, expressed as percentage of dry tissue weight*(Five infant and five adult brains examined. Results given as mean $\pm$ S.E.M.)

	Grey matter			White matter		
	Infant (%)	Adult (%)	Adult/infant ratio	Infant (%)	Adult (%)	Adult/infant ratio
Cerebroside	5.64 $\pm$ 0.91	5.54 $\pm$ 0.80	0.98 : 1	6.21 $\pm$ 0.39	16.28 $\pm$ 0.99	2.61 : 1
Total cholesterol	5.09 $\pm$ 0.37	6.28 $\pm$ 0.14	1.23 : 1	7.00 $\pm$ 0.44	14.33 $\pm$ 0.56	2.04 : 1
Free cholesterol	5.03 $\pm$ 0.35	6.17 $\pm$ 0.20	1.23 : 1	6.70 $\pm$ 0.33	14.08 $\pm$ 0.55	2.10 : 1
Ester cholesterol	0.06 $\pm$ 0.04	0.10 $\pm$ 0.05	—	0.30 $\pm$ 0.07	0.26 $\pm$ 0.22	—
Total phospholipin	19.56 $\pm$ 1.39	21.27 $\pm$ 0.49	1.08 : 1	22.04 $\pm$ 0.59	23.84 $\pm$ 0.73	1.08 : 1
Monoaminophospholipin	18.26 $\pm$ 1.36	17.96 $\pm$ 0.59	0.98 : 1	20.64 $\pm$ 0.62	17.01 $\pm$ 1.15	0.82 : 1
Lecithin	7.81 $\pm$ 0.45	6.25 $\pm$ 0.63	0.80 : 1	9.07 $\pm$ 0.28	4.63 $\pm$ 0.34	0.51 : 1
Sphingomyelin	1.30 $\pm$ 0.46	3.03 $\pm$ 0.29	2.33 : 1	1.40 $\pm$ 0.58	6.82 $\pm$ 0.67	4.87 : 1
Kephalin	10.46 $\pm$ 1.08	11.71 $\pm$ 0.40	1.11 : 1	11.57 $\pm$ 0.58	12.39 $\pm$ 0.83	1.07 : 1

matter of the adult brain was balanced by a lesser concentration of lecithin (Table 3). This point is well illustrated in Table 4, where the individual phospholipins are expressed as a percentage of the total phospholipin. For white matter the percentage of kephalin was about the same (52%) for both infant and adult brain. Lecithin accounted for 41% of the total phospholipin for infant brain and only 19% for the adult, whereas sphingomyelin accounted for 6% of the total phospholipin for infant brain and 29% for the adult.

by the numerous publications of Donaldson (see Donaldson & Hatai, 1931, for references). This raises the question of whether infant brain should be compared with adult brain on a wet-weight basis, as has been done by many workers. For instance, Backlin (1930) found that the concentration of total phospholipin in the whole brain of the adult rabbit was greater than that of a newborn rabbit in terms of wet weight, but that there was no difference when dry weight was used as a reference standard. We have found the same to be true for the white

Table 4. *Concentration of phospholipins in grey and white matter of infant and adult brain, expressed as percentage of total phospholipid*

(Five infant and five adult brains examined. Results given as mean $\pm$ S.E.M.)

	Grey matter			White matter		
	Infant (%)	Adult (%)	Adult/infant ratio	Infant (%)	Adult (%)	Adult/infant ratio
Lecithin	40.28 $\pm$ 2.31	29.16 $\pm$ 2.46	0.72 : 1	41.22 $\pm$ 1.85	19.42 $\pm$ 1.35	0.46 : 1
Sphingomyelin	6.62 $\pm$ 2.37	15.68 $\pm$ 1.40	2.37 : 1	6.28 $\pm$ 2.61	28.80 $\pm$ 3.31	4.57 : 1
Kephalin	53.10 $\pm$ 2.48	55.20 $\pm$ 2.55	1.04 : 1	52.50 $\pm$ 1.94	51.78 $\pm$ 2.17	0.99 : 1

DISCUSSION

Frankel & Linnert (1910) found that the concentration of total lipid in the whole brain of the adult was greater than that in the brain of the newborn infant. Using the classical differential solubility techniques they showed that the increase in lipid concentration in adult brain was not confined to one lipid fraction only. This work was confirmed by Schiff & Stransky (1921) and recently by Schuwirth (1940). Raske (1886) found that the whole brain of foetal calves contained no cerebroside, a result confirmed by Mendel & Leavenworth (1908) using pigs. Noll (1899), when studying the 'protagon' fraction of human brain, observed that with the beginning of myelination there appeared an ethanol-soluble substance from which a reducing sugar was split off on hydrolysis. Koch & Koch (1913) showed that cerebroside was not present in the brain of newborn rats, but that the concentration of cerebroside, phospholipin and cholesterol increased with age. A similar finding was reported for the dog (Smith & Mair, 1912-13a), the rabbit (Backlin, 1930) and man (Koch & Mann, 1907; MacArthur & Doisy, 1919). Less comprehensive work on total phospholipin has been done for rat brain (Lang, 1937; Fries, Entenman, Changus & Chaikoff, 1941), and human brain (Bergamini, 1925; Singer & Deutschberger, 1928; Cattaneo, 1932). Similar studies on total cholesterol have been reported for rat brain (Lang, 1937; Fries *et al.* 1941), dog brain (Mansfeld & Liptak, 1913) and human brain (Rosenheim, 1914; Bergamini, 1925; Page & Menschick, 1931; Cattaneo, 1931). In addition, McConnell & Sinclair (1937) using rats, reported that the lecithin and kephalin fatty acids increased during growth. They did not estimate the individual phospholipins, nor did they distinguish between white and grey matter; hence from these studies very little can be deduced about the chemical nature of myelin.

That foetal brain contains more water than does adult brain was known to most of the above authors and the subject has been well reviewed for the rat

matter of human brain. In terms of wet weight (Table 1) the concentration of total phospholipin was greater in the adult than in the newborn infant, but when referred to unit dry weight there was no such difference (Table 3). Previously (Johnson *et al.* 1948a, b), lipids have been expressed as a percentage of 'essential lipid', i.e. as a percentage of the sum of the cerebroside, total cholesterol and total phospholipin. The values for the adult and infant brains were also calculated on an 'essential lipid' basis, but this method provided no further information than that already given in Table 3.

The recent report of Williams, Galbraith, Kaucher, Moyer, Richards & Macy (1945) on the lipids of the brains of rats of different ages is of interest for they estimated the individual phospholipins. It is difficult, however, to make a strict comparison of their results with our own, for, besides the species difference, the method which they used to distinguish the individual phospholipins was not the same as ours, and the white and grey matter were not separated. Williams *et al.* (1945) found that with increasing age, in addition to the well established greater concentrations of phospholipin, cerebroside and cholesterol, there was a large increase in kephalin, a lesser increase in sphingomyelin and a decrease in lecithin. In our study on the white matter of the human brain there was a lesser concentration of lecithin in the adult than in the infant, the same concentration of kephalin and a much greater concentration of sphingomyelin.

Many of the pioneers of brain chemistry noted the great difference between the lipids of white and those of grey matter. For instance, both Petrowsky (1873) and Thudichum (1901) found considerably more cholesterol and

cerebroside in white than in grey matter, and less lecithin. These findings have subsequently been confirmed (Smith & Mair, 1912-13*b*; Frankel & Linnert, 1910; Kirschbaum & Linnert, 1912; Yasuda, 1937; Randall, 1938; Johnson *et al.* 1948*a*). In addition, Schmidt, Benotti, Hershman & Thannhauser (1946) showed that the concentration of sphingomyelin in the white matter of ox brain was greater than that in grey matter. Many of these workers also noted that the concentration of water was greater in grey than in white matter.

That the lipids of white matter of foetal brain resemble those of grey matter was first pointed out by Raske (1886) and later by Frankel & Linnert (1910) and Smith & Mair (1912-13*b*). Not only have we confirmed this similarity for cerebroside, cholesterol and total phospholipin, but also for lecithin, sphingomyelin and kephalin.

Since the white matter of adult brain is distinguished from that of the brain of the newborn by greater concentrations of cerebroside, cholesterol and sphingomyelin, and, since it has been shown histologically that myelination of the various tracts is complete in the white matter of the adult brain and not in the brain of the newborn infant, it is perhaps reasonable to assume that these are the principal lipids of the myelin sheath. However, a word of caution is necessary. Waelsch, Sperry & Stoyanoff (1941) have pointed out that after birth lipid is deposited in the brain of the rat as a result of two processes, (a) growth and (b) myelination. Immediately after birth and before myelination is complete there is an active deposition of brain lipids as shown by isotope studies. The incorporation of radioactive phosphorus in the phospholipin fraction (Fries, Changus & Chaikoff, 1940) and of deuterium in both the fatty acid and non-saponifiable fraction of the brain lipids (Waelsch *et al.* 1941) was greatest in young rats immediately after birth, when growth was greatest, and decreased sharply with increasing age, even although myelination was proceeding rapidly. Such experiments show that much new lipid material is deposited in the brain after birth as a result of growth, and the differences between the adult and the newborn need not be necessarily the result of myelination.

In general, the lipid distribution is similar in both white and grey matter of infant brain and closely resembles that of grey matter of adult brain. The white matter of the adult is distinguished from that of the newborn by a much greater concentration of cerebroside, free cholesterol and sphingomyelin. This observation, together with the finding that these lipids are present in high concentrations in peripheral medullated nerves, makes it likely that cerebroside, free cholesterol and sphingomyelin, rather than lecithin and kephalin, go to make up the lipids of 'myelin'.

SUMMARY

1. The concentration of cerebroside, free cholesterol, total cholesterol, total phospholipin, lecithin, sphingomyelin and kephalin has been determined in both the grey and white matter of the brains of five infants and five adults.

2. The water content of both white and grey matter of infant brain was greater than that of adult brain, and for the adult the water content of grey matter was much greater than that of white matter.

3. The distribution of lipids in the white matter of infant brain resembled that of the grey matter and also the grey matter of adult brain.

4. The distribution of lipids in the white matter of adult brain differed from that of the white matter of infant brain in that there was a higher concentration of cerebroside, free cholesterol and sphingomyelin.

5. Although, in terms of dry weight, the white matter of adult brain had about the same total phospholipin content as white matter of infant brain, it contained more sphingomyelin and less lecithin.

6. The data reported here, taken in conjunction with those of previous studies, suggest that cerebroside, free cholesterol and sphingomyelin are the principal lipid components of the 'myelin' sheath of nerve fibres.

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REFERENCES

- Backlin, E. (1930). *Beiträge zur quantitativen Kenntnis der Gehirnlipide*. Uppsala: Almqvist and Wiksell.
 Bergamini, M. (1925). *Ber. ges. Physiol.* **31**, 281.
 Cattaneo, L. (1931). *Ann. Ostet. Ginec.* **53**, 1755.
 Cattaneo, L. (1932). *Ann. Ostet. Ginec.* **54**, 577.
 Donaldson, H. H. & Hatai, S. (1931). *J. comp. Neurol.* **53**, 263.
 Frankel, S. & Linnert, K. (1910). *Biochem. Z.* **26**, 44.
 Fries, B. A., Changus, G. W. & Chaikoff, I. L. (1940). *J. biol. Chem.* **132**, 23.
 Fries, B. A., Entenman, C., Changus, G. W. & Chaikoff, I. L. (1941). *J. biol. Chem.* **137**, 303.
 Johnson, A. C., McNabb, A. R. & Rossiter, R. J. (1948*a*). *Biochem. J.* **43**, 573.
 Johnson, A. C., McNabb, A. R. & Rossiter, R. J. (1948*b*). *Biochem. J.* **43**, 578.
 Kirschbaum, P. & Linnert, K. (1912). *Biochem. Z.* **46**, 253.
 Koch, W. & Koch, M. L. (1913). *J. biol. Chem.* **15**, 423.
 Koch, W. & Mann, S. A. (1907). *J. Physiol.* **26**, xxxvi.
 Lang, A. (1937). *Hoppe-Seyl. Z.* **246**, 219.

- MacArthur, C. G. & Doisy, E. A. (1919). *J. comp. Neurol.* **30**, 445.
- Mansfeld, G. & Liptak, P. (1913). *Pflüg. Arch. ges. Physiol.* **152**, 68.
- McConnell, K. P. & Sinclair, R. G. (1937). *J. biol. Chem.* **118**, 131.
- Mendel, L. B. & Leavenworth, C. S. (1908). *Amer. J. Physiol.* **21**, 99.
- Noll, A. (1899). *Hoppe-Seyl. Z.* **27**, 370.
- Page, I. H. & Menschick, W. (1931). *Biochem. Z.* **231**, 446.
- Petrowsky, D. (1873). *Pflüg. Arch. ges. Physiol.* **7**, 367.
- Randall, L. O. (1938). *J. biol. Chem.* **124**, 481.
- Raske, K. (1886). *Hoppe-Seyl. Z.* **10**, 336.
- Rosenheim, M. C. (1914). *Biochem. J.* **8**, 82.
- Schiff, E. & Stransky, E. (1921). *Jb. Kinderheilk.* **96**, 245.
- Schmidt, G., Benotti, J., Hershman, B. & Thannhauser, S. J. (1946). *J. biol. Chem.* **166**, 505.
- Schuwirth, K. (1940). *Hoppe-Seyl. Z.* **263**, 25.
- Singer, K. & Deutschberger, O. (1928). *Biochem. Z.* **198**, 328.
- Smith, J. L. & Mair, W. (1912-13a). *J. Path. Bact.* **17**, 123.
- Smith, J. L. & Mair, W. (1912-13b). *J. Path. Bact.* **17**, 418.
- Thudichum, J. L. W. (1901). *Die chemische Konstitution des Gehirns des Menschen und der Tiere*. Tübingen: F. Pietzcker.
- Waelsh, H., Sperry, W. M. & Stoyanoff, V. A. (1941). *J. biol. Chem.* **140**, 885.
- Williams, H. H., Galbraith, H., Kaucher, M., Moyer, E. Z., Richards, A. J. & Macy, I. G. (1945). *J. biol. Chem.* **161**, 475.
- Yasuda, M. (1937). *J. Biochem., Tokyo*, **26**, 203.

Effects of Amidines on Oxidases of *Escherichia coli* and of Animal Tissues

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Interest in the diamidines as antiprotozoal and antibacterial agents led us to consider their possible mechanism of action, and a study of their effects on oxidation systems offered a method of approach. Bernheim (1943, 1944) reported that certain amidines inhibited the oxidation of D-proline and of both isomers of alanine by *Escherichia coli*, but were without effect on the oxidation of glucose, succinate or pyruvate at similar concentrations.

We considered that further valuable information would possibly be revealed by studying the effects of amidines at higher concentrations, using also a wider range of substrates. Accordingly, we have carried out an investigation along these lines, with *Esch. coli* as the test organism. Propamidine (1:3-di-(4'-amidinophenoxy)propane) was mainly employed, but a number of experiments were also carried out with hexamidine (1:6-di-(4'-amidinophenoxy)hexane) and two halogenated derivatives, i.e. dibromopropamidine (1:3-di-(2'-bromo-4'-amidinophenoxy)propane) and monoiodohexamidine (1:4'-amidinophenoxy-6-(2'-iodo-4'-amidinophenoxy)-hexane). Some parallel experiments on the oxidation systems of rat tissues were included. A recent communication (Wien, Harrison & Freeman, 1948), in which some of our results were quoted, has dealt with the diamidines as bactericides.

EXPERIMENTAL

Measurement of respiration

The Warburg manometric technique was used for following O_2 uptakes at 37°. The bacterial suspension or tissue preparation was contained in the main vessel in 0.033M-phosphate buffer (for tissue preparations, phosphate-Locke medium was used), with or without the amidine salt in appropriate concentration. A 6% KOH solution (0.2 ml.) was introduced into the central tube of the vessel, and soaked into a small piece of rolled Whatman no. 41 filter paper. The substrate solution (0.2 ml.) was contained in the side arm and was tipped into the main vessel after a suitable equilibration or incubation period. The total volume of vessel contents, including those of the side arm and central tube, was always 3 ml.

Organisms

Esch. coli (National Collection of Type Cultures no. 4144) was used. The organisms were sown on trypsin digest-agar plates, and after 18-24 hr. growth the cultures were washed off the plates, using 3 ml. of distilled water for each plate. The collected suspension was centrifuged, was twice washed by resuspending in distilled water and recentrifuging, and finally diluted to 7.15 mg. dry wt./ml. by the use of Wellcome standard opacity tubes. Of this suspension 0.5 ml. was used for each vessel. It was confirmed that no appreciable change in the suspension occurred during the time occupied by the experiments. The O_2 uptake due to the organisms alone, without substrate, was usually not greater than 20 μ l./hr.; since this was small in comparison with the O_2 uptake in the presence of most substrates employed, it is permissible to regard the difference as representative of the O_2 used in the

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