Schmitt & Gasser (1933) were able to correlate the increase in metabolism, brought about in nerve by electrical stimulation in the presence of veratrine, with an increase of the negative after-potential. It is not unlikely that the potentiation of the metabolic effect of brief electrical stimuli, brought about in sliced cerebral cortex by protoveratrine, is attended by a corresponding electrical change. In the nerve fibre, protoveratrine produces a lesser, but longer-lasting augmentation of the negative after-potential than does veratrine (Graham & Gasser, 1931). However, nothing is known as yet about the action of these or of other veratrum alkaloids on electrical activity in the higher parts of the nervous system. This also makes it all the more difficult to explain why the accelerating effects of protoveratrine and of high-frequency electrical stimuli do not sum up, although (see Wollenberger, 1955) they are strikingly similar in many respects. It seems that, as in the case of the veratrine effect on heat production in nerve (Hill, 1933), a period of rest between electrical stimuli is required in order that the protoveratrine effect can take its course.

SUMMARY

1. Simultaneous exposure of cerebral-cortex slices to protoveratrine and to brief condenser pulses, delivered at a rate of 100 per sec., did not

bring about a greater increase in the rate of respiration than did exposure to the drug or to the pulses alone.

2. Simultaneous exposure of cerebral-cortex slices to protoveratrine and to brief condenser pulses, delivered at a rate of 1–2 every 2.6–2.9 sec., brought about an increase in the rate of respiration which was greater than the sum of the increases caused by the drug and by the pulses individually.

3. The rate of respiration of isolated white matter of brain (corpus callosum) was increased both by electrical pulses and by protoveratrine.

I wish to express my thanks to Dr H. McIlwain for his hospitality and for his help and advice during this work which was undertaken during the tenure of a Life Insurance Medical Research Fund Fellowship.

REFERENCES

- Ayres, P. J. W. & McIlwain, H. (1953). *Biochem. J.* **55**, 607.
Dixon, T. F. & Meyer, A. (1936). *Biochem. J.* **30**, 1577.
Graham, H. T. & Gasser, H. S. (1931). *J. Pharmacol.* **43**, 163.
Hill, A. V. (1933). *Proc. Roy. Soc. B*, **113**, 386.
Kratzing, C. C. (1951). *Biochem. J.* **50**, 253.
McIlwain, H. (1951). *Biochem. J.* **49**, 382.
McIlwain, H. (1954). *J. Physiol.* **124**, 117.
Schmitt, F. O. & Gasser, H. S. (1933). *Amer. J. Physiol.* **104**, 320.
Wollenberger, A. (1955). *Biochem. J.* **61**, 68.

The Resynthesis of Glycogen by Guinea Pig Cerebral-Cortex Slices

By F. N. LEBARON*

Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, S.E. 5

(Received 29 December 1954)

Some time ago Kerr (1936) showed that the glycogen in the brain is very labile and that its determination during *in vivo* experiments required special quick-freezing techniques. This lability has since been confirmed by others (Chesler & Himwich, 1943; Albaum, Tepperman & Bodansky, 1946; Klein & Olsen, 1947), and recently Chance & Yaxley (1950) showed that the brain glycogen of mice dropped markedly within 1 min. after death and had effectively disappeared in 4–5 min.

* U.S. Public Health Service Postdoctorate Research Fellow of the National Institute of Neurological Diseases and Blindness. Present address: Department of Biochemistry, McLean Hospital, Waverley, Massachusetts, U.S.A.

Consequently, as prepared for *in vitro* experiments, brain slices, unlike liver slices or hemidiaphragm preparations, contain practically no glycogen, and to the best of our knowledge resynthesis of glycogen by such slices has not previously been demonstrated.

In the studies of labile brain metabolites conducted in this laboratory, conditions for resynthesis of glycogen by surviving guinea pig cerebral-cortex slices were sought and are now reported, together with some studies of the effects of changes in concentration of potassium and phosphate ions, of added 2:4-dinitrophenol, and of electrical pulses, on the resynthesis.

METHODS

Metabolic techniques. In all experiments thin slices of guinea pig cerebral cortex were prepared and incubated in an oxygen atmosphere by the same methods that have been used previously in this laboratory (McIlwain, 1951*a*). Three slices of about 100 mg. moist weight were cut successively from each cerebral hemisphere and incubated in 3.5 ml. saline in separate conical manometric vessels of about 15 ml. capacity. The standard saline, buffered with phosphate at pH 7.4, was made as described by Rodnight & McIlwain (1954) and was similar to that found to promote the resynthesis of labile tissue phosphates (McIlwain, Buchel & Cheshire, 1951). Glucose was used as substrate throughout, and any additions or modifications are described in the individual experiments. Respiratory rate was measured in all experiments as a criterion of the metabolic condition of the slices. In experiments testing the effects of agents and changes in the incubating medium, the three slices from one hemisphere of a brain were incubated under normal conditions and served as control for the slices of the other hemisphere of the same brain which were incubated under the chosen experimental conditions.

The time between death of the animal and final equilibration at 37° was ordinarily about 20 min. Glycogen synthesis in slices rapidly prepared from one hemisphere was compared with that occurring in slices prepared more slowly from the companion hemisphere, the time of preparation being varied from 10 to 30 min. No difference was found and therefore the normal procedure of slice preparation was not altered.

Estimation of glycogen. The method of estimating glycogen was a micromodification of Kerr's (1936) method. Experiments were terminated by transferring each slice as rapidly as possible from the manometric flask to a centrifuge tube calibrated at 2 ml. and containing 1 ml. of ethanolic KOH (2 vol. abs. ethanol + 1 vol. 60% w/v, aqueous KOH). A small stainless-steel spatula was used for this transfer which took 5–10 sec. Each tube was immediately shaken until the solvent had penetrated the slice, and, after all slices had been transferred, the tubes were loosely stoppered with glass bulbs and heated in a hot-water bath at 80–85° for 10 min. with frequent shaking. They were then cooled in cold water and centrifuged at 3000 rev./min. for 30 min. The ethanolic KOH was decanted and drained by inverting the tubes on a filter paper pad. To wash the residue free of cerebrosides, it was suspended in 1 ml. CHCl_3 -MeOH (1:4, v/v), the suspension briefly brought to boiling in a hot-water bath, and centrifuged for 10 min. The supernatant fluid was then decanted and the remaining liquid drained away by inverting the tube. This washing was repeated twice. The residue A was then dried by putting the tubes in a boiling-water bath for 10 min.

The glycogen in A was hydrolysed by adding 1 ml. $\text{N-H}_2\text{SO}_4$ to each tube and heating in a boiling-water bath for 2 hr. The solutions were then cooled, roughly neutralized to a pH between 4.5 and 9.5 with NaOH, and diluted to the 2 ml. mark with water. Reducing substance was determined on 0.5 ml. portions by the colorimetric method of Nelson (1944), using 0.5 ml. each of copper reagent and colour reagent and a final vol. of 6 ml. Optical density was measured in a Spekker absorptiometer, using Ilford filter no. 608 (620 $\text{m}\mu$. onwards). A glucose standard was run with each set of glycogen hydrolysates. It should be noted

that HCl cannot be substituted for H_2SO_4 in hydrolysis since the chloride ion interferes with the colorimetric method in this low range.

Because of the rapid *in vivo* loss of brain glycogen it was feared that the resynthesized glycogen in the slices might also be labile. Accordingly, slices from some hemispheres were placed on the side of the centrifuge tubes for varying times before being submerged in the ethanolic KOH. No decrease in the glycogen level below that of the corresponding slice in the rapidly fixed hemisphere was noted in the slices for which fixing was delayed up to 1 min. Varying losses did occur in long intervals depending upon how well the slice was drained of saline. In all experiments reported subsequently in this paper the transfer of slices was done as quickly as possible, i.e. within 5–10 sec., and thus no loss is indicated during transfer.

Electrical pulses. In experiments using electrical pulses the methods reported previously from this laboratory were employed, using alternating condenser pulses of 18 v peak potential and 0.4 msec. duration at 100/sec. in all cases. The apparatus and holders used in long stimulation experiments were described by McIlwain (1951*a*), and that used for short stimulation and rapid transfer by Heald, McIlwain & Nelligan (1954). Slices from control, unstimulated hemispheres were put in identical holders or apparatus and treated exactly as were the stimulated slices, except for the actual passage of current.

RESULTS

The rate of resynthesis of glycogen

Glycogen was determined in cortical slices after varying incubation times (Table 1). Mean values for each time of incubation are given and also separate results for the three slices cut successively from the cortex. The maximum rate of synthesis was about 1 μmole glucose equiv./g. moist tissue/hr. There was no significant difference in synthesis between the three layers, although the top slice had a consistently higher initial glycogen content. Glycogen had reached maximum level in the slices from some hemispheres in 2–3 hr. and in most by 4 hr. Levels attained after 6 hr. were never higher than those obtained with shorter incubations.

Evidence indicating the substance synthesized is glycogen

The method used for isolation of the substance to be analysed demonstrated that it had certain of the physical and chemical properties of liver glycogen, i.e. insolubility in ethanol or CHCl_3 -MeOH, solubility in water, and stability to hot alkali. To further characterize this material supposedly containing glycogen, the following tests were performed on the solid matter which remained in the centrifuge tubes after decanting the ethanolic KOH, washing with hot CHCl_3 -MeOH and drying (residue A, preceding column).

Iodine test. A drop of dilute Lugol's iodine solution (50 mg. I_2 + 100 mg. KI/100 ml.) was put on

the dried residue *A* obtained from incubated and unincubated slices. No colour was obtained from residues of unincubated slices, but with residues from slices incubated 4 hr. a marked red-purple colour was obtained which was similar to that given by an equivalent amount of glycogen under similar circumstances.

Anthrone test. The test as described by Kahan (1953) gave a substantial colour with the reagents employed in separating the glycogen. A darker colour was obtained with residues from slices incubated 4 hr., but not with residues from unincubated slices.

Lack of reducing action without hydrolysis. The dried residue *A* from a 4 hr.-incubated slice was dissolved directly in 2 ml. water without hydrolysis, and reducing substance was determined by the usual procedure. None was found.

Destruction of reducing substance by fermentation. A number of residues *A* from incubated slices were

hemisphere of the same brain were incubated in phosphate-free saline buffered with 25 mM aminotrihydroxymethylmethane at pH 7.4. Synthesis by slices (Table 2) in the two salines was equal within the normal variation between slices.

Glycogen synthesis and agents which stimulate respiratory rate

2:4-Dinitrophenol. 2:4-Dinitrophenol was added to the standard saline to give concentrations varying from 2.6×10^{-6} to 4.5×10^{-5} M and these salines were used for incubating the experimental slices (Table 3). The synthesis of glycogen was reduced by very low concentrations of this agent and eliminated at concentrations lower than that which maximally stimulates respiration. At a concentration of 1.8×10^{-4} M, which is reported (Peiss & Field, 1948) to inhibit respiration of cerebral cortex slices, we found no synthesis of glycogen. However, in contrast to the findings of the previous

Table 1. *Glycogen content of guinea pig cerebral-cortex slices incubated for various times in standard saline*

Top, second and third slices were cut successively from guinea pig cerebral cortex and incubated aerobically at 37°. Values given are means followed by standard deviation, the number of determinations in parentheses, and, in the last line only, the range of values.

Slice	Glycogen content (μmoles glucose/g. moist tissue) after incubation for			
	0	1 hr.	2 hr.	4 hr.
Top	1.0±0.4 (4)	1.4±0.5 (10)	2.6±0.9 (21)	3.4±0.6 (30)
Second	0.3±0.1 (12)	0.8±0.3 (14)	2.2±0.8 (15)	2.8±0.7 (22)
Third	0.4±0.2 (22)	1.0±0.3 (10)	2.0±0.4 (8)	2.7±0.9 (18)
Mean for all slices	0.4±0.2 (38) 0-1.6	1.1±0.3 (34) 0.3-2.0	2.3±0.8 (44) 0.9-3.7	3.0±0.7 (70) 1.3-4.6

hydrolysed and analysed in the normal manner. The solutions remaining after portions were taken for analysis were pooled to obtain sufficient material for yeast fermentation (Raymond & Blanco, 1928). For fermentation, 2.5 ml. of this was treated with 1 ml. of washed yeast suspension (0.25 g. yeast/ml. water) and the process stopped with 0.5 ml. (5%, w/v) tungstic acid. The fermented solution and appropriate yeast- and reagent-blanks were analysed for glucose by Nelson's (1944) method. Results indicated that the reducing substance released on hydrolysis of residue *A* was at least 90% fermentable by yeast.

Glycogen synthesis and phosphate concentration

The slices of one hemisphere were incubated in the usual phosphate-buffered saline which was 11.7 mM in phosphate while slices from the other

Table 2. *Comparison of glycogen synthesis by slices from companion cerebral hemispheres in phosphate-free and phosphate-buffered salines*

Phosphate-buffered saline was the standard saline. In phosphate-free saline KH_2PO_4 was replaced by equimolar KCl and phosphate buffer was replaced by 25 mM aminotrihydroxymethylmethane buffer at pH 7.4.

Expt. no.	Saline	Incubation time (hr.)	Glycogen content (μmoles glucose/g. moist tissue)
1	PO_4 -free	2	1.7, 1.3
		4	1.9, 1.9
	PO_4 -buffered	2	1.8, 2.1
		4	2.1, 2.2
2	PO_4 -free	2	2.2, 2.5
		4	1.8, 1.9
	PO_4 -buffered	2	3.7, 3.3
		4	2.5, 2.5

workers, in our experiments the respiration was observed to be initially rapid and then to decrease steadily to negligible values.

To ascertain whether or not glycogen already formed in incubated slices was removed by subsequent addition of 2:4-dinitrophenol, experiments were done in which this agent was tipped into the flasks from the side-arms after 3 or 3.5 hr. incubation. In these cases the final 2:4-dinitrophenol concentration was $4.5 \times 10^{-5} \text{ M}$ and the glycogen content dropped nearly to its initial level within 10 min. after tipping in the 2:4-dinitrophenol. It was completely eliminated in 20 min. Control hemispheres synthesized the normal amount of glycogen.

potassium ion, although the oxygen consumption was markedly increased. The apparent increase of 26 % is less than the observed variation of $\pm 28 \%$ shown (Table 1) by normal slices incubated for 1, 2 and 4 hr. Also the glycogen levels of individual slices in high-potassium saline were not consistently higher than corresponding slices from control hemispheres.

Electrical pulses. It was found that the increased respiratory rate obtainable with electrical pulses in the present experiments was not maintained more than 1.5–2 hr., and that the tissue did not respond well to the pulses after 4 hr. incubation for the purpose of obtaining maximum glycogen synthesis. Consequently, the study of effects of

Table 3. *Effect of agents stimulating respiratory rate on glycogen synthesis by slices of guinea pig cerebral cortex*

In each experiment control slices were from one cerebral hemisphere and treated slices from the other hemisphere of the same brain. 2:4-Dinitrophenol was added to standard saline and was present throughout the incubation. Increase in K^+ was made by replacing NaCl in the standard saline with KCl. Electrical pulses were applied to the slices in holders type D (McIlwain, 1951a).

Stimulating agent	Incubation time (hr.)	Average stimulation of respiratory rate (%)	Average glycogen content ($\mu\text{moles glucose/g. moist tissue}$)	
			Control slices	Treated slices
$2.6 \times 10^{-6} \text{ M}$ 2:4-dinitrophenol	1	15	1.0	0
	2	4	2.7	1.6
	4	15	3.8	2.9
$5.2 \times 10^{-6} \text{ M}$ 2:4-dinitrophenol	1	30	0.8	0.4
	2	28	1.6	1.3
	3	21	2.3	0.6
	4	46	3.2	2.4
$6.5 \times 10^{-6} \text{ M}$ 2:4-dinitrophenol	1	40	0.8	0
	2	29	3.4	1.4
	4	33	3.6	2.1
$1.3 \times 10^{-5} \text{ M}$ 2:4-dinitrophenol	1	88	1.3	0.5
	2	84	1.5	0.4
	4	74	3.2	0.8
$4.5 \times 10^{-5} \text{ M}$ 2:4-dinitrophenol	1	140	1.1	0.1
	2	117	2.0	0.2
	3	152	2.1	0.1
26 mM K^+	4	38	3.1	3.9
45 mM K^+	2	72	1.9	2.4
Electrical pulses for 1 hr., after 1 hr. incubation	2	60	1.7	0.9
Electrical pulses for 2 hr., after 15 min. incubation	2.25	92	2.6	1.1
Continuous electrical pulses without previous incubation	1.75	70	1.5	0.9

Potassium ion. To test the effect of increased K^+ content of the incubating medium on glycogen synthesis, test salines were made in which part of the NaCl was replaced by equimolar KCl. The results of experiments using these salines (Table 3) show that there was no significant change in glycogen synthesis by slices in salines containing as much as seven times the normal amount of

electrical pulses on glycogen synthesis was limited to shorter experiments. Application of electrical pulses (Table 3) lowered the level to which glycogen was synthesized, whether stimulation was continuous from the start or an incubation period up to an hour was allowed first. The decreases of 40 and 58 % are beyond the variation between normal slices, and stimulated slices were consistently lower

in glycogen content than corresponding control slices.

In an attempt to see whether a rapid, transient rise in glycogen level, similar to that reported *in vivo* in convulsed mice (Chance & Yaxley, 1950), could be obtained after the glycogen level had been built up by incubation of the slices, experiments were run using the apparatus described by Heald *et al.* (1954) for rapid stimulation and transfer of slices. The slices were incubated for 3.5 hr. in the usual manometric vessels, and then subjected to pulses for 10 sec. and fixed rapidly. No difference was found between slices which received pulses and those which did not.

DISCUSSION

Glycogen from the brain fixed *in vivo* has been shown by isolation (Kerr, 1938) to be chemically indistinguishable from liver glycogen, i.e. it is a polysaccharide of glucose. As further evidence for this Brante (1954) recently reported that cerebral glycogen exhibited only one spot when hydrolysates were subjected to chromatography for sugars. The results of the present paper show that a chemically similar material is obtainable *in vitro* from cerebral cortex slices. The maximum amount of glycogen synthesized by the slices was about 75 $\mu\text{g.}/100\text{ mg.}$ moist tissue, although a few higher values were obtained. No data have been found for *in vivo* levels of glycogen in guinea pig brain but this figure is closely similar to values for normal mouse cerebral cortex (Chance & Yaxley, 1950) and for adult dog and cat cerebral cortex (Chesler & Himwich, 1943).

The rate of glycogen synthesis in these studies is only about one-tenth the rates of synthesis reported *in vitro* in diaphragm (Stadie & Zapp, 1947) and liver (Ostern, Herbert & Holmes, 1939) and is also much less than the maximal rate of glycogenolysis in the brain *in vivo*. Relatively slow fall and still slower resynthesis in the brain *in vivo* have also been reported (Gaevskaya, 1953). In view of the present concepts of the structure of glycogen macromolecules, the low rate found in the present work may perhaps be explained by the fact that the initial level of glycogen, at the start of incubation, is very much lower in cerebral-cortex slices than in diaphragm or liver slices. A synthesis of limit dextrins may first be necessary in the cerebral tissue and this reaction has been shown to be slower than simple extension of existing peripheral glucose chains (Stetten & Stetten, 1954). The rather large variation in amount of synthesis in normal brain tissue may also be partly accounted for by this initial very low level, since it has been shown (Kerly & Ottaway, 1954) with liver slices that the extent of glycogen synthesis depended upon the

initial level, and this effect could be expected to be accentuated at very low levels. Variation with diet, also reported by these workers with liver and diaphragm, would be minimal with cerebral glycogen (Kerr & Ghantus, 1936).

That no change in glycogen synthesis occurred with variation in phosphate-ion concentration when the rest of the ionic environment was unchanged, is similar to findings with diaphragm (Stadie & Zapp, 1947) and liver (Buchanan, Hastings & Nesbitt, 1949).

In comparing the effects on glycogen synthesis of the three different agents stimulating respiratory rate, a number of factors must be considered. In several respects the metabolic effects of these agents on cerebral tissue have been shown to be similar but not additive (McIlwain & Gore, 1951; Gore & McIlwain, 1952; McIlwain, 1952). However, it has been found (McIlwain, 1951*b*) that atropine acts differently on the metabolism of cerebral tissues stimulated by electrical pulses and by 2,4-dinitrophenol or by potassium ions. Also, potassium salts specifically stimulate glycogenesis in liver (Buchanan *et al.* 1949) although not in diaphragm (Stadie & Zapp, 1947). In the experiments reported in this paper it would seem that the lowered glycogen levels obtained when respiration was increased by electrical pulses or 2,4-dinitrophenol were associated with the lower available high-energy phosphate. With increased potassium ions this could have been offset by a stimulation of glycogenesis as occurs in liver.

It should be noted that our results on the effect of electrical pulses *in vitro* are more nearly in agreement with the *in vivo* work of Klein & Olsen (1947), who found a decrease in brain glycogen after convulsions produced by electrical stimulation, than with that of Chance & Yaxley (1950) who found an increase. However, all three sets of data were obtained by quite different techniques.

SUMMARY

1. The resynthesis of glycogen by surviving slices of guinea pig cerebral cortex has been demonstrated, and the synthesis found to depend on the conditions under which the slices were incubated.

2. Glycogen was resynthesized to a maximum level of about 3 $\mu\text{moles glucose/g.}$ moist tissue and, under the conditions used, the rate of resynthesis was much slower than that of post-mortem breakdown, being about 1 $\mu\text{mole glucose/g.}$ moist tissue/hr.

3. Evidence from qualitative colour tests with I_2 and anthrone, and from fermentation experiments, supports the conclusion that the material resynthesized was glycogen.

4. The rate or amount of glycogen resynthesis was not affected by wide variation in phosphate concentration.

5. Of agents stimulating respiratory rate, 2:4-dinitrophenol and electrical pulses reduced or eliminated glycogen resynthesis while increased potassium-ion concentration had no effect.

The author is very grateful to Dr H. McIlwain, at whose suggestion this problem was undertaken, for guidance, advice and criticism throughout the work. He also wishes to express his gratitude to Mr R. Rodnight for valuable advice and criticism, to Dr P. J. Heald for help and advice with the quick-transfer technique, and to Mr T. B. P. Claypole for technical assistance.

REFERENCES

- Albaum, H. G., Tepperman, J. & Bodansky, O. (1946). *J. biol. Chem.* **164**, 45.
 Brante, G. (1954). *1st Int. Neurochem. Symp.* Oxford (in the Press).
 Buchanan, J. M., Hastings, A. B. & Nesbitt, F. B. (1949). *J. biol. Chem.* **180**, 435.
 Chance, M. R. A. & Yaxley, D. C. (1950). *J. exp. Biol.* **27**, 311.
 Chesler, A. & Himwich, H. E. (1943). *Arch. Biochem.* **2**, 175.

- Gaevakaya, M. S. (1953). *Zhur. Vysshei Nervnoi Deyatel.* **3**, 617. (From *Chem. Abstr.* 1954, **48**, 12 273.)
 Gore, M. B. R. & McIlwain, H. (1952). *J. Physiol.* **117**, 471.
 Heald, P. J., McIlwain, H. & Nelligan, D. (1954). *Biochem. J.* **56**, xlviii.
 Kahan, J. (1953). *Arch. Biochem. Biophys.* **47**, 408.
 Kerly, M. & Ottaway, J. H. (1954). *J. Physiol.* **123**, 516, 534.
 Kerr, S. E. (1936). *J. biol. Chem.* **116**, 1.
 Kerr, S. E. (1938). *J. biol. Chem.* **123**, 443.
 Kerr, S. E. & Ghantus, M. (1936). *J. biol. Chem.* **116**, 9.
 Klein, J. R. & Olsen, N. S. (1947). *J. biol. Chem.* **167**, 747.
 McIlwain, H. (1951*a*). *Biochem. J.* **49**, 382.
 McIlwain, H. (1951*b*). *Brit. J. Pharmacol.* **6**, 531.
 McIlwain, H. (1952). *Biochem. J.* **52**, 289.
 McIlwain, H., Buchel, L. & Cheshire, J. D. (1951). *Biochem. J.* **48**, 12.
 McIlwain, H. & Gore, M. B. R. (1951). *Biochem. J.* **50**, 24.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Ostern, P., Herbert, D. & Holmes, E. (1939). *Biochem. J.* **33**, 1858.
 Peiss, C. N. & Field, J. (1948). *J. biol. Chem.* **175**, 49.
 Raymond, A. L. & Blanco, J. G. (1928). *J. biol. Chem.* **79**, 649.
 Rodnight, R. & McIlwain, H. (1954). *Biochem. J.* **57**, 649.
 Stadie, W. C. & Zapp, J. A. (1947). *J. biol. Chem.* **170**, 55.
 Stetten, M. R. & Stetten, D. (1954). *J. biol. Chem.* **207**, 331.

The Component Fatty Acids of the Fat of *Aspergillus nidulans*

By J. SINGH AND T. K. WALKER

College of Technology, University of Manchester,

AND M. L. MEARA

Department of Organic Chemistry, University of Liverpool

(Received 11 November 1954)

Considerable interest has been shown from time to time in the production of fats on an industrial scale by micro-organisms, with the object of alleviating the possible world shortage of fats produced by the more usual agricultural methods. It is now well known that a considerable number of micro-organisms when cultured on suitable media exhibit high carbohydrate into fat-conversion rates. It is also known that in many cases the percentage of free fatty acid (expressed as oleic acid) present in the fat produced is of an order too high to permit of economical refining.

The mould fungus *Aspergillus nidulans*, grown under suitable conditions, gives rise to a high yield of fat, the free acidity of which is low, and of the order of that usual in a commercial seed fat. The results of a preliminary nutritional investigation of this fat, which was carried out at the Dunn Nutritional Laboratory, Cambridge (private com-

munication from Dr T. Moore and Mr R. J. Ward), were deemed sufficiently promising to warrant more extensive nutritional trials. At the same time it was considered highly desirable to investigate the component fatty acids and glycerides of *A. nidulans* fat.

EXPERIMENTAL

The fat was produced by culturing *A. nidulans* at 25° under static conditions on a medium of the composition (g./100 ml.): sucrose, 34; NH_4NO_3 , 0.6; K_2SO_4 , 0.044; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.016; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.73; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.50; made up with distilled water, the pH adjusted to 3.8. After 8 days, when fat accumulation had reached its maximum, the mycelial felts were removed, sterilized in free steam for 15 min., washed and pressed on a pad of filter paper. The felts were then dried in air for 2 days at room temperature and finally in an air-oven at 70° for 3 hr. The dried material was broken up, ground in a coffee-mill and extracted thoroughly (Soxhlet apparatus)