interest for several reasons. It is the first cholinesterase of cold-blooded animals to be so identified; it occurs in a secretion, and not, as do other members of the class, in intimate association with structural elements of the cell; it is present in high activity in venoms whose neurotoxic properties are much in evidence. Much further work on this and the many other enzymes present in this secretion will, however, be necessary before a clear understanding of their biological significance is attained.

SUMMARY

1. The hydrolysis of choline and non-choline esters by cobra venom has been studied: all hydrolysis is attributed to cholinesterase.

2. It is suggested that the enzyme is inactivated at interfaces giving an erroneous idea of the specificity pattern when sparingly soluble esters are used. The enzyme can, however, be protected by the addition of gum acacia or inert proteins.

3. The protected enzyme gives a specificity pattern similar to those found for other acetocholinesterases. Acetates are hydrolysed faster than the corresponding propionates; butyrates are not hydrolysed. The rate of hydrolysis of aliphatic esters increases as the acetylcholine configuration is approached, giving a maximum with 3:3-dimethylbutyl acetate.

I am greatly indebted to Dr V. P. Whittaker for his interest and advice in this work; also to Dr H. Blaschko for the gift of cobra venom and to the Medical Research Council for a personal grant.

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Observations on the Formation and Structure of Bacterial Cellulose

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Sisson & Clark (1933) and Sisson (1936), employing X-ray technique, studied cellulose produced from sugar by *Acetobacter xylinum*. Sisson's conclusions confirm and supplement earlier observations by Mark & Susich (1929), Hibbert & Barsha (1931) and Khouvine, Champetier & Sutra (1932). Later, bacterial cellulose produced from glucose by *A. xylinum* was examined by means of the electron microscope by Franz & Schiebold (1943), while Frey-Wyssling & Mühlethaler (1946) applied the same technique to the study of cellulose formed by *A. xylinoides*. Mühlethaler (1949) has continued these studies using cellulose formed by *A. xylinum* in a beer-sucrose medium.

Kaushal & Walker (1947, 1951) reported the ability of certain species of *Acetobacter* other than

A. xylinum and *A. xylinoides* to form cellulosic membranes when cultivated in specified carbohydrate media. In particular, a strain of *A. acetigenum* isolated in an East African vinegar brewery was noteworthy in this respect. Observations made, when membranes formed by *A. acetigenum* from a number of substrates were examined by X-rays and by the electron microscope, are now recorded.

EXPERIMENTAL AND RESULTS

X-ray examination of samples of cellulose formed by Acetobacter acetigenum *grown on different substrates*

The cultures employed were *A. acetigenum* (Henneberg) Bergey, National Collection of Type Cultures 5346, and two strains of the same species isolated from East African vinegar brews. They were grown on media consisting of the several carbohydrates or related substances (from 2 to 5 %, w/v) in yeast-water (prepared by boiling 75 g. of brewer's pressed yeast in 1 l. of water for 30 min.), or in the solution of nutrient inorganic salts recommended by Henneberg (1926) in which $(NH_4)_2SO_4$ is the source of nitrogen. Growth in the last-mentioned medium was enhanced by addition of corn-steep liquor (1 %, v/v) or of Witte peptone (0·2 %, w/v). The media were usually contained in large conical flasks which had wide necks or in the flat, circular, glass dishes originally devised for penicillin production (Clayton, Hunwicke, Hanes, Robinson & Andrews, 1944). Later, larger areas of cellulose were developed in a rectangular glass trough fitted with a flat glass cover which rested on a pad of sterile non-absorbent cotton wool to admit air. Each medium was inoculated from tube cultures prepared from the same liquid medium, the whole contents (10 ml.) of one tube, which had been inoculated from a platinum loop and incubated for 48 hr. at 25°, being employed to inoculate 200 ml. of solution in a flask. The depth of liquid in the flasks was usually from 1 to 1·5 cm. The surface pellicles were examined when the cultures had been developing for about 14 days at 25°, but in some cases incubation was conducted at room temperature (20–22°). Treatment with cold NaOH (2 %, w/v) for 10 hr., followed by washing with water, then with dilute acetic acid, and finally with distilled water for 12 hr., freed the cellulose from traces of protein, salts and other matter. The purified pellicles after drying at room temperature were obtained as very thin, almost colourless, films.

Usually these films were folded several times before X-ray examination, and the time of exposure varied from 4 to 6 hr. Table 1 shows the several substrates and the respective interplanary spacings in the cellulose from these substrates. These spacings were calculated by measuring the ring diameters as described by Clark (1940).

Since X-ray photographs of cellulose produced by the action of *A. xylinum* on glucose, on sucrose and on certain other substrates have already been published by Barsha & Hibbert (1934), we submit only our X-ray photographs of cellulose from cultures on media containing ethylene glycol, glycerol and maltose, respectively. These are reproduced as Figs. 1–3 and may be compared with the X-ray photograph of non-absorbent cotton (Fig. 4). In each photograph, *D*, the distance from the plate, was 3 cm. Non-absorbent cotton was used in mistake for absorbent cotton when preparing Fig. 4. The non-absorbent material contains 0·16 % (as Al_2O_3) of an aluminium compound added to confer non-absorptive properties, but this small quantity is insufficient to interfere with the typical X-ray photograph of cotton cellulose.

Rotation photograph of a cellulose membrane from a glycerol medium

Hitherto, the literature has not contained any reference to examination of bacterial cellulose by means of a rotation photograph, and to obtain evidence from this source we have photographed in a 19 cm. powder camera, a membrane (from a glycerol medium) 1·5 cm. in breadth and rolled into a cylinder of about 0·7 mm. in diameter. The results are given in Table 2 and the photograph is reproduced as Fig. 5. The photographs depicting the X-ray diffraction rings of bacterial cellulose and the data given in Tables 1 and 2 render it clear that the calculated interplanary spacings are in close agreement with those observed for cotton. The interplanar spacings of the three principal planes 101, 10$\bar{1}$, and 002, agree very closely with those calculated on the Meyer and Misch model of the

Table 1. *Interplanar spacings in cellulose from different substrates*

(Readings are measured in Ångström units.)

Ethylene glycol	Glycerol	Mannitol	Glucose (*a*)	Glucose (*b*)*	Fructose	Maltose	Non-absorbent cotton
6·18	6·12	6·12	6·12	6·12	6·12	6·18	6·20
5·45	5·45	5·45	5·45	5·48	5·48	5·45	5·45
4·35	4·40	4·35	4·40	4·35	4·35	4·40	4·35
3·95	3·92	3·95	3·92	3·95	3·92	3·95	3·95
3·20	—	3·15	—	3·15	3·20	3·25	3·20
—	2·95	2·90	2·95	2·95	2·95	2·95	—
2·70	2·68	2·68	2·66	2·68	2·66	2·68	2·70

* The values given under (*b*) in the glucose column were obtained by examination of a stretched membrane.

Table 2. *Data from rotation photograph of cellulose synthesized in a glycerol medium*

(Time of exposure, 36 hr. X-ray film corrected to standard length, 270·1 mm.)

Distance of arcs from one end, corrected for contraction (mm.)	$\log \sin^2 \theta$ from curve	$\log \sin \theta$	$d = \lambda/2 \sin \theta$
7·6	$\bar{2}$·204	$\bar{1}$·102	6·09
10·6	$\bar{2}$·311	$\bar{1}$·155	5·39
16·8	$\bar{2}$·493	$\bar{1}$·246	4·36
21·1	$\bar{2}$·584	$\bar{1}$·292	3·93
30·2	$\bar{2}$·766	$\bar{1}$·383	3·19
34·5	$\bar{2}$·842	$\bar{1}$·421	2·92
38·7	$\bar{2}$·909	$\bar{1}$·454	2·70

monoclinic unit cell of cellulose, namely 6·05, 5·45 and 3·95 A. respectively (compare Clark, 1940).

In one experiment (see Table 1, column 4, *b*) the purified membrane was folded and dried under tension before examination, and its X-ray diagram showed uniaxial preferred orientation in agreement with the observations of Sisson and others. Previous workers, employing *A. xylinum*, studied membranes derived usually from growth on glucose and on sucrose. From sucrose Eggert & Luft (1930) obtained a membrane which yielded an X-ray pattern similar to that given by cotton cellulose. Finally, it is necessary to add the qualification that while the essential agreement between the spacings observed by us for cellulose synthesized from various carbon sources would indicate that the unit cell is the same in all cases, this need not necessarily imply that the molecules within the cell, or their distribution, are identical in all cases.

Examination of bacterial cellulose by the electron microscope

Preparation of the membranes. The media were inoculated with liquid taken on a platinum loop from liquid cultures in malt wort which had been developing for about 48 hr. at 27°. Incubation was then carried out at room temperature (20–22°). Material was removed from the cultures for examination, in some cases as early as 18 hr. after inoculation. If such young cultures of *A. acetigenum* are observed carefully, it will be found that when cellulose first becomes visible to the naked eye it is not seen floating on the surface of the liquid but appears as a submerged and very fine mainly gelatinous membrane which has a diaphanous appearance. Later, this delicate structure rises to the surface, and on further development forms the characteristic pellicle. This early growth of material was taken up on a grid and was washed. In some cases the material was transferred first to a test tube, washed in the latter, and then taken up on the grid. After drying *in vacuo* the material was examined in the usual manner and Figs. 6–10 represent a selection of the electron micrographs which were prepared.

If *A. acetigenum* is grown in a test tube on a liquid medium containing glucose, or other suitable carbon source, together with a little kieselguhr (the latter being added for the purpose of producing turbidity when the tube is disturbed), after 10 days the cellulosic pellicle will be about 2 mm. thick and will adhere so firmly to the glass that the tube can be inverted and still retain its contents. We have observed that when this is done the kieselguhr sets up a turbidity which, however, does not extend downwards to what appears to be the undersurface of the pellicle, but stops along an interface which follows the line of the underside of the visible pellicle at a distance of about 2 mm. from the latter. Thus, between the turbid liquid and the opaque pellicle, there is revealed a zone or layer consisting of a transparent colourless gel which, otherwise, would escape observation. It would seem not unreasonable to suggest that this transparent layer consists either of lower polymer or of cellulose at a stage before crystallization has commenced.

Finally, Fig. 11, kindly prepared for us by Mr J. A. Lord, represents a sectional photograph of cellulose produced by *A. acetigenum* on a medium containing starch hydrolysate as source of carbon. After purification the specimen was photographed with an optical microscope-microcamera. The figure shows that during the formation of bacterial cellulose layering of fibrils takes place and, further, that the thickness of the fibrils appears to be of the same order, namely, 250 A., as is the thickness of many of those revealed by the electron microscope.

DISCUSSION

Franz & Schiebold's (1943) electron micrographs show cells of *A. xylinum* from 10,000 to 20,000 A. in length and from 3000 to 5000 A. in breadth. Surrounding these cells are submicroscopic strands of cellulose, the orientation of which is random, and the authors point out that these strands appear to be interwoven. Further, they state that the visible cellulose threads are ribbon-like, of length often 40 μ., with a minimum breadth of about 200 A. and thickness 100 A. Bands of up to 5000 A. in width and consisting of a number of narrower bands were also observed.

Frey-Wyssling & Mühlethaler's electron micrographs illustrate the crystallization of submicroscopic cellular strands from multimolecular cellulose films formed by *A. xylinoides*. These authors state that all the cellulose threads have the same diameter (200 A.) and the micrographs demonstrate the presence of 'dark threads embedded in a thin

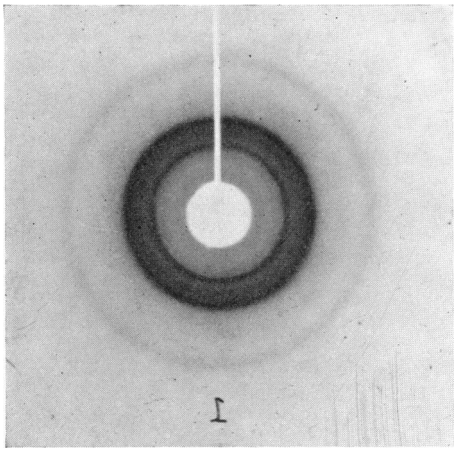

Fig. 1.

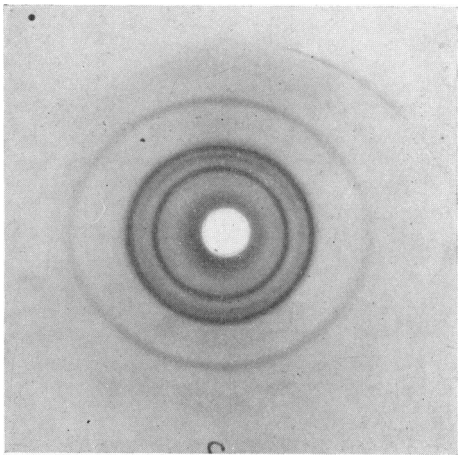

Fig. 2.

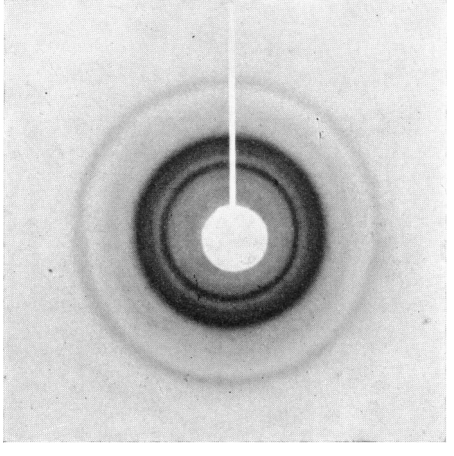

Fig. 3.

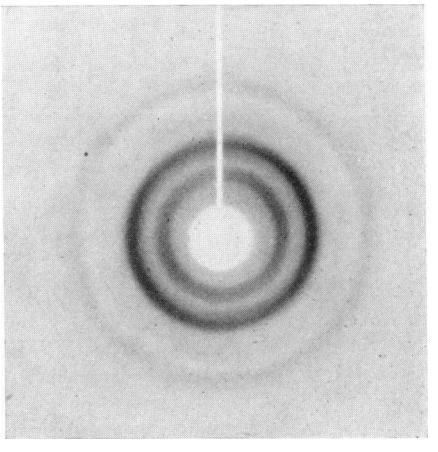

Fig. 4.

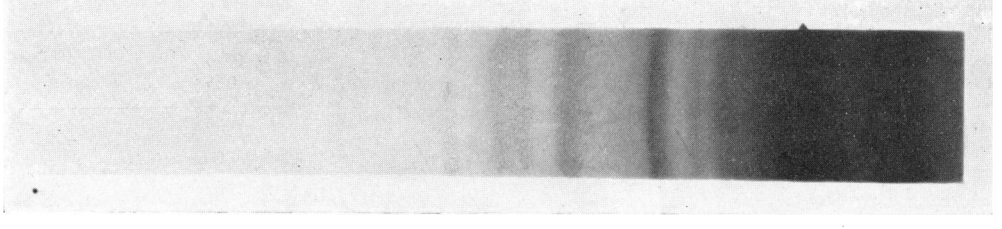

Fig. 5.

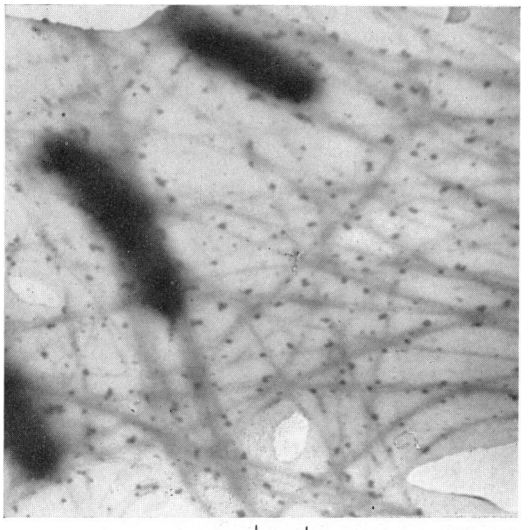

Fig. 6.

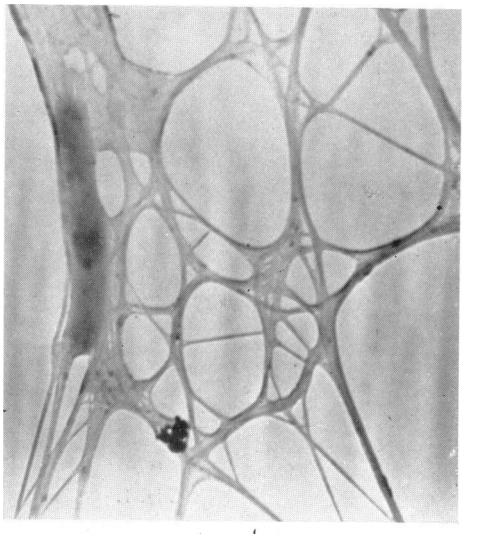

Fig. 8.

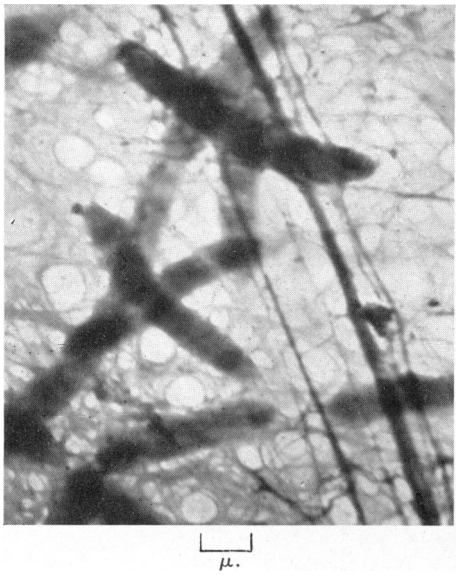

Fig. 7.

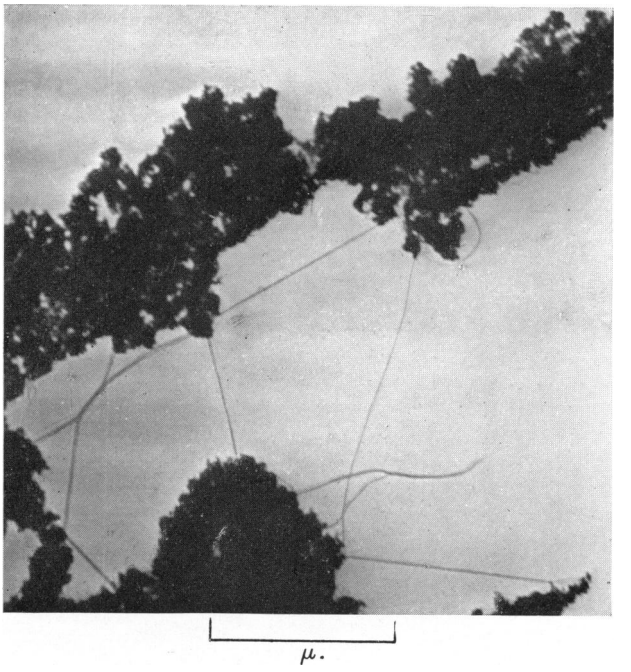

Fig. 9.

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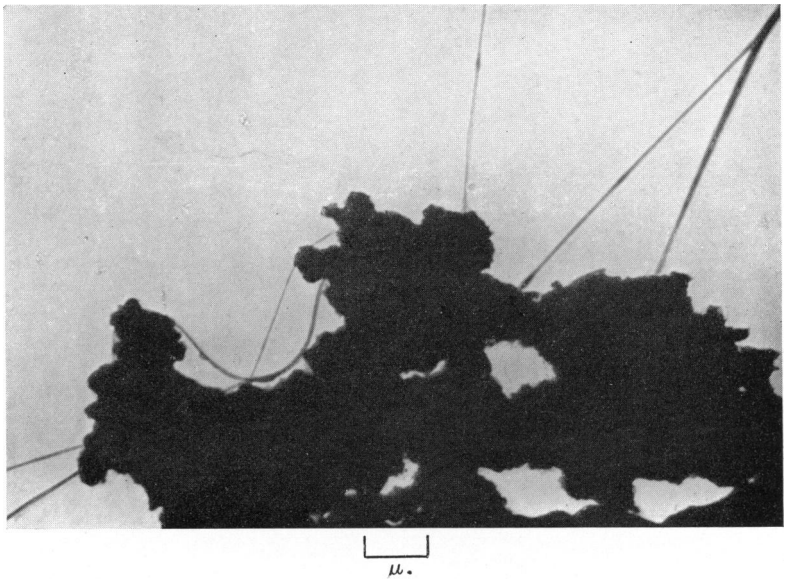

Fig. 10.

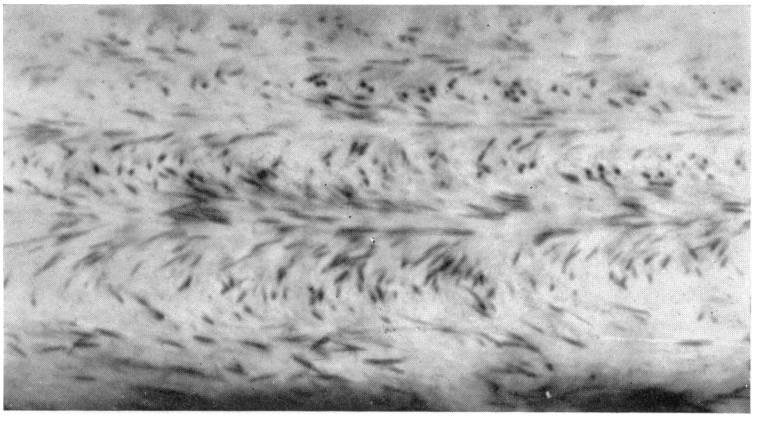

Fig. 11.

grey film (less than 100 A. thick) which apparently is pure cellulose. This is a surface film of the insoluble cellulose on the culture liquid. From this diffuse film the visible threads are crystallized.'

The cells of *A. acetigenum* as depicted on our electron micrographs are considerably larger than the cells of *A. xylinoides*, the former measuring from 43,000 × 10,000 A. to 22,000 × 8000 A., whereas the cells of *A. xylinoides* as shown by Frey-Wyssling & Mühlethaler vary from 20,000 × 5000 A. to 10,000 × 5000 A. Apart from these differences in the sizes of the typical cells of the two species our electron micrographs of preparations from glucose media show cellulose formations having a general resemblance to those depicted in the electron micrographs of Frey-Wyssling & Mühlethaler. The latter state that: 'the equal thread diameter (about 200 A.) in old cellulose films of *A. xylinum* as well as in new films of *Bacterium xylinoides* is rather surprising. Whether this conformity is accidental or whether there is a preferred strand width (which would indicate that the possibility of crystallization is limited) is a problem which should be investigated.'

The uniformity in thread diameter (about 200 A.) reported by Frey-Wyssling & Mühlethaler is not apparent in our preparations, but many of the threads in our own specimens of cellulose from glucose media had a width of approximately 250 A. and, in his latest publication on this topic, Mühlethaler (1949) observes that cellulose fibres produced by *A. xylinum* 'have had from the very first a diameter of about 250 A.'. The 50 A. difference between these two estimated widths (namely, 250 and 200 A.) may perhaps be capable of explanation when account is taken of the fact that, when measuring the threads, the accuracy with which their edges can be defined will depend upon (*a*) the resolving power of the electron microscope used, (*b*) the resolving power of the photographic emulsion used, and (*c*) the conditions of development of the photograph. Our observations lead us to the view that the bacteria produce round their cells mucoid carbohydrate material which is built up to the dimensions of cellulose, whereupon crystallization begins in the manner shown in our figures, as also in those of Frey-Wyssling & Mühlethaler. The latter, in his latest communication, expressed a similar opinion. The electron micrographs of our preparations from media containing glycerol and glycol, respectively, support this opinion, since the amorphous masses shown in these pictures might well consist of lower carbohydrate polymers in process of elaboration to cellulose.

SUMMARY

1. Cellulose membranes produced by *Acetobacter acetigenum* yield an X-ray pattern similar to that revealed by X-ray examination of cotton cellulose.

2. Membranes from very young cultures of *Acetobacter acetigenum* on media containing glucose, glycerol and ethylene glycol, respectively, as sources of carbon, have been examined in the electron microscope.

3. The preparations from the glucose media show cellulose films in which are threads usually of a width either a little less than 250 or approximately 500 A., the latter being in the majority.

4. The preparation from a glycerol medium shows mainly amorphous material with a very few threads or bands of cellulose, of width approximately 500 A. The preparation from the ethylene glycol medium also shows amorphous material with not many fibrils.

5. It is suggested that the amorphous material consists of carbohydrate at a stage of polymerization lower than cellulose.

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EXPLANATION OF PLATES

Figs. 6–10. Electron micrographs of cellulose formed by *A. acetigenum*

PLATE 1

Fig. 1. Cellulose from ethylene glycol medium.

Fig. 2. Cellulose from glycerol medium.

Fig. 3. Cellulose from maltose medium.

Fig. 4. Cotton cellulose.

Fig. 5. Cellulose from glycerol medium. Rotation photograph.

PLATE 2

Fig. 6. Cellulose formed in a glucose yeast-water medium and examined in an unwashed condition. Magnification, ×8400. The majority of the threads are about 500 A. in width.

Fig. 7. Washed material from a glucose yeast-water medium. Magnification, ×8000. Threads mostly about 500 A. wide with a minority measuring a little less than 250 A. Two threads were found to be not greater than 200 A. in width.

Fig. 8. Washed material from a medium consisting of a solution of glucose and inorganic salts. Magnification, ×9000. Most of the threads have a width of approximately 500 A., but one or two threads embedded in film are approximately 250 A. wide.

Fig. 9. Preparation from a medium containing ethylene glycol and inorganic salts. Magnification, ×24,000. This shows what appears to be amorphous matter with a very few fine threads, the narrowest of which has a width of less than 80 A. Others measured less than 200 A. and one band was found to be 250 A. in width.

PLATE 3

Fig. 10. Preparation from a medium containing glycerol and inorganic salts. Magnification, ×8500. Masses of what apparently is amorphous material, together with a very few fine threads, can be seen. The narrowest thread has a width of 250 A., others are 500 A. wide, while yet other bands may be noted varying in width from 750 to 1000 A.

Fig. 11. Sectional photograph of cellulose from a starch hydrolysate medium. Magnification, ×1600.

A Means of Metabolic Investigation of Small Portions of the Central Nervous System in an Active State

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A method is described below by which it is possible for the first time to study the metabolism of small isolated fragments of the central nervous system of higher animals, in a state of electrically induced activity. Increase in the metabolism of slices of cerebral cortex on electrical stimulation *in vitro* was reported recently (McIlwain, 1951*a*). In the apparatus which was used then, direct contact was made throughout the experiment between the slice of tissue and two stimulating electrodes. The tissue weighed about 50–120 mg. It was realized that the scope of such investigations would be greatly increased if they could be applied to smaller fragments of tissue and without fixing them to electrodes. Regional differences in biochemical responses to stimulation, and in the action of drugs on such responses, could then easily be explored. It has now been found possible to study in this way fragments weighing a mg. or less.

EXPERIMENTAL

Guinea pigs and rats of various breeds and ages were used. They were killed by a blow on the neck or by decapitation, the skull opened and the brain removed. Tissue preparations were normally ready for placing in the thermostat (37 or 39°) 15–30 min. after death of the animal. Salines and means of electrical stimulation were those described previously (McIlwain, 1951*a*). A commercial diathermy apparatus was also used. This depended on charging and discharging a condenser from a.c. mains, through a coil and spark gap. A secondary coil, adjustable in relation to the first, gave the impulses which were taken through a high-frequency meter