in the absence of glucuronic acid. This corresponded to about 5 μ g. glucuronic acid. Obviously, by using more dilute ceric sulphate in the burette, with consequent sacrifice of stability, and less ferri-cyanide, the lower range of the method could be extended.

The error in recovery for any given initial pH appeared to be constant in amount over most of the glucuronic acid range studied. When the initial pH was 5, the standard deviation of a single observation from the mean was 2.4 μ g.

SUMMARY

1. A method is described for the cerimetric determination of 10–300 μ g. glucuronic acid in

tissue preparations, using the Conway micro-burette. Protein is removed with copper tungstate and barium carbonate.

2. The error appeared to be constant in amount over most of the range of glucuronic acid values studied. Of 99.5 μ g. glucuronic acid added, the mean recovery was 95.0 μ g., the standard deviation of a single observation from the mean being 2.4 μ g.

3. Various factors in the determination of reducing sugars were examined.

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The Recognition of Material Present in Horse Muscle Affecting the Formation of α -Toxin by a Strain of *Clostridium welchii*

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The α -toxin of *Cl. welchii* type A (*Cl. perfringens*) was shown to be a lecithinase by Macfarlane & Knight (1941); fairly good yields of α -toxin/ml. of culture filtrate were obtained by growing strain S107 of the organism in a medium composed essentially of peptone (Evans Sons, Lescher and Webb Ltd.), glucose, and a protein-free muscle extract (Deutsch, Eggleton & Eggleton, 1938). The presence of the muscle extract appeared to increase α -toxin formation/ml. of culture filtrate. Various published observations had already indicated that the incorporation of muscle (as a solid) in culture media favoured the production of high potency α -toxin by *Cl. welchii* type A, although the function of the muscle remained undetermined. In order to investigate the factors which influence toxin formation by an organism it is necessary to take into account the alterations in the potency of culture

filtrates/unit volume, due simply to variations in the mass of bacterial growth which may have occurred. Hence, in order to find whether substances which really enhance α -toxin formation (henceforth called toxigenic factors, or T.F.) were present in the muscle extracts, apart from substances directly affecting nutrition and growth, the amount of lecithinase (α -toxin) produced/ml. of medium should be related to the amount of bacterial growth which had occurred.

This paper records the examination of various materials for the presence of so-called toxigenic factors, i.e. material which increases the yield of α -toxin/unit weight of bacterial matter formed, and the attempt to characterize and purify such material from protein-free muscle extracts.

During the course of this work we freely interchanged information with Dr A. M. Pappenheimer, Jr., who for a time studied the same problem. Such liaison between scientists in the U.S.A. and Great

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Britain was arranged by the American Organization for Scientific Research and Development (O.S.R.D.) and by the Medical Research Council in this country. We have received Dr Pappenheimer's encouragement to publish this paper as it is unlikely that he will publish his own observations separately. We would like to record therefore that many of our observations were complementary and mutually confirmatory (cf. Adams & Hendee, 1945).

EXPERIMENTAL

Analytical methods

(i) *Estimation of α -toxin (lecithinase) of *Cl. welchii*.* The assay was carried out by the method of van Heyningen (1941), which depends on estimation of the turbidity produced in egg-yolk emulsion by the action of the lecithinase upon the lecithin of the emulsion. The method was modified by the addition of 0.5 ml. of borate-calcium chloride buffer to each toxin + egg-yolk emulsion mixture (McClean, Rogers & Williams, 1943). The results of toxin estimations by this method are expressed in egg units (E.U.) (van Heyningen, 1941). The estimations were done on samples of the supernatant liquid after removal of the bacteria from cultures by centrifuging.

(ii) *Amount of bacterial growth.* This was measured by determination of total N (micro-Kjeldahl) on twice-washed cells from 10 ml. samples of culture. The cells were washed with water by centrifuging and resuspending.

(iii) *Various estimations.* Amino-sugars were estimated by the method of Elson & Morgan (1934); total carbohydrate by a modification of the orcin method of Tillmans & Phillipi (1929); phosphate by the method of Fiske & Subbarow (1925); pH was determined with the use of a glass electrode.

Method of cultivation

Organism. The organism used throughout was derived from a culture of *Cl. welchii*, S107, obtained from the current stock of this strain as used for toxin production at the Lister Institute, Elstree. This was maintained in sealed tubes of Robertson's bullock-heart medium (cf. Lepper & Martin, 1929).

Inoculation. An 18 hr. culture (0.5 ml.) in Robertson's bullock-heart medium was used to inoculate 50 ml. of test medium contained in 6 x 1 in. boiling-tubes. The inoculum was introduced, by means of a Pasteur pipette, into the bottom of the tube containing the freshly heated and cooled medium; no further anaerobic precautions were needed and growth rapidly ensued.

Attempts were made to use an inoculum obtained from serial subculture in a simplified medium (see later), but these cells produced only low yields of toxin in a medium which was capable of producing larger yields when inoculated with cells from a heart-medium culture. Control experiments showed that the heart-medium inoculum did not introduce substances enhancing toxin formation, the indication being therefore that the cells obtained by subculture on the simplified medium were potentially less effective toxin producers, the serial subculture having given rise to cells modified at least in their

ability to produce toxin in the media used. In order, therefore, to avoid as much as possible variations of toxin yield due to variations in the cells employed, inoculations of test media were always carried out with cells from heart-medium cultures. In work of this kind, variation in the organisms used is a constant source of difficulty, and steps must be taken to ensure that the organisms used are capable of responding to the stimulus of toxin-promoting conditions. Suitable controls using the same cells inoculated into basal medium alone (negative control) and into the basal medium + standard muscle extract (positive control), were always included to check the toxin-producing capacity of cells used as inocula, when testing for the presence of toxin factor in the various preparations to be described.

Cultivation and sampling. The basal medium (40 ml. samples), contained in 6 x 1 in. Monax boiling tubes (cleaned by chromic-sulphuric acid mixture) was sterilized by autoclaving at 110° for 20 min. Immediately before inoculation the tubes of medium were heated for 30 min. in a boiling water-bath to expel dissolved oxygen, and then cooled rapidly to 37°. One ml. of sterile (filtered) 10% (w/v) glucose solution and 9 ml. of sterile distilled water, or of a sterile Seitz-filtered solution to be tested for activity, were then added aseptically, and the tubes inoculated and incubated at 37°. Samples were taken aseptically at hourly (or other) intervals during growth, and cooled quickly by immersion in ice-water to stop further action. The organisms were removed from samples by centrifuging, α -toxin being estimated (as lecithinase) in the supernatant fluid, and bacterial-N in the deposit of cells after washing. In calculating the effect of any material upon toxin formation the values for the samples giving the maximum ratio, toxin/mass of growth, for the supplemented and unsupplemented test media, respectively, were compared. This precaution is necessary since the toxin already formed may be destroyed after multiplication of the organisms has ceased; and this rate of destruction is very variable.

The design of a test medium for examining toxin formation

In order to study the conditions which influence toxin synthesis it is necessary to use a medium of controllable composition. Little knowledge of the exact nutrient requirements of *Cl. welchii* was available and the design of a chemically defined medium (cf. Pappenheimer & Shaskan, 1944) would have entailed considerable preliminary work which we were unable to undertake. Consequently we compromised by designing a simplified medium which, although not chemically defined, would be reproducible and controllable in composition. It may be emphasized that we sought a test medium suitable for use in studying those substances in muscle which influence the formation of α -toxin by *Cl. welchii* S107, and were not attempting necessarily to design a medium which would yield high α -toxin

titres. The medium, however, had to be capable of producing sufficiently large amounts of toxin to make the measurements reasonably accurate. The conditions we tried to establish were such as would enable us to recognize and to follow through subsequent fractionation procedures, material which could be looked upon as 'toxigenic' in the sense previously discussed.

Gladstone & Fildes (1940) showed that their acid hydrolysate of casein + yeast extract medium, 'CCY', would support the growth of *Cl. welchii* type A. Preliminary tests showed that too little α -toxin was produced on unmodified 'CCY' medium supplemented by 10% (v/v) of protein-free muscle extract (Macfarlane & Knight, 1941). Investigation showed that for toxin formation this medium was insufficiently buffered and contained an unsuitable concentration of acid hydrolysate of casein. By increasing the concentration of sodium glycerophosphate to 2.5 g./100 ml. and the amount of acid hydrolysate of casein until the final N content of the medium was 1.4 mg./ml., reasonable yields of lecithinase could be obtained. The final test medium was designed in accordance with these findings and with those of Dr Pappenheimer. It had the following composition:

'Dephosphated' acid-hydrolyzed casein solution (1 ml. = 7.4 mg. N)	150 ml.
Hot-water extract of yeast (1 ml. = 1 g. pressed brewers' yeast)	25 ml.
50% sodium lactate syrup (British Drug Houses Ltd.)	10 ml.
MgSO ₄ ·7H ₂ O (AnalaR)	0.2 g.
L-(-)-tryptophan	0.01 g.
Distilled water to 1 l.; pH adjusted to 7.4.	

(The 'dephosphated' hydrolysate was the acid hydrolysate of Gladstone & Fildes (1940) treated with CaCl₂ solution at pH 8.0 and 80° until no further precipitate formed on addition of more CaCl₂ solution. The precipitated calcium salts were removed by filtration and the filtrate readjusted to pH 7.4. This process was necessary since excess of inorganic phosphate had been shown to inhibit toxin formation (cf. Tamura, Tytell, Boyd & Logan, 1941).)

The medium was tubed in 40 ml. quantities and sterilized by autoclaving at 115° for 10 min. Before use, sufficient sterile glucose solution (10 g./100 ml.) was added to give a final glucose concentration of 0.5 g./100 ml. and other supplements as required for testing. The final total volume/culture tube was adjusted to 50 ml. with sterile distilled water.

The effect of the protein-free muscle extract (M.E.) upon α -toxin formation

The most convenient material to study for the presence and properties of the suspected toxigenic factor (T.F.) was the protein-free muscle extract used by Macfarlane & Knight (1941) in the preparation of culture media for α -toxin production by *Cl. welchii*. This extract, prepared from horse muscle by the method of Deutsch *et al.* (1938) is a concentrated solution of all the water-soluble materials,

including those that are heat-labile (e.g. phosphocreatine), expressed from the tissue after addition of anhydrous Na₂SO₄ to full saturation at 32°. Under these conditions the protein is completely precipitated and only those substances soluble in saturated sodium sulphate solution are extracted from the

Table 1. *The effect of protein-free muscle extract (M.E.), added at a level of 10% (v/v) to culture medium, upon α -toxin production by Cl. welchii type A, S107*

The results are the mean of 13 separate experiments.

Medium	E.U.*/ml.		E.U./ml. of culture mg. bacterial-N/ 10 ml. of culture	
	Mean	Variation	Mean	Variation
Unsupplemented	5.1	1.6-8.10	49	15-64
Supplemented [+10% (v/v) M.E.]	13.5	4.1-19.8	93	27-132

* E.U. = egg units (van Heyningen, 1941).

Fig. 1. *The effect upon α -toxin (lecithinase) formation of various concentrations of three different preparations of protein-free muscle extract, added to the test medium.*

tissue. On cooling to 0°, much Na₂SO₄·10H₂O crystallizes out, thus further concentrating the extract. A single extraction gave an average yield of 143 ml. of extract/kg. of horse muscle. This extract (M.E.) was the reference standard and was used in the medium at a concentration of 10% (v/v). Table 1 shows the average results taken from

13 separate experiments in which toxin production/unit weight of bacterial growth was compared in supplemented (i.e. plus 10% (v/v) M.E.) and unsupplemented test medium. Each pair of cultures was inoculated from a different 18 hr. seed culture of *Cl. welchii* type A (strain S107).

The average stimulation of toxin formation caused by the M.E. was 98%. This value is calculated from the results for the toxin/growth ratio recorded in column 4 of Table 1 (i.e. $\frac{93-49}{49} \times 100 = 98\%$). The

average variation between the individual stimulation values for the 13 pairs of cultures was only 10–20%. Thus the larger variations shown in columns 3 and 5 of Table 1 were due to the different levels of toxin production and are probably due to differences in the toxin-producing capacity of the cells from the different seed cultures.

Fig. 1 shows the effect upon toxin formation of adding various amounts of three preparations of M.E. to the test medium. Between concentrations of 0–15% (v/v) the amount of toxin produced/unit weight of organisms is approximately proportional to the amount of M.E. present in the medium.

The association of toxigenic factor with proteins in biological materials

A survey of various animal and plant sources for the presence of T.F. was made using extracts prepared by the sodium sulphate method of Deutsch *et al.* (1938). Horse muscle, sheep ileum and brain, and human urine, all gave active extracts. (A concentrated hot-water extract of yeast was also active.) Inactive extracts were obtained from the yolk and white of hens' eggs, milk, grass and mangolds.

The lack of activity of the milk extract is noteworthy because Dr Pappenheimer had already found 'Tryptone', a commercial tryptic digest of casein, to be a good source of T.F. for his strain of *Cl. welchii*. We confirmed this result for tryptic digest of casein, using our strain and conditions of test. Likewise, ether-extracted whole milk was slightly active. Thus the T.F. in milk was apparently precipitated with the casein during the sodium sulphate treatment. Similarly, no T.F. was found in the filtrate from a T.F.-active, protein-containing commercial meat juice (Allen and Hanbury Ltd.), after removing protein with saturated sodium sulphate at 35°, yet animal tissues yielded active extracts. A possible explanation appears to be that the T.F. is liberated by an autolytic change occurring before application of the sodium sulphate treatment.

To test this, fresh horse muscle was allowed to autolyse for 48 hr. under benzene at 37°, before the sodium sulphate treatment. The benzene was

removed from the final filtrate *in vacuo* and the activity of this extract compared with a control prepared from muscle kept at 0–4° for 48 hr. before treatment. The extract from the autolyzed muscle was 40% more active than the control. It was not practicable, however, to introduce deliberate pre-autolysis into the large scale routine production of muscle extracts; the autolysis which normally took place during the time between slaughter and the processing of the horse muscle was relied upon to give sufficient free T.F. for reasonable yields on extraction.

Some properties of the toxigenic factor present in protein-free horse muscle extract

The extract could be concentrated *in vacuo* (at 25°) to a tenth or less of the initial volume without loss of T.F. Such concentrated extracts were used to characterize the following properties of T.F. material therein:

- (1) Freely dialyzable through cellophan.
- (2) Not extracted from the concentrated extracts at any pH by ether, chloroform, benzene, or *n*-butanol.
- (3) Not precipitated by neutral or basic lead acetate; recoverable after removal of Pb, although 20–30% of the activity was lost by this treatment.
- (4) Not precipitated as a calcium or barium salt either from aqueous solution or from 50% (v/v) aqueous ethanol.
- (5) Stable to drastic treatment with acids (e.g. heating in a boiling water-bath with 8N-HCl for 6 hr.) but easily inactivated by treatment with alkalis (e.g. 1 hr. heating in boiling water-bath at pH 9.0).
- (6) Mercuric acetate in weakly alkaline solution (Neuberg & Kerb, 1912) completely destroyed T.F. Mercuric sulphate in acid solution destroyed about 60–70%.
- (7) Phosphotungstic acid precipitation of the concentrated extract yielded inactive filtrates, but no T.F. could be recovered from the precipitate after decomposition of the phosphotungstates by grinding with Ba(OH)₂.
- (8) Treatment at 0° with nitrous acid for 1 hr. inactivated the T.F.
- (9) Active charcoal adsorbed small amounts of T.F. which could be eluted at pH 9.0 in the presence of citrate or glycerophosphate.
- (10) No adsorption of T.F. took place on alumina or benzoic acid.

The effect of the inorganic iron concentration in the medium upon toxin synthesis

A preliminary test in which various amounts of inorganic iron (0.12–4.0 mg. Fe/ml. of medium) were added to the medium containing 10% (v/v) of

muscle extract showed no marked effect on lecithinase synthesis under our conditions. Dr Pappenheimer (personal communication), however, when using his medium and strain found that the concentration of inorganic iron was an important factor in lecithinase synthesis. Attempts to repeat his observation, using strain S107 and crude M.E. as a source of T.F., failed. Later, however, when lecithinase formation was examined in the presence of acid-treated muscle-extract (i.e. material heated in a boiling water-bath with 8N-HCl for 6 hr.), the iron concentration in our own medium was found to be equally important under these conditions. Likewise the iron concentration was important when a partially purified solution of T.F. was used instead of crude muscle-extract. Fig. 2 shows the effect on formation of lecithinase when the inorganic iron concentration is varied in a medium containing partially purified T.F. (Fraction E—see later).

Fig. 2. The effect on α -toxin (lecithinase) formation of different iron concentrations added to the test medium containing a partially purified solution of T.F., viz. Fraction E.

In the light of these observations some of the fractions obtained in earlier work were again tested in media containing various amounts of inorganic iron. No fractions previously tested and found to be inactive were found to possess activity when the iron concentrations were varied over the critical range observed with the preparation Fraction E. The important effect of iron on the glucose metabolism of *Cl. welchii*, studied by Pappenheimer & Shaskan (1944), is discussed later (cf. Adams & Hendee, 1945).

Partial purification of T.F. from the concentrated extracts

(1) *Removal of salts by ethanol.* Ethanol was added to the concentrated (evaporated *in vacuo*) muscle extract to give a final concentration of 15 %

(v/v) and the mixture was cooled to -20° overnight. The crystalline precipitate was removed on a cold Buchner funnel. The filtrate was adjusted to a concentration of 30 % (v/v) ethanol and the process repeated. The ethanol concentration of this filtrate was finally raised to 50 % (v/v) and again cooled. The precipitates from each stage were combined and recrystallized at -20° from 50 % (v/v) ethanol. The final filtrate from the ethanol precipitation and the liquor from the recrystallization of the salts were combined and the ethanol removed *in vacuo* to give Fraction B.

(2) *Removal of fermentable carbohydrate.* About 5 % of the dry weight of the concentrated extract consisted of carbohydrate fermentable by yeast. Almost all of the sugar persisted in the ethanol-treated material, Fraction B. Its removal appeared to assist further purification of the T.F. by rendering the concentrates easier to handle in anhydrous solvents.

50 g. of pressed bakers' yeast which had been washed 3 times with distilled water were added to 450 ml. of the ethanol-treated muscle concentrate, Fraction B, warmed to 38° . The mixture was maintained for 20 min. at this temperature, after which none of the 'fermentable' material remained in the concentrated extract. The concentrate was cooled and the yeast cells removed by centrifuging, yielding Fraction C, the material from the yeast treatment.

(3) *Removal of bases by picric acid.* Picric acid precipitated none of the T.F. activity but removed some inactive bases. 500 ml. of the ethanol- and yeast-treated material (Fraction C) were cooled to 0° and mixed with 50 ml. of a saturated ethanolic solution of picric acid. The sides of the flask were scratched with a glass rod until a precipitate of crystalline picrates formed, and the mixture set aside for 16 hr. at 0° to complete precipitation. The insoluble picrates were removed on a cold filter, and the ethanol from the filtrate distilled off *in vacuo*. The filtrate was acidified with 10 ml. of 2N- H_2SO_4 and excess picric acid removed by successive extractions with *n*-butanol. (This solvent proved more efficient for removing picric acid than ether.) The picric acid-free solution was then neutralized with cold saturated baryta, the barium sulphate being centrifuged off and washed. Finally the filtrate and washings were concentrated *in vacuo* to 450 ml.; the material remaining in solution was called Fraction D.

Extraction of the active substance from Fraction D with dry methanol

The Fraction D material contained very hydrophilic substances which rendered drying difficult. A dry preparation, however, was secured by spraying the solution of Fraction D into a 2 l. flask

attached to an efficient condensing system and evacuated to a pressure of 13 mm. Hg. The flask was immersed in a water-bath at 80–90°. Occasional batches of Fraction D solution did not yield dry products even with this treatment. In such cases repeated (10–12 times) washing with dry absolute ethanol achieved the desired result. The dry Fraction D material was extracted with 500–1000 ml. of dry absolute methanol added in 100 ml. quantities. A light brown precipitate separated from the methanol extract and was removed by centrifuging. The supernatant methanol solution was evaporated *in vacuo* leaving a heavy brown oil, which was redissolved in 450 ml. distilled water; this was called Fraction E. This concentrate possessed about 75 % of the T.F. activity originally present in the M.E., provided the iron concentration in the medium was optimal when testing its activity. Only about 10–20 % of the *growth-promoting* factors (G.F. in Table 2) present in the original M.E. remained. Thus additional evidence is provided for the presence in M.E. of substances which specifically influence α -toxin production without influencing the mass of bacterial growth. It is clearly unlikely, both from the method of fractionation which had to be used and from the results of chemical analysis that Fraction E was a single substance.

Table 2. *The partial purification of T.F. from horse-muscle extracts; analyses and activities of some preparations*

Material	Dry weight (mg./ml.)	Yield of T.F. (%)	Yield of G.F.* (%)	Total N* (%)	Total carbo-hydrate (%)	'Hexosamine'† (%)
Original M.E.	387	100	100	6.1	12.9	0.12
Fraction D	190	80–90	90–100	—	17.4	—
Fraction E	9.5	70–80	10–20	7.6	3.1	0.40

* Calculated on the solids in the fraction.

† G.F. is a measure of the relative increase in bacterial-N, i.e. growth-promoting effect.

‡ 'Hexosamine' by method of Elson & Morgan (1934).

The behaviour of T.F. in the protein-free muscle extract towards various reagents allowed the following conclusions to be drawn about its general chemical properties:

- (1) The T.F. is of relatively small molecular size.
- (2) Activity is associated with hydrophilic substances.
- (3) T.F. activity is dependent upon the presence of primary amino-groups.
- (4) The T.F. was stable to drastic treatment with acids but unstable in alkaline solutions.

Amino-hexoses have properties very similar to those of the T.F. Accordingly, glucosamine, *N*-acetylglucosamine and chondrosamine were separately added to tubes of the test medium containing

various amounts of inorganic iron. The concentration of amino-hexose used in the medium (0.60 mg./100 ml.) corresponded to the amount that would normally be added as an analytically-determined constituent of the M.E. Glucosamine and *N*-acetylglucosamine both showed an activity at this concentration which corresponded to about 30 % of that caused by 10 % (v/v) of M.E. The concentration of inorganic iron was then very important. Chondrosamine was completely inactive.

DISCUSSION

It thus seems probable that labile 'toxigenic' material takes part in the formation of α -toxin by *Cl. welchii* strain S107. The formation of small amounts of toxin in the unsupplemented test-medium may be due to the traces of T.F. shown to be present in the hot-water yeast extract which was a constituent of this medium. This is supported by Dr Pappenheimer's results using a medium containing purified growth factors in which only negligible amounts of toxin were formed by his strain of organism in the absence of added T.F. (see Pappenheimer & Shaskan, 1944). The properties of the material which increases α -toxin (lecithinase) production by *Cl. welchii* cells multiplying in our medium and which is present in protein-free muscle extract, preclude the possibility that inorganic salts, e.g. Fe salts, are the only active substances involved. The stability to acids and instability to alkalis, combined with the hydrophilic nature of the T.F., and its inactivation by gentle treatment with nitrous acid, all suggest that an amino sugar-like substance might be involved. The association of T.F. with animal proteins is not incompatible with this. Glucosamine and its *N*-acetyl derivative both showed a slight activity, while chondrosamine was inactive.

The difficulties encountered in attempting to isolate the T.F. may be partly due to the inherent complexity of the process of α -toxin formation by *Cl. welchii*, together with the variability of the organism. Whereas the concentration of inorganic iron in all growth media is important for toxin formation by, for example, *C. diphtheriae* (Pappenheimer & Johnson, 1936), it is only so for *Cl. welchii* in certain media. In the presence of crude, untreated muscle extract, up to 4.0 mg./l. of iron may be added without suppressing toxin formation. As the muscle extract is purified, however, or if it is treated with acids, the concentration of inorganic iron becomes very important (cf. Adams & Hendee, 1945). The optimal concentration varies also according to the condition of the organism, i.e. biological variation may play a large part in affecting the response, in toxin formation, to the cultural conditions.

The study by Pappenheimer & Shaskan (1944) of the effect of iron concentration on the glucose metabolism by *Cl. welchii* strikingly illustrates one function of iron in the growth of this organism. These workers found that the products of glucose breakdown depended upon the iron content of the cells; as the iron content decreased the reaction shifted from a predominantly acetic-butyric acid type with production of large amounts of carbon dioxide and hydrogen, towards a more purely lactic acid type of fermentation with slight gas formation. Under the conditions of their experiments the iron concentration for maximum growth, optimum toxin production and minimum lactic acid production were identical. But the relationship between α -toxin (lecithinase) production and the carbohydrate metabolism was not a direct one, since the shift to a lactic acid type fermentation at low iron concentrations occurred equally well under conditions otherwise unsuitable for α -toxin formation, i.e. in the absence of the toxigenic factor. The toxigenic factor studied in the present work is only one factor which influences toxin formation. The nature of the relation between iron concentration and toxigenic factor in toxin formation, which again may be indirect, remains to be discovered.

SUMMARY

1. Protein-free horse muscle extract contains labile material which enhances α -toxin (lecithinase) formation by *Cl. welchii* type A (strain S107).

2. Some general properties of the partially purified toxigenic factor have been studied, which suggest that the active component may be of the nature of an amino-sugar.

3. The inorganic iron concentration, in some media, is very important in its effect on α -toxin formation by *Cl. welchii*.

The work recorded in this paper was begun during 1941 as part of a study of toxin formation by bacteria of the gas-gangrene group. Later this was incorporated into a programme of research initiated by a group of workers, eventually organized as the Toxoid team, Anaerobe Subcommittee of the War Wounds Committee of the Medical Research Council. The work described here lay outside the main programme of the Toxoid team as it eventually developed, and was discontinued when the authors took up other aspects of the problem; it is thus suitable for publication separately. Our sincere thanks are due to Dr J. W. Trevan, F.R.S., Wellcome Physiological Research Laboratories, Beckenham, and to Mr R. L. Grant, Wellcome Chemical Works, Dartford, for supplying us with large quantities of the horse-muscle extract.

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The Metabolism of Sulphapyridine in the Rabbit

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Although the metabolism of sulphapyridine has been the subject of many investigations, much of the work has been concerned only with the isolation and identification of its excretion products or with single aspects of its metabolism. The earlier studies were carried out before it was appreciated that the oxidation of sulphapyridine occurred as a normal

metabolic process, but recent investigations, such as those of Gilligan (1945), in which the metabolism of several sulphonamides in man was investigated; of Seudi & Jelinek (1944), which was concerned with the role of urinary excretion products of these compounds in urolithiasis, and of Maher (1944), which deals with the part played by the reticulo-