of approximately 200 in². without any symptoms of histamine poisoning. Evidently, a diffusible substance like histamine was not liberated in any quantity sufficient to account for the massive wheal.

(5) A histamine wheal may be readily formed (electrophoresis or injection) over an irradiated area of the skin which has responded by whealing. (Fig. 1.) In other words, the whealing response to light does not prevent histamine whealing in the same area. Similarly, a light wheal may be superimposed on a wheal formed by electrophoresis of 1:10,000 histamine.

Summary. (1) The spectral response to light in a case of *Urticaria solare* may be connected with the pigmentation mechanism. (2) A process which produces a relatively non-diffusible whealing substance is probably responsible for the whealing in the case in question.

The preceding observations, which will be reported in detail elsewhere, do not support the point of view that histamine or a readily diffusible H-substance of low molecular weight is responsible for the skin response to ultraviolet irradiation.

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Toxin Production by *C. diphtheriae* Types: Mitis, Gravis and Intermedius.

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The differentiation of *C. diphtheriae* into 3 groups, gravis, intermedius and mitis, and the observation that the case death rate was highest and the incidence of paralysis greatest with gravis type infections, less with intermedius and least with mitis infections,[1, 2] coupled with reports of the inability of gravis strains to produce

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1 Anderson, Happold, McLeod and Thomson, *J. Path. and Bact.*, 1931, **34**, 667.

2 Cooper, Happold, McLeod and Woodcock, *Proc. Roy. Soc. Med.*, 1936, **29**, 5.

potent toxins,[3] have led to the postulation of two theories to explain the apparent anomaly: (a) That gravis strains have a more rapid invasive power than other strains.[4] (b) That the gravis type is more toxigenic *in vivo* than *in vitro* compared with the intermedius and mitis types.

The studies of Mueller[5] and Mueller and Pappenheimer[6] resulted in the production of chemically-defined media for the production of diphtheria toxin, and their findings have been applied in comparative studies of toxin formation. It is conceivable that modification of media may affect the toxin production by different types to different degrees and if this is so, it would give support to the second hypothesis. In addition the knowledge which is accumulating in the studies which are in progress at Harvard and at Leeds, that the nutritional requirements of gravis and intermedius types are not as simple as are those of the mitis, increases the possibility that additional toxic elements not previously recognized may be produced by gravis and intermedius strains.

Experimental. Media. The basal medium used was prepared as described by Mueller.[5] All glassware was cleaned with chromic acid before use. Media was sterilized by autoclaving at 10 lb pressure for 10 min.

Strains. Ten gravis, 8 intermedius, 4 mitis and 3 "Park Williams 8" strains were used. Of these 7 gravis and 4 intermedius strains were known to be from fatal or severe infections.

Flasks were inoculated from 24-hour peptone cultures since the inoculation from plate cultures results in poor and irregular toxin formation. Duplicate cultures were tested for toxin after 3, 5, 7 and 9 days' growth. The growth of mitis strains was good, of gravis, moderate, of intermedius infinitesimal. The latter produced less than 2½ Fl units of toxin.

Gravis—Six strains (severe) produced 5-10 Fl units per ml.
Four strains produced <2½ units per ml.

Mitis—One strain (severe) produced 10 Fl units per ml.
Three strains produced <2½ units per ml.

Park 8 (Toronto) produced 60 Fl units per ml and the *δL3* and *δ1058* strains 17 units.

Toxin production in complex media. Strains have been tested for toxin production in a further variety of media, after 3, 5, 6 and 8

[3] Parish, Watley and O'Brien, *Brit. Med. J.*, 1932, ii, 915.

[4] Orr, McLeod and Woodcock, *J. Path. and Bact.*, 1939, **48**, 99.

[5] Mueller, *J. Bact.*, 1938, **36**, 499.

[6] Pappenheimer, Mueller and Cohen, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 795.

days. (a) Parke Davis Peptone water (corrected for Fe content), (b) Wheeler and Wadsworth's Medium, and (c) Media (9) and (7) modified by the addition of sterile rabbit's serum (1 ml per 30 ml) both before and after heat sterilization. Only the 3 Park 8 strains produced toxin on these media and their titer of toxin was inferior to that produced on medium (7).

Neither free growth nor considerable pellicle formation in cultures of pathogenic strains of *C. diphtheriae* necessarily leads to the production of toxin even when there is an absence of wide pH fluctuation during growth.

Iron Requirements. The iron content of the basal medium was varied to contain 6 γ, 9 γ, 12 γ, and 24 γ $FeSO_4 \cdot 7H_2O$ per 30 cc. (The Park 8 strains had left a considerable proportion of their inorganic iron in the media after reaching the point of optimum toxin production, whereas gravis and mitis strains rapidly cleared the medium of inorganic iron.) PWδ1058 produced most toxin with 6 γ $FeSO_4 \cdot 7H_2O$ per 30 cc, δL3 and Toronto with 12 γ.

Addition of Mineral Constituents. In view of the findings of Evans, Happold and Handley that additional mineral requirements were necessary for the growth of intermedius strains, a salt mixture was prepared, containing those inorganic constituents which appeared in small concentrations in the original medium devised in this investigation for these strains.

The growth was increased by the addition of concentrations of this mixture equivalent to their content in (7), but only 2, Shore and T626, gave toxin in this new media (5-10 Fl per ml) and that with irregularity.

Energy Sources. The following energy sources were substituted in variable concentrations for the 2% Maltose used in medium (9), *viz.*, glucose, sodium citrate, glycerol, sodium lactate and sodium pyruvate. Toxin formation was inhibited by the lactate, pyruvate and citrate, perhaps because the cultures developed a strong alkalinity. Toxin formation was lessened by the substitution of the glucose and glycerol. These cultures developed an increased acidity in comparison to the maltose cultures.

The effect of balancing the increased acidity due to the fermentation of the glucose and glycerol with the alkalinity from the sodium lactate was now tried. Better growth of cultures was obtained for intermedius strains but the "non-toxin"-producing strains were not induced thereby to produce toxin.

Effect of Certain Amino Acids. Tryptophan. In the preparation of medium (9) all but traces of tryptophan must be destroyed. The effect of added tryptophan (1-5 mg per 10 ml of media) was tested on

the 3 Park 8 strains. The toxin production of the Toronto strain was unaffected by such addition, δ13 and δ1058 produced more toxin subsequent to such addition, the effect being greatest with δ1058.

Isoleucine was also shown to be an essential amino acid for the growth of the intermedius type.[7] Its effect on toxin production was studied. The intermedius strain "Shore" produced more toxin after such enrichment. The Park 8 strains were unaffected by such addition.

Basal medium	5 Fl
" " + 3 mg isoleucine per 30 cc	5-7 Fl
" " + 30 " " " " "	10-15 Fl

Pantothenic Acid. It was shown[8] that pantothenic acid was an essential growth factor for gravis strains (unessential for all mitis strains tested). It is also an essential for certain intermedius strains. Its optimum effect lies between 1 and 5 γ pantothenic acid per 10 cc medium. The addition of pantothenic acid to cultures of gravis and intermedius strains inoculated from 18-hour peptone cultures into medium[9] or the same enriched with tryptophan or isoleucine, increases the rate of growth but does not cause greater toxin production.

Conclusions. The Park 8 strains are the only potent toxin producers from the gravis, intermedius, mitis and Park 8 strains tested. All strains were virulent, the Park 8 and Freeman strains but feebly so. The cultural requirements of the Park 8 strains for optimum toxin production vary with the strain in its iron and tryptophan requirements. The same is true for the intermedius strain "Shore", whose toxigenic capacity is also increased by the addition of isoleucine.

Whilst all markedly pathogenic strains of *C. diphtheriae* tested were poor toxin producers under the experimental methods applied, there is indication that optimum conditions may not have existed for toxin production by these strains.

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7 Evans, Happold and Handley, *Brit. J. Exp. Path.*, 1939, **20**, 41.

8 Evans, Handley and Happold, *Brit. J. Exp. Path.*, 1939, **20**, 396.

9 Mueller, *J. Immunol.*, 1939, **37**, 103.