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LYMPHO-RETICULAR STIMULATORY PROPERTIES OF *CORYNEBACTERIUM PARVUM* AND RELATED BACTERIA

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KILLED suspensions of the anaerobic diphtheroid *Corynebacterium parvum* were shown by Halpern *et al.* (1964) to possess a marked capacity for stimulating the lympho-reticular system. Treated mice showed large increases in spleen and liver weight and increased ability to clear intravenously injected carbon from the circulation. It has since been shown that *C. parvum* acts as an adjuvant (Neveu, Branellec and Biozzi, 1964; Biozzi *et al.*, 1966) and that treatment of animals with this organism can protect them from tumour-cell (Halpern *et al.*, 1966; Woodruff and Boak, 1966), protozoal (Nussenzweig, 1967) and bacterial challenge (Adlam, Broughton and Scott, 1972). Protection is also afforded against graft-versus-host disease (Howard *et al.*, 1967). In addition to these properties, *C. parvum* resembles *Bordetella pertussis*, an unrelated organism that may also act as an adjuvant (Kind, 1957) and possesses the ability to induce splenomegaly (Morse, 1965), in being able to sensitise susceptible strains of mice to endotoxin (Howard, 1968) and to histamine (Adlam *et al.*).

The work of Prévot and Tran Van Phi (1964), who showed that several diphtheroid species other than *C. parvum* stimulate the reticulo-endothelial system to take up injected carbon more rapidly, suggested that the properties possessed by *C. parvum* might be common to several related species. This possibility was also indicated by the work of Farber and Smith (1969), who showed that a strain of *C. acnes* possessed reticulo-endothelial stimulatory properties similar to *C. parvum*. In addition, both *C. rubrum* (Katsh, 1960) and *C. avidum* (Giambanco, Armani and Falcone, 1966), like *C. parvum* (Neveu *et al.*), may be satisfactorily substituted for the mycobacterial component of Freund's complete adjuvant.

In the present work, several species of corynebacteria were quantitatively

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tested for lympho-reticular stimulatory activity and an attempt has been made to relate their biochemical reactions to their stimulatory ability. The following parameters of lympho-reticular stimulation were examined: increase of spleen and liver weight; carbon clearance; adjuvant effect; and sensitisation of mice to histamine.

METHODS

Bacterial strains. Strains obtained from various sources (table I) were maintained as freeze-dried seed cultures in the Wellcome Culture Collection.

Media. Bacterial growth media were Wellcome Reagents, unless otherwise indicated.

TABLE I
Culture collection numbers of strains of corynebacteria

Strains	Culture collection numbers				
	NCTC*	ATCC†	Pasteur Institute	Wellcome	Sources‡ of strains
<i>C. acnes</i>	...	11828	...	CN 6280	(1)
<i>C. acnes</i>	...	...	...	CN 6276	(1)
<i>C. anaerobium</i>	...	...	4685	CN 6134	(2)
<i>C. betae</i>	...	...	...	CN 4229	(3)
<i>C. diphtheriae</i> §	7429	296	...	CN 2000	(3)
<i>C. diphtheroides</i>	...	...	2764	CN 6295	(1)
<i>C. equi</i>	...	...	...	CN 4686	(3)
<i>C. granulosum</i>	...	...	3024-B	CN 6292	(1)
<i>C. laevaniformans</i>	...	15923	...	CN 6366	(4)
<i>C. liquefaciens</i>	...	...	3044-B	CN 6290	(1)
<i>C. lymphophilum</i>	...	...	...	CN 6294	(1)
<i>C. parvum</i> (a)	10387	11829	...	CN 5888	NCTC
(b)	10387	11829	...	CN 6293	(1)
(c)	10387	11829	...	CN 6156	(5)
<i>C. parvum</i>	10390	12930	643-C	CN 5936	NCTC
<i>C. pyogenes</i>	...	...	637-B	CN 6291	(1)

* National Collection of Type Cultures, Central Public Health Laboratories, Colindale Avenue, London, N.W.9.

† American Type Culture Collection, Rockville, Md, USA.

‡ Sources of strains: (1) Professor R. F. Smith, Department of Microbiology, Temple University School of Pharmacy, Philadelphia, Pa., USA. (2) Professor M. Raynaud, Institut Pasteur, Annexe de Garches, Garches, 92 Hauts-de-Seine, France. (3) Wellcome Culture Collection. (4) Dr J. Miranda, Wellcome Research Laboratories. (5) Dr K. C. Milner, Department of Health, Education and Welfare, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana, USA.

§ Park Williams no. 8 strain.

Growth of bacteria. Strains of *C. acnes*, *C. anaerobium*, *C. diphtheroides*, *C. granulosum*, *C. liquefaciens*, *C. lymphophilum*, *C. parvum* and *C. pyogenes* were grown anaerobically. Organisms from freeze-dried vials were sub-cultured in Robertson's meat broth (20 ml) containing 5 per cent. horse serum and 1 per cent. glucose. After static incubation for 7–10 days at 37°C organisms were transferred to 1 per cent. glucose broth (100 ml) and re-incubated for 7–10 days. For bulk growth this culture was used to seed 2-l bottles containing glucose broth which were then statically incubated at 37°C for 7–10 days.

C. betae and *C. laevaniformans* strains were grown aerobically on nutrient agar slopes for 48 hr at 20°C before subculture into 2-l bottles of brain heart infusion broth (Oxoid Ltd) and incubation with shaking at 20°C for 3 days. The *C. equi* strain was subcultured in brain heart infusion broth (20 ml) and incubated at 37°C for 5–7 days. For bulk growth

this culture was used to seed 2-l bottles containing brain heart infusion broth which were then incubated with shaking at 37°C for 5–7 days. The *C. diphtheriae* strain was grown in 100-ml bottles of papain digest of beef (Ramon, Pochon and Amoureux, 1941) supplemented with ferric citrate (10 µg per ml) to abolish toxin formation (Edwards and Seamer, 1960). For bulk growth this culture was used to seed 300-ml bottles of media which were shaken at 37°C for 48 hr.

Killing and harvesting bacteria. Only killed organisms were used for animal inoculation. All cultures were killed by addition of formalin to 0.5 per cent.; after standing overnight, they were centrifuged and washed three times in pyrogen-free physiological saline before being suspended in the same medium with 0.01 per cent. thiomersalate as preservative.

Dry weight of bacteria. Organisms were washed three times in filtered glass-distilled water (Sartorius filter, 0.2 µm pore size; V. A. Howe and Co. Ltd, 88 Peterborough Road, London, S.W.6). Samples (1.0 ml) of known optical density were dispensed in triplicate into weighed bottles and dried at 60°C to constant weight.

Carbohydrate fermentation. A dense suspension of organisms was inoculated into 1 per cent. peptone sugars and the formation of acid noted over a 3-wk period. Incubation was at 37°C except in the case of *C. betae* and *C. laevaniformans* strains which were incubated at 20°C. For anaerobic strains, 0.2 per cent. nutrient agar was added to the peptone medium.

Gelatin liquefaction. Tubes of nutrient gelatin were stab-inoculated and examined periodically over 3 weeks by placing in the refrigerator at 4°C for 30 min.

Catalase production. A dense suspension of organisms was mixed on a microscope slide with a drop of freshly prepared hydrogen peroxide (5 per cent.). The production of effervescence was noted.

Bromocresol purple milk. Tubes of milk medium containing indicator (Cowan and Steel, 1965, p. 160) were inoculated with organisms and examined daily for 14 days for acid and clot production.

Indole production. This was tested for by the method of Cowan and Steel (p. 158) using Ehrlich's reagent as indicator.

Nitrate reduction. This was tested for by the method of Cowan and Steel (p. 161). Inoculated broth was incubated for 5 days and the reaction elicited by the addition of sulphanilic acid and α-naphthylamine.

Measurement of lympho-reticular stimulation

Increase in organ size. Groups of ten female W-Swiss mice (16–20 g, S. Schofield and Co. Ltd, Intake Head, Delph, Oldham, Lancs) received a single intravenous injection of organisms in varying doses. Animals were killed by cervical dislocation at varying days after treatment and their spleens, livers and lungs weighed immediately; body weights were also measured. Mean values for organ weights were corrected for body weights so that they could be expressed as mg per 20 g body weight. Control animals that had received thiomersalate saline were included in each experiment.

Carbon clearance. Groups of eight female CBA/T6 mice (16–20 g, reared at the Wellcome Laboratories) received a single intravenous injection of organisms (1.4 mg dry weight). At intervals thereafter animals were assayed for their ability to clear intravenously injected colloidal carbon from the circulation. Phagocytic indices (K values) were calculated by the method of Biozzi, Benacerraf and Halpern (1953). Control animals injected with thiomersalate saline were inoculated in each experiment.

Adjuvant effect. Groups of ten male CBA *p* mice (16–20 g, reared at the Wellcome Laboratories) received a single intravenous injection of organisms (1.4 mg dry weight). Four days later the animals were given intravenous injections of 0.25 ml of a 10 per cent. suspension of washed sheep red cells (Wellcome Reagents, Ltd) in saline (5×10^8 cells) and were afterwards bled (0.05 ml per mouse) at intervals. Sera pooled from ten animals were assayed for their haemagglutination titre by microtitration in conical plastic wells (Linbro Chemical Co. Inc., 681 Dixwell Avenue, Newhaven, Conn. 06511, USA). Serial two-fold dilutions of sera in physiological saline were added in 0.05-ml volumes to an equal volume

FIG. 1.—The weights of internal organs of mice at different times after injection of varying doses of *Corynebacterium anaerobium*.

Groups of ten mice received killed organisms intravenously; numbers identifying the curves are dry weights (mg) of organisms injected. Animals were then killed at intervals and organ and total body weights determined. Organ weights were corrected for body weights; standard errors are shown for the animals that received 2.4 mg of bacteria. The solid bar indicates organ weights and standard errors for all control animals studied during the course of the experiments.

of washed sheep red cells (1 per cent.); duplicate titrations were made. After incubation (1 hr at 37°C, then overnight at 4°C), titres were recorded as the reciprocal dilution of the last well showing agglutination.

Histamine-sensitising effect. Groups of ten female W-Swiss mice (16–20 g, Bromfield Ltd, 81 Windsor Avenue, Newton Abbot) received a single intravenous injection of organisms at varying doses. Each animal was challenged 7 days later with an intraperitoneal injection of histamine phosphate (1 mg histamine base; B.D.H. Ltd, Poole, Dorset). Deaths were recorded over a 24-hr period. Control animals that had received intravenous thiomersalate saline were included in each experiment and were challenged with 5 mg histamine base per mouse.

RESULTS

Increase in organ size. After a single intravenous injection of an active strain, the spleen, liver and lung weights were markedly increased. As an

TABLE II

Spleen, liver and lung weights of mice after the injection of different strains of corynebacteria

Strain CN number	Species	Spleen index* at 14 days	Liver index* at 10 days	Lung index* at 10 days
6134	<i>C. anaerobium</i>	5.09±0.26	2.49±0.09	1.45±0.08
6280	<i>C. acnes</i>	5.06±0.35	2.55±0.09	1.53±0.10
6276	<i>C. acnes</i>	4.84±0.19	2.41±0.11	1.36±0.06
6295	<i>C. diphtheroides</i>	4.78±0.28	2.49±0.13	1.38±0.05
6291	<i>C. pyogenes</i>	4.69±0.20	2.36±0.04	1.32±0.03
6290	<i>C. liquefaciens</i>	4.67±0.17	2.46±0.10	1.40±0.09
6294	<i>C. lymphophilum</i>	4.58±0.32	2.46±0.10	1.71±0.08
6293†	<i>C. parvum</i>	4.10±0.36	1.89±0.08	1.12±0.04
5936	<i>C. parvum</i>	3.94±0.33	2.17±0.14	1.27±0.06
6292	<i>C. granulorum</i>	3.40±0.39	2.29±0.11	1.28±0.05
4686	<i>C. equi</i>	2.73±0.21	1.89±0.09	1.01±0.02
6156†	<i>C. parvum</i>	2.52±0.19	1.41±0.09	1.26±0.12
6366	<i>C. laevaniformans</i>	1.83±0.16	1.04±0.03	1.16±0.04
5888†	<i>C. parvum</i>	1.37±0.09	1.01±0.03	1.24±0.02
2000	<i>C. diphtheriae</i>	1.21±0.10	1.00±0.02	1.17±0.03
4229	<i>C. betae</i>	1.12±0.05	1.10±0.01	1.10±0.04

* Groups of ten mice received a single intravenous injection of killed organisms (2.4 mg dry wt). Animals were killed and organs weighed after 10 or 14 days. Figures in the body of the table are organ indices with standard errors. Organ index = $\frac{\text{mean organ weights of treated animals}}{\text{mean organ weights of control animals}}$.

† The same strain obtained from three different sources.

example, positive responses to varying doses of *C. anaerobium* are shown in fig. 1. After an injection of 2.4 mg (dry weight) of this organism, a five-fold increase in spleen weight and a three-fold increase in liver weight occurred. Maximum weights of spleen and liver were reached 10–14 days after treatment. A small but significant increase in lung weight was observed between 7 and 14 days after injection.

The extent of increase in organ weights produced by all organisms tested is shown in table II. For ease of comparison, strains are arranged in approximate order of maximum activity. Organ-weight increase is expressed as an index by comparison with control values. The kinetics of organ-weight increase were similar for all active strains, and a marked increase in spleen weight was always accompanied by an increase in liver and lung weights. Although

greatest activity was shown by anaerobic strains, not all were active and not all aerobic strains were inactive since *C. equi* showed moderate activity. A culture of *C. parvum* (CN 5888) obtained from the National Collection of Type Cultures was inactive, although two cultures of the same strain (CN 6293 and 6156) obtained from American laboratories possessed some activity.

Carbon clearance. Experiments with *C. anaerobium* showed that maximum carbon clearance was produced 4 days after bacterial inoculation, the kinetics of the response (fig. 2) being similar over a wide range of dose of organisms.

FIG. 2.—Enhancement of carbon clearance from the circulation by *Corynebacterium anaerobium*. Groups of eight mice received different doses of killed organisms intravenously. Carbon clearance indices (K values) were determined at intervals subsequently. Standard errors of values are shown for the animals that received 1.2 mg dry weight of organisms. The single control point shows the mean value and standard error of control animals throughout the experiment. K values: ○—○ after 1.2 mg; ●—● after 0.9 mg; △—△ after 0.3 mg of organisms; —▲— in controls.

Fig. 3 shows the results of carbon-clearance assays for eight of the strains of corynebacteria. K values of phagocytosis are shown in the form of a histogram with high carbon clearance strains on the left. Whereas *C. anaerobium* and *C. acnes* (CN 6134 and 6276) were most active and *C. granulosum* and *C. lymphophilum* (CN 6292 and 6294) also showed activity, *C. parvum* and *C. diphtheriae* (CN 5936 and 2000) were doubtfully active. *C. betae* and another *C. parvum* strain (CN 4229 and 5888) were inactive.

Adjuvant effect. Experiments with *C. anaerobium* showed that peak adjuvant activity to sheep red-cells was produced by injecting the organisms 4 days before the red cells. Table III shows the results of an experiment in which nine of the strains of corynebacteria were used in this way. Results have been expressed as log₂ haemagglutination titres, strains being arranged in the order of highest titre attained. Strains CN 6294, CN 5936, CN 6290, CN 6292, CN 6134, CN 6295 and CN 6276 showed marked activity that reached a peak

6-8 days after injection of sheep cells. Strains CN 5888 and CN 2000 were inactive.

Histamine sensitisation. Table IV shows the combined results of two experiments in which strains were tested for their ability to sensitise mice to histamine. Results have again been grouped in order of activity of the strains.

FIG. 3.—The ability of different strains of corynebacteria to enhance carbon clearance from the circulation. Groups of eight mice received a single intravenous injection of killed organisms (1.4 mg dry weight). Four days later carbon clearance assays were performed and the phagocytic indices (K values) so obtained are indicated by the heights of columns in the histogram, with standard errors. The Wellcome Collection (CN) number of each strain is shown at the foot of the relevant column (see table I for identity of strains). NaCl = K value in control mice receiving saline. The numbers in parentheses are the probabilities (P) of significance of differences between test results and controls. N.S. = Not significant.

In terms of dry weight of organisms required to sensitise, *C. lymphophilum* possessed the highest activity and strains of *C. acnes*, *C. anaerobium*, *C. diphtheroides*, *C. granulosum* and *C. liquefaciens* were quantitatively similar in activity. *C. pyogenes* and *C. parvum* (CN 5936) were low in activity and the remaining strains were inactive or caused a low-grade irregular sensitisation unrelated to dose of organisms injected.

Taxonomy. The results of the biochemical tests are shown in table V. All corynebacteria tested fermented glucose and produced catalase. In addition, all strains with the exception of *C. equi* fermented galactose, mannose and fructose. *C. equi* was exceptional, not only in its inability to ferment any

TABLE III

*Adjuvant activity of corynebacteria: haemagglutinin titres in mice given injections of sheep red cells after pre-treatment with different strains of corynebacteria**

Strain CN number	Species	Log ₂ haemagglutination titre on day†				
		2	4	6	8	10
6294	<i>C. lymphophilum</i>	2.0	9.5	13.5	15.5 ‡	11.5
5936	<i>C. parvum</i>	2.0	10.5	14.5	13.5	13.5
6292	<i>C. granulosum</i>	3.5	10.0	14.0	12.5	11.5
6290	<i>C. liquefaciens</i>	2.0	10.5	13.5	13.5	13.5
6134	<i>C. anaerobium</i>	2.0	9.0	13.0	13.0	13.0
6295	<i>C. diphtheroides</i>	2.0	10.0	13.0	13.0	12.0
6276	<i>C. acnes</i>	2.0	10.0	13.0	13.0	12.0
5888	<i>C. parvum</i>	2.0	8.5	10.0	11.5	11.5
2000	<i>C. diphtheriae</i>	2.0	8.0	10.0	10.0	10.5
Saline control		2.0	9.0	10.0	10.0	10.0

* Groups of ten mice received a single intravenous injection of killed organisms (1.4 mg dry wt); 4 days later they were given 5×10^8 sheep red cells; then they were bled at intervals.

† After the injection of sheep red cells.

‡ In bold type, peak titre.

TABLE IV

*Histamine sensitisation by corynebacteria: death rates of mice given injections of various strains of corynebacteria and subsequently challenged with histamine**

Strain CN number	Species	Number of deaths† in mice given the following doses of organisms (mg dry weight)			
		0.80	0.27	0.09	0.03
6294	<i>C. lymphophilum</i>	20	19	18/19	17
6295	<i>C. diphtheroides</i>	18/19	16	12	9
6134	<i>C. anaerobium</i>	16/19	19	8	10
6276	<i>C. acnes</i>	19	17	10	4
6290	<i>C. liquefaciens</i>	19	18	9	3
6280	<i>C. acnes</i>	19	14	12	3
6292	<i>C. granulosum</i>	19	13	8	8
6291	<i>C. pyogenes</i>	17	11	5	1
5936	<i>C. parvum</i>	17	10	1	2
4686	<i>C. equi</i>	14	9	3	3
5888‡	<i>C. parvum</i>	7/19	5	6	6
6293‡	<i>C. parvum</i>	4/16	8	6	9/19
2000	<i>C. diphtheriae</i>	5	6	5	7
6366	<i>C. laevaniformans</i>	4	3	2	2
6156‡	<i>C. parvum</i>	3	3	1	2
4229	<i>C. betae</i>	0	1	5/19	5
Saline control		3/40	...	...	...

* Groups of mice received varying doses of organisms intravenously. Seven days later, animals were challenged with 1 mg histamine base intraperitoneally (saline-control animals received 5 mg base). Deaths were recorded 24 hr after challenge.

† Number of deaths per 20 mice, unless otherwise indicated.

‡ The same strain obtained from three different sources.

TABLE V
Biochemical reactions of the strains of corynebacteria

Organism	Wellcome number (CN)	† Fermentation of																							NO ₃ reduction	BCP milk*	Indole production	Gelatin liquefaction	Catalase production
Anaerobic species		Glucose	Lactose	Sucrose	Maltose	Mannitol	Starch	Galactose	Trehalose	Mannose	Fructose	Dulcitol	Dextrin	Sorbitol	Rhamnose	Raffinose	Arabinose	Salicin	Inositol	Inulin	Xylose	Adonitol	Glycerol						
<i>C. acnes</i>	6276	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. acnes</i>	6280	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. anaerobium</i>	6134	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. granulorum</i>	6292	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. liquefaciens</i>	6290	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. lymphophilum</i>	6294	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. parvum</i>	5936	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. parvum</i> † (a)	5888	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. parvum</i> † (b)	6293	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. parvum</i> † (c)	6156	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. pyogenes</i>	6291	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Aerobic species		Glucose	Lactose	Sucrose	Maltose	Mannitol	Starch	Galactose	Trehalose	Mannose	Fructose	Dulcitol	Dextrin	Sorbitol	Rhamnose	Raffinose	Arabinose	Salicin	Inositol	Inulin	Xylose	Adonitol	Glycerol						
<i>C. betae</i>	4229	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. diphtheriae</i>	2000	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. equi</i>	4686	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>C. laevaniformans</i>	6366	++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		

* Bromocresol purple milk.

† + = Positive, ± = variable, and — = negative reaction.

of the sugars tested (except for weak glucose fermentation), but also in its production of a bright pink pigment. *C. betae* and *C. laevaniformans* were the only strains possessing bright yellow pigmentation.

As a group, the anaerobic strains showed similar biochemical reactions. Particularly close patterns of reaction were observed amongst the two *C. acnes* strains, *C. anaerobium*, *C. liquefaciens*, *C. pyogenes* and the *C. parvum* strains. All three cultures of *C. parvum* NCTC 10387 (CN 5888, CN 6293 and CN 6156), although obtained from different sources, gave very similar reactions.

TABLE VI

Publications by other authors describing the properties of the same strains of corynebacteria as those used in this study and their conclusions about taxonomy

Species	Wellcome number (CN)	Reference	Authors' conclusions and recommendations
<i>C. acnes</i>	6280	{ Smith and Bodily (1968)* Zierdt, Webster and Rude (1968) Reid and Joya (1969)†	Used as reference <i>C. acnes</i>
<i>C. diphtheriae</i>	2000	Lampidis and Barksdale (1971)	"Mitis" strain
<i>C. diphtheroides</i>	6295	Zierdt <i>et al.</i>	Transfer to <i>C. acnes</i>
<i>C. granulosum</i>	6292	{ Zierdt <i>et al.</i> Moss <i>et al.</i> (1969)	Transfer to <i>C. acnes</i>
<i>C. laevaniformans</i>	6366	Dias and Bhat (1962, 1964)	New species producing laevan
<i>C. liquefaciens</i>	6290	{ Zierdt <i>et al.</i> Moss <i>et al.</i> Werner and Mann (1968)	Transfer to <i>C. acnes</i>
<i>C. parvum</i>	5888		Mixed culture of <i>C. parvum</i> and <i>C. acnes</i>
<i>C. parvum</i>	5936	{ Reid and Joya Werner and Mann	Transfer to <i>C. acnes</i>
<i>C. pyogenes</i>	6291	{ Moore, Holdeman and Cummins (1968) Zierdt <i>et al.</i> Moss <i>et al.</i>	Not a typical <i>C. pyogenes</i> Transfer to <i>C. acnes</i>

* Strain termed *Propionibacterium acnes* by authors.

† Strain termed *C. liquefaciens* by authors.

DISCUSSION

The taxonomy of the corynebacteria remains a matter of considerable controversy. Several workers are of the opinion that some of the anaerobic species described by Prévot (1966), e.g., *C. anaerobium*, *C. avidum*, *C. diphtheroides*, *C. granulosum*, *C. liquefaciens*, *C. lymphophilum* and *C. parvum* should be re-classified as *C. acnes* (Zierdt, Webster and Rude, 1968; Moss *et al.*, 1969; Reid and Joya, 1969). Others disagree with this view (Moore, Holdeman and Cummins, 1968), and some consider that the *C. acnes* species contains two distinct groups of organisms (Brzin, 1964; Voss, 1970). To add further confusion, there seems little doubt that the type species *C. diphtheriae* differs markedly from these anaerobic species (Barksdale, 1970), and that *C. pyogenes* and the plant diphtheroids do not resemble *C. diphtheriae* (Cummins, 1962; Barksdale). Table VI gives literature references to work in which the

strains of corynebacteria studied were identical with those used in the present work.

The question arises as to how many of these species possess the lympho-reticular stimulatory activity described for a strain of *C. parvum* by Halpern *et al.* (1964). In addition it may be asked how far other stimulatory strains possess the attributes of *C. parvum*. The present work has confirmed that several species of corynebacteria besides *C. parvum* possess lympho-reticular stimulating properties. The increase in lymphoid organ size produced by active strains was similar to that previously described for *C. parvum* (Halpern *et al.*, 1964) and the uniformity of kinetics of organ-weight increase produced by active strains suggests that these organisms contain similar if not identical stimulating material. Organisms that were able to act as adjuvants to sheep red cell agglutinin formation also increased lymphoid organ size and possessed the ability to sensitise mice of the strain used to histamine. Conversely corynebacteria lacking adjuvant activity also failed to cause histamine sensitisation or to produce marked increases in lymphoid organ size.

In the present study, enhancement of carbon clearance, even by strains highly active in organ weight-increasing ability, was lower than that originally observed with a strain of *C. parvum* (Halpern *et al.*, 1964). However, different strains of mice have been shown to vary in response to *C. parvum* treatment in this respect. A large increase in organ size was not always paralleled by concomitant enhancement of carbon clearance (Stiffel *et al.*, 1970). Nevertheless, in the present work, only those strains that increased organ size and possessed adjuvant and histamine-sensitising effects also caused a marked increase in carbon clearance.

The biochemical reactions exhibited by the strains confirm the close relationships amongst the anaerobic corynebacteria noted by other workers. As found by Zierdt *et al.* and Reid and Joya, gas production and gelatin liquefaction proved to be unreliable criteria for distinguishing between species. For example, the *C. anaerobium* strain when grown in bulk occasionally produced gas from glucose broth, which would place it in the *C. parvum* group of organisms according to Prévot. Also *C. parvum* strain CN 6156 did not liquefy gelatin, but is surely the same species as strains CN 5888 and CN 6293.

It must be concluded that the ability to stimulate the lympho-reticular system cannot, at this stage, be linked with any specific biochemical test. This is shown particularly well in the case of *C. equi* which possessed moderate capacity to increase spleen and liver weight and to sensitise to histamine, but was exceptional in its lack of biochemical reactivity. In addition, the three strains of *C. parvum* (NCTC 10387) had almost identical biochemical reactions but differed markedly in ability to increase organ weight.

The strain of *C. pyogenes* used in these studies is probably atypical since it produces catalase and indole and reduces nitrate (Moore *et al.*; Roberts, 1968). Its cell-wall amino acid composition is similar to that of other *C. pyogenes* strains but different from that of *C. acnes* (Cummins and Harris, 1956). This would suggest that the ability to stimulate the lympho-reticular system does not depend on the qualitative nature of these cell-wall amino acids. In view

of the work of Moss *et al.*, the cell-wall fatty acids may be a more important guide to the possession of stimulatory activity by any individual strain, but this remains a subject for further study.

SUMMARY

Several aerobic and anaerobic species of corynebacteria were examined for their ability to stimulate the lympho-reticular system of mice. Whereas active strains produced marked increases in spleen, liver and lung weight, possessed adjuvant activity, and were capable of sensitising mice to histamine, inactive strains possessed none of these attributes. Enhancement of carbon clearance was observed only in strains that possessed the other stimulatory properties but was not as great as previously observed by other workers for *Corynebacterium parvum*. The majority of active strains were anaerobic and possessed quantitatively similar stimulating activities. No correlation was observed between biochemical reactions of strains and lympho-reticular stimulation.

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