Table 2. *Mobilities of the components of various cobra venoms*

(Expressed as 10^{-5} cm.^{2}/sec./V. at pH 6·2 and 2°.)

Cobra	Components									
	A	*B*	*C*	*T*	*D*	*E*	*F*	*G*	*H*	*I*
Naja nigricollis	—	—	8·02	6·21	5·24	4·12	—	—	2·11	0·63
Naja haja (var. *angulosa*	—	—	8·5	6·40	5·4	4·0	3·6	—	2·1	0·3
Naja haja	—	—	8·6	6·6	5·6	4·4	3·6	—	2·07	0·65
Naja naja	11·7	9·3	8·1	6·52	5·3	4·3	—	3·01	—	0·74
Naja flava	—	9·00	8·0	6·44	5·6	—	3·6	—	—	1·1
Sepedon haemachates	—	9·3	—	6·46	—	—	—	2·94	—	1·4

mass of the *T* component shown in the electrophoretic diagram, then *N. nigricollis* venom should be only slightly inferior to *N. flava*. *N. naja* venom should be at best only slightly superior to *Sepedon haemachates* venom and markedly inferior to the others. It would be of interest to show whether *Naja naja* venom is superior as an antigen to *N. flava* or whether the greater mass of the *T* component is counterbalanced by the greater complexity as shown by the presence of an additional well-marked component.

SUMMARY

1. The electrophoresis of a number of cobra venoms is described, together with the method of identification of the toxic component in the electrophoresis diagram.

2. The venoms of *Naja nigricollis*, *N. haja*, *N. naja*, *N. flava* and *Sepedon haemachates* were examined electrophoretically, and the respective diagrams obtained under identical conditions at pH 6·2 are shown.

3. The neurotoxic and haemolytic activity is found to be associated with the *T* component having a mobility of about $6{\cdot}4 \times 10^{-5}$ cm.^{2}/sec./V.

4. The various components of the different venoms fall into groups having the same mobility.

5. *Sepedon haemachates* venom is the least complex and *Naja naja* venom the most complex.

6. The contention that the efficiency of an antigen may be gauged by the mass of the *T* component is discussed.

One of us (F.J.J.) expresses his gratitude to the Director, Dr P. J. du Toit, for the opportunity of working at his Institute. We are indebted to the South African Institute for Medical Research, Johannesburg, for the supply of desiccated venoms.

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The Metabolism and Functioning of Vitamin-like Compounds

2. A COMPARISON OF PANTOTHENATE METABOLISM BY PROLIFERATING AND BY NON-PROLIFERATING BACTERIA

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Suspensions of non-proliferating haemolytic streptococci were previously found (McIlwain & Hughes, 1944) to destroy the growth-promoting powers of pantothenate, for streptococci and for other organisms, during glycolysis. Such pantothenate inactivation was inhibited by pantoyltaurine, which inhibited also the growth of streptococci; the activities of pantoyltaurine in these two respects towards a series of organisms were quantitatively correlated, as were also the effects of a series of compounds structurally similar to pantoyltaurine, upon a given organism (McIlwain & Hughes, 1945). Preformed

pantothenate was necessary for the growth of the organisms concerned, and a knowledge of the manner of connexion between its participation in the two processes of metabolism and growth might give knowledge of reactions underlying the biological functioning of a vitamin-like substance.

The present paper examines an hypothesis concerning this connexion, based on the following argument. Pantothenate is normally assimilated during the growth of haemolytic streptococci, but the possibility of such assimilation is limited in non-proliferating suspensions of the organisms; reactions which in growing cultures led to assimilation of pantothenate might then, in non-proliferating cells, lead to catabolism of pantothenate through lack of co-ordination between pantothenate metabolism and reactions which in growing organisms result in the synthesis of new bacterial substance. This suggestion led to the expectation that pantothenate inactivation would not occur to the same extent in growing cultures as in suspensions of non-proliferating organisms. It appeared especially desirable to find whether this was the case, as few comparable studies of the metabolism of growth essentials in growing and non-growing cells have been encountered. Though the 'resting-cell' technique is widely applicable to studies of microbial metabolism, vitamin-like substances, through their special connexion with growth, may well be in a special category in this respect; certain of their reactions cannot occur without concomitant growth. Also, an assessment of bacterial economy demands an understanding of the inactivation of pantothenate by streptococci, for in a given environment this activity would appear to restrict their potential growth.

Streptococcal inactivation of pantothenate, in concentrations comparable to those of natural environments of the organisms, has been found nearly proportional to time and expressed as a metabolic quotient Q_P (in mμ mol./mg. dry wt./hr.) for comparison with the reaction as it occurred during growth. To obtain this latter value the pantothenate present initially and at a few intervals during growth was determined, and the bacterial activity with which the changes were associated was obtained by graphical integration of the growth curve of the culture. The activity was expressed in mg.-hr. (the product of the dry weight of bacteria in mg., and the time in hr. during which they were acting). The growth curve was obtained from optical density measurements of samples taken from the culture during growth; a logarithmic plot of the optical density was made to characterize the different phases of growth of the culture, whose pantothenate metabolism were in a few cases compared. To approach the wider problems indicated above, the scope of the investigation has been extended by applying similar methods to strains of *Proteus morganii*, whose inactivation of pantothenate was more rapid than that of the streptococci, and also to *Escherichia coli* and *Pseudomonas aeruginosa* (*Ps. pyocyanea*) which synthesized pantothenate.

EXPERIMENTAL

Organisms. The following bacterial strains were examined: (R), the 'Richards' strain of *Streptococcus haemolyticus*, No. 5631 of the National Collection of Type Cultures, passaged in mice before and during the investigations; (G), a β-haemolytic streptococcus of group G; (F) a *Streptococcus faecalis*, isolated from human faeces, being strain II of McIlwain & Hughes (1945); two strains of *Proteus morganii*, N.C.T.C. no. 2818 and strain 2 of McIlwain & Hughes (1945); *Pseudomonas aeruginosa* (strains 3 and 4) isolated from an infected wound and given by Dr L. Colebrook; and two recently isolated strains of *Esch. coli* from faeces.

The β-haemolytic streptococci were maintained, after passage, by weekly subculture in blood broth, from which subcultures to serum-broth-agar slopes were made for daily use. Other organisms were subcultured on broth-agar slopes.

Growth media for streptococci. The *casein medium* of Table 1 contained a hydrolysate of casein prepared by acid in the presence of titanium salts, glucose, phosphate, and small quantities of many growth-promoting substances. It was prepared by adding aseptically to the basal medium (70 ml.) of McIlwain & Hughes (1944), M-glucose (5 ml.); 0·02M-arginine and 0·02M-glutamine (2 ml.); 0·5M-sodium bicarbonate (0·2 ml.); group A of McIlwain (1944) (5 ml., containing $\frac{1}{25}$ of the concentration of Fe salts there described); N-NaOH to neutralize the acid of group A (1 ml.) pantothenate as required; an inoculum containing *c.* 10^7 organisms in 0·1 ml. of the medium itself, and water to 100 ml. *Casein-yeast* and *casein-broth* media were prepared in the same manner as the casein medium except that the pantothenate-free yeast preparation of McIlwain (1944) was added (1 ml./100 ml.) in the former case and an ordinary bacteriological infusion broth (10 ml.) in the latter, in place of equal volumes of water.

Growth media for Pr. morganii. The casein medium was the 'assay medium' of McIlwain & Hughes (1945) with added pantothenate and prepared from the same reagents as for streptococci but containing fewer accessory factors; the *casein-yeast* medium was again prepared from it by addition of 1% of the pantothenate-free yeast preparation.

Ps. aeruginosa was grown in the ammonium lactate and inorganic salts of Fildes (1940); for *Esch. coli* either this medium, or one in which glucose (final concentration, M/80) replaced the lactate, was used.

Pantothenate metabolism during growth

Media were prepared in volumes slightly in excess of those of the intended cultures (e.g. 320 ml. for three cultures of 100 ml.) and portions of uninoculated media kept for the assay of their original pantothenate concentration, for preparation of the inoculum, and to serve as control in the photometric measurement of subsequent growth. The remainder was inoculated and portions of 100 ml. pipetted to 250 ml. conical flasks which were shaken at about two

oscillations of 5 cm./sec. in a thermostat at 37°. Portions of c. 1·5 ml. were taken aseptically at intervals (usually of ½ hr.) during growth by Pasteur pipettes, after manually shaking the flasks. After the desired growth, the cultures were no longer handled aseptically; their volume was measured, a portion taken for determination of the dry weight of bacteria present, the organisms of the bulk collected by centrifuging, and a portion of the culture fluid filtered to sterilize. Its pantothenate content, and that of the organisms after digestion (McIlwain & Hughes, 1944) were determined by growth of *Pr. morganii* (McIlwain & Hughes, 1945). With the streptococci and *Pr. morganii*, the pantothenate of the bacteria was a small fraction only, $<1\%$, of that of the initial solution and values for bacterial pantothenate are recorded separately in only a few cases; the pantothenate content of cultures after growth, in Table 2, include the values for that of the bacterial cells. Change in the pantothenate of the bacterial cell during growth is not considered unimportant, but is being studied separately. Changes in pH during growth under the present conditions were $<0·2$ pH unit.

Measurement of growth

The optical densities of samples taken during growth were compared with that of the original medium in a Hilger photoelectric absorptiometer. Neutral filters and cells taking 0·5 ml. of liquid were used; the 1·5 ml. samples were adequate for washing a cell between consecutive readings. Significant readings were not obtained from specimens taken earlier than 1 hr. after inoculation, but the initial point of each growth curve was obtained accurately by measuring the optical density of a portion of the suspension used as inoculum; this had been prepared in the medium itself and was diluted to a known extent in inoculation. The course of growth during the initial period could usually be deduced with reasonable certainty from such data (see Fig. 2); the bacterial mg.-hr. during this period was a very small fraction of the whole. Culture fluids were not found to change in light absorption as a result of growth; the use of titanium salts during preparation of the casein hydrolysate gave media with small initial light-absorbing capacities. By examination of a series of dilutions prepared from two bacterial suspensions, in their growth media, the densities given by the instrument were shown to be proportional to the quantity of bacteria present. The ratio between bacterial mass and optical density was determined in each experiment by finding the dry weight of bacteria present in a sample from one of the cultures.

Pantothenate metabolism of bacterial suspensions

The suspensions were of organisms grown in the above manner, and also of some grown in a more normal and convenient bacteriological fashion: in similar media but from smaller inocula, overnight, in an incubator, and without shaking. They were washed twice in 0·5% NaCl by centrifuging, and suspended in this solution for use. Reactions were carried out at 37° and pH 7·6 in Warburg vessels of c. 20 ml., usually containing the reactants (Table 1; cf. also McIlwain & Hughes, 1945) in 3·3 ml., and lasting ½–2 hr. The reactions of streptococci and *Pr. morganii* towards pantothenate, in the presence of excess glucose, were found to be independent of oxygen and most experiments with these organisms were performed anaerobically, in a N_2-CO_2 atmosphere forming a buffer with $NaHCO_3$ in solution. Those with *Esch. coli* and *Ps. aeruginosa* were carried out aerobically, as were the growth experiments, and in phosphate buffer.

Pantothenate before and after metabolism was determined as described above. The response of the assay organism to pantothenate has already been found (McIlwain & Hughes, 1945) to be independent of various constituents present during the metabolic experiments; in addition to control experiments described below, the assay of small quantities of pantothenate in the presence of β-alanine and pantoic acid was examined, and found to be unaffected by them.

RESULTS

β-Haemolytic streptococci as non-proliferating suspensions

Comparison of the metabolism of pantothenate by growing and non-growing bacteria necessitated the use of different media in the two cases, if normal organisms were to be used and in one case they were not to grow. Also, the composition of a medium would change during growth. To find whether significant values could be obtained for pantothenate metabolism under such circumstances, the effects of different constituents of the growth media and of other variable factors on the reaction were first examined. Excess of glucose was added in all cases as such addition was necessary both for growth and for pantothenate metabolism in the absence of growth.

(1) *Pantothenate concentration and time of reaction.* Fig. 1A shows the progress of pantothenate inactivation by the 'Richards' strain of streptococcus to be roughly proportional to time, but to fall slightly in rate as the reaction progressed. The first three points correspond to a value for Q_P of $-1·31$ and the last three, $-1·16$. Similar results with the group G streptococcus have been given previously (McIlwain, 1944). The range of pantothenate concentrations in these experiments was relatively large, 0·3–6·6, and $0·4–2·2 \times 10^{-6}$ M, and covered those of most animal tissues and of the present experimental media. The time occupied by the present experiments with streptococcal suspensions was not greater than that of Fig. 1A. It is therefore considered that the rate of pantothenate metabolism by the present suspensions is sufficiently stable to be evaluated as a mean Q_P over periods of about an hour.

(2) *Bacterial suspensions.* Table 1 shows the largest variation in Q_P of a given streptococcal strain to be associated with the growth and handling of the suspension examined. Q_P varied little with age of suspension until cultures were long past their period of maximal growth rate. Organisms collected at 5 hr. when growth was rapid and the bacterial mass was about one-fifth of the maximum obtainable in the medium, were found to be of

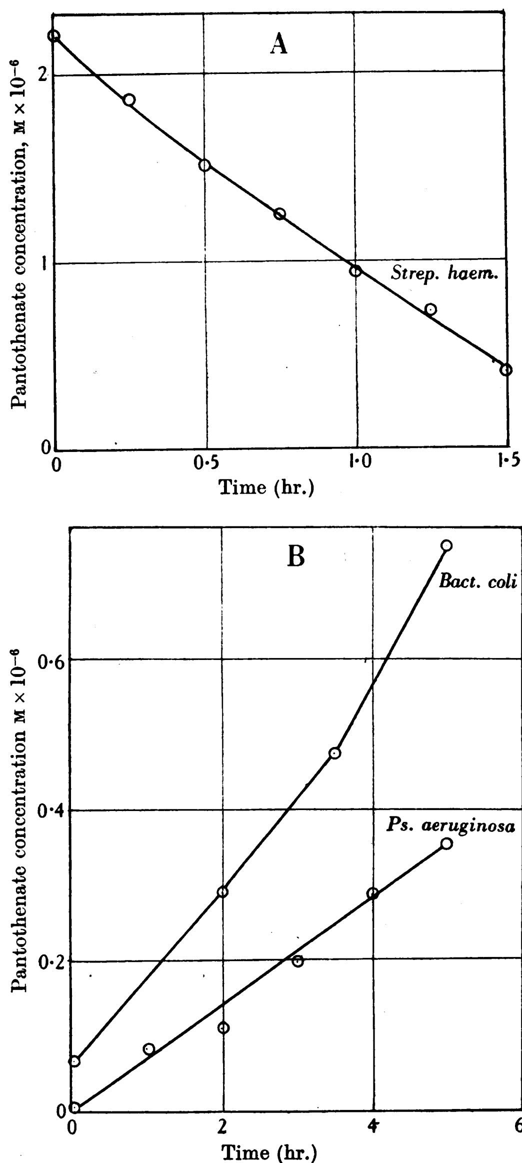

Fig. 1. Kinetics of pantothenate metabolism by bacterial suspensions. (A) Inactivation by the 'Richards' streptococcus. Experimental procedure as described by McIlwain & Hughes (1945). The organisms (3·5 mg. dry wt.; parent culture, 24 hr. old) were added to glucose-bicarbonate-saline (final volume 3·5 ml.) and gave a mean value for Q_P of $-1·25$. (B) Pantothenate production. *Esch. coli* (3·35 mg. dry wt.; parent culture, 24 hr. old) was suspended in 25 ml. glucose-phosphate-saline; Q_P during the first $3\frac{1}{2}$ hr., $+0·88$. A 6% increase in optical density occurred during the latter part of the experiment. *Ps. aeruginosa* (10·3 mg. dry wt.; strain 3, parent culture 7 hr. old) was suspended in 100 ml. lactate-phosphate-saline. No growth, but a 5% diminution in optical density occurred during the experiment. Mean Q_P: $+0·70$.

approximately the same Q_P as organisms collected at 18 hr., when the cells had been metabolizing for some time without much increase in bacterial substance. Between 18 and 24 hr. both glycolyzing activity and Q_P fell. Harvested organisms did not decrease in Q_P if they were kept cold for 2 hr., but Q_P and rate of glycolysis fell after glycolysis in a simple medium and resuspension of the organisms in another portion of the same medium. This fall was more rapid than in the richer media used for growth. The decrease in both cases was presumed to be due to exhaustion of various systems directly or indirectly connected with glycolysis, such as may be illustrated by the effect of glutamine studied previously (McIlwain, 1946) with streptococcal strains which included those of Table 1.

(3) *Medium constituents other than glucose and pantothenate.* The effects of such constituents were investigated most fully in the cases of the two β-haemolytic streptococci, and were found to be small. The casein preparation, representing the greater mass and number of substances of any constituent present during growth, caused a small acceleration in pantothenate inactivation. Folic acid and biotin were examined as they had been considered to be involved in pantothenate metabolism in rats (Wright & Welch, 1943; Emerson & Wurtz, 1944). One interpretation of the observations of Wright & Welch (1943) could be that the two compounds decreased pantothenate-inactivation; but their effect upon the reaction in streptococci was negligible. The preparation of folic acid (*L. casei* factor, 80% pure) which was used was that of Hutchings, Stokstad, Bohonos & Slobodkin (1944) and I am greatly indebted to Dr E. L. R. Stokstad for providing it. Sevag & Green's (1944) opinion that a function of pantothenate in staphylococci was in the synthesis of tryptophan suggested that pantothenate-usage might be altered by the presence of excess tryptophan; but little effect of tryptophan on streptococcal inactivation of pantothenate was found. Mg salts and adenosine triphosphate (examined on account of their relationship to glycolysis) and glutathione were also inactive in this respect. The results suggest that no considerable change would be expected in Q_P on account of the differences in media in which proliferating and non-proliferating organisms were examined.

β-Haemolytic streptococci during growth

The changes in pantothenate which were observed during growth of several cultures are recorded in Table 2, together with quantitative descriptions of the course of growth. The derivation of these descriptions from experimental data is illustrated in Fig. 2, which gives the growth curve and logarithmic growth curve of a culture of the group G streptococcus. In this case, growth was initiated in two

Table 1. *Rates of pantothenate metabolism by non-proliferating bacterial suspensions*

Vessels initially contained saline suspensions of the organisms (2–10 mg. dry wt.) in a side arm, and in the main compartment a buffering solution and substrate. Quantities (per vessel) and further details of the reagents, indicated below, are: glucose (present in all experiments with streptococci), 200 μmol.; $NaHCO_3$, 150 μmol.; saline, the mixture of Krebs & Eggleston (1940), with however only 3 μmol. of phosphate; phosphate-saline, the preceding with 80 μmol. of phosphate; casein hydrolysate, that of McIlwain & Hughes (1944) (75 mg.); $MgSO_4$, 0·5 μmol.; adenosine triphosphate and adenylic acid, 0·7 μmol. Experiments with streptococci and *Pr. morganii* (except where indicated) were performed anaerobically in an atmosphere of $N_2 + 5\%$ CO_2 and with yellow phosphorus in a centre well. Those with *Esch. coli* and *Ps. aeruginosa* were aerobic (except where indicated otherwise): gas phase, air; NaOH in a centre well.

		Pantothenate		
Organism; age of culture	Reagents	Initial ($M \times 10^{-8}$)	Final ($M \times 10^{-8}$)	Q_P (mμmol./mg. dry wt./hr.)
Str. haem. (G); 23 hr.	Saline; or phosphate-saline; or saline with $MgSO_4$ and either adenosinetriphosphate or adenylic acid	325	126–130	−1·21
	Casein hydrolysate and saline	325	122	−1·25
Str. haem. (G); 17 hr.	Saline-bicarbonate, anaerobically	585	325	−1·72
	Saline-bicarbonate, aerobically	585	310	−1·84
	Casein hydrolysate with and without glutathione (10 μmol.), anaerobically	585	291	−1·96
Str. haem. (G); 23 hr.	Saline-bicarbonate with or without tryptophan (1, 10 μmol.)	500	351	−1·24
	Saline-bicarbonate with folic acid concentrate (0·01, 0·1 mg.) and biotin (0·1, 0·5 μg.), with or without tryptophan (1, 10 μmol.)	500	366	−1·15
Str. haem. (G); 5½ hr.	Saline-bicarbonate	281	150	−1·62
Str. haem. (R); 22 hr.	Saline-bicarbonate; or phosphate-saline, $MgSO_4$, and adenosine triphosphate	348	252	−2·25
	Saline-bicarbonate and casein hydrolysate	348	250	−2·3
Str. faecalis; 18 hr.	Saline-bicarbonate with or without $MgSO_4$ and adenosine triphosphate	504	354	−1·96
Str. faecalis; 24 hr.	Saline-bicarbonate	627	273	−1·43
Pr. morganii (N.C.T.C. 2818); 16 hr.	Saline-bicarbonate-glucose with or without casein hydrolysate and yeast preparation	340	206	−6·9
Pr. morganii (laboratory strain 2); 4 hr.	Saline-bicarbonate-glucose	336	269	−1·1
	Saline-bicarbonate-glucose with casein hydrolysate and yeast preparation	336	81	−4·1
Esch. coli; 18 hr.	Saline	6·7	12	+0·26
	Saline-glucose	6·7	75	+2·8
	Saline-glucose with β-alanine (10 μmol.); 0–2 hr.	6·7	310	+30·3
	Saline-glucose with β-alanine (10 μmol.); 2–5 hr.	310	940	+43·5
Ps. aeruginosa (strain 3); 40 hr.	Phosphate-saline	0·1	0·28	+0·04
	Phosphate-saline with lactate (200 μmol.)	0·1	4·0	+0·92
Ps. aeruginosa (strain 3); 48 hr.	Phosphate-saline with lactate (200 μmol.)	0·3	2·5	+0·54
	Phosphate-saline with lactate (200 μmol.) and β-alanine (1 μmol.)	0·3	2·65	+0·57
Ps. aeruginosa (strain 3); 48 hr.	Phosphate-saline with lactate (200 μmol.)	0·3	2·72	+0·47
	Phosphate-saline with lactate (200 μmol.) and pantoic acid (10 μmol.)	0·3	8·90	+1·67

portions of the same medium, one culture being continued for 3 hr. and the other for 5 hr. and the initial and final pantothenate of each determined. The logarithmic plot of optical density readings of specimens taken from the cultures at different times, showed the cultures to grow with negligible lag and with two phases of logarithmic increase. The rate of growth during these phases has been described in Table 2 by their slope, expressed as a mean generation time (m.g.t.; cf. Dagley & Hinshelwood, 1938) or time taken for the readings to double in value. The point of transition from the more rapid to the slower phase was obtained from the logarithmic curve, and this time, together with the

arbitrary time at which growth of the first culture was stopped, divided the bacterial activity into the three parts indicated by different shading in Fig. 2. The shaded areas were measured and the values converted to mg.-hr. by a factor derived from the optical density and dry weight of a measured sample of the second culture. The disappearance of pantothenate from the first culture is thus associated

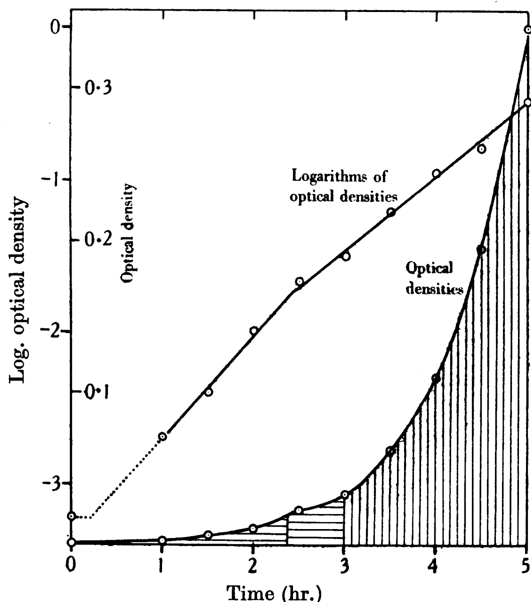

Fig. 2. Growth curve and logarithmic growth curve of the group G streptococcus in the casein-yeast medium, shaken aerobically in a thermostat at 37°. Inocula were added to portions of the warmed medium at a time taken as zero in the figure. Of duplicate cultures, one was removed at 3 hr. for determination of its pantothenate content and the other at 5 hr. Data derived from this figure are given in the asterisked experiment of Table 2; the mg.-hr. of bacterial activity performed in the first culture was proportional to the horizontally shaded area (comprising two portions differing in mean generation time), and the further activity in the second culture, to the vertically shaded area. The mean generation times of the culture were obtained from the slopes of the logarithmic plot; the dotted extrapolation of this indicates the method of computing the length of the lag phase. When this method gave values for the lag phase of <15 min., as in the figure, the existence of this phase was not considered sufficiently well defined to record in Table 2.

partly with growth of m.g.t. 28 min. and partly with that of m.g.t. 38·4 min.; it was small, and was not determined accurately. The further disappearance of pantothenate found to have occurred in the second flask was associated entirely with growth at the slower rate, but with much greater bacterial activity; the greater pantothenate change was determined more accurately.

The rate of pantothenate inactivation during growth is unmistakably of the same order of magnitude as that of non-proliferating suspensions of the organism. A more accurate value for Q_P during the early phase of growth has been obtained in a separate experiment by using a lower initial concentration of pantothenate (Table 2). Further, the Q_P of organisms after growth has been compared with that of the same organisms, during the period of growth immediately preceding their harvesting, with the following result. The group G organism, grown in a casein medium, gave a m.g.t. of 39 min. and Q_P of $-1{\cdot}43$; in a casein medium and without growth, a Q_P of $-1{\cdot}15$ was found. Different determinations of Q_P during growth in different media have given values associated with growth both slower and quicker than that of Fig. 1, but all values for Q_P have fallen into a relatively narrow range. Tables 1 and 2 show the behaviour of the 'Richards' streptococcus to be similar.

Other organisms inactivating pantothenate

The behaviour of a strain of *Str. faecalis* towards pantothenate was closely similar to that of the β-haemolytic streptococci (Tables 1, 2). Values of Q_P during growth fell within the same range and were lower during slower growth. The values in non-proliferating suspensions approximated to those during growth.

Proteus morganii inactivated pantothenate during growth at rates notably greater than those of the preceding organisms. Of two strains, one attained a high rate of glycolysis (Q_{CO_2}, 450–650 μl./mg. dry wt./hr.) and a rate of pantothenate metabolism approximating to that in culture, when metabolizing in a relatively simple medium. Glycolysis of the other strain was slow (Q_{CO_2}, *c.* 150) in a simple medium, and its rate of inactivation of pantothenate was also much lower than in culture. The rates of both processes were increased by addition of the casein and yeast preparations present during growth, and the Q_P then approximated to that found to accompany growth. The addenda did not include all substances used in growth of the organisms, and growth did not occur during the metabolic experiment. In an experiment in which the rate of pantothenate inactivation during growth was compared with that given by the same organisms immediately afterwards, as a non-proliferating suspension, Q_P during growth of m.g.t. 42 min. in casein-marmite-glucose was $-3{\cdot}8$ and of the separated organisms, $-4{\cdot}1$ mμmol./mg. dry wt./hr.

Organisms synthesizing pantothenate

Esch. coli. Growth of the present strain in the medium of glucose and inorganic salts was not obviously affected by addition of 10^{-5} or 10^{-7} M-pantothenate, but this substance was found to be

Table 2. *Rates of pantothenate metabolism during growth of bacteria*

One or more flasks containing 100 ml. of the media indicated were inoculated, and specimens taken at intervals to give growth curves such as that of Fig. 2, from which were obtained the data of the second column of the table. The asterisked experiment is that of Fig. 2. Solution and cells were separated after growth and the pantothenate of each determined. In a few cases in which the pantothenate change was a small fraction of that present, the large possible error is noted.

	Phases of growth			Pantothenate		
Organism; medium of growth	Description (m.g.t. in min.)	Duration (min.)	Bacterial activity (mg.-hr.)	Initial (mμmol.)	Final (mμmol.)	Q_P (mμmol./mg. dry wt./hr.)
Str. haem. (G); casein hydrolysate	log., m.g.t. 45	270	40·0	100	38	−1·55
	log., m.g.t. 62	210				
Str. haem. (G); casein hydrolysate + yeast preparation	lag	90	34	100	20	−2·35
	log., m.g.t. 29	270				
	log., m.g.t. 60	90				
Str. haem. (G)*; casein hydrolysate + yeast preparation	log., m.g.t. 26	144	1·62	94	87	−2·0 (±0·5)
	log., m.g.t. 37	36	1·92			
	log., m.g.t. 37	120	32·5	87	50	−1·14
Str. haem. (G); casein hydrolysate + broth	log., m.g.t. 29	270	4·08	120	108	−3·0 (±0·5)
	log., m.g.t. 29	27	4·7	108	33	−3·35
	log., m.g.t. 57	63	17·7			
Str. haem. (G); casein hydrolysate + yeast preparation	lag	65	2·08	9·0	3·3	−2·7
	log., m.g.t. 24	135				
Str. haem. (R); casein hydrolysate + broth	log., m.g.t. 41	277	6·0	109	96	−2·1 (±0·3)
	log., m.g.t. 87	81				
	log., m.g.t. 87	180	19	96	53	−2·3
Str. faecalis; casein hydrolysate + broth	log., m.g.t. 25	120	10·2	86	58	−2·7
	log., m.g.t. 35	120				
	log., m.g.t. 35	20	19·8	58	26	−1·6
	Growth slowing to m.g.t. 65	40				
Pr. morganii (2818); casein hydrolysate + yeast preparation	log., m.g.t. 25·5	180	6·34	100	62	−6·0
Pr. morganii (2818); casein hydrolysate + yeast preparation	log., m.g.t. 28·5	175	1·64	30	23	−4·3
	log., m.g.t. 42	15				
	log., m.g.t. 42	60	4·5	23	6	−3·8
Esch. coli; glucose-inorganic salts	log., m.g.t. 44	330	16·6	0·05	25·6	+1·54
	log., m.g.t. 56					
	Gradual retardation from m.g.t. 56 to 210	90	38·6	25·6	89·4	+1·65
Esch. coli; glucose-inorganic salts	log., m.g.t. 61	240	0·96	0·2	7·1	+7·0
	log., m.g.t. 61	80	9·8	7·1	22·1	+1·53
Ps. aeruginosa, strain (4); lactate-inorganic salts	lag	85	5·1	0	6·1	+1·20
	log., m.g.t. 37	125				
	log., m.g.t. 37	90	12·9	6·1	25·0	+1·46
Ps. aeruginosa, strain (3); casein hydrolysate	log., m.g.t. 34·5	285	8·5	0	5·9	+0·70
	log., m.g.t. 58	75				
Ps. aeruginosa, strain (3); lactate-inorganic salts	lag	60	1·84	0	11·2	+6·1
	log., m.g.t. 30	180				
	log., m.g.t. 30	62	45·5	11·2	44·5	+0·73
	log., m.g.t. 108	118				

present in the cells and solution after growth in pantothenate-free media. Suspensions of cells which had been washed twice in 0·5% NaCl grew little if at all during 3 hr. in a mixture of glucose and inorganic salts lacking NH_4^+ ions. Under these conditions, pantothenate was produced at a steady rate (Fig. 1) which corresponded to a Q_P of +2 to +3 mμmol./mg. dry wt./hr. Without glucose, pantothenate production was much slower. Under either of these conditions, the organisms were presumably supplying a nitrogenous compound for synthesis of pantothenate; when β-alanine was

added as well as glucose, Q_P was greatly increased, to values of $+30$ or $+40$ mμmol./mg. dry wt./hr.

Values for Q_P during growth of *Esch. coli* in the glucose-inorganic salt medium varied between $+1{\cdot}5$ and $+2$ while the bulk of growth was taking place, and thus approximated to the rate of the reaction caused by non-proliferating suspensions in comparable media. The values were not markedly affected by rate of growth. In several instances, however (and of these Table 2 gives an example), Q_P during the production of the first few mμmol. of pantothenate was found unusually high. This did not represent carry-over of pantothenate from the previous culture used as inoculum; the inoculum was not washed but its pantothenate (in cells and solution) was estimated and included in the value quoted for the initial pantothenate of the experimental culture. The high rate of production could be due to transfer from the previous culture of substances other than pantothenate; Q_P remained below that observed in the presence of β-alanine. The proportion of the total pantothenate which was present in the bacterial cells varied considerably, from 3 to 12%. The quantity per unit weight of cell was also variable, values of 0·07, 0·09, 0·15 and 0·42 mμmol./mg. dry wt. having been obtained with two strains in cultures of varying age. The high initial rate of pantothenate production was not associated with an unusual distribution of the substance between cells and solution.

Pantothenate was also produced by strains of *Esch. coli* in solutions of lactate and inorganic salts, but growth in ammonium lactate media followed a lag of some hours, or required large inocula for prompter growth, which made conditions less suitable for comparisons of the present type than those employing glucose.

Pseudomonas aeruginosa. Results with strains of this organism resembled those with *Esch. coli* but since a lactate medium supported a good growth, the majority of experiments were carried out with lactate as carbon source. Pantothenate production by washed cells in lactate and inorganic salts was approximately proportional to time (Fig. 1) and corresponded in different experiments to Q_P values of $+0{\cdot}5$ to $+1$. The production was very slow in absence of lactate and in its presence was not much affected by β-alanine; pantoic acid however increased it.

During growth of the bulk of the organisms in media of ammonium lactate + inorganic salts, the rates of pantothenate production approximated to those found in non-proliferating suspensions. Thus a value for Q_P of $+0{\cdot}72$ was obtained with suspensions of organisms (strain 4), immediately after growth, which had been associated with Q_P of $+0{\cdot}95$; with strain (3) the corresponding values were $+0{\cdot}70$ and $+0{\cdot}73$. Again, however, the first few mμmol. of pantothenate were produced at a greater rate.

DISCUSSION

The commonest method of expressing relationships between micro-organisms and vitamin-like substances which they need, preformed, for their growth, has been to state a minimum quantity or concentration of the substance which is found to be needed for good or optimum growth; this may be amplified by determining the quantity of organisms produced with varying quantity of the growth essential. In the case of β-haemolytic streptococci the smallest concentration of pantothenate for optimal growth is about 10^{-7} M. In artificial media such as those of the present investigation, pantothenate concentrations between 10^{-7} and 10^{-5} M or more have little influence on the mass of bacterial growth (see, for example, McIlwain, 1944) and the mass obtained is about that produced in tissue fluids or ordinary bacteriological media. These are usually 10^{-6} to 10^{-5} M with respect to pantothenate, and the reaction of the bacteria to concentrations of pantothenate of this order of magnitude therefore reflects more closely their normal relationship to pantothenate, than does their behaviour to limiting quantities of the substance.

It has been shown above that the interaction between haemolytic streptococci and 10^{-6} to 10^{-5} M-pantothenate is a much more vigorous one than would be anticipated from their minimal requirements for the substance: the organisms inactivated in 1 hr. some 10–25 times the pantothenate with which they could be grown, and some 50–100 times the pantothenate present in their cells. The inactivation was independent of most media constituents but required glucose, a substance present in the normal environments of the organism in a host or a laboratory medium. The inactivation was found also to be to a large extent independent of growth; in comparing different phases in the history of a culture, that of most rapid growth tended to be associated with most rapid inactivation of pantothenate, but the inactivation remained at a relatively high level in the absence of any growth. This finding of a large turnover or usage of pantothenate which could not be assessed by determining its quantity in the total bacterial mass at different times, nor by finding the minimal quantity of pantothenate necessary for growth, emphasizes the dynamic aspect of the interaction of organism and vitamin. It has been shown that a similar relationship holds between streptococci and glutamine which they require, preformed, for growth (McIlwain, 1944); data given incidentally and without comment by Thompson (1942, Tables X, XII) suggest that during growth under certain conditions *Aero-*

bacter aerogenes also inactivates considerable quantities of pantothenate. Lwoff & Lwoff (1937) and Morel (1941) concluded that coenzymes I and II were inactivated by *Haemophilus influenzae* and *Proteus vulgaris* during reactions which occurred in growth, and also independently of it; the coenzymes functioned as growth factors for the organisms, though they could be replaced by simpler substances. This type of relationship between micro-organism and vitamin-like substance is thus fairly common. With respect to its origin, the possibility that pantothenate inactivation occurred in resting cells through restraint of their growth while they were in the presence of excess of a single growth factor has been disproved by the present investigation.

Relationships between vitamin-like substances and bacteria of the intestinal flora of animals have been the subject of many studies but have been considered largely in terms of the possible synthesis of vitamins by such organisms. An exception was the observation of Benesch (1945) that nicotinamide could be destroyed by mixed caecal flora under conditions in which *Clostridium welchii* was the predominant organism. The further potentialities of intestinal organisms in this connexion are illustrated by the present findings with *Streptococcus faecalis* and *Proteus morganii*.

Synthesis of vitamin-like substances by micro-organisms has also usually been expressed in terms of the quantities finally found in cultures rather than in terms of the specific activity of the organisms in producing the substances, such as is provided by metabolic coefficients. The latter method has now enabled comparisons to be made between the synthesis of pantothenate by some organisms and its inactivation by others, and also the detection of the unusual activity of *Esch. coli* and *Ps. aeruginosa* towards pantothenate, in the early phases of cultures. Data given by Thompson (1942, Table XI and Fig. 2) for the production of biotin by *Proteus vulgaris* can in part be treated in this manner, and calculation shows the rates of biotin production during the first two periods of 4 hr., when the m.g.t. was approximately 90 min., to be roughly constant (but of unknown absolute value), with a considerable fall in m.g.t. and rate of production of biotin after that period.

Comparison of pantothenate synthesis by proliferating and non-proliferating organisms can be expected on theoretical grounds to be less straightforward than comparison of the inactivation of the substance under the two conditions. Thus the substrates for the synthesis are not known and the overall pantothenate exchange which is measured may well be a balance between pantothenate production and inactivation. Nevertheless, the values of Q_P observed during production of the bulk of the pantothenate in culture were paralleled by the values obtained in non-growing suspensions of organisms, containing as added compounds only glucose or lactate, and inorganic salts. The organisms, or associated materials, were then supplying at least a nitrogenous compound. The higher values for Q_P in earlier phases of growth were approached or exceeded by the addition of compounds which might be expected to be, or to be related to, the substrates of the synthesis, namely, β-alanine and pantoic acid. Evidence that these substances may be precursors of pantothenate in micro-organisms has previously been given by their replacing pantothenate in the growth of certain organisms requiring pantothenate or such a surrogate for optimal growth. The present observations suggest that the substances may be related also to pantothenate production in *Esch. coli* and *Ps. aeruginosa*, whose growth is independent of added pantothenate.

SUMMARY

1. (*a*) Inactivation and synthesis of pantothenate by non-proliferating suspensions of various bacteria proceeded with definite velocities which could satisfactorily be expressed as metabolic quotients, that adopted, Q_P, being mμmol. change in pantothenate/mg. dry wt./hr.

(*b*) Q_P, during the inactivation of pantothenate by haemolytic streptococci which accompanied their glycolysis, was $-1\cdot2$ to $-2\cdot5$; corresponding values with *Str. faecalis* were $-1\cdot5$ to -2 and with *Proteus morganii*, -4 to -7. The values were relatively little affected by the addition of various vitamin-like substances or other materials present in growth-media.

(*c*) Production of pantothenate by *Esch. coli* suspended in solutions of inorganic salts was slow (Q_P, $+0\cdot3$), but increased by addition of glucose (Q_P, $+1$ to $+3$) and still further (Q_P, $+30$ to 40) by β-alanine. *Pseudomonas aeruginosa* also gave low values in salt solutions, increased by lactate and by pantoic acid.

2. (*a*) These activities of non-proliferating organisms were compared with the organisms' metabolism of pantothenate during growth. Values for Q_P in this case were calculated from (i) the mg.-hr. of bacterial activity (obtained from growth curves) during different phases of a culture's growth and (ii) the associated changes in pantothenate content of the cultures.

(*b*) Rates of inactivation of pantothenate by the streptococci and by *Pr. morganii* were largely independent of phase of growth, and were close to the values observed in non-proliferating suspensions of the corresponding organisms.

(*c*) Production of pantothenate by *Ps. aeruginosa* and *Esch. coli* was rapid during a short initial phase; during the greater part of the culture's growth, Q_P

values approximated to those observed in non-proliferating suspensions of the organisms in the presence of the lactic acid or glucose present during growth.

3. Pantothenate inactivation by non-proliferating bacteria is thus not induced by an imbalance in metabolism resulting from the presence of a growth factor without opportunities for growth. Pantothenate synthesis may proceed from β-alanine or pantoic acid.

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Pyruvate Oxidation in Pigeon Brain Catalyzed by Fumarate

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Small amounts of C_4-dicarboxylic acids, when added to finely minced or ground animal tissues, respiring *in vitro*, are well known to stimulate the O_2 uptake; the effect was first noted by Szent-Györgyi and his co-workers, using pigeon breast muscle (Gözsy & Szent-Györgyi, 1934; Annau, Banga, Gözsy, Huszak, Laki, Straub & Szent-Györgyi, 1935). It was found to be a characteristic of these acids that, unlike true substrates, they preserved the respiration rate of the tissue instead of raising it above its initial level. Stare & Baumann (1937) showed that the effect was truly catalytic; using pigeon breast muscle, they found that the increased O_2 consumption was greater than could be accounted for by the complete oxidation of the added C_4-dicarboxylic acid. Annau, Banga, Blazso, Brukner, Laki, Straub & Szent-Györgyi (1936) and Annau & Erdös (1939), using rabbit liver and pig kidney, respectively, have demonstrated that catalysis by succinate and fumarate is markedly greater when pyruvate is being oxidized. Other investigations (Banga, Ochoa & Peters, 1939; Krebs & Eggleston, 1940; Smyth, 1940) have shown fumarate catalysis to be active in other tissues and have confirmed its relation to the pyruvate oxidation system.

Although the stimulation by fumarate of the O_2 uptake of tissues respiring in a medium containing pyruvate is now well recognized, it is suprising to find lack of agreement as to whether the rate of pyruvate utilization is also increased. Unfortunately, most determinations of pyruvate disappearance have been made in the presence of malonate and the literature contains few observations in the absence of this inhibitor. In those cases where malonate has been excluded and the rate of pyruvate disappearance estimated in the presence and absence of fumarate, the observations are conflicting. Thus in pigeon brain, Banga *et al.* (1939), in two experiments quoted, found an increased pyruvate utilization in the presence of fumarate. On the other hand, Annau *et al.* (1936), using rabbit liver, consistently found a decreased rate of disappearance of pyruvate when fumarate was present, while Krebs & Eggleston (1940), working with pigeon breast muscle, reported an increased disappearance in one case and a decreased disappearance in another.

The present investigation is a continuation of previous work (Long, 1943) in which the behaviour of a dialyzed preparation of ground pigeon brain, respiring in a medium containing pyruvate, inorganic phosphate and adenine nucleotide, was quantitatively investigated. With this enzyme system, which is essentially the same as that of Banga *et al.* (1939), the effect of fumarate on the oxidation of pyruvate has now been re-examined. The increased O_2 uptake due to fumarate catalysis, first reported by the last mentioned authors, has been amply confirmed, but their further claim to have observed an increased rate of disappearance of pyruvate could not be supported. Indeed, a slightly decreased rate of pyruvate disappearance has always been found,