varies with different amino acids. In all cases studied, inhibition ceases at a plateau value which again varies with the individual amino acid concerned.

We are indebted to Dr B. A. Newton for taking the electronmicrographs shown in Pl. 1.

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The Assimilation of Amino Acids by Bacteria

21. THE EFFECT OF NUCLEIC ACIDS ON THE DEVELOPMENT OF CERTAIN ENZYMIC ACTIVITIES IN DISRUPTED STAPHYLOCOCCAL CELLS

BY E. F. GALE AND JOAN P. FOLKES
Medical Research Council Unit for Chemical Microbiology, Department of Biochemistry, University of Cambridge

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The rate of protein synthesis has been associated with the nucleic acid content of cells in a number of studies (Brachet, 1941; Caspersson, 1941, 1947; Jeener & Brachet, 1944; Malmgren & Heden, 1947; Caldwell, Mackor & Hinshelwood, 1950; Mitchell & Moyle, 1951; Jeener, 1952*a*, *b*; Jeener & Jeener, 1952; Northrop, 1952; Price, 1952; Wade, 1952; Gale & Folkes, 1953*a*), and a number of authors have suggested that nucleic acid plays a role, possibly as a template, in the synthesis of protein (Haurowitz, 1950; Caldwell & Hinshelwood, 1950; Dounce, 1952; Gale, 1953, 1955; Rich & Watson, 1954). In the present paper a direct effect of nucleic acids on the 'development' of certain enzyme systems in disrupted staphylococcal cells is shown.

In washed suspensions of intact *Staphylococcus aureus*, protein synthesis will occur only if a complete mixture of the amino acids essential for growth is provided; the rate of synthesis can be markedly stimulated by the addition of a mixture of purines and pyrimidines to the medium and is directly proportional to the nucleic acid content of the cells (Gale & Folkes, 1953*a*). If a single amino acid is added to the incubation medium, then no synthesis of protein can be demonstrated, but accumulation of the free amino acid takes place within the cell; if the amino acid is labelled with ^{14}C, then incorporation of the label into the protein of the cell can be shown whether protein synthesis is demonstrable or not (Gale & Folkes, 1953*c*). In the previous paper of this series (Gale & Folkes, 1955) it has been shown that incorporation of [^{14}C]glutamic acid into the protein of disrupted staphylococcal cells is dependent upon, and activated by, nucleic acid derived from staphylococci. It was also shown that the rate of incorporation of a single labelled amino acid cannot be taken as a measure of protein synthesis and that, even under conditions in which protein synthesis is occurring, the effect of nucleic acids upon incorporation varies with the particular amino acid whose incorporation is being measured. Consequently, such studies are not suitable for the investigation of protein synthesis as such, and the

present communication describes the effect of nucleic acids on the development of enzyme activities in disrupted cells.

Of the enzymes whose formation is studied below, β-galactosidase is an induceable enzyme whose formation in *Escherichia coli* has been the subject of detailed investigation by Monod & Cohn (see reviews by Monod & Cohn, 1952, 1953). The enzyme in intact *Staph. aureus* differs somewhat in properties from that in *Esch. coli* and has been studied by Creaser (1955), who finds that galactose is the most efficient inducer of β-galactosidase formation in staphylococci.

METHODS

Preparation of nucleic acid-depleted disrupted cells. Disrupted cell preparations were made as described in the previous paper (Gale & Folkes, 1955). Nucleic acid was extracted from the preparations by incubation for 1 hr. at 37° with M-NaCl; in the previous paper it was shown that the degree of depletion of nucleic acid by this treatment was largely controlled by the time of exposure to supersonic vibration and three types of preparation, corresponding to stages *B*, *C* and *D* of Table 1 (Gale & Folkes, 1955) have been used. In general, protein synthesis will occur in all three types of preparation but, whereas it is unaffected by nucleic acid at stage *B*, it becomes increasingly dependent upon nucleic acid at stages *C* and *D*, as shown below.

Incubation mixtures. In general, the conditions of incubation corresponded to condition 2 of the previous paper with amounts and concentrations of ATP, HDP, amino acid mixture A and nucleic acids as there described. Cozymase solution (0·1 ml.) containing 100 μg. DPN was added to the incubation mixture in all experiments. In some cases, nucleic acid has been replaced by 0·1 ml. of a purine–pyrimidine mixture (PP) containing 1·0 mg. of each of the following/ml.: adenine, guanine, hypoxanthine, uracil, xanthine, thymine.

Development of enzymes. In general, disrupted cell preparations were incubated for 90–120 min. at 37° in the incubation mixture in 15 ml. centrifuge tubes. The disrupted cells were then centrifuged down for 15 min. at 4000 *g*, washed once in buffered saline and suspended in water at a concentration suitable for estimation of enzyme activity.

Estimation of 'glucozymase' activity. Warburg manometers were set up containing 1·0 ml. 0·03 M-$NaHCO_3$ in the main cup and 0·5 ml. 1% (w/v) glucose in the side bulb; the disrupted cell preparation (1·5 ml.) containing approx. 3–4 mg. dry wt. of preparation was added to the main cup and the manometers filled with N_2 containing 5% CO_2 before equilibration at 37°. After the glucose was mixed with the contents of the main cup, the evolution of gas was studied for 60 min. The 'glucozymase' activity is expressed as μl. CO_2 evolved from glucose in this system (=acid production)/hr./mg. dry wt. of preparation.

Estimation of catalase activity. Warburg manometers were set up containing 1·0 ml. 0·05 M phosphate buffer pH 6·2 in the main cup and 0·5 ml. '9 volume' hydrogen peroxide solution in the side bulb; the test preparation (1·0 ml.) containing approx. 0·005 mg. disrupted cells was added to the main cup. The manometers were equilibrated in a bath at room temp., the peroxide added to the main cup and readings taken every 2 min. for 10 min. The gas evolution was generally linear from 2–8 min. after tipping in the substrate and the catalase activity calculated from this linear evolution. When the activity of the centrifuged and washed preparation was compared with that of an equivalent amount in the incubation medium, it was found that a loss of 10–15% occurred during the washing procedure; consequently a sample of incubation mixture was diluted with water until 1·0 ml. suspension contained 0·005 mg. disrupted cells, and activity was estimated directly on this diluted suspension. The presence of traces of components of the incubation mixture had no effect on catalase activity during estimation, and this procedure was therefore adopted for most of the experiments recorded.

Estimation of β-galactosidase activity. Ledeberg (1950) showed that β-galactosidase activity can be estimated readily by the action of the enzyme in liberating *o*-nitrophenol from *o*-nitrophenyl β-galactoside (NPG). This method, as modified by Creaser (1955), has been used in the present studies. For each estimation the following reagents are put into 15 ml. centrifuge tubes: 2·0 ml. 0·2 M phosphate buffer pH 7·5, 1·0 ml. cysteine (10 mg./ml.) and 1·0 ml. *o*-nitrophenyl β-galactoside (10 mg./ml.). After equilibration at 37°, 1·0 ml. of disrupted cell preparation (approx. 1 mg. dry wt.) is added and incubation continued for 30 min. The tubes are then cooled in ice, the preparation centrifuged down and the colour developed estimated at 420 mμ. in a Beckman spectrophotometer. The colour developed is standardized against nitrophenol, and β-galactosidase activity expressed as μmoles NPG hydrolysed/hr./mg. dry wt. of preparation.

Incorporation of [^{14}C]*uracil.* In certain experiments, the mixture PP was replaced by an equivalent purine–pyrimidine mixture containing [^{14}C]uracil. The radioactive uracil was worked up from residues of *Chlorella* grown in $^{14}CO_2$ and supplied by the Radiochemical Centre, Amersham. After incubation, the disrupted cell preparation was precipitated with 5% trichloroacetic acid (TCA) in the cold, left in the ice-chest for 2 hr., centrifuged down, washed with cold TCA and acetic acid as described by Gale & Folkes (1953*b*) and the dried preparation counted. Extraction of the precipitate with 5% TCA at 90° for 15 min. removed 98% of the radioactivity.

Expression of activities. In the majority of the experiments described, an increase in the protein content of the cell preparation occurs in the course of the incubation. Enzymic activities are expressed in terms of the initial dry weight of preparation used.

RESULTS

Development of 'glucozymase' activity

Disrupted cell preparations retain the ability to produce acid from glucose; preparations corresponding to stages *B*, *C* and *D* have 'glucozymase' activities ($Q_{CO_2}^{acid}$) of the order of 60, 30 and 15 respectively. These activities can be increased to 100–140 by incubation for 90–120 min. under appropriate conditions. The activity of stage *B* preparations can be developed by incubation with amino acid mixture A and the addition of nucleic acids has little or no effect; at stages *C* and *D* the addition of nucleic acids markedly accelerates the

development. Fig. 1 summarizes the conditions affecting the development in a typical stage *D* preparation and shows that stimulation of 'glucozymase' formation is given by the addition of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) or PP. Of the three additions, PP is the most effective and the further addition of RNA or DNA does not increase its stimulative effect. Whether PP or nucleic acids are present or not, the development of activity is abolished by omission of the amino acid mixture or by addition of chloramphenicol at a concentration (30 μg./ml.), which inhibits protein synthesis (Gale & Folkes, 1953*b*).

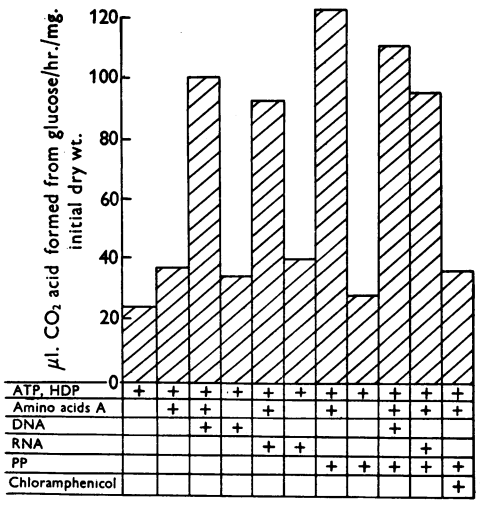

Fig. 1. Development of 'glucozymase' activity in disrupted cell preparations. Disrupted cell preparation: fraction 2, stage *D* extracted with M-NaCl, incubated at suspension density 0·4 mg. dry wt./ml. in buffered salt solution pH 6·25 with additions as shown for 90 min. at 37°; preparation then centrifuged down, washed once and 'glucozymase' activity estimated. Concentrations during incubation: ATP, 2 μmoles; HDP, 18 μmoles; DNA, 0·4 mg.; RNA, 0·4 mg.; PP, 100 μg. each component; chloramphenicol, 105 μg./3·5 ml. CO_2 acid = CO_2 liberated from bicarbonate buffer by acid produced in the fermentation.

Fig. 2 shows the effect of concentration of RNA or DNA on 'glucozymase' development under these conditions; the concentrations giving maximal effect are similar to those required for maximal activation of the incorporation of [^{14}C]glutamic acid in disrupted cell preparations (Gale & Folkes, 1955). Fig. 3 shows that the development is approximately linear for 2 hr. although the stimulation produced by PP and RNA decreases with time.

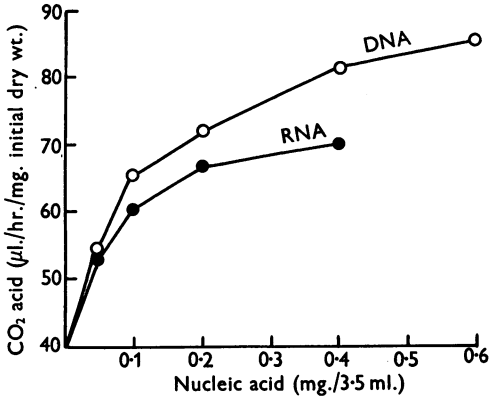

Fig. 2. Effect of concentration of nucleic acid on development of 'glucozymase'. Conditions as for Fig. 1 with nucleic acid concentrations as shown, ATP, HDP and amino acid mixture A present in all cases.

Development of catalase activity

The conditions controlling the development of catalase activity in disrupted cell preparations again differ according to the degree of nucleic acid depletion of the preparations. At stage *B* the catalase activity can be doubled by incubation for 90 min. with amino acid mixture A, and nucleic acids have little or no effect. Fig. 4 shows the effects obtained at stages *C* and *D*; at both stages there is little or no increase in activity as a result of incubation with amino acids alone and, in contrast with 'glucozymase' development, the addition of PP has no stimulatory effect although, in some experiments, it has been found to have a small inhibitory effect. At stage *C* catalase development is markedly stimulated by RNA, while DNA has little effect either alone or in the presence of RNA;

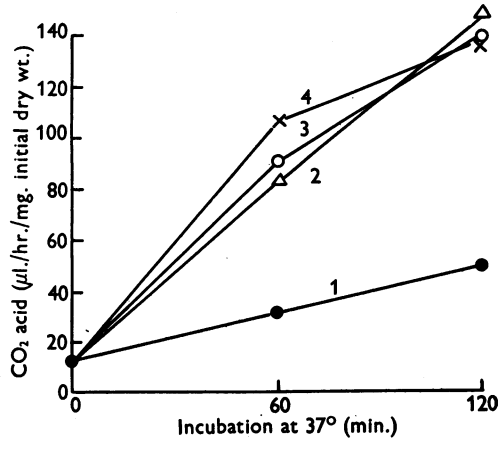

Fig. 3. Course of 'glucozymase' development in disrupted cells. Conditions as for Fig. 2 with nucleic acid additions as: 1, no addition; 2, 0·2 mg. DNA/3·5 ml.; 3, 0·4 mg. RNA/3·5 ml.; 4, PP mixture, 100 μg. each component/3·5 ml.

Fig. 5 shows the time course of the development under these conditions, and it can be seen that whereas the stimulation by RNA is constant throughout the 3 hr. incubation, DNA has no marked effect until the third hour of incubation, by which time many alterations may have occurred in the basic system. PP is found to reduce the effect of RNA but Fig. 4 shows that PP + DNA are more effective than either alone; on two out of eleven tests under these conditions, PP + DNA proved more effective than RNA alone. Omission of the amino acid mixture A or addition of 30 μg. chloramphenicol/ml. to the incubation mixture abolishes the increase in catalase activity under any condition tested.

As the nucleic acid content of the disrupted cell preparation decreases, RNA becomes less effective in stimulating catalase development. Fig. 4 shows that, at stage *D*, DNA shows a significant stimulation, greater than that of RNA, while RNA and DNA together show an increased, possibly additive, effect. At this stage, concentrations of added nucleic acid become critical and it is necessary to test effects over a range of nucleic acid concentrations; Fig. 4 shows optimum effects in each case, while Fig. 6 shows the variation in these effects with nucleic acid concentration. The optimum concentration of DNA appears to be critical and varies between 0·01 and 0·06 mg./ml. with different preparations; in the particular experiment shown in Fig. 6, 0·17 mg. dry wt. of disrupted cells/ml. was best activated by 0·03 mg. DNA/ml. while 2–3 times that concentration of DNA was without effect. Low concentrations of DNA (0·02–0·04 mg./ml.) increase the stimulation due to RNA, although the

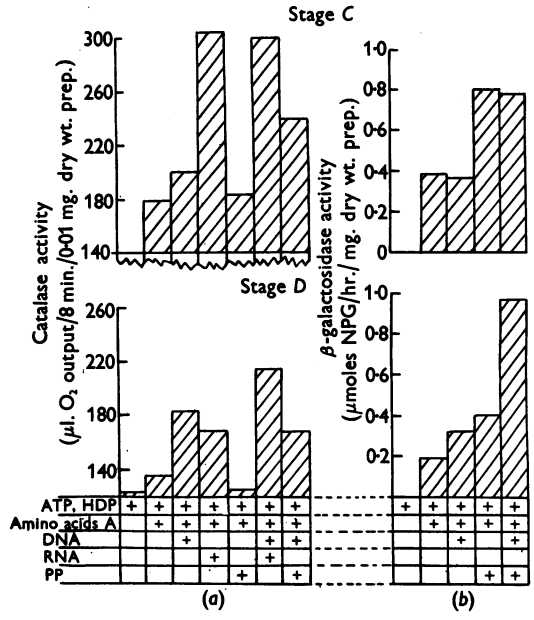

Fig. 4. Development of (*a*) catalase and (*b*) β-galactosidase activity in disrupted cell preparations at progressive stages of nucleic acid depletion. Conditions as for Fig. 1 except that DNA concentration was 0·05 mg./3·5 ml. and medium for β-galactosidase development contained 2% (w/v) galactose. Initial dry wt. of preparation/3·5 ml.: stage *C*, 0·65 mg. for catalase, 1·2 mg. for β-galactosidase; stage *D*, 0·4 mg. in both cases. Disrupted cell preparations extracted with M-NaCl before incubation.

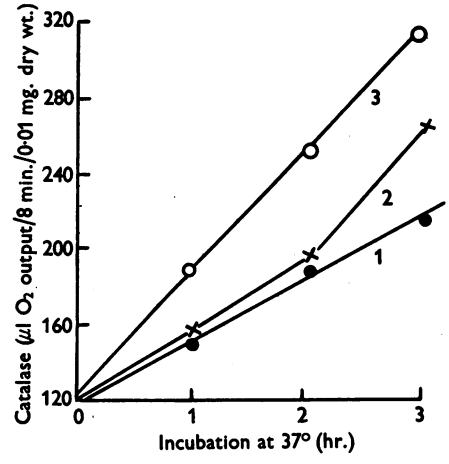

Fig. 5. Time course of catalase development in disrupted cells. Conditions as for Fig. 4 with stage *C* preparation extracted with M-NaCl. Incubation medium contained ATP, HDP and amino acid mixture A in all cases, with following additions: curve 1, none; 2, 0·05 mg. DNA/3·5 ml.; 3, 0·4 mg. RNA/3·5 ml. Dry wt. of preparation used, 0·63 mg./3·5 ml.

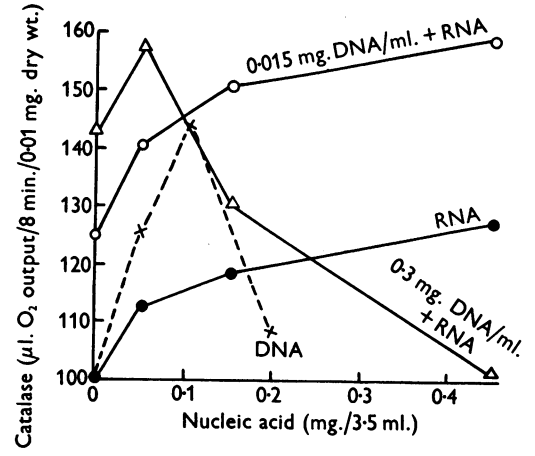

Fig. 6. Effect of nucleic acids on development of catalase in disrupted cell preparations highly depleted of nucleic acid. Preparation: disrupted cells, fraction 2 stage *D*, extracted with M-NaCl, incubated at 0·6 mg./3·5 ml.; conditions as for Fig. 4 with nucleic acid additions as shown.

action of RNA and DNA together is almost additive, 0·015 mg. DNA/ml. + 0·15 mg. RNA/ml. being 26 % more effective together than the sum of their separate effects. In the presence of higher concentrations of DNA, RNA + DNA become less effective than RNA alone. At stage *D*, the response of the preparation to either RNA or DNA is abolished by the presence of either ribonuclease or deoxyribonuclease (10–30 μg./ml.) in the incubation mixture. At stage *C*, however, the response to RNA is reduced by 30–40 % but not abolished by the presence of ribonuclease, and a mild alkali digest of RNA is as effective as RNA itself. Ox liver RNA was without effect on catalase development, while RNA prepared from another strain of *Staph. aureus* had less than 10 % of the activity of RNA from strain Duncan used for the preparation of the disrupted cells.

Development of β-galactosidase

Disrupted cell preparations made from cells grown in the usual medium in the absence of galactose, lactose or other inducer have no β-galactosidase activity. Incubation with amino acid mixture A with or without nucleic acids or PP leads to no development of activity unless an inducer is added to the incubation mixture. The most effective inducer of β-galactosidase activity in intact *Staph. aureus* is galactose (Creaser, 1955) and incubation of stage *B* disrupted cell preparations with amino acid mixture A and galactose leads to development of the enzyme. Fig. 7 shows that the concentration of galactose required for maximal enzyme development is high and a final concentration of 2 % has been chosen for later experiments.

As the nucleic acid depletion of the disrupted cells increases, the development of β-galactosidase in response to amino acids alone decreases. The addition of neither RNA nor DNA has any effect on stage *C* preparations but Fig. 4 shows that the addition of PP markedly stimulates development. RNA has been prepared from cells grown in galactose but this is also without effect on the development in the presence of amino acid mixture A; in the presence of amino acid mixture A and PP a high concentration of the 'adapted' RNA (0·3 mg./ml.) has increased the effect of PP on three occasions (once by 75 %) and been without effect on nine occasions. Fig. 8 shows the time course of β-galactosidase development at this stage; in the absence of added PP, the development shows a lag period of 20–30 min. and the addition of PP shortens the lag period and increases the rate of development. Once the lag period is over, enzyme development continues at a steady rate for up to 3 hr. incubation. The concentration of PP is not critical, the lag period is almost abolished by addition of PP at a final concentration of 100 μg. each component/3·5 ml. but the rate of enzyme development is significantly the same if the mixture is reduced to a concentration of 1 μg. each component/3·5 ml. Omission of the amino acid mixture or addition of 30 μg. chloramphenicol/ml. to the incubation mixture abolishes development of the enzyme.

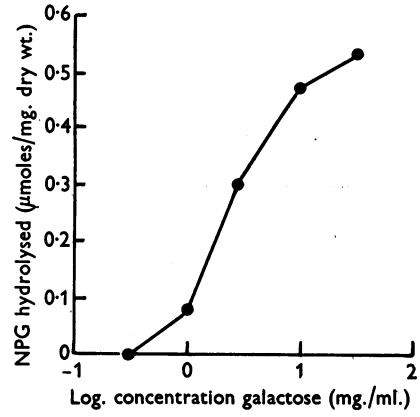

Fig. 7. Effect of galactose concentration on development of β-galactosidase in disrupted cell preparations. Disrupted cell preparation: stage *C* extracted with M-NaCl, incubated at 1·2 mg. dry wt./3·5 ml. for 90 min. at 37° in presence of ATP, HDP, amino acid mixture A and purine–pyrimidine mixture PP (100 μg. each component/3·5 ml.) and galactose as shown; preparation centrifuged down and washed before determination of β-galactosidase activity.

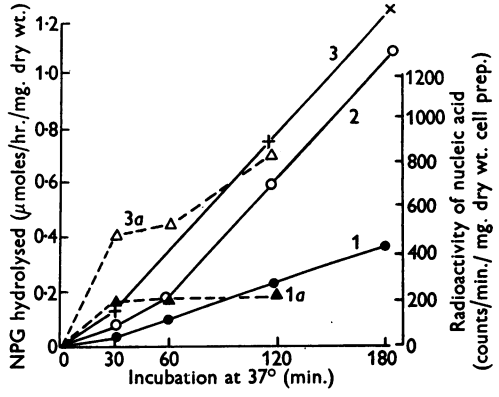

Fig. 8. Course of β-galactosidase development and [^{14}C]-uracil incorporation in disrupted cell preparations. Disrupted cell preparation: stage *C* extracted with M-NaCl, incubated at 0·7 mg. dry wt./3·5 ml. in presence of purine–pyrimidine mixture (PP) as follows: curves 1, 1*a*, no PP; curve 2, PP at concentration of 1 μg. each component/3·5 ml.; curves 3 and 3*a*, PP at concentration of 100 μg. each component/3·5 ml. Continuous lines, β-galactosidase activity; broken lines, radioactivity of nucleic acid in cell preparations from parallel experiment in which PP mixture contained [^{14}C]uracil (specific activity 0·7 mc/m-mole) at 0·1 μmole/ml.

Table 1. *Effect of DNA on development of β-galactosidase by disrupted cell preparations*

Disrupted cell preparations, fraction 2, incubated for 2 hr. at 37° in buffered salt pH 6·25 containing 2% (w/v) galactose and amino acid mixture A either alone or with PP at final conc. 100 μg. each component/ml. DNA added at final conc. 0·012 mg./ml. DNA (gal), preparation from cells grown in galactose; DNA(glu), preparation from cells grown in glucose. At end of incubation, cells centrifuged down, washed once and β-galactosidase activity determined as μmoles NPG hydrolysed/hr./mg. dry wt. of disrupted cell preparation.

Disrupted cell preparation	DNA added	β-Galactosidase developed after incubation with: Amino acid mixture A	β-Galactosidase developed after incubation with: Amino acid mixture A + purine–pyrimidine mixture (PP)
Stage *C*, extracted with M-NaCl	None	0·48	1·00
	DNA(glu)	0·38	0·85
Stage *D*, extracted with M-NaCl	None	0·18	0·44
	DNA(glu)	0·29	1·07
Stage *D*, incubated 1 hr. with 30 μg. deoxyribonuclease/ml.	None	0·02	0·07
	DNA(glu)	0·24	—
	DNA(gal)	0·58	0·78

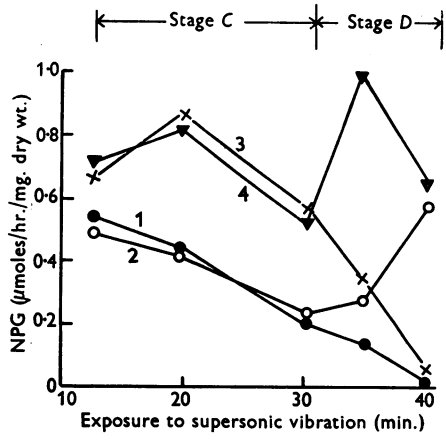

Fig. 9. Effect of duration of exposure to supersonic vibration on the conditions affecting development of β-galactosidase in disrupted cell preparations. Disrupted cell preparations, fraction 2, at each stage extracted with M-NaCl before incubation for 2 hr. at 37° in the presence of ATP, HDP, amino acid mixture A, galactose and additions as follows: curve 1, no addition; curve 2, 0·05 mg. DNA/3·5 ml.; curve 3, PP mixture to concentration of 100 μg. each component/3·5 ml.; curve 4, DNA + PP as in (2) and (3).

The response of stage *D* disrupted cell preparations to amino acids and PP is much less than for stage *C* preparations. Fig. 4 shows that DNA has a significant stimulation at stage *D* and that DNA + PP are markedly more effective together than either alone. At this stage all responses, whether to DNA or PP, are abolished by addition of either ribonuclease or deoxyribonuclease (10–30 μg./ml.) to the incubation medium. Table 1 shows that treatment of stage *D* preparations with deoxyribonuclease before incubation abolishes subsequent response to amino acid mixture A with or without PP but that these responses can be restored to normal by addition of 0·012 mg. DNA/ml., DNA prepared from cells grown in galactose being 2–3 times more effective than DNA from glucose-grown cells.

Fig. 9 shows how the factors controlling the development of β-galactosidase in disrupted cell preparations change with the period for which the preparations were exposed to supersonic vibration. It has been shown previously that the nucleic acid content of the preparations decreases with the length of exposure (Gale & Folkes, 1955). The development of enzyme in response to incubation with amino acid mixture A decreases with length of exposure and although the response is increased by the further addition of PP at all stages, the degree of PP stimulation also decreases with exposure time. In the series shown in Fig. 9 DNA has no significant effect in preparations made by exposure of 30 min. or less but for longer exposures (giving stage *D* preparations) a marked response to DNA is obtained. At 40 min. exposure time, the preparations are little affected by PP whether in the presence or absence of DNA and it is probable that preparations at this stage have lost the ability to carry out the necessary metabolism of purines and pyrimidines. At the penultimate stage, given by 35 min. exposure, DNA markedly increases the effect of PP in the presence of amino acid mixture A. In this particular series 40 min. was the longest exposure practicable as complete disintegration occurred shortly after this time.

Other systems. The disrupted cell preparations do not possess many enzyme systems sufficiently active for the type of studies described above. Other systems tested but proving unsuitable have included galactozymase, invertase, arginine di-

hydrolase, lactic dehydrogenase, pyrophosphatase and nitratase.

Changes in protein-nitrogen. Disrupted cell preparations at stage *D* have been incubated for 90 min. under the usual conditions with (*a*) amino acid mixture A alone, (*b*) *A* + 0·1 mg. RNA/ml., (*c*) A + 0·1 mg. DNA/ml., and (*d*) A + PP, and the protein-nitrogen content determined before and after incubation. Increases obtained in a typical case were (*a*) 2, (*b*) 31, (*c*) 21 and (*d*) 20 %.

Actions of antibiotics

Chloramphenicol. The development of the three enzyme systems studied above is inhibited by chloramphenicol; 30 μg./ml. gives complete inhibition and 10 μg./ml. gives 95–98 % inhibition in all cases. The action is thus strictly comparable with the action previously described (Gale & Folkes, 1953*b*) for the inhibition of net protein synthesis in intact *Staph. aureus*.

Penicillin and bacitracin. These antibiotics appear to be very similar in their inhibitory activities and both effect an inhibition of the incorporation of specific amino acids into the protein fraction of either intact or disrupted cells of *Staph. aureus* (Gale & Folkes, 1953*b*, 1955). Fig. 10 shows the action of bacitracin and penicillin on the development of β-galactosidase and catalase in disrupted cells as described above; β-galactosidase formation is inhibited by 10 μg. of either antibiotic/ml., while catalase formation is less than 30 % inhibited by 300 μg. antibiotic/ml. The inhibitions have been studied with stage *C* preparations of disrupted cells and there is no significant difference in the percentage inhibition by penicillin or bacitracin whether the β-galactosidase development is in response to amino acid mixture A or to A + PP. A difference is found between bacitracin and penicillin in that, whereas inhibition by the former reaches 100 % at 100–300 μg./ml., inhibition by the latter reaches a plateau value at less than 100 %. In Fig. 10 the plateau inhibition by penicillin is 80 % but it has been found that the position of the plateau value varies from preparation to preparation between limits of 60 and 85 % inhibition. A similar situation is found in the effect of penicillin on amino acid incorporation.

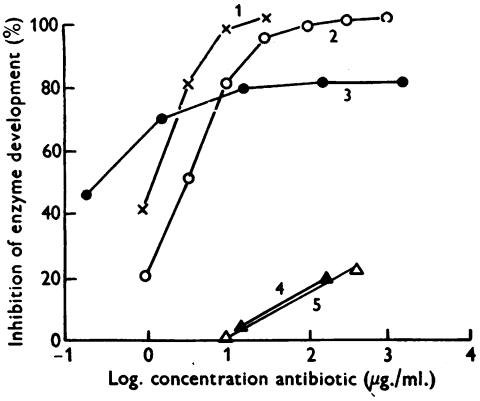

Fig. 10. Effect of antibiotics on development of catalase and β-galactosidase in disrupted cell preparations. Disrupted cell preparations used at stage *C* extracted with M-NaCl and incubated for 90 min. under conditions optimum for enzyme development (catalase with and without RNA; β-galactosidase with and without PP) with and without antibiotics as shown; inhibition expressed on basis that increase in enzyme activity in absence of antibiotic = 100. Inhibitions proved independent of presence or absence of RNA or PP. 1, chloramphenicol inhibition of catalase or β-galactosidase development; 2, bacitracin inhibition of β-galactosidase development; 3, penicillin inhibition of β-galactosidase development; 4 and 5, penicillin and bacitracin inhibition of catalase development.

Creaser (1955) finds that β-galactosidase formation in intact *Staph. aureus* is similarly sensitive to penicillin but Hahn & Wisseman (1951) report that lactase formation in *Esch. coli* is unaffected by penicillin at 50 times the growth-inhibitory level, although inhibited by the growth-inhibitory level of chloramphenicol. The activity, as opposed to the development, of the enzymes studied is unaffected by penicillin, bacitracin or chloramphenicol in the concentrations used.

[^{14}C]*Uracil incorporation*

The effect of PP on the development of 'glucozymase' and β-galactosidase might be due to formation of nucleic acid from the mixture in the presence of amino acids, as occurs with intact *Staph. aureus* (Gale & Folkes, 1953*a*). To test this possibility [^{14}C]uracil has been added to the incubation media and the incorporation of radioactivity into the nucleic acid fraction of the disrupted cells measured. Insufficient [^{14}C]uracil was available for detailed investigations and Table 2 gives results of preliminary studies. Little incorporation took place when the disrupted cells (stage *C*) were incubated with ATP, HDP, amino acids and [^{14}C]uracil alone, but addition to the incubation medium of either PP or galactose increased the incorporation, while the presence of both PP and galactose gave a marked increase, occurring under conditions optimal for β-galactosidase formation in the preparation. Fig. 8 shows the time course of [^{14}C]uracil incorporation during the development of β-galactosidase, the values being corrected for incorporation occurring in the absence of galactose; it can be seen that the incorporation is most rapid during the early stages of incubation corresponding to the lag period in the enzyme development.

Effect of penicillin. Table 2 also shows the effect of penicillin on the incorporation of [^{14}C]uracil. In the

Table 2. *Incorporation of [^{14}C]uracil by disrupted cells; effect of penicillin*

Disrupted cell preparation, stage *C* extracted with M-NaCl, incubated for 2 hr. at 37° in buffered saline pH 6·25 containing 0·1 μmole [^{14}C]uracil (specific activity 0·7 mc/m-mole)/ml. and other additions as below, with and without 30 i.u. penicillin/ml. After incubation cells centrifuged down and either washed before β-galactosidase determination, or precipitated in the cold with 5% TCA; precipitate washed with cold 1% acetic acid and dried for counting.

Components of incubation mixture				Radioactivity incorporated (counts/min./mg. dry wt. prep.) penicillin		Percentage inhibition of incorporation by penicillin	Increase in incorporation due to galactose			Percentage inhibition of β-galactosidase formation by penicillin
ATP, HDP	Amino acids A	Purines–pyrimidines (PP)	Galactose 2% (w/v)	Penicillin absent	Penicillin present		Penicillin absent	Penicillin present	Inhibition (%)	
+	−	−	−	46	—	—	—	—	—	—
+	+	−	−	128	138	0	—	—	—	—
+	+	−	+	394	212	46	266	74	72	73
+	+	+	−	350	280	20	—	—	—	—
+	+	+	+	1216	620	49	866	340	61	76

absence of galactose, penicillin has little effect on the incorporation and this may be correlated with the absence of effect on development of catalase and glucozymase. Penicillin has a marked inhibitory effect on incorporation in the presence of galactose and reduced by 60–70% the stimulation of incorporation produced by the addition of galactose; this inhibition can be correlated with the inhibition of β-galactosidase development.

DISCUSSION

It is clear that removal of nucleic acid decreases the ability of disrupted cell preparations to develop certain enzymes, and that this ability can be restored by supplying the preparations with appropriate nucleic acids or their precursors. The development of the enzyme systems studied is dependent upon a supply of amino acids, is accompanied by an increase in the protein-nitrogen of the preparations, and is inhibited by chloramphenicol at a concentration known to prevent protein synthesis in intact cells. It seems reasonable that the development of the enzymes occurs as a result of protein synthesis, although in a complex system such as 'glucozymase' other factors may clearly be involved. At least two stages can be recognized in the requirement of preparations for enzyme development or protein synthesis: when the disrupted cells are partially depleted of nucleic acid, they can be stimulated by RNA or PP in specific cases; when nucleic acid depletion has become severe, DNA becomes an additional requirement for stimulation. In this latter stage, enzyme development is abolished by the action of either ribo- or deoxyribo-nuclease.

Of the single enzymes which have been studied, catalase is constitutive and preparations are highly active at the beginning of the experiments, β-galactosidase is an induceable enzyme and the preparations are devoid of initial activity. Catalase activity is increased in the presence of RNA, while the addition of PP has no effect; the reverse is true for the development of β-galactosidase activity. Since the effect of PP on β-galactosidase 'development' is abolished by the presence of ribonuclease and is accompanied by a marked incorporation of uracil, it seems probable that RNA synthesis underlies the effect in this case, but it would seem that the RNA formed must be unstable since it has not been possible to promote β-galactosidase formation by RNA prepared from cells grown in glucose or galactose, Pardee (1954) has shown that the formation of β-galactosidase in purine-less mutants of *Esch. coli* is dependent upon the supply of purines but that, if the supply of purines is suboptimum, then enzyme formation ceases when the purine supply is exhausted. Pardee therefore suggests

that it is the synthesis of RNA which is involved in enzyme formation, and that the RNA once synthesized is inert. However, the organisms in Pardee's experiments are growing, and a possible alternative explanation of his results would be that the RNA required for β-galactosidase formation is unstable and, in the absence of a sufficient supply of purines, its breakdown products are used for the synthesis of other more stable RNA structures required by the growing cell. In the present experiments, RNA clearly has an effect on catalase 'development'; it is possible that the RNA undergoes a cycle of breakdown and resynthesis which is undetected by the experiments as at present designed. It may be that different mechanisms are involved in the synthesis of different proteins. Spiegelman, Halvorson & Ben-Ishai (1955) have shown that the formation of induceable enzymes in yeast can be inhibited by the addition of purine analogues and it seems clear that, in induceable systems at any rate, protein synthesis is accompanied by, if not dependent upon, RNA synthesis, and that some factor in the process requires stabilization by the presence of the substrate. Deken-Grenson (1953), studying the rate of protein synthesis and RNA renewal in a variety of bird and mouse tissues, found that the rate of RNA renewal was considerably less than that of protein synthesis, and concluded that RNA plays a catalytic role if any.

The experiments with stage *D* disrupted cells show clearly that RNA is not the sole controlling factor in protein synthesis but that DNA is also involved. The results are compatible with various hypotheses that have been put forward (Gale, 1955; Rich & Watson, 1954) suggesting that DNA is responsible for the organization of RNA residues or protein-RNA residues which are, in turn, responsible for the organization of amino acid residues before combination into peptide bonds. The mechanism of DNA and RNA function and their inter-relationships remains in the realm of speculation.

The site of action of penicillin is gradually becoming clearer. It is known to inhibit the incorporation of certain amino acids into the protein of intact and disrupted staphylococcal cells (Gale & Folkes, 1953*b*, 1955), and from the facts that the inhibition is incomplete and that such incorporation is dependent upon reaction between the proteins and nucleic acids (Gale & Folkes, 1955) it might be deduced that the antibiotic prevents the reaction of certain proteins with their corresponding nucleic acids. Such an action might be expected to interfere with synthesis of both nucleic acid and protein when conditions become suitable for cell growth, and this might be reflected in impairment of the synthesis of certain enzymes. It is shown above that low concentrations do, in fact, act as differential inhibitors of enzyme development. Further, in the case of β-galactosidase formation, the inhibition by penicillin is accompanied by parallel inhibition of the incorporation of [^{14}C]uracil that accompanies enzyme development. It is probable that these findings are connected with those of Park (1952) who showed an accumulation of nucleotide-like substances containing uridine in penicillin-treated staphylococci. The unusual plateau effects found in studies of inhibition by penicillin cannot be explained at present unless there are two paths of synthesis of proteins such as β-galactosidase of which only one is sensitive to the antibiotic. The mechanism of action of penicillin awaits elucidation of the relationship between the syntheses of nucleic acids and proteins.

SUMMARY

1. The development of certain enzymic activities, 'glucozymase', catalase and β-galactosidase, in disrupted cell preparations of *Staphylococcus aureus* is described. Preparations *B*, *C* and *D* correspond to progressive stages in removal of nucleic acid from the disrupted cells.

2. Preparations at stage *B* develop 'glucozymase' and catalase activities when incubated with a complete mixture of amino acids, and β-galactosidase if galactose is added to the incubation medium. Nucleic acids have little or no effect on enzyme development at this stage.

3. Preparations at stage *C* develop little enzymic activity unless nucleic acid or its derivatives are added: catalase development is strongly stimulated by ribonucleic acid (RNA) but not by a mixture of purines and pyrimidines (PP); β-galactosidase development is stimulated by PP but not by RNA whether derived from glucose- or galactose-grown cells. Deoxyribonucleic acid (DNA) has little or no effect at this stage.

4. Preparations at stage *D*, highly depleted of nucleic acid, respond less markedly to RNA or PP than stage *C* preparations, but DNA is now found to be stimulatory both for catalase and β-galactosidase development. 'Glucozymase' development is stimulated by RNA, DNA or PP.

5. Development of all three enzyme activities is abolished by chloramphenicol at a concentration of 10–30 μg./ml. Penicillin and bacitracin at a concentration of 30 μg./ml. strongly inhibit the formation of β-galactosidase but are without action on catalase development.

6. Incubation of disrupted cells with [^{14}C]uracil under conditions suitable for enzyme development results in incorporation of radioactivity into the nucleic acid fraction; this incorporation is markedly increased by the presence of galactose in the medium and the increase due to galactose is 75% abolished by penicillin in concentrations inhibiting β-galactosidase formation.

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Hydrocarbons in Pyrethrum Cuticle Wax

By G. G. WANLESS, W. H. KING JUN. AND J. J. RITTER
Department of Chemistry, New York University, Washington Square, New York, and Standard Oil Development Company, Linden, N.J.

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During the period 1928–37, there were many contributions to this *Journal* on the cuticle waxes, as found on the petals of flowers, the protective coatings of fruit and on the leaves of vegetables. For references see Chibnall, Piper, Pollard, Williams & Sahai (1934), Chibnall & Piper (1934), and also Kreger (1948).

In general these cuticle waxes are found to consist of long-chain carboxylic acids of even number, and their esters; even-numbered primary alcohols; and odd-numbered secondary alcohols, ketones and paraffin hydrocarbons. The proportions vary from plant to plant, and the carbon numbers range from C_{24} to C_{36}. These findings caused considerable speculation about the sequence of formation of these materials in the plants, and the subject is still not clarified.

Good methods are available for characterizing pure samples. For hydrocarbons one may mention the transition-point data and the X-ray data of Piper *et al.* (1931), and Piper, Chibnall & Williams (1934). But the real difficulty in this work is the separation of members of a homologous series of these materials; this limits the applicability of the analytical procedures.

The purpose of this work was to examine the hydrocarbon portion of pyrethrum wax, and to attempt to accomplish some resolution of the mixtures found therein.

EXPERIMENTAL

All melting points are corrected.

Materials

Two samples of the crude pyrethrum wax were supplied by the U.S. Industrial Chemicals Corp., these being factory samples from different periods of operation. The crude wax is precipitated by chilling the hexane extract of the dried pyrethrum flowers. These two samples, *A*, and *B*, were found to contain hydrocarbon portions of 46 and 17% respectively, as measured by recovery from the light petroleum eluate, of a chromatogram on activated alumina. It is with these hydrocarbon portions of the wax that this communication is principally concerned.

Sample *A*, once-crystallized from acetone melted at 61·5–64·5°. The average molecular weight of this sample was 370 ± 15, as determined by the micro-Rast procedure. Carbon and hydrogen analyses (C 82·08, 82·16; H 13·99, 14·07%) accounted for 96·1% of the same sample. The infrared curves confirmed the presence of oxygen by the carbonyl