values, losses in iodine value, and rather erratic changes in refractive index.

3. Marked parallelisms between the alterations of the characteristics for any one sample suggested that these alterations proceed from a single general cause, most probably aerial oxidation. Increases in acidity, in particular, exactly paralleled loss in iodine value for any particular batch of samples, suggesting an essentially oxidative mechanism producing free acidity in butterfat as opposed to a mainly lipolytic one in butter.

4. The mixed free fatty acids from each batch of pooled rancid samples have been extracted thoroughly with ethanol and analyzed for component acids. Important features were: the presence of *n*-octane-1-carboxylic acid to the extent of 7–9 mol. % (proved by the preparation of its *p*-toluidide derivative, a series of such derivatives from pure fatty acids being also incidentally prepared for reference purposes) and of normal homologous saturated acids from butyric to *n*-nonane-1-carboxylic in roughly similar amounts; the, perhaps adventitious, presence of acetic acid (as shown both by chromato-

graphic and direct fractionation methods); the occurrence of azelaic acid, with traces of unidentified higher and lower homologous dicarboxylic acids; the presence of about 10 % of palmitic acid; the absence of any great quantity of oleic acid, but the presence of fragments of uncharacterized lower unsaturated acids in small amounts, and of non-volatile residues of low equivalent and low iodine value to the extent of 10–20 % (accounting for nearly half of the total unsaturation).

5. Most of the above features are considered to be explicable as resulting from the autoxidation, probably as a glyceride, of oleic acid by recently proposed mechanisms. Polymerization also emerges as an important feature of rancidification in these comparatively saturated fats. Glyceride lipolysis also occurs, but to smaller extents than the two effects just enumerated.

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The Use of the Tyrosine Apodecarboxylase of *Streptococcus faecalis* R for the Estimation of Codecarboxylase

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The bacterial L-lysine and L-tyrosine decarboxylases have been shown to contain a coenzyme known as codecarboxylase; this substance was found to be widely distributed in nature (Gale & Epps, 1944a, b; Epps, 1944). Gale & Epps have used the apoenzyme of the L-tyrosine decarboxylase of *Streptococcus faecalis* for the estimation of codecarboxylase; the rate of decarboxylation of tyrosine was a function of the amount of codecarboxylase added; the apoen-

zyme was prepared by the dissociation of the holoenzyme. A method of estimating codecarboxylase by means of tyrosine apodecarboxylase has also been described by Umbreit, Bellamy & Gunsalus (1945). They used, as source of apoenzyme, suspensions of freeze-dried cells of *Strep. faecalis* R, which had been grown in a vitamin B₆-free medium. Such preparations also contained an enzyme system which formed codecarboxylase from pyridoxal and adenosinetri-

phosphoric acid (ATP). Therefore, in the presence of excess ATP the rate of decarboxylation of tyrosine was a function of the amount of pyridoxal added.

The present paper describes experiments in which acetone-dried preparations of vitamin B₆-free cells of *Strep. faecalis* R were used for the estimation of codecarboxylase in tissues. In the course of this work, some new observations were made on the coenzyme-apoenzyme relationship of the tyrosine decarboxylase system of *Strep. faecalis* R.

METHODS

The methods used were similar to those described by Umbreit *et al.* (1945). The preparation of intact and acetone-dried cells of *Strep. faecalis* R has been described elsewhere (Sloane-Stanley, 1949). Cells grown in a vitamin B₆-free medium contained tyrosine apodecarboxylase but no codecarboxylase; they will be called deficient cells. Cells grown in the same medium but in the presence of pyridoxal contained the enzyme saturated with codecarboxylase; they will be referred to as complete cells.

The manometric procedure was similar to that already described (Sloane-Stanley, 1949). Unless otherwise stated, the total fluid volume in each flask was 3.0 ml. The side bulbs contained 0.4 ml. of a 0.04 M-L-tyrosine suspension. The contents of the main compartments differed with the type of cell preparation used, as shown in Table 1. The cell preparations

Table 1. *Contents of main compartments of the manometer flasks*

(Total vol. of fluid 2.6 ml.)

(a)	(b)	(c)
Dried deficient cells	Dried complete cells	Intact deficient cells
0.2 ml. M-Acetate buffer (pH 5.5)	0.2 ml. M-Acetate buffer (pH 5.5)	1.0 ml. 0.075 M-Phthalate buffer (pH 5.0)
1.0 mg. Cells	10 mg. Cells	0.5 ml. Cell suspension, from 3 ml. medium
Source of codecarboxylase	—	Pyridoxal

were always added last. The bath temperature was 28.5°. In experiments with acetone-dried deficient cells and pyridoxal, 0.1 ml. of a sulphate-free solution of ATP, containing 1 mg. of the free acid, was added to each flask. The calcium pyridoxal-5-phosphate used in some of the experiments was kindly given by Dr K. Folkers; this substance will also be called synthetic codecarboxylase. For the experiments with acetone-dried cells, the gas phase was N₂, because it was found that the rate of decarboxylation of tyrosine was 25% lower in air than in N₂. On the other hand, air was used with intact cells as the rate of the reaction was the same in air as in N₂. The course of the reaction was followed by taking readings every 5 min. In some experiments the rate was low in the first few minutes after tipping, but subsequently increased and became steady. In every experiment the steady rate of evolution of CO₂ was measured and assumed to represent the true enzymic activity of the system; it was expressed in μ l.

of CO₂/hr., and is represented by V . The maximum value of V obtained with any given preparation of deficient cells, in the presence of enough codecarboxylase to saturate the apoenzyme, is given as $V_{\max}$. (Umbreit *et al.* 1945); the value of $V_{\max}$ was assumed to be a measure of the concentration of apodecarboxylase in the preparation.

RESULTS

Estimation of codecarboxylase

Acetone-dried deficient cells did not decarboxylate tyrosine at all in the absence of any source of codecarboxylase nor did the addition of ATP to these preparations, in the absence of pyridoxal, cause any decarboxylation. Aqueous suspensions of these cells, stored in the refrigerator, retained their full tyrosine apodecarboxylase content for at least 24 hr.

Preparation of tissue suspensions. At first, the procedure described by Bellamy, Gunsalus & Umbreit (1945) was used. A weighed piece of tissue was homogenized in 0.1 N-NaOH; the mixture was heated for 5 min. in boiling water and stored in the refrigerator. Before each estimation some of the homogenate was acidified with N-HCl to pH approx. 5.5. Samples of the resulting suspension were then added to manometer flasks, as shown in Table 1 (a). Under these conditions V was approximately proportional to the volume of tissue suspension used, up to a value about 60% of $V_{\max}$. The relation between V and the amounts of tissue added was similar to that illustrated in Fig. 2, curve A.

It was found that the codecarboxylase in homogenates of tissue in 0.1 N-NaOH was not stable; for instance, in one experiment, a homogenate of rat liver had lost 50% of its original codecarboxylase activity after 18 hr. storage in the refrigerator. In borate buffers, however, the codecarboxylase activities of tissue homogenates were found to be more stable (Table 2). In another, similar experiment, a rat-liver

Table 2. *Effect of pH on stability of codecarboxylase in rat liver homogenates*

(Contents of manometer flasks as in Table 1 (a); suspension equivalent to 0.5 mg. of rat liver (homogenized in distilled water, and samples heated in the solutions below, for 4 min. at 100°) added to each flask as source of codecarboxylase.)

Solution used for preparation of homogenate (final conc., 0.1 M)	Values of V (μ l. CO ₂ /hr.) after storage in refrigerator for		
	0 hr.	24 hr.	72 hr.
Borate (pH 8.0)	120	130	100
Borate (pH 9.0)	110	100	90
Borate (pH 10.0)	110	110	80
NaOH	110	80	40

homogenate in 0.01 N-NaOH lost its codecarboxylase activity even more rapidly. In both these experiments, the loss of activity was least in the borate buffers of pH 8 and 9; in all subsequent assays of codecarboxylase, therefore, the following method was adopted.

A weighed piece of tissue was homogenized in distilled water and 4 ml. of 0.25 M-borate buffer (pH 8.5) were added to each 6 ml. of homogenate; the mixture was heated for 4 min. in boiling water and stored in the refrigerator. Before use,

a sample of this mixture was diluted with an equal volume of m-acetate buffer of pH 5.5; the resulting suspension was diluted with a suitable volume of distilled water. The pH of suspensions thus prepared was found to be 5.5 when measured with the glass electrode. The 0.25M-borate buffers used in these experiments were prepared from H_3BO_3 and NaOH in the proportions given by Cole (1933), but without KCl.

The codecarboxylase in these borate-buffered homogenates was found to be stable. For instance, in one experiment, the equivalent of 1 mg. of rat liver gave the following values of V : immediately after preparation, 140; after 120 hr. in the refrigerator, 150.

tions of acetone-dried deficient cells synthetic codecarboxylase was a suitable standard for the estimation of codecarboxylase in tissues. In these experiments, the amount of apoenzyme was kept constant and varying amounts, either of rat liver (crosses, Fig. 1) or of calcium pyridoxal-5-phosphate (circles, Fig. 1) were added. The rate of decarboxylation was the same when either 2 mg. of rat liver or 0.03 μ g. of calcium pyridoxal-5-phosphate were added; Fig. 1 has, therefore, been drawn so that these two points coincide. It will be seen that all the points for other values of V lie on or very near the same curve, irrespective of whether the source of codecarboxylase was rat liver or calcium pyridoxal-5-phosphate.

Fig. 1. Rates of decarboxylation of tyrosine by acetone-dried vitamin B_6 -free cells of *Strep. faecalis* R, with varying amounts of natural and synthetic codecarboxylase. +, rat liver suspension; O, calcium pyridoxal-5-phosphate; (a), $\times$, pyridoxal (10 μ g.) with ATP (1 mg.)

Synthetic codecarboxylase was found to be unstable in distilled water; however, solutions in borate buffer, which had initially the same codecarboxylase activities as solutions in distilled water, were more stable. For instance, a solution of calcium pyridoxal-5-phosphate (0.1 μ g./ml.) in distilled water lost 60 % of its activity in 48 hr. at -2° , but a similar solution (0.16 μ g./ml.) in 0.1M-borate buffer (pH 8.5) kept its full activity for 100 hr. at 0° . All subsequent experiments with synthetic codecarboxylase were therefore done with solutions in borate buffer.

Estimation of codecarboxylase with calcium pyridoxal-5-phosphate as standard. The experiments illustrated in Fig. 1 showed that with our prepara-

As a result of these experiments the following procedure was adopted for the estimation of codecarboxylase in tissues. Determinations were carried out with at least two different volumes of tissue suspension, so chosen that the resulting values of V were approximately proportional to the amounts of tissue used; the equivalent quantities of calcium pyridoxal-5-phosphate were found from the calibration curve of the sample of cells used (e.g. Fig. 1), and the mean of the results was taken as the concentration of codecarboxylase in the suspension.

Table 3 gives the results of the assay of three different rat livers; these results were obtained with the sample of cells for which the calibration curve is given in Fig. 1. The maximum enzymic activity

($V_{\max}$) of this preparation did not change in six weeks, but others have been less stable. With the small amounts of codecarboxylase used in these assays, however, small decreases in the tyrosine apodecarboxylase content of a sample of cells are of little importance. For instance, in an experiment with 1 mg. of rat liver, V was 150 when 1 mg. of cells was used; with 2 mg. of cells, V was 180; therefore, doubling the amount of apoenzyme had only increased V by 20 %. With a constant quantity of apoenzyme, V depends on the amount rather than on the concentration of codecarboxylase added: in an experiment with 1 mg. of cells and 0.02 μ g. of calcium pyridoxal-5-phosphate, V was 240 when the volume of liquid in the manometer flask was 3 ml.; when the concentration of codecarboxylase was doubled by reducing the volume to 1.5 ml., V increased by only 8 %, to 260. These results suggest that nearly all the added codecarboxylase combined with the apoenzyme.

Table 3. *Estimation of codecarboxylase in rat liver, with acetone-dried deficient cells calibrated with calcium pyridoxal-5-phosphate*

Animal used as source of liver*	Amount of liver (mg.)	V (μ l. CO_2 /hr.)	Codecarboxylase content of liver (m μ g. calcium pyridoxal-5-phosphate/mg.)
Deficient in vitamin B ₆	0.5	80	11.0
	1.0	150	11.0
		Mean	11.0
Control receiving vitamin B ₆	0.5	110	16.0
	1.0	195	15.0
		Mean	15.5
Control receiving vitamin B ₆	0.5	130	19.0
	1.0	210	16.0
		Mean	17.5

* See Blaschko, Carter, O'Brien & Sloane-Stanley (1948).

Estimation of codecarboxylase with pyridoxal as standard. Before synthetic codecarboxylase became available, assays were made using pyridoxal, with excess ATP as suggested by Umbreit *et al.* (1945). The relation between V and amounts of pyridoxal added to the acetone-dried deficient cells in the presence of ATP was similar to that reported by Umbreit *et al.* for freeze-dried cells; but difficulties were encountered when attempts were made to express the codecarboxylase activities of tissues in terms of pyridoxal, for two reasons. First, the curves obtained by plotting V against amounts of tissue suspension added to the cells could not be superimposed on those obtained with pyridoxal in the presence of ATP. It was found that V was nearly proportional to the volume of tissue suspension added up to a value of about 60 % of $V_{\max}$, whereas V was proportional to the amount of pyridoxal

added up to a value of only 25 % of $V_{\max}$; yet the values of $V_{\max}$ obtained with tissue suspension and with pyridoxal were almost identical (Fig. 2). Secondly, the relation between V and the amount of pyridoxal added to a given preparation of cells was not constant, but varied from one experiment to another; the result of the experiment described below suggests that this may have been due to the time taken for the phosphorylation of added pyridoxal. In this experiment, the manometer flasks were shaken in the bath for varying times before the addition of the tyrosine to the main compartments. With 0.05 μ g. of pyridoxal, V increased more than threefold when the time of equilibration was increased from 10 to 130 min.; with 10 μ g. of pyridoxal, the increase in V was less than 25 %.

Fig. 2. Rates of decarboxylation of tyrosine by acetone-dried vitamin B₆-free cells of *Strep. faecalis* R, with varying amounts of rat liver suspension (curve A) and of pyridoxal in the presence of 1 mg. ATP (curve B) as sources of codecarboxylase.

Synthetic pyridoxal acetal-3-phosphate was tested as a possible source of codecarboxylase, using 1 mg. of acetone-dried cells. With 20 μ g. of pyridoxal acetal-3-phosphate, V was about 30; with 20 μ g. of this substance plus 1 mg. of ATP, V was about 60; with 0.01 μ g. of pyridoxal plus 1 mg. of ATP, V was 120; the value of $V_{\max}$ for this sample of cells, determined with 10 μ g. of pyridoxal plus ATP, was 600. These results do not support the view that pyridoxal acetal-3-phosphate has codecarboxylase activity; they suggest that the sample used may have contained a trace of codecarboxylase and of pyridoxal.

Properties of the tyrosine decarboxylase system

(a) Acetone-dried deficient cells

Effect of sulphate. It was found that sulphate inhibited the decarboxylation of tyrosine by preparations of acetone-dried deficient cells in the presence of codecarboxylase (Table 4). The inhibition increased with increasing sulphate concentration,

when the amount of synthetic codecarboxylase added was kept constant and was almost enough to saturate the apoenzyme. When the concentration of sulphate was kept constant, the inhibition was most marked with small amounts of codecarboxylase, but very nearly disappeared with large

the flask in which sulphate was added from the side bulb together with the tyrosine at the moment of tipping, the codecarboxylase had been in contact with the dried-cell preparation for about 30 min. Under these conditions the addition of sulphate had an almost negligible effect.

Table 4. *Effect of sulphate concentration on the decarboxylation of tyrosine by acetone-dried deficient cells*

(Source of codecarboxylase: 0.07 μ g. calcium pyridoxal-5-phosphate.)

Concentration of Na_2SO_4 (M)	V (μ l. CO_2 /hr.)	Inhibition (%)
0	565	—
3.3×10^{-4}	535	5
1×10^{-3}	460	19
3.3×10^{-3}	300	47
1×10^{-2}	190	66
3.3×10^{-2}	100	82

Table 5. *Effect of varying the amount of codecarboxylase on the inhibition by sulphate of the decarboxylation of tyrosine by acetone-dried deficient cells*

(Concentration of Na_2SO_4 , 10^{-2} M.)

Calcium pyridoxal-5-phosphate added (μ g.)	V without sulphate (μ l. CO_2 /hr.)	V with sulphate (μ l. CO_2 /hr.)	Inhibition (%)
0.025	285	75	74
0.07	520	170	67
0.2	630	375	40
0.7	645	590	8.5
7.0	715	655	8.5

amounts of codecarboxylase (Table 5). The degree of inhibition by sulphate depended on the order in which sulphate and synthetic codecarboxylase were added to the apoenzyme preparation (Table 6). In

Sulphate also inhibited the reaction in experiments in which pyridoxal and ATP were used as source of codecarboxylase. For instance, in an experiment in which 10 μ g. of pyridoxal were added, V was 500 in the absence of sulphate; in the presence of 0.033 M-sodium sulphate, V was 110. In another similar experiment the corresponding figures were: without sulphate, 580; with 0.0033 M-sodium sulphate, 390.

Other salts. Tables 7 and 8 give some results obtained with other substances which were found to inhibit the reaction when acetone-dried deficient cells were used. The experiments of Table 7 were done with synthetic codecarboxylase, those of

Table 7. *Inhibition of tyrosine decarboxylase system in acetone-dried deficient cells by phthalate, phosphate, and pyrophosphate*

(Source of codecarboxylase: 0.07 μ g. calcium pyridoxal-5-phosphate.)

Salt added	V with salt (μ l. CO_2 /hr.)	V without salt (μ l. CO_2 /hr.)	Inhibition (%)
10^{-2} M-phthalate	30	600	95
buffer (pH 5.5)			
3.3×10^{-2} M- KH_2PO_4	290	600	52
3.3×10^{-3} M- KH_2PO_4	480	475	0
3.3×10^{-2} M- $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$	230	455	50
3.3×10^{-3} M- $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$	490	475	0

Table 8 with pyridoxal and ATP. It will be seen that phthalate was a stronger inhibitor than sulphate, whereas phosphate and pyrophosphate had a much weaker action. Some chlorides were also tested: NaCl, KCl and BaCl_2 , each in a final concentration of 0.033 M, did not affect the rate of decarboxylation of tyrosine by 1 mg. of acetone-dried deficient cells in the presence of 0.07 μ g. of calcium pyridoxal-5-phosphate.

(b) *Acetone-dried complete cells*

With complete cells, the inhibitory effects of the salts tested as described above were either absent or less marked. There was no inhibition with sulphate, phosphate, or pyrophosphate. With phthalate the

Table 6. *Inhibition by sulphate: effect of varying order of addition of constituents of system*

(Main compartments of manometer flasks contained acetone-dried deficient cells and acetate buffer, as in Table 1(a). Source of codecarboxylase: 0.025 μ g. calcium pyridoxal-5-phosphate (added in 0.25 ml.). Concentration of sulphate 10^{-2} M, added as 0.3 ml. 0.1 M- Na_2SO_4 . Codecarboxylase in main compartment.)

Manometer no.	1	2	3
Sulphate ...	Not added	In main compartment	In side bulb
V (μ l. CO_2 /hr.)	285	75	265

Table 8. *Effects of phthalate and phosphate with acetone-dried deficient cells, and pyridoxal and ATP*

Pyridoxal added (μ g.)	Salt added	V with salt (μ l. CO_2 /hr.)	V without salt (μ l. CO_2 /hr.)	Inhibition (%)
10	2.5×10^{-2} M-Phthalate buffer (pH 5.5)	180	330	45
0.1	3.3×10^{-2} M-Phthalate buffer (pH 5.5)	20	170	88
0.1	3.3×10^{-2} M- KH_2PO_4	100	170	41

degree of inhibition was only 22 %. All these substances were tested in a final concentration of 0.033 M.

(c) Intact cells

With intact deficient cells, substitution of 0.067 M-acetate buffer (pH 5.0) for the phthalate buffer normally used had no effect on the value of V . Using intact deficient cells in the same acetate buffer, the value of V was not affected by the addition of 0.033 M-sulphate, phosphate or pyrophosphate.

It was found that codecarboxylase did not restore the complete tyrosine decarboxylase system in intact deficient cells. Experiments were done with both synthetic and natural codecarboxylase. For instance, the addition of 3.7 μ g. of calcium pyridoxal-5-phosphate to a washed suspension of intact deficient cells did not cause any significant decarboxylation of tyrosine ($V > 8$); yet V equalled 430 in the same experiment when 10 μ g. of pyridoxal were added. In an experiment with rat-liver suspension as source of codecarboxylase, the equivalent of 110 mg. of rat liver was added to a sample of intact deficient cells; this did not cause any decarboxylation of tyrosine ($V = 0$), as compared with $V = 320$ when 10 μ g. of pyridoxal were added. The amount of codecarboxylase in 110 mg. of rat liver is equivalent to about 1.65 μ g. of calcium pyridoxal-5-phosphate, which was more than enough to saturate the apoenzyme in 1 mg. of an active sample of acetone-dried deficient cells (Fig. 1).

DISCUSSION

Difficulties were encountered in the attempt to estimate codecarboxylase in terms of pyridoxal, using acetone-dried preparations of *Strep. faecalis* R. The experiments described above suggest that this was mainly due to an impairment in such cells of the system which forms codecarboxylase from pyridoxal and ATP. The difference between our results and those of Umbreit *et al.* (1945) is probably explained by our use of acetone-dried instead of freeze-dried cells. It has been shown, however, that acetone-dried preparations of vitamin B₆-free cells (deficient cells) permit the estimation of tissue codecarboxylase in terms of the synthetic substance, with a probable error of ± 10 %. Our observations support the findings of Gunsalus & Umbreit (1947) that synthetic pyridoxal acetal-3-phosphate, which was claimed by Karrer & Viscontini (1946) to be an active form of codecarboxylase, has in fact little or no codecarboxylase activity.

The finding that codecarboxylase is unstable in distilled water agrees with observations of Umbreit *et al.* (1945). Borate buffer (0.1 M, pH 8.5) has proved useful in preserving the codecarboxylase activities of solutions of both natural and synthetic codecarboxylase. This effect of borate is of interest since there

is evidence for the formation of a stable complex between borate and pyridoxine (Scudi, Bastedo & Webb, 1940).

The experiments on the inhibitory action of sulphate on the tyrosine decarboxylase system suggest that sulphate has no effect on the complete tyrosine decarboxylase, but that it acts by preventing the combination of codecarboxylase with tyrosine apodecarboxylase. This is supported by the observation that the inhibition occurred only with acetone-dried deficient cells whatever the source of codecarboxylase used, while sulphate had no effect on acetone-dried complete cells. The finding that, with acetone-dried deficient cells, the inhibitory effect of sulphate was most marked with small amounts of codecarboxylase but was small with large amounts of codecarboxylase, suggests that sulphate competes with codecarboxylase for the apoenzyme. The finding of sulphate inhibition is of practical importance in the estimation of codecarboxylase in terms of pyridoxal in the presence of ATP, since it makes it necessary to ensure that the ATP solutions used are free of sulphate.

Our observations on the inhibitory effects of phosphate and phthalate are in agreement with those of Epps (1944). With intact deficient cells, no inhibitory effect of sulphate, phthalate, phosphate or pyrophosphate on the decarboxylation of tyrosine in the presence of pyridoxal was observed.

It has been reported that in growing cells of *Strep. faecalis* R, synthetic codecarboxylase has no vitamin B₆ activity (Rabinowitz & Snell, 1947). This is of interest in view of the inability of intact deficient cells to decarboxylate tyrosine in the presence of large amounts of codecarboxylase. It seems that in the cells of *Strep. faecalis* R the apoenzyme is inaccessible to added codecarboxylase. Our results suggest that intact deficient cells of *Strep. faecalis* R could be used to detect pyridoxal in the presence of codecarboxylase. The absence of any decarboxylation of tyrosine when rat-liver suspensions were used with intact deficient cells shows that the pyridoxal content of the suspensions was negligible.

SUMMARY

1. The use of acetone-dried cells of *Streptococcus faecalis* R, grown in a vitamin B₆-free medium, as a source of tyrosine apodecarboxylase for the estimation of codecarboxylase, has been investigated.

2. Satisfactory assays of codecarboxylase in animal tissues can be made with such preparations, using calcium pyridoxal-5-phosphate (synthetic codecarboxylase) as standard.

3. Solutions of both synthetic and natural codecarboxylase in 0.1 M-borate buffer (pH 8.5) keep their full initial codecarboxylase activities for several days in the cold.

4. With acetone-dried deficient cell preparations, pyridoxal in the presence of ATP is not a satisfactory standard. This is probably due to the low activity of the pyridoxal-phosphorylating system in such preparations.

5. Sulphate, phthalate, phosphate and pyrophosphate inhibit the decarboxylation of tyrosine by acetone-dried deficient cells in the presence of small amounts of codecarboxylase; the inhibition by sulphate is reduced or abolished by an increase in the amount of codecarboxylase added. These substances do not inhibit the decarboxylation of tyrosine by intact deficient cells in the presence of pyridoxal; with acetone-dried preparations of cells grown in a medium containing excess pyridoxal they have little or no inhibitory effect. The action of these salts is, therefore, interpreted not as an inhibition

of the holoenzyme, but as an interference with the reaction between codecarboxylase and the apoenzyme.

6. Vitamin B₆-free intact cells of the organism do not decarboxylate tyrosine in the presence of either synthetic or natural codecarboxylase.

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A Quantitative Study of Complex Formation in Heated Protein Mixtures

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When proteins are heated together under certain conditions, the heat-denatured particles of different proteins can combine to form complex aggregates. Previous work on such complexes has been solely qualitative. It has shown that complexes are formed (Kleczkowski, 1945) and that they can differ from their components in solubility and precipitability by salts (Kleczkowski, 1941*a*), electrophoretic mobility (Van der Scheer, Wyckoff & Clarke, 1941), isoelectric point (Kleczkowski, 1946) and serological behaviour (Kleczkowski, 1941*b*; Bawden & Kleczkowski, 1941, 1942*a, b*), but there has been no quantitative work on their formation or properties. The present paper describes (i) the effect of varying the concentrations and ratios of the components in heated mixtures on the constitution of the resulting complexes, and (ii), the effect of variations in the constitution of the complexes on their solubility and serological behaviour.

MATERIALS AND METHODS

The proteins used were tobacco mosaic virus (TMV), tomato bushy stunt virus (BSV) and human-serum albumin. The viruses were purified by the methods described by Bawden & Pirie (1943). For convenience the heat-denatured viruses will be referred to as viruses, although they are non-infective and heat-denatured TMV has also lost its serological activity and its nucleic acid. The albumin was a crude unfractionated preparation from human serum. After removal of the globulin by half saturation with (NH₄)₂SO₄ and filtration, the filtrate was saturated with the salt. The precipitated albumin was collected by filtration, dissolved in water and dialyzed against water. Toluene was added as an antiseptic.

In all experiments designed to test the effect of heat, the protein solutions were made with M/15 phosphate buffer at pH 6.8 and heated in thin-walled test tubes immersed in a water bath. Except when otherwise stated, the time of heating was 10 min. and the temperature of the water bath 83°. Under these conditions, when heated alone, both viruses