Among several reports, the results by Ehrismann et al. (*Biochem. Z.*, **284**, 476 (1936)) coincide to some extent with the present results in the form of absorption curves, though all the absorption bands except at 520 mμ do not coincide with those of the author. On the other hand, α-oxyphenazine ($C_{12}H_8N_2O$) is found to be an impurity accompanied by pyocyanine. Therefore it is significant to examine the absorption spectrum of α-oxyphenazine in order to test its effect on the determination of pyocyanine.

It is found that α-oxyphenazine shows a slight absorption band at 51mμ in visible region only when its solution is kept alkaline. Accordingly, the effect of the contamination of α-oxyphenazine on the photometric determination can be concluded to be negligible. Pyocyanine chloroplatinate was observed to be rather easily purified and it was considerably stable, so that chloroplatinate was used for the estimation of molecular extinction coefficient (ε) of pyocyanine ($C_{13}H_{10}N_2O$:210.09) and found to be as follows:

$\varepsilon=4300$ at 690 mμ with pyocyanine aqueous solution, or $\varepsilon=2400$ at 520 mμ with pyocyanine-HCl aqueous solution.

Using this coefficient, amount of pyocyanine in the medium mentioned in the previous paper (This Bulletin, **25**, 71 (1951)) was calculated as 0.2835 g. per liter. This amount attains to about thirty times to the yield reported by other authors (Fr. Wrede u. E. Strack: *Z. f. physiol. Chem.*, **181** 59 (1929)).

---

23. Studies on the *Propionibacterium*. (V)

Hideo KATAGIRI and Yoshio ICHIKAWA
(Katagiri Laboratory)

Researches on the mechanism of propionic acid fermentation by *Propionibacterium* were hitherto undertaken on anaerobic conditions, and the chemical changes in the fermentation are suggested as follows: 1) pyruvic acid is produced from sugars by EMBDEN-MEYERHOF-PARNAS schema; 2) pyruvic acid is converted into acetic acid by oxidative decarboxylation; 3) succinic acid is formed by successive reduction after fixation of CO_2 to pyruvic acid; 4) propionic acid accumulates by decarboxylation of succinic acid.

The fermentation under aerobic condition (shaking culture) was carried out in the present paper and found that the products differed from those of anaerobic condition (under reduced pressure) as will be seen in Table 1; 1) crop yield of the bacteria was increased and the total acids produced in the medium was decreased; 2) greater amount of acetic acid, but less amount of propionic acid were formed, although succinic acid was always recognized in each case.

Table 1. Fermentation of Glucose by *Propionibacterium arabinosum* and *Propionibacterium freudenreichii*.

Bacteria	Manner of fermenta-tion	Glucose Consumed	Crop Yield	Acid Produced			
				Acetic	Propio-nic	Succi-nic	Malic
		(gr.)	(gr.)	(gr.)	(gr.)	(gr.)	
P. arabinosum	Anaerobic	1.97	2.0	0.225	0.976	0.103	
	Aerobic	1.97	3.0	0.514	0.029	0.116	
P. freuden-reichii	Anaerobic	1.97	2.2	0.300	0.940	0.026	
	Aerobic	1.82	2.9	0.393	0.090	0.146	+

With resting cells or dried cells of the bacteria, enzymatic decomposition of pyruvic and α-ketoglutaric acids were tested and found that pyruvic acid was decomposed into CO_2 and acetic acid as was already concluded, and CO_2 and succinic acid were detected as decomposition products of α-ketoglutaric acid in the presence of malonic acid. It is worth to note that acetic acid was never changed by this enzymatic reaction.

Production of malic acid was verified by paper chromatography as shown in Table 1, therefore it suggests that complete oxidation of pyruvic acid may be achieved according to Krebs tricarboxylic acid cycle.

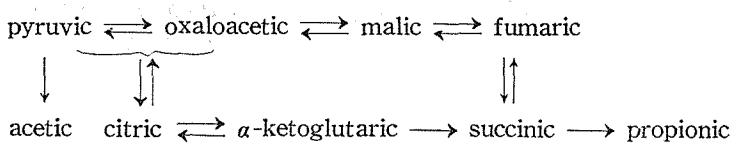
Experiments on oxygen uptake of some members of Krebs cycle were carried out with Warburg's apparatus.

4 ml. of mixture was used in each cup. Unless otherwise stated the reaction mixture contained 1 ml. each of substrate, cell suspension, 0.1 M phosphate buffer and water (cf. Remarks). The temperature of the bath was kept 30°. The results will be seen in in Table 2.

Table 2. O_2-uptake of some members of Citric acid cycle.

Substrate (μM)	pH	Cell added	O_2-uptake in		Remarks
			10min.	30min.	
α-keto glutarate 15	6.0	mg 20(dry)	μl 24	μl 54	Reaction mixture contains 0.8 ml. of 0.2 M malonate.
Citrate 25	6.0	20(dry)	12	40	
Succinate 17	7.2	200(wet)	46	70	
Fumarate 17	7.2	200(wet)	7	30	
Malate 17	7.2	200(wet)	10	35	

From these results, we suggest that under aerobic condition succinate will be converted to fumarate instead of propionate, The whole pathway of pyruvic decomposition may be as follows.



24. Relation of the Drugs Acting on the Autonomic Nervous System to the Effect of Analgesics

Hajime FUJIMURA

(Ogiu Laboratory)

In order to investigate the above mentioned theme, the effect of various drugs in combined use with drugs acting on the autonomic nervous system was studied using a modification of Hardy's radiant heat technique in man.

The pain threshold elevating effects of (I) Morphine, (II) Dolantin, (III) Ohton (1,1-Dithienyl-3-dimethylamino-butene-1), (IV) *l*-Ephedrine, (V) *dl*-Desoxyephedrine, (VI) *d*-Isolan (3.4-Methylendioxyphenyl-isopropylamine), (VII) Benadrin (Diphenhydramine), (VIII) Parpon-M (Dimethylaminoethylbenzylate) and (IX) Avacan-M (Isoamyl ester of α-[N-(β-Dimethylaminoethyl)] -Aminophenylacetate) were markedly reduced by Priscolin or Regitine (adrenolytics), and also by Prostigmine (cholinergics). On the contrary, they were potentiated by *l*-Ephedrine or *d*-Desoxymethylephedrine (adrenergics). For example, Figs. 1 and 2 show the influence of various autonomic drugs to VII and II respectively. (Curves represent average of the

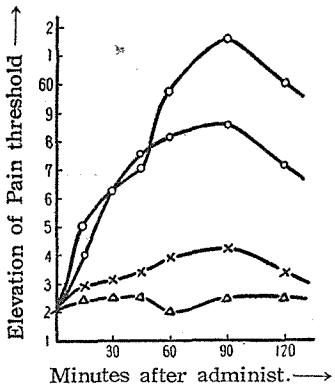


Fig. 1. —○—,(VII 25mg.+IV 25mg.) P.O.
—●—,VII 50mg. P.O.
—×—,(VII 50mg.+Priscoline 50mg.) P.O.
—△—,VII 50mg. P.O.+Prostigmine 0.5mg. S.C.

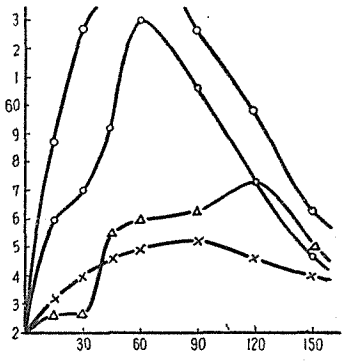


Fig. 2. —○—,(II 50mg.+IV 50mg.) P.O.
—●—,II 50mg. P.O.
—△—,II 50mg. P.O.+Prostigmine 0.5mg. S.C.
—×—,(II 50mg.+Regitine 50mg.) P.O.