Chromogenicity of SAKAGUCHI Products of Monosubstituted Guanidines and Histidine

The chemistry of the SAKAGUCHI reaction – between arginine, 1-naphthol and alkaline hypochlorite – is not known. As originally described forty years ago[1] this sensitive reaction fortuitously revealed itself as a red coloration which slowly developed and faded. By substituting hypobromite[2] for hypochlorite, almost instantaneous colour development was effected, and more recently SZILAGYI and SZABO[3] achieved an improvement in the reproducibility of the reaction with the aid of N-bromosuccinimide (BSI) – a more stable source of available bromine.

SAKAGUCHI[4] also observed that the 1-naphthol could be replaced by 8-hydroxy quinoline (OHQ) in which case an orange-yellow colour is produced, and using OHQ, SATAKE and LUCK[5] were able to develop reasonably stable colours from arginine. However, I have found that at normal laboratory temperatures a reagent blank reaction slowly develops, which compensates for the slow disappearance of the SAKAGUCHI chromogen.

In the absence of SAKAGUCHI-reactive compounds, bromination of 1-naphthol or OHQ in N NaOH results in a yellow coloration. This reaction is inhibited by an increase in (Br^+), a decrease in 1-naphthol concentration or a low temperature. If a strongly alkaline solution of OHQ (2.8 μmoles) and aqueous BSI (5.6 μmoles) are mixed together and allowed to stand for a few seconds no colour is obtained on adding the mixture to an arginine solution, although the addition of more BSI will result in a pink colour. Apparently OHQ (or 1-naphthol) competes with the guanidine derivative for Br^+, and the higher the temperature the more successfully does it do so (see Table I). For this reason, a fairly stable and low temperature (0–5°C) is essential for accurate and reproducible arginine analysis. This was stipulated by SAKAGUCHI[1,4], although the condition has been ignored in more recent attempts[5,6] to adapt the reaction for quantitative analysis.

Amino acids have previously been shown to inhibit the SAKAGUCHI reaction[7]. It can be demonstrated, however, that small amounts (0.05–0.2 μmoles) of many amino acids *intensify* the colour reaction developed from 1 ml 0.01 m*M* arginine with 1-naphthol (2 ml 0.25 m*M*) and BSI (1 ml 2.5 m*M*) or with OHQ (2 ml 1.4 m*M*)[5] and BSI (1 ml 5 m*M*). The increase in colour occurs with glycine and with taurine, and is most marked with lysine and ornithine. It is also found with ethylene diamine. No such effect on the arginine reaction is seen with similar small amounts of cystine, glutamine, citrulline, glucosamine, creatinine, urea or EDTA. The group responsible for the colour-enhancing effect appears to be $-CH_2 \cdot NH_2$. A larger amount (1–5 μmoles) of any amino acid and also of glutamine, cytosine, guanidine and bilirubin may remove bromine and thereby decrease colour production from 1 ml 0.01 m*M* arginine; adenine, EDTA and hematin are without influence.

Histidine also reacts under similar conditions with alkaline 1-naphthol and BSI to give a rose-red colour (λ_{max}: 495 nm). This reaction appears not to have been previously reported. It is about twenty times less sensitive than the arginine reaction. It occurs when hypochlorite is used as the oxidizing agent, as in the original technique of SAKAGUCHI[1]. It does not occur in the absence of 1-naphthol (or alternatively OHQ), and thus it differs from the KNOOP reaction[8] in which bromination of histidine at pH 1 produces a similar coloured product. The histidine colour, but not the arginine colour, is intensified by the addition of a few micromoles of a cupric salt. Neither urocanic acid nor histamine, which gives a positive KNOOP reaction[9] yields colour in the SAKAGUCHI reaction.

With high concentrations of OHQ (e.g. 2.8 μmoles per 5 ml reaction mixture) lower blank colours develop than do with similar concentrations of 1-naphthol[5]. However, with smaller amounts of 1-naphthol (0.25–0.5 μmoles), i.e. about one-fifth that employed by previous workers,

1 S. SAKAGUCHI, J. Biochem., Tokyo, *5*, 25 (1925).
2 C. J. WEBER, J. biol. Chem. *86*, 217 (1930).
3 I. SZILAGYI and I. SZABO, Nature *181*, 52 (1958).
4 S. SAKAGUCHI, Jap. Med. J. *1*, 278 (1948).
5 K. SATAKE and J. M. LUCK, Bull. Soc. Chim. Biol. *40*, 1743 (1958).
6 H. KRAUT, E. SCHRADER-BEIELSTEIN, and M. WEBER, Hoppe Seyler's Z. *286*, 248 (1950).
7 L. E. THOMAS, J. K. INGALLS, and J. M. LUCK, J. biol. Chem. *129*, 263 (1939).
8 F. KNOOP, Beitr. chem. Physiol. Pathol. *11*, 356 (1908).
9 E. RACKER, Biochem. J. *34*, 89 (1940).

Table I. Extinction coefficients of SAKAGUCHI reaction mixtures

	Arginine (μmoles)				
	0	0.025	0.05	0.10	0.15
Method A Temp. (°C)	Readings at 5 min				
4	3	70	178	350	520
18	40	103	179	338	500
23	69	124	190	350	490
30	80	139	198	325	482
Method B Temp. (°C)					
4	0	141	278	550	780
19	30	166	276	540	740
30	81	190	288	475	615

Method A: arginine solution (1 ml); 1.0 m*M* OHQ (2 ml); 5.0 m*M* BSI (1 ml). Method B: arginine solution (1 ml); 0.25 m*M* 1-naphthol (2 ml); 2.5 m*M* BSI (1 ml).

Table II. Chromogenicity of SAKAGUCHI-reactive compounds

Compound	No. of guanidine groups	No. of nanomoles of compound required to give colour[a]
Albumin, human	24	1.5
Salmine (molecular weight: 4000)	20	2.8
Methyl guanidine	1	11
Arginine	1	18
1-Guanidino acetate	1	36
1-Guanidino butyrate	1	36
Octopine	1	36
Streptomycin	2	40
Dicyandiamidine	1	140
Histidine	0	440

[a] Optical density of 0.100 at 510 nm, 1.5 cm given by arginine solution (2 ml), alkaline 1-naphthol solution (2 ml), bromosuccinimide solution (1 ml) at 5°C.

I find that there is no blank colour. Furthermore, at the lower 1-naphthol reagent levels the pink arginine colour is always more intense than the yellow colour given by OHQ for the same concentration of arginine, and a two- to threefold increase in the sensitivity of the original reaction may readily be achieved.

The monosubstituted guanidine compounds, methyl guanidine, 1-guanidino acetate, 1-guanidino butyrate and octopine (N-[1-carboxyethyl]arginine) are known to show a similar colour reaction to arginine[1,2]. The molar chromogenicity of these and some other compounds is indicated in Table II. Not all monosubstituted guanidine derivatives are 'SAKAGUCHI-reactive', e.g. canavanine yields no coloured product. Under optimum reaction conditions, methyl guanidine is the most chromogenic of the SAKAGUCHI-reactive compounds. It is of interest that the SAKAGUCHI products of human and bovine albumin have equal molecular extinction per guanidine group and each is equal to that produced from 1-guanidino acetate (Table II). Trace amounts of copper have no effect on the SAKAGUCHI reaction of albumins. Furthermore, mild alkaline hydrolysis (2N KOH, 1 h, 20°C) of albumin has no appreciable enhancing effect on the subsequent SAKAGUCHI colour yield, so that the reaction may yet have analytical applications in the chemistry of macromolecules that contain monosubstituted guanidines[10].

Zusammenfassung. Es werden neue Beobachtungen über die Zweckmässigkeit der SAKAGUCHI-Farbreaktion für Arginin mitgeteilt: Aminosäuren, die die Farbreaktion gewöhnlich hindern, wirken in niedriger Quantität (0,05 bis 0,2 μM) verstärkend. Histidin ergibt eine positive SAKAGUCHI-Reaktion.

D. WATSON
Biochemistry Department, Royal Columbian Hospital, New Westminster, British Columbia (Canada), September 20, 1965.

[10] These observations were made at the Regional Urological Centre, Liverpool, England, during the course of a study of blood serum amidine transferases, full details of which will be published elsewhere. The work was supported by a grant from the Department of Surgery, University of Liverpool.

Evidence for an Intracytoplasmic Membrane in the Core of Spores of *Bacillus popilliae*

Fine structural studies reveal bacterial spores to be cells of singular complexity. Particularly striking is the elaborate layering in the integument. Few published electron micrographs, however, show much structural detail in the so-called 'core', the protoplast[1] of bacterial spores. Experiments on the fine structure of *Bacillus popilliae* have recently yielded a surprisingly detailed spore core, prominent in which is a membranous element described herewith.

Spores of *Bacillus popilliae*, harvested from the hemolymph of infected larvae of Japanese beetles, were fixed

[1] C. L. HANNAY, J. biophys. biochem. Cytol. *9*, 285 (1961).

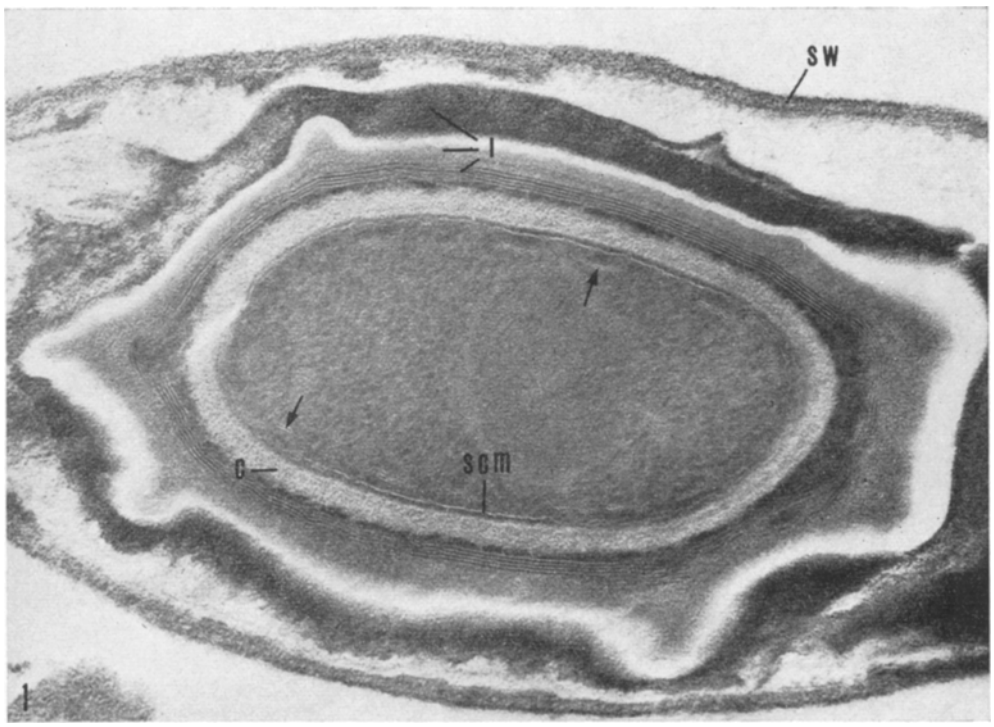

Fig. 1. Longitudinal section of a spore of *Bacillus popilliae*. Note the sporangial wall (SW) and the various layers of the spore wall (I) peripheral to the cortex (C). The core is bounded by the spore core membrane (SCM) parallel to which runs interruptedly another membrane (arrows) of comparable dimensions. × 93,500.