Table 1. TOXICITY OF ERYTHROMYCIN FOR GUINEA PIGS

Preparation	Route of administration	Daily dosage (mgm./kgm.)	Average weight of animals (gm.)	No. of days treatment	No. of animals used	No. of animals dead
Aqueous suspension tablets 'Ilotycin'	Oral	110	180	6	5	4
	,,	65	180	3	5	2
	,,	33	180	3	10	4
	,,	16	180	3	5	0
Aqueous sol. pure crystalline erythromycin	Intraperitoneal	33	180	3	5	5
'Ilotycin heptonate' saline sol.	,,	5·5*	180	1	5	2
		0·5*	180	1	5	2
		6·2*	800	1	5	4
		0·6*	800	1	5	1
,,	Intravenous	5·5*	180	1	1	1
		0·6*	180	1	1	1

* Single dose.

for guinea pigs is reported as 413·4 ± 51·7 mgm./kgm. [1]. It may be recalled that penicillin is toxic for guinea pigs, although there is little positive evidence regarding the nature of this action.

We wish to thank Dr. J. E. French for his assistance in the interpretation of the histological sections.

The erythromycin used as 'Ilotycin' in this work was supplied by Eli Lilly and Co., Ltd., the crystalline erythromycin by Abbott Laboratories, Ltd.

W. J. KAIPAINEN
S. FAINE
(Nuffield Dominions Demonstrator)

Sir William Dunn School of Pathology,
University of Oxford. July 23.

[1] Anderson, R. C., Harris, P. N., and Chen, K. K., *J. Amer. Pharm. Assoc.*, **41**, 555 (1952).

Potent Diphtheria Toxin within the Cells of *C. diphtheriae*

ALL the exotoxins except diphtheria toxin have been demonstrated within the bacterial cells in a very early stage of culture. So far as diphtheria toxin is concerned, it has been postulated, but never proved, that the exotoxin is formed within the cell. I have, however, previously deduced the possibility of temporary existence of the toxin within the cell from the morphological and biochemical characteristics of this organism[1,2]. I believe that the failure to show the presence of the toxin was due to the fact that physiologically young cells of *C. diphtheriae*, however early they might be harvested, could not be obtained when static culture was used.

By using shaking cultures, I have obtained physiologically young cells containing the highest toxicities ever found. The shaker rotated at the rate of approximately 130 cycles per min. with an amplitude of 13 cm. Each flask of 200 ml. capacity contained 20 ml. of Pope's medium. A series of flasks was sampled at the three stages of growth: logarithmic phase, that of negative acceleration, and maximum stationary phase. The bacilli harvested from each flask were washed with water, suspended in 0·01 per cent merthiolate solution and then tested for toxicities by subcutaneous or intraperitoneal injection. Toxicities of the bacilli harvested at the three stages, such as, for example, 12, 15 and 35 hr. intervals, are compared in Table 1.

It was found that both the very young and the very old bacilli contained little toxin within the cells,

Table 1

	Incubation time (hr.)	Bacterial growth (mgm. *N*/20 ml.)	*Lf*/ml. (in culture filtrate)	*MLD*/mgm. *N*
No. 1	12	7·7	0	< 25
No. 2	15	11·8	15	>300
No. 3	35	13·9	55*	< 25

* Maximum titre produced in this medium. As the toxin titre in the filtrate already reached to 50 *Lf* at the 24th hour, almost all the toxin produced within the cell is thought to be excreted outside in 24 hr.

Table 2

MLD/gm. (dry weight)	Authors
75	Prigge, R. (ref. 3)
1	Eisler, M. (ref. 4)
21,300*	(in this report)

* Cell suspension of *C. diphtheriae* corresponding to 1 mgm. nitrogen was dried at 60° C., measured for its weight, and proved to be 14 mgm.; *MLD* 300 in Table 1 was multiplied by 1,000/14.

and the toxin content of the bacilli was highest in the period of decelerating rate of multiplication. This result was different from that for other exotoxins, because the bacilli harvested in the logarithmic phase, for example, at the 12th hour (see Table 1), showed remarkably lower toxicity than in the period of decelerating growth-rate. About 200–350 *MLD* per mgm. nitrogen was usually obtained from the bacilli during the period of decreasing growth-rate, but once the value of 750 *MLD* per mgm. nitrogen was obtained.

It is inevitable that the toxin content of the bacilli in this period is variable because toxin production and excretion occur together for such a short time. My titre is compared with the highest toxicities reported by other authors in Table 2. The toxicity I obtained was identified by the findings at adrenal glands of injected guinea pigs and by the neutralization test by diphtheria antitoxin. Thus, this work shows that diphtheria toxin is formed within the cell.

I thank Prof. T. Tani for his encouragement and guidance.

SHOKI NISHIDA

Microbiological Department,
Faculty of Medicine, Kanazawa University,
Kanazawa, Japan. July 29.

[1] Nishida, S., *Nissin Igaku Zassi*, **39**, 552 (1952); **40**, 277 (1953) (in Japanese).
[2] Nishida, S., *Jap. J. Med. Sci. Biol.* (in the press).
[3] Prigge, R., *Z. Immunit. Forsh.*, **77**, 421 (1932).
[4] Eisler, M., *Z. Immunit. Forsh.*, **56**, 209 (1928).

Growth- and Inhibiting-Substances in Relation to the Rest Period of the Potato Tuber

IN an investigation of the growth- and inhibiting-substances in the potato tuber in relation to its rest period, Hemberg[1] has shown that potato peelings contain an acid and a neutral growth-substance in addition to acid and neutral growth-inhibiting-substances. On the basis of molecular weight determinations by diffusion through agar, and sensitivity towards acid and alkali, he concluded that the acid growth-substance is in all likelihood indole-3-acetic acid. In addition, he was able to show that the neutral growth-substance could be converted to the acid growth-substance by enzyme action, and concluded that the former is probably indole-3-acetaldehyde. The disappearance of the acid growth-inhibiting