data indicate that plasma phospholipids turnover is also retarded.

After these experiments were completed, a paper from STEIN and STEIN[13] showed direct experimental evidence that microsomal phospholipase activity is depressed in the rat liver after phenobarbital treatment, confirming the data of turnover rate of HOLTZMAN[5] and our own results, in the sense of a decrease in the phospholipid catabolism[14].

Résumé. Après administration de phénobarbital chez le rat le renouvellement des phospholipides microsomiaux étudiés par double marquage ^{14}C-^{32}P n'est pas augmenté alors que celui des phospholipides plasmatiques est ralenti. L'hypertrophie des membranes endoplasmiques n'est pas le résultat d'une augmentation de leur biosynthèse et semble être la conséquence d'un ralentissement du catabolisme.

R. INFANTE, D. PETIT,
J. POLONOVSKI and J. CAROLI

Unité n° 9 de l'I.N.S.E.R.M. and Laboratoire de Biochimie, Faculté de Médecine Saint-Antoine, Paris XIIe (France), 18 December 1970.

[13] Y. STEIN and O. STEIN, Israel J. med. Sci. *5*, 985 (1969).

[14] This work has been subsidized for the purchase of radiochemicals by a grant from the Commissariat à l'Energie Atomique (France).

[15] Acknowledgments. Dr. SCHWARZMANN and Miss N. BERTHAUX have kindly made the enzymatic determination. Misses C. REY and D. CATALA have skilfully contributed to this work.

Mechanism of Action of Boseimycin

Boseimycin[1,2], a new streptothricin-like antibiotic, was isolated from culture broth of an unidentified *Streptomyces* sp Ac_6 569. It is effective against a number of gram positive and gram negative bacteria, fungi and yeast in vitro. The mechanism of action of boseimycin, investigated with *Bacillus subtilis*, is reported here.

Experimental. A sterile aqueous solution of boseimycin hydrochloride was obtained by membrane filtration. Nutrient medium containing bacto peptone, 5 g/l; beef extract (Difco) 10 g/l and NaCl, 5 g/l in distilled water was used in all growth experiments excepting those with radioactive precursors, when a semisynthetic medium containing peptone, 2.5 g/l; NaCl, 5 g/l and glucose 5 g/l in distilled water was used. The pH of the media was maintained at 7.4 before sterilisation.

For growth studies, the culture grown overnight was diluted 20-fold with fresh medium. Liquid cultures were incubated at 37 °C by agitation. The cells were harvested in logarithmic growth phase (turbidity, 0.5 at 600 nm), centrifuged below 4 °C, washed twice with 0.05 *M* phosphate buffer and resuspended in liquid medium. The cells were incubated for 90 min in the medium prior to antibiotic addition. Absorbancy was measured at 600 nm with a Bosch and Lomb Spectronic colorimeter. Viable cell counts were obtained by use of a spreading plate method on nutrient agar.

Nucleic acids and protein syntheses were determined on 10 ml aliquots removed at required intervals. DNA, RNA and protein fractions were obtained after a modified SCHMIDT-THANHAUSSER[3] method and estimated colorimetrically by standard diphenylamine[4], orcinol[5] and LOWRY's[6] methods respectively.

Cells at early logarithmic phase were used for the incorporation of ^{14}C-leucine and carrier free ^{32}P-phosphoric acid as precursors for the syntheses of cellular protein and nucleic acids. Aliquots were taken at intervals and the reaction was stopped with ice-cold 5% perchloric acid. The protein and nucleic acids were fractionated as above and the rate of incorporation was expressed as cpm per ml of the suspended culture.

Results and discussion. The effect of boseimycin on growth and macromolecular syntheses of *B. subtilis* are shown in Figures 1 and 2. In the presence of 0.2 µg/ml of the antibiotic, the turbidity of the culture continued to increase but at a slightly lower rate than that of the control. Macromolecular syntheses paralleled to the growth of bacteria. On the other hand, an immediate cessation of bacterial growth was observed after the addition of the antibiotic at 2 µg/ml. Protein synthesis was concurrently inhibited, which could be correlated with the growth inhibition. DNA and RNA syntheses were levelled off comparatively at a later stage. Similar results were observed in another set of experiments where

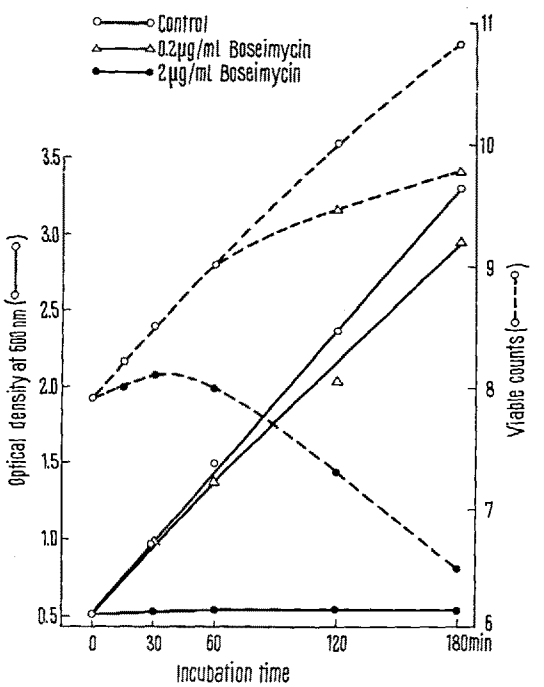

Fig. 1. Effect of boseimycin on the growth of *B. subtilis*. Boseimycin was added after 90 min of incubation under agitation. The dotted and solid lines represent changes in the viable cell counts and optical density respectively.

[1] R. K. SINHA and P. N. NANDI, Experientia *24*, 795 (1968).

[2] R. K. SINHA, J. Antibiotics, Tokyo *23*, 360 (1970).

[3] G. SCHMIDT and S. J. THANHAUSSER, J. biol. Chem. *161*, 83 (1945).

[4] K. BURTON, Biochem. J. *62*, 315 (1956).

[5] G. ASHWELL, *Methods in Enzymology* (Academic Press Inc., New York 1957), vol. 3, p. 87.

[6] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

Table I. Effect of boseimycin on the incorporation of ^{14}C-leucine into cellular protein of *B. subtilis*

Boseimycin (μg/ml)	15 min		30 min		60 min		120 min		180 min	
	cpm/ml	% I[a]	cpm/ml	% I[a]	cpm/ml	% I[a]	cpm/ml	% I[a]	cpm/ml	% I[a]
0	55	–	140	–	262	–	465	–	787	–
0.2	55	0	141	0	258	0	426	8	577	27
2.0	25	55	39	71	39	85	39	91	39	95

[a] % I, % inhibition.

Table II. Effect of boseimycin on the incorporation of ^{32}P-phosphoric acid into the cellular components of *B. subtilis*

Incubation time (min)	Boseimycin (μg/ml)	Acid soluble		RNA		DNA	
		cpm/ml	% I[a]	cpm/ml	% I[a]	cpm/ml	% I[a]
15	0	101	100	600	100	65	100
	0.2	99	98	588	98	64	98
	2.0	84	83	151	25	65	100
30	0	256	100	1154	100	151	100
	0.2	240	94	1127	98	150	100
	2.0	168	65	253	22	101	66
60	0	684	100	2301	100	300	100
	0.2	681	99	1813	79	296	99
	2.0	216	32	352	15	105	35
120	0	1766	100	4308	100	608	100
	0.2	1610	91	3516	81	606	100
	2.0	268	15	554	12	104	17

[a] % I, % incorporation.

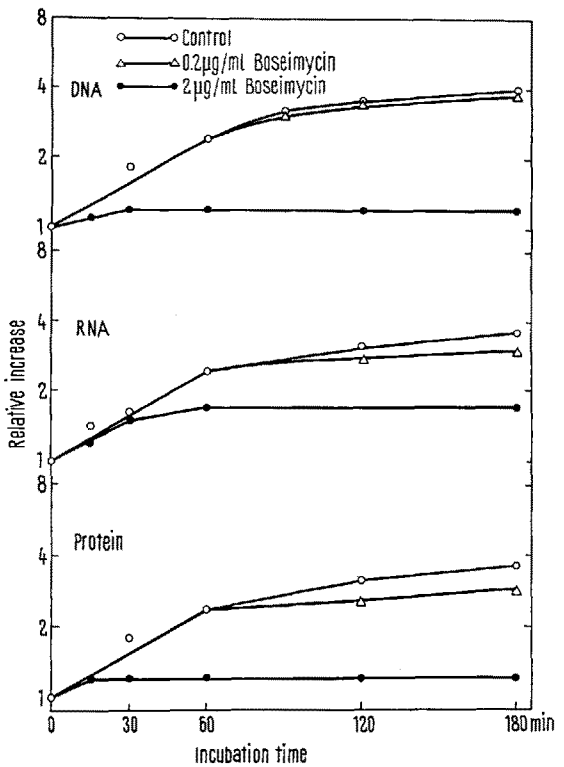

Fig. 2. Effect of boseimycin on the syntheses of protein and nucleic acids in *B. subtilis*. The values are expressed as relative increase per unit volume of culture to the initial amounts.

the effect of boseimycin was tested on the incorporation of ^{14}C-leucine and ^{32}P-phosphoric acid into protein, DNA and RNA fractions in *B. subtilis*. A comparison of the results given in Tables I and II indicates that the minimum inhibitory concentration level (2.0 μg/ml) affects more severely the assimilation of amino acid than that of phosphate.

The antibiotic was found to cause no major damage to the bacterial cell wall or cell membrane (details of the report will be published elsewhere). The growth kinetics of boseimycin treated cells (5 μg/ml of boseimycin for 30 min) returns to normal when the antibiotic was removed by washing the cells with phosphate buffer (0.05 *M*, pH 7.2).

The primary effect of boseimycin on bacteria appears to be directed on a site or sites closely associated with the protein synthesis. Formation of DNA and RNA are appreciably affected after 30 to 60 min of incubation. However, the apparent synthesis of RNA in the early phase in absence of protein synthesis may be due to accumulation of m-RNA owing to its slower rate of degradation or a stimulation of ribosomal RNA synthesis in antibiotic treated cells since the ratio of amino-acyl s-RNA/s-RNA is increased as a result of inhibition of protein synthesis. The above ratio controls ribosomal RNA synthesis in bacteria was proposed by KURLAND

[7] C. G. KURLAND and O. MAALOE, J. molec. Biol. *4*, 193 (1962).
[8] F. GROS, J. M. DUBERT, A. TISSIERES, S. BOURGEOIS, M. MICHELSON, R. SOFFER and L. LEGAULT, Cold Spring Harb. Symp. quant. Biol. *28*, 299 (1963).

and MAALOE[7] and is supported by the findings of GROSS et al.[8] in a cell-free system.

The growth rate, protein and RNA syntheses of *B. subtilis*, exposed to boseimycin (0.2 µg/ml), were affected keeping DNA synthesis unimpaired even during 3 h of treatment. It is suggested that the drug affects the translational level of the protein synthesizing system. The normal growth of the bacteria restored immediately after the removal of the drug suggests only a transient inhibition of macromolecular synthesis rather than any irreparable damage caused to cellular components.

Zusammenfassung. Unmittelbar nach Zugabe von Boseimycin zu *B. subtilis* werden Wachstum und Proteinsynthese verhindert. DNA und RNA werden erst später beeinflusst. Die hemmende Wirkung von Boseimycin auf die behandelten Zellen ist nach Waschung mit Phosphatpuffer reversibel.

T. K. MISRA and R. K. SINHA[9]

Department of Microbiology, Bose Institute, Calcutta 9 (India), 7 August 1970.

[9] Present address: Department of Molecular Biology and Microbiology, School of Medicine, Tufts University, Boston (Massachusetts 02111, USA).

Widespread Occurrence of Inhibitors of Melanoma Tyrosinase in Plant and Mammalian Tissues

Inhibitors of tyrosinase have been detected in melanotic melanomas[1–5]. Partial purification of some of these inhibitors has been reported[4]. These studies have implied that these inhibitory factors have a limited distribution being rather specific for melanoma. They have also been considered to have a role in regulating melanogenesis. It was thought of interest to study the natural distribution of inhibitors of tyrosinase to see whether they are specific to melanoma tissue.

Materials and methods. B16 melanoma was transplanted in C57 BL/6J mice as previously described[6]. Cloudman S91 melanoma obtained from Jackson Laboratories, Bar Harbor, was found to be mostly amelanotic. This tumour was maintained by transplantation into DBA/1J mice.

Tyrosinase activity was determined as previously described[7]. The major source of tyrosinase for these experiments were homogenates (15% prepared in water) of previously frozen B16 melanoma. Serum from mice bearing B16 melanoma and an extract of mushroom was also used. The tissue extracts were homogenates (90% prepared in water).

Results. Table I shows the effect of extracts from a variety of tissues on tyrosinase activity. It can be seen that homogenates of liver, kidney, spleen and brain from rats and mice inhibited the tyrosinase activity. This inhibitory effect varied from 20 to 70% depending on the tissues. Heated extract of mushroom also decreased the tyrosinase activity. Extract from an amelanotic Cloudman S91 melanoma also inhibited the tyrosinase activity. High concentrations of the tissue extracts produced increasingly greater inhibition. Human sera and similarly prepared extracts from human spleen, liver, skin, kidney and breast gave similar results.

Table II shows some of the properties of the inhibitory factor(s) in rat liver. Either heating at 100 °C for 15 min or dialysis partially abolished the inhibitory effect, whereas when the extract was dialyzed and then heated, the inhibitory effect was absent. Similar results were also obtained with the following tissues – liver, kidney, spleen and brain from mice and rats and Cloudman S91 melanoma. These tissues were also found to inhibit tyrosinase activity of purified mushroom tyrosinase (Sigma Chemical Laboratory) and of serum from mice bearing B16 melanoma.

Discussion. The above results demonstrate the widespread distribution of inhibitor(s) of tyrosinase in a variety of sources. That there is more than one inhibitory factor is shown by the observation that either heating or dialysis alone only partially decreases the inhibitory effect, whereas the combination of dialysis and heating almost completely abolishes any inhibitory effect.

Table I. Effects of extracts from various tissues on tyrosinase activity of B16 melanoma homogenate

Experiment No.	Animal	Tissue	Tyrosinase activity[a]	Decrease (%)
1	–	None	18.2	–
	Rat	Liver	7.2	60
	Rat	Kidney	7.2	60
	Rat	Spleen	11.2	39
	Rat	Brain	6.8	62
2	–	None	15.2	–
	Mouse	S91 melanoma	9.8	35
4	–	None	3.9	–
	Mouse	Liver	2.6	33
	Mouse	Kidney	1.2	69
	Mouse	Spleen	2.9	25
	Mouse	Brain	1.3	33
5	–	None	11.2	–
	Mouse	Serum	6.8	39
6	–	None	19.6	–
	Mushroom	Shoot	14.6	25
7	–	None	15.2	36
	Mouse	Cloudman S91 melanoma	9.8	

[a] nmoles of tyrosine oxidized.

1 M. COOPER and Y. MISHIMA, Nature, Lond. *216*, 189 (1967).
2 E. DERNALOWICZ, J. TROJANOWSKI, A. BOMIRSKI and T. DOMINICZAK, Nature, Lond. *215*, 188 (1967).
3 G. J. Z. SATOH and Y. MISHIMA, J. Invest. Dermat. *48*, 301 (1967).
4 G. F. WILGRAM, P. LYNCH, J. A. MABEL and D. A. EVANS, Fedn Proc. *29*, abst. 413 (1970).
5 S. SILAGI, Abstracts of the VIIth International Pigment Cell Conference, Seattle 1969, p. 23.
6 I. A. MENON and H. F. HABERMAN, Cancer Res. *28*, 1237 (1968).
7 I. A. MENON and H. F. HABERMAN, Arch. Biochem. Biophys. *137*, 231 (1970).