

effective, yielding in comparable order and concentration 76, 51 and 27 per cent moult.

As summarized by Gilmour<sup>8</sup>, most insects require choline for growth. We conclude that acetylcholine or possibly other choline salts must be added in sufficient amount to semisynthetic basal diets for satisfactory growth. Insufficient levels are evidenced by inability to moult. By the addition of sufficient acetylcholine we have succeeded in rearing silkworms on an artificial diet from hatching to spinning without mulberry leaves or their extracts.

We thank Dr. K. Naito, Miss K. Matsuura, Mr. J. Nishida and Miss M. Kumazawa for their assistance, and Dr. M. Kamata (Takeda Chemical Industry Co.) for his advice. This work was supported by a grant from the Ministry of Education.

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<sup>1</sup> Fukuda, T., Suto, M., and Higuchi, Y. L., *Nature*, **187**, 669 (1960).

<sup>2</sup> Ito, T., *J. Sericult. Sci. Japan (Nippon Sanshigaku Zasshi)*, **31**, 1 (1962).

<sup>3</sup> Hayashiya, K., Naito, K., Matsuura, K., Nishida, J., and Hamamura, Y., *J. Agr. Soc. Japan (Nippon Noeikagaku Zasshi)* in the press.

<sup>4</sup> Ito, T., *J. Jap. Soc. Food and Nutrition (Eiyo to Syokuryo)*, **14**, 1 (1961).

<sup>5</sup> Henscher, D., *Naturwissenschaften*, **6**, 41 (1954).

<sup>6</sup> Watanabe, T., *J. Pharm. Soc. Japan (Yakugaku Zasshi)*, **75**, 86 (1955).

<sup>7</sup> Hamamura, Y., Hayashiya, K., and Naito, K., *Nature*, **190**, 879 (1961).

<sup>8</sup> Gilmour, D., *Biochemistry of Insects* (Academic Press: New York and London, 1961).

## MICROBIOLOGY

### Stimulation of Transformation by Thalidomide

At present congenital abnormalities caused by thalidomide are engaging the attention of a number of laboratories. However, only a few papers concerning the effect of the drug on protozoa and micro-organisms have been noticed so far<sup>1-3</sup>. In some cases it is difficult to analyse the effects of thalidomide because of its high instability. In neutral and aqueous solutions thalidomide is unstable with a tendency to open the phthalimide and glutarimide rings, with hydrolysis of the amide groups of phthalimide and glutarimide<sup>3</sup>. In the work recorded here the influence of thalidomide on transformation of the indole marker in *Bacillus subtilis* was examined.

An aqueous solution of thalidomide was prepared by dissolving the drug in 0.1 N KOH and then immediately neutralizing with diluted HCl so that concentration was 100 mg/ml. Directly after its dissolution thalidomide was added to the medium to a final concentration of 200 µg/ml. One general approach was made in this investigation. An attempt was made to examine the effect of thalidomide on recipient cells using the procedure of transformation by Spizizen<sup>4</sup>. To a series of tubes containing cultures of the recipient cells thalidomide was supplemented 120, 60 and 30 min before, together with and 60 min following the addition of transforming DNA. Aliquots of the culture were taken for determinations of transformation frequency 4 h after DNA was added.

Table 1

Time of treatment with thalidomide 200 µg/ml.	No. of recipient cells per ml.	Average No. transformants per ml.	Per cent transformation
0	5 × 10 <sup>6</sup>	0.2 × 10 <sup>2</sup>	0.004
3	4 × 10 <sup>6</sup>	2.4 × 10 <sup>2</sup>	0.6
4	4 × 10 <sup>6</sup>	0.2 × 10 <sup>2</sup>	0.005
4.5	3 × 10 <sup>6</sup>	3.1 × 10 <sup>2</sup>	0.1
5	4 × 10 <sup>6</sup>	5.3 × 10 <sup>2</sup>	0.066
6	8 × 10 <sup>6</sup>	5.5 × 10 <sup>2</sup>	1.37

Similar results were obtained in 4 repetitions of the foregoing experiment. Whereas in control tubes the frequency of transformation ranged from 0.002 to 0.005 per cent, in some aliquots with thalidomide it was higher than 2 per cent. To obtain a high percentage of transformants, it was found essential to supplement the drug 2 h before or 1 h following the addition of DNA to the recipient culture. The mechanism of the thalidomide effect is still obscure. It is not related to the mutagenic action of the drug. Significant differences in the number of transformants depending on the time of the drug addition suggest that the promotion of the transformation is not connected with the selection process either as a nutritional effect of thalidomide or its breakdown products. It may be assumed that the stimulation of the transformation is related to the specific effect of thalidomide on the competence of the recipient cells. Moreover, we have observed in our preliminary investigations the stimulating effect of thalidomide on the frequency of the colicinogenic factor transfer.

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<sup>1</sup> Frank, O., Baker, H., Ziffer, H., Aaronson, S., Hutner, S. H., and Leevy, C. M., *Science*, **139**, 110 (1963).

<sup>2</sup> Nyström, C., *Scand. J. Clin. Lab. Invest.*, **151**, 102 (1963).

<sup>3</sup> Frank, O., Baker, S., Hutner, S. H., and Sobotka, H., *Proc. Soc. Exp. Biol. and Med.*, **114**, 326 (1963).

<sup>4</sup> Spizizen, J., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1072 (1958).

### Conversion of DDT to DDD by *Proteus vulgaris*, a Bacterium isolated from the Intestinal Flora of a Mouse

THE dechlorination of 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) to 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethane (DDD) by various animals has been reported recently<sup>1,2</sup>. The same detoxification reaction appears to be carried out by yeasts<sup>3</sup>. DDT administered intraperitoneally to mice was dechlorinated after, but not before, the animals were killed<sup>4</sup>. We undertook to test the hypothesis that the degradation of DDT to DDD in animals is due, at least in part, to the activity of the microflora of the gut.

Several types of micro-organism were isolated from the gut of a DDT-resistant female mouse<sup>5</sup> by serial dilution and streakplate methods on agar-brain-heart-infusion media. An ethanol solution of pure *p,p'*-DDT was placed in sterile test-tubes and evaporated over a hot-water bath. Sterile brain-heart infusion medium was then introduced into the cooled test-tubes. These preparations were inoculated with several isolates obtained from the mouse gut and the cultures were incubated for 5 days at 30° C. The contents of the tubes were extracted with methanol and chloroform<sup>6</sup>. The mixture was permitted to stand long enough to give a very clear chloroform layer. The extract was sufficiently clean to produce very clearly defined spots on paper chromatography. Paper chromatographic analysis was performed quantitatively for DDT on these extracts<sup>7</sup>.

One isolate, which was identified by morphological and biochemical tests<sup>8,9</sup> as *Proteus vulgaris*, was thus shown to dechlorinate DDT to DDD.