

Resin	Aniline	CHCl <sub>3</sub>	React. time.	React. temp.	Product
2.7g.	11g.	20g.	2½ hr.	b. p. of CHCl <sub>3</sub>	3.6g.

According to the above details, light yellow and brittle resin was formed. Theoretical value of N contents calculated from the weight which increased in the product was 6.11%, and analytical value was 6.14%. From this result, it is apparent that 63% of Cl was replaced by aniline.

## 17. Studies on Cobalt Metabolism of *Bacillus subtilis* by Using Co<sup>60</sup>

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Among many microbes, *Streptomyces griseus* and *Bacillus subtilis* are reported to be the good producers of vitamin B<sub>12</sub>. Microorganisms are expected that they absorb cobaltous ion from the culture media and synthesize this vitamin. These investigations were undertaken with the intention of explaining physiological functions of vitamin B<sub>12</sub> and other cobalt containing substances for microorganisms.

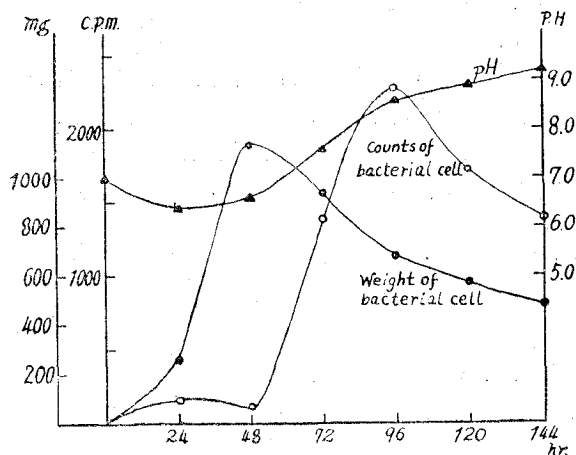


Fig. 1. Absorption of Co<sup>60</sup> by *bacillus subtilis* (in 250 ml. medium).

The chemical constituents of *bacillus subtilis*, grown on Waksman's media containing 2 p. p. m. radioactive Co<sup>60</sup>, and of the remaining media were precisely analysed during the growth. Total cobalt content in the unit weight of bacterial cells was found to increase with the growth. Co<sup>60</sup> in the 75 % alcoholic extract, however, showed the maximum after 48 hr. of inoculation. The amount of vitamin B<sub>12</sub> in bacterial cells and remaining media were also estimated by means of colorimetric determination of cyanide in cyanoco-

cyanocobalamin. The following facts were observed. The content of cyanocobalamin increased in the bacterial cells at their growing stage and was excreted into the media when autolysis began to take place. The autolysed cells, however, was found to contain still much  $\text{Co}^{60}$ . This  $\text{Co}^{60}$  was designated to be inorganic by means of chemical method.

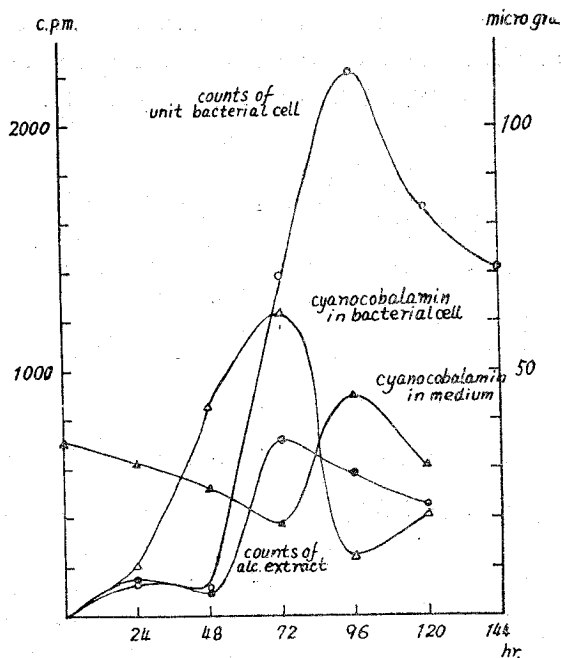


Fig. 2. Amounts of cyanocobalamin (in 250 ml. medium).

## 18. Relation between Analgesic Effect of Drugs and Chemical Structures

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In the previous report it was mentioned that  $(\text{CH}_3)_2\text{N}$ -radical on the side chain seemed to play more important role than  $(\text{C}_2\text{H}_5)_2\text{N}$ -radical in potentiating the analgesic action of morphine (This Bulletin, 36-49, 25, 1951).

This experiment was carried out in order to estimate the analgesic action of various drugs using Haffner's method in mice and Hardy's radiant heat method in man.

Analgesic actions of N-dimethylsalicylamide (I), N-dimethylphenetidine (II), dimethylaminoacetophenetidine (III), 4-dimethylaminoacetaminoantipyrine (IV), N-dimethylaminoethylphenothiazine (V), and dimethylaminoethyldiphenylglyco-