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Study of the Effect of Chloramphenicol on Photochemical Formation of Self-Sustaining Coacervates in Presence of Low Concentration of Biological Minerals

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ABSTRACT. The number of self-sustaining particles formed in sterilised aqueous mixture, containing ammonium molybdate, diammonium hydrogen phosphate and formaldehyde in presence of small concentration of biological minerals, increase on exposure to sunlight, if a small concentration of chloramphenicol is added in the mixture. On prolonged exposure, the number of particles does not increase in the mixture which has high concentration of chloramphenicol and the inhibition in the formation of particles is proportional to the concentration of chloramphenicol in the irradiated mixture.

Thorough investigations of the origin of life have been undertaken during the last two decades based upon the theories of chemical evolution suggested by Oparin (1) and Haldane (2). The underlying idea is that the first molecule which formed the earliest living systems came about by a process of molecular evolution to form the earliest living system. A lot of data has been collected during this period to suggest the natural processes under which the biochemicals forming the earliest living cells are synthesised. Reviews on abiogenesis have appeared (3,4).

Another important step in the investigation of the processes of "life synthesis" is the organisation of specific molecular associations, which show the properties of biological order. In this field the work on microspheres by Fox (5) and coacervates by Oparin (6), concerns the structures which could be synthesised under natural specific conditions.

The work on 'Jeewanu' reported in 1963 by Bahadur et. al. (7,8,9,10) describes the formation of microstructures from sterilised aqueous mixtures of formaldehyde, ammoniacal nitrogen and biological minerals, on exposure to light. The presence of various biochemicals in these mixtures have been reported (3,11). These particles have a boundary wall and distinct internal structures. The particles consist of a number of amino acids in free and combined form, nucleic acid bases such as adenine, guanine, cytosine, uracil and thymine, sugars such as ribose, deoxyribose glucose, fructose, a number of organic acids and material with enzyme-like activity. The work has been confirmed in a number of laboratories (11,12).

In 1970 Bahadur and Ranganayaki synthesised self-sustaining coacervates by exposing sterilized aqueous mixtures containing ammonium molybdate, diammonium hydrogen phosphate, biological minerals consisting of sodium chloride, potassium sulphate, magnesium sulphate, calcium acetate and potassium dihydrogen phosphate and formaldehyde to sunlight or artificial light (13). These particles have a boundary wall and internal structures and a number of biochemicals as amino acid in free and combined state, sugars, nucleic acid bases and enzyme-like materials. They "grow" from within, multiply by budding and have metabolic activities. The boundary wall is composed of phospholipids (14). The particles have been fixed with biological fixatives and stained with a number of biological dyes (15).

As these particles appeared to show a number of life-like properties, it was of interest to observe the effect of an antibiotic, viz. chloramphenicol when mixed before exposure to radiation, on the formation of these microstructures. It has already been shown that these particles are antibiotic sensitive (16). Tetracycline inhibits the formation of these particles (17). Although a high concentration of antibiotic in the irradiated mixture inhibits the formation of particles, smaller concentrations act as activators.

EXPERIMENTAL

Aqueous solutions of ammonium molybdate 4% (W/V) and diammonium hydrogen phosphate 3% (W/V) were prepared. The mineral solution was made up by dissolving 20 mg each of sodium chloride, potassium sulphate, magnesium sulphate, calcium acetate and potassium dihydrogen phosphate in 100 ml distilled water. 2 ml of 36% formaldehyde solution was used in each mixture. Chloramphenicol solution was prepared by dissolving 50 mg of chloramphenicol in 5 ml distilled water.

Into each of six test tubes, 2 ml of ammonium molybdate solution, 4 ml of diammonium hydrogen phosphate solution and 2 ml mineral solution were added. The tubes were cotton wool plugged and sterilised in an autoclave at 15 lb pressure for 30 minutes. The test tubes were cooled to room temperature and then 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of chloramphenicol solution were added to five of the test tubes. The sixth one was left as a control. The total volume of each mixture was made up to 9.0 ml by adding sterilized distilled water aseptically followed by 2 ml of formaldehyde solution. The test tubes were shaken gently and the mixtures were then exposed to sunlight.

The mixtures became blue when exposed to sunlight and turbidity developed shortly thereafter. The turbidity increased with time and a large number of microstructures formed in the mixture.

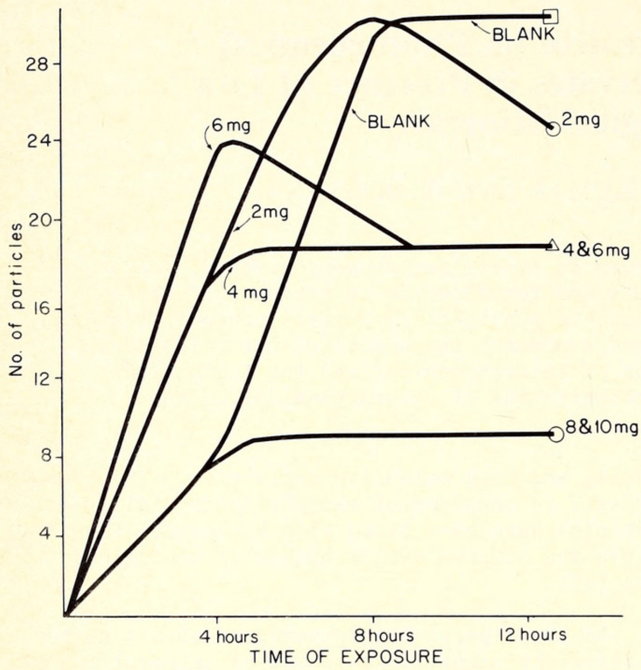

Figure 1. Effect of different concentration of Chloramphenicol on the formation of self-sustaining coacervates.

COUNTING OF PARTICLES

After exposure for four hours to sunlight all the mixtures were stored and the particles were examined microscopically. The particles were counted with a haemocytometer. Four slides of each mixture were prepared by taking one drop of the mixture aseptically from the test tube by glass rod. The counting of the particles are done under oil immersion microscope at 1,000 magnif-ication. The number of particles in 10 different views at different places on the slide were counted within a specific area as marked in the eye piece. Thus, 40 counts in four slides for each mixture were taken. The counting of the particles formed in each mixture was performed after each four hours of sunlight exposure of each day.

OBSERVATIONS.

The results are shown in Table 1.

RESULTS

There was an increase in the number of part-icles after 4 hours exposure in the mixtures which had 2, 4 and 6 mg of chloramphenicol. In the mix-ture which had 2 mg of chloramphenicol, this in-crease continued and became comparable with the number of particles in the control mixture, but the number of particles did not increase on further exposure in the mixture which contained 4 mg of chloramphenicol. The number of particles in the mixture which had 6 mg of chloramphenicol was maximal at 4 hours exposure, but subsequently

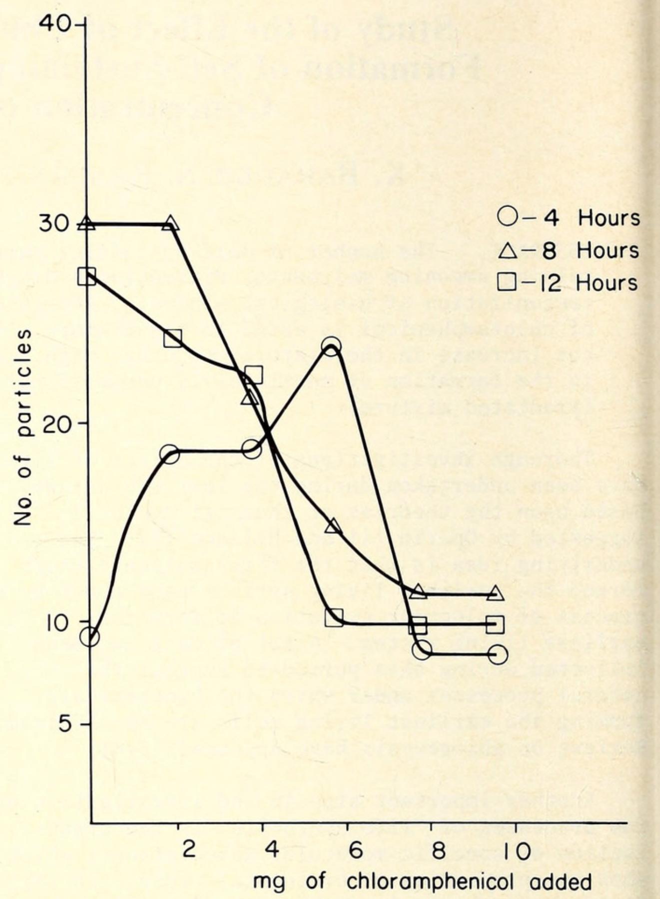

Figure 2. Number of particles as observed after 4, 8 and 12 hours of exposure.

the number of particles decreased rapidly (Figures 1 and 2). In the mixture containing high con-centrations of chloramphenicol, i.e., 8 mg or 10 mg, the number of particles is the same as in the control during the first 4 hours of exposure. After this there was no significant increase in the number of particles with further exposure.

DISCUSSION

Thus, it has been observed that low concen-tration (i.e. up to 6 mg) of chloramphenicol in the mixtures which form self-sustaining coacer-vates on irradiation to sunlight increases the formation of these particles while larger con-centrations (8 mg - 10 mg) do not affect the formation of particles during the first 4 hours of exposure. On further exposure, the number of particles became equal to the control in the mix-ture which had just traces (2 mg) of chloram-phenicol. In the mixture having higher concen-trations of antibiotic, the number of particles did not increase and was much less than the con-trol.

TABLE 1

EFFECTS OF CHLORAMPHENICOL CONCENTRATION AND TIME OF EXPOSURE ON NUMBER OF PARTICLES

Reaction mixtures: 2 ml 4% ammonium molybdate solution + 4 ml 3% diammonium hydrogen phosphate solution + 2 ml mineral salts solution + 0.2 - 1.0 ml 1% chloramphenicol solution + distilled water to 9 ml total volume + 2 ml 36% formaldehyde solution.

Weight for chlor-amphenical in reaction mixture (mgs)	Number of Particles at different exposure times		
	4 hours	8 hours	12 hours
0	9.3 ± 1.08	30.35 ± 2.03	27.8 ± 1.13
2	18.9 ± 1.02	31.95 ± 1.9	24.6 ± 1.4
4	17.1 ± 2.4	21.3 ± 1.69	22.6 ± 2.1
6	24.3 ± 2.08	15.2 ± 1.8	10.3 ± 1.0
8	10.6 ± 1.4	13.6 ± 1.1	13.1 ± 0.8
10	13.9 ± 1.04	16.1 ± 1.8	16.2 ± 3.5

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