The same technique of tissue culture was used for the cultures of *A. ægypti* cells. The growth pattern of these cells was different from that of *A. albopictus*. Three days after the cultures were set up, small hollow vesicles developed at the cut ends of the tissue fragments and continued to increase in size and number (Fig. 4). These vesicles appeared to consist of monolayers of epithelium like cells. Very few cells were found attached to the glass surface and therefore very little growth on the glass wall of the containers was observed. Eight to ten days after the cultures were set up, the floating tissue fragments with hollow vesicles were removed and cut into small pieces and seeded into new bottles. Within two days a large proportion of the cell masses attached themselves to the glass wall but the floating tissue fragments again developed hollow vesicles. Over the next week the numbers of the cells sticking to the glass as well as those of the hollow vesicles increased. Two weeks after the seeding of the cultures the floating tissue fragments with hollow vesicles were removed and the first subculture of the attached cells was made. Subsequent cultures were made at intervals of one to two weeks depending upon the growth of the cells. The cells attached themselves to the glass immediately and were found proliferating within 24 hours.

Four such cultures of cells of *A. ægypti* larvæ were set up. One was lost due to contamination in the 11th passage and another in the 7th passage. Of the remaining, one is in the 15th passage and the other in the 4th passage. These cultures mainly consisted of an epithelial type of cells (Fig. 5) and even after many subcultures still have a tendency to form hollow vesicles and occasionally tube-like structures.

Many mitotic figures, some showing diploidy or polyploidy, were seen in cultures of both species (Figs. 3 and 6).

These established cell cultures of *A. albopictus* and *A. ægypti* can be maintained with 10% fetal bovine serum in the medium. The cultures are now being adapted to medium 199 and minimum essential medium (Eagle) with 10% fetal bovine serum. A few subcultures have been made withiut any apparent ill-effect on the cells. Further studies on the growth of these cell cultures in different media and their susceptibility to different viruses are in progress.

I thank Drs. T. Ramachandra Rao and C. R. Anderson for their advice. The technical assistance of Mr. U. K. Murty Bhat is acknowledged.

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MANGANESE IN BIOLOGICAL SAMPLES*

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DETERMINATION of manganese by conversion of Mn into MnO_4^- has been known since 1845.[1] Of the many oxidising agents tested for this reaction,[2-4] potassium periodate[5,6] and ammonium persulphate[7-9] are more commonly used with biological samples, however, with varying success. The sensitivity with potassium periodate is reported to be 2 to 7 μg. Mn/ml. and with ammonium persulphate 1 to 2 μg. Mn/ml. The reaction with potassium periodate in strongly acid solution is said to be autocatalytic and more reliable; however, the reaction time is about 30 minutes and the colour is unstable for lower Mn levels. Reaction with ammonium persulphate is considered erratic,[5] and particularly unsuitable for soil samples,[10] but is recommended by others as satisfactory and more economical.[8,11] Discrepancies in values of Mn have been reported with all the oxidising agents tried hitherto on account of interfering substances such as chlorides, iron and titanium, as well as due to acidity, organic impurities, and lack of sufficient oxidiser for stabilising the reaction.[2] Preference for any oxidising agent seems to be based on the degree

* Memoir No. 41 from Centre for Advanced Studies in Botany.

of control of interfering factors as well as the ease with which the final reaction could be carried out. Obviously, more critical attention is indicated in the preparation of samples for the dependability and uniformity of the colorimetric reaction. The procedure tested in this laboratory and found to be critically reproducible with an improved sensitivity of 0·1 μg. Mn/ml. for a wide range of biological samples is reported here.

Reagents.—Analytical grades of concentrated sulphuric acid, nitric acid, perchloric acid, orthophosphoric acid, 0·15% aqueous silver nitrate solution, and 20% aqueous ammonium persulphate solution.

Glass distilled water redistilled with potassium permanganate and a little potassium hydroxide was used throughout.

Standard Series.—50 mg. of electrolytically purified manganese metal was dissolved with minimum quantity of concentrated nitric acid. The flask was warmed carefully to expel the nitrous fumes completely, and the solution was made up to 50 ml. with redistilled water. Further, dilutions were made to give 100 μg. Mn/ml. and 10 μg. Mn/ml. respectively. Appropriate aliquots from the stock solutions were transferred to 100 ml. Pyrex conical flasks to give 5, 10, 20, 30, 40, 50, 60, 70, 80, and 100 μg. Mn. To this were added 10 ml. of redistilled water, 5 ml. of 0·15% silver nitrate solution, 2 ml. of ortho-phosphoric acid and heated to boiling prior to addition of 1 ml. of *freshly prepared* ammonium persulphate solution. The colour reaction was instant. The contents were boiled for a minute and an excess of few drops of ammonium persulphate solution was added, cooled and made up to 50 ml. Using 10 mm. quartz cells, optical density readings were taken at 525 mμ wavelength in UVISPEC spectrophotometer. Readings taken after 24 hours showed no variations. The relationship between Mn concentrations and the optical density readings was linear (Fig. 1). This was true for an extended range up to 10 μg. Mn/ml.

Preparation of Biological Samples.—Half to one gram of samples (whole or powdered) were weighed into 100 ml. Pyrex conical flasks; 15 ml. of concentrated nitric acid were added and left overnight for cold digestion. If heated directly, there was a tendency for violent reaction with the contents boiling over resulting in loss of material. After the initial cold digestion, the flasks were placed on an aluminium plate over an electric hot plate. When the brown fumes subsided, 0·5 ml. of sulphuric acid and 0·5 ml. of perchloric acid were added. It was a good precaution to add perchloric acid dropwise at short intervals. The digestion now proceeded at a rapid rate; if necessary more nitric acid was added. The contents became gradually pale yellow to colourless. The contents were evaporated to dryness, the residue dissolved in 10 ml. of redistilled water and evaporated to dryness. This was repeated once over. Finally the contents (pale yellow or colourless, and crystalline) were dissolved in 10 ml. of redistilled water while the flasks were still hot. To this were added silver nitrate solution and phosphoric acid, heated to boiling and the oxidation was effected with ammonium persulphate as for the standards. In 3 to 4 hours the samples were ready for optical density readings.

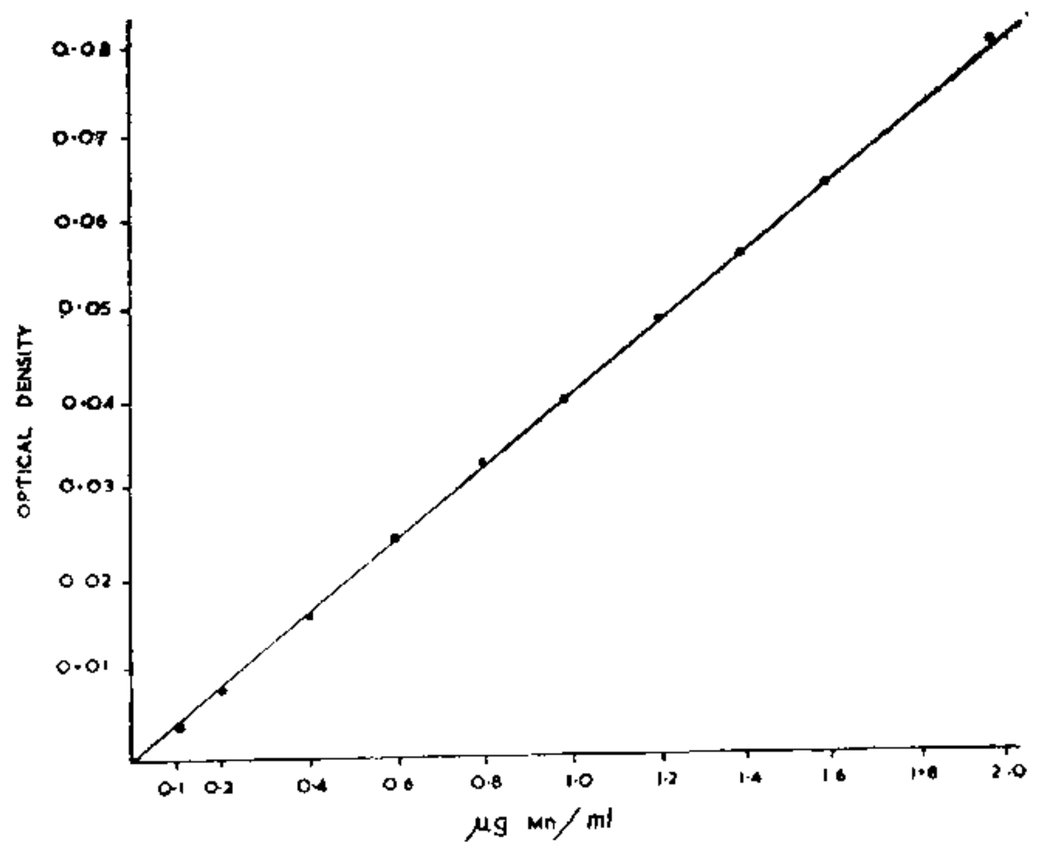

FIG. 1. Relationship between Mn concentrations and optical density readings at 525 mμ.

An acid blank was prepared alongside. No colour was obtained in the case of acid- and water blanks. Routine recovery tests were also made for quantitative checks. The experimental error was less than 2%. The colour was stable at all concentrations of Mn and in the cases of a variety of samples tested. Wet digestion was preferred in order to avoid any possible loss of material in handling; further the entire procedure was carried out in the same container. Where samples were small, lesser quantities of acids were used for digestion and the final volume was made up to 5 to 10 ml.

Standards and digestates were also oxidised with potassium periodate for comparison. Closely comparable values were obtained when the volume of sulphuric acid was carefully limited with reference to the Mn concentrations. The time taken for colour

development, however, was considerable with potassium periodate.

Total and exchangeable Mn,[12,13] in a large number of samples of *Lathyrus* field soils were satisfactorily estimated by this wet-ashing procedure followed by oxidation with ammonium persulphate. In the case of soils, however, filtration during sample preparation was necessary.

This procedure was usefully employed for Mn estimations in various crop plants lathyrism endemic areas in Central In[illegible] tissues of experimental animals fed *La* diet, and in sera, CSF fluids and fecal [illegible] of lathyrism patients.

This work was carried out during the tenure of a scheme on Lathyrism sponsored by the Indian Council of Medical Research.

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INFLUENCE OF SIMAZINE ON CHLOROPLAST METABOLISM

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A CHLOROPLAST, the site of photosynthesis, acts as energy capturing, storing and transferring device in carbon assimilation. The photosynthetic pigment, the chlorophyll and carotenoids, are concentrated within the dense lamellæ of the grana containing chloroplasts and in the lamellar chloroplasts. In addition to these pigments it consists of protein and lipid layers. It has been reported that simazine [2-chloro-4, 6-*bis* (ethylamino)-*s*-triazine] a selective herbicide inhibits the photochemical activity of isolated chloroplasts.[4-6] Ashton *et al.*[2] reported the destruction of chloroplast structure as the result of atrazine treatment. As no result is available to indicate the changes in the concentration of chlorophyll and protein of the chloroplast the present investigation was undertaken to study the influence of simazine on the chlorophyll and protein metabolism in this very important organelle of the plant.

Methods and Materials

In this investigation, seminole variety of oats (*Avena sativa* L.) which is susceptible to simazine was grown in the acid washed sand treated with 2 ppm of simazine with the use of Hoagland-Arnon complete nutrient solution. The plants were grown under controlled temperature, photoperiod and light intensity for 12 days before their desiccation.

Determination of Chlorophyll.—Total chlorophyll, chlorophyll *a* and chlorophyll *b* of simazine treated and control plants were determined by the method of Association of Official Agricultural Chemists.[3] In all four samples in three replications were collected at two-day intervals starting six days after the treatment. The following equations were used to determine these chlorophylls:

Total chlorophyll (mgm per litre) = 7·12 A 652 + 16·8 A 634·5

Chlorophyll *a* (mgm per litre) = 9·93 A 652 − 0·777 A 634·5

Chlorophyll *b* (mgm per litre) = 17·6 A 634·5 − 2·81 A 652.

where A = Absorbance.

Estimation of Chlorplast Protein.—Triplicate samples of plants from simazine and control treatments were collected at two-day intervals starting six days after treatment. The tissue was homogenized in 0·5 *M* glucose, 0·02 *M*