mately) emits middle C. As the temperature at the junction is increased the pitch is raised; but it has not been determined whether this is due to the temperature alone or to the gradual shrinking of the bulb, as the temperatures required are above that at which pyrex softens. To avoid this difficulty, it is planned to continue the investigation using tubes of quartz.

F. L. ROBESON
VIRGINIA POLYTECHNIC INSTITUTE

A SIMPLE MICROSCOPE EYEPIECE POINTER

THE use of an eyepiece pointer to augment the value of a demonstration under the microscope is usually appreciated by both student and instructor in the laboratory. The customary procedure of gluing a short hair to the rim of the ocular diaphragm is simple and effective. When, however, the eyepiece is in demand both with and without a pointer, the necessity of having to adjust the hair each time is highly inconvenient.

To meet the need for a pointer that could be readily inserted and removed from the ocular, the writer has devised the accessory here described.

A round 18-mm coverglass, free from imperfections, is selected and cleaned with acid alcohol. This forms a base upon which a pointer may be mounted. The pointer itself is drawn from a thin glass rod to a fiber-like thickness. With a little care and practice the glass can be drawn to a diameter appreciably less than that of even a fine human hair.

The tapered end of the pointer is then placed on the base, a drop of Canada balsam added followed by a second coverglass, likewise perfectly clean. By means of the protruding end of the pointer its tip may be centered and its axis adjusted parallel to the radius of the two coverglasses. In this way the fine rod is sealed, free from disturbance between the two coverglasses. After the protruding end of the pointer is snapped off the finished product results.

If actually embedded in the balsam the glass pointer appears highly refractory when viewed through the microscope. If this is objectionable, the pointer can be held in place by applying the cement only to the edges of the coverglasses. When so mounted it is seen as a black line.

A dozen or so of these pointers can be made and mounted in half an hour and they may then be kept permanently on hand for instant use when needed.

JAMES A. LOUNSBURY
DEPARTMENT OF BOTANY,
MARQUETTE UNIVERSITY

SPECIAL ARTICLES

OBSERVATIONS CONCERNING THE CAUSATIVE AGENT OF A CHICKEN TUMOR[1]

IN early publications on the chicken tumor group, some of the properties of the filterable agents causing these neoplasms were described. Recently additional observations have been reported from this laboratory which may be summarized as follows: The agent of Chicken Tumor I, a spindle-cell sarcoma, is selectively adsorbed and fixed by certain mesodermal tissues from susceptible animals, but not by similar tissues from non-susceptible animals. The plotted curve of the amount of ultraviolet light of selected wave lengths required to inactivate the tumor agent shows a significant qualitative and quantitative variation from the curves for bacteria, typical viruses and bacteriophage. The tumor producing activity of the tumor filtrates can be precipitated out with a protein fraction and somewhat purified.

Certain extensions of the work will now be recorded.

Steps in Purification of the Tumor Agent

Precipitation. As already reported, the agent active in a tumor filtrate can be precipitated out by electrodialysis or by increasing the hydrogen-ion concentration with acid or buffer. The pH at which the precipitate comes down is between 4.4 and 4.8. It carries all of the agent with it and can be dissolved in alkali and reprecipitated repeatedly without destruction of the agent.

The average amount of nitrogen in the precipitate is about 12 per cent. and varies little with the method of preparation of the extract. The phosphorus ranges from 0.16 per cent. to 0.69 per cent., being lower when the extract is prepared with water and higher when an alkali or Ringer's solution extract is used. Hydrolysis of the precipitate shows the constant presence of a considerable amount of reducing substance in all the active precipitates tested. The Feulgen reaction is positive, becoming more intense with each reprecipitation of the material. With the Mallory connective tissue stain the first precipitates give generally a maroon red, tending more to yellow red with the specimens showing a stronger Feulgen reaction.

Purification by Adsorption. Adsorption on colloidal aluminum hydroxide, a method already employed by other investigators, was utilized in attempts to purify the agent. The results were disappointing in that so little of the agent could be released after adsorption that inoculation produced at best tumors much smaller and less vigorous in their growth

[1] From the laboratories of the Rockefeller Institute for Medical Research.

than those resulting from injections of comparable amounts of the original extracts. The point of importance which developed from this study was that, after centrifuging out the aluminum hydroxide with its adsorbed materials, the supernatant fluids proved to be far more active than the full strength extracts. In fact, it is the most active material so far obtained, and this in spite of the fact that an appreciable quantity of the agent is taken down with the aluminum hydroxide.

When there has been a proper ratio of aluminum hydroxide (Willstätter Type C) to tumor extract, the supernatant fluid just mentioned is viscous, generally opalescent but often water clear. No precipitate is produced by acetic, tungstic, tannic or chloracetic acids. It gives negative Biuret, Millon and Xanthoproteic tests and only a slightly positive Ninhydrin reaction. Analysis shows an average nitrogen content of 0.050 mg per cc and a reducing substance figured as glucose of 0.175 mg per cc. The form in which the nitrogen occurs in this fluid is as yet undetermined, but the failure to induce sensitization in guinea pigs by the injection of large amounts suggests that it is either non-protein in nature or is a protein lacking antigenic properties.

Evidence exists that the viscosity of the supernatant fluids is due to the presence of a substance resembling chondroitin sulphuric acid. An attempt has been made to remove this substance by combining it with a basic protein. When the latter is precipitated out it takes with it all of the viscous material, leaving a water-clear, limpid fluid, which retains a tumor producing activity at least equal to that of the original supernatant fluid before removal of the viscous material, and far more so than the original concentrate. Chemical study of it is not yet complete.

Antigenic Properties of the Tumor Agent

The literature on the antigenic properties of the tumor producing agents will not be reviewed, since our study is concerned only in relation to the steps in the purification of the active principle. By the injection into rabbits of a concentrated Berkfeld filtrate of a water extract of the chicken tumor a good precipitating serum was obtained, which proved capable of neutralizing the tumor producing activity of a tumor extract. It was then found that the protein fraction of such a tumor filtrate, prepared as already described, was also effective in calling out precipitating and neutralizing antibodies in rabbits. A Berkfeld filtrate of an extract of tumor in Ringer's solution induced the formation of precipitins and neutralizing bodies, but the active protein fraction of this Ringer's extract failed to induce definite precipitins, though the neutralizing power of this serum was as good as that of the other sera. That the neutralizing power of these various sera was not referable to ordinary anti-chicken protein antibodies was shown by the failure of a strong anti-chicken serum of the rabbit to neutralize the activity of the tumor agent.[1]

After the development of the method of preparing highly active tumor extracts practically free of protein, the antigenic properties of such material were tested, the full strength concentrate being employed as control material. The sera of the rabbits injected with this latter contained precipitins and complement fixing antibodies, and they neutralize active tumor filtrates. The sera from rabbits immunized with the purified material showed no precipitins, no complement fixing antibodies, gave negative Ramon flocculation t ut were more strongly neutralizing to the act mor extracts then the sera developed against the full extract. The interpretation of this result must await further study.

Evidence of an Inhibiting Principle in the Chicken Tumor

The occasional occurrence of an inactive tumor filtrate or extract of dry tumor material has been noted by a number of workers. Several such inactive preparations were encountered in the course of our study of the acid precipitates. It was noted in these cases that the protein fraction gave a negative or faintly positive Feulgen reaction, and showed an excess of blue colored material with the Mallory stain. This observation suggested the possibility that the inactivity of an extract might be due to the presence of an inhibiting substance. An experiment was undertaken to test the possibility that this inhibitor might be more soluble than the active material. The dry powder of the chicken tumor was thoroughly extracted with water, centrifuged and the sediment extracted a second time with water. The second extract proved more active in the production of tumors than the first. The sediment of the second extract treated a third time with water yielded an extract even more active than the second. The result might mean simply that the active material was difficultly soluble, more of it going into solution after the long treatment with water. That this is not the correct interpretation is indicated by the observation that the residue inoculated after the first extraction was less active than the residue after the second washing, and this in turn less active than the residue after the third washing. In fact the residue reached maximum activity only after being extracted four times with water. In this connection the finding should again be mentioned that the fluid left

[1] These studies were carried out with the assistance of Dr. D. C. Hoffman.

after the adsorption with aluminum hydroxide as above described is markedly more active in tumor production than the most concentrated filtrate, although some of the tumor producing material is carried down with the aluminum. No other explanation seems possible than that both tumor producing principle and some substance or condition inhibiting its activity existed in the fluid prior to adsorption with aluminum hydroxide, the process removing far more of the inhibitor than of the principle. While there is less activity in the aluminum supernatant fluid than in the original extract, yet, unhampered by the inhibitor, it is more active.

The details of the experiments and a discussion of the results will be published later.

JAMES B. MURPHY
O. M. HELMER
ALBERT CLAUDE
ERNEST STURM

THE DEHYDROGENATION OF CHLOROPHYLL AND THE MECHANISM OF PHOTOSYNTHESIS

IN a recent paper,[1] it was shown that the allomerization of chlorophyll is essentially a dehydrogenation (oxidation) reaction. We have now been able to obtain additional evidence in favor of this view in a study of the dehydrogenation of the magnesium-free compound methyl phaeophorbide *a*. In a pyridine-acetone solution this compound is oxidized by potassium molybdicyanide; approximately two equivalents of reagent are required per mole. The product (methyl dehydrophaeophorbide *a*) yields the same hydrolysis products with hot alkali as allomerized phaeophorbide, and like this substance is not further oxidized by molybdicyanide. The difference between the spectra of methyl phaeophorbide *a* and methyl dehydrophaeophorbide *a* is slight in the visible range, but considerable in the near ultraviolet.

These facts, which prove that the chlorophyll molecule contains an easily dehydrogenated group, suggest at once a possible mechanism for photosynthesis. Emerson's recent work[2] has proved that chlorophyll is involved in the so-called Blackman dark reaction, and hence must be a participant in some strictly chemical step in the photochemical process. We suggest that this step is the reduction of carbon dioxide by chlorophyll itself, the other product being dehydrochlorophyll. In order to make the system chlorophyll-dehydrochlorophyll mobile, an enzyme would undoubtedly be necessary; this would account for the sensitivity of the Blackman reaction to hydrocyanic acid. The regeneration of chlorophyll would require energy furnished by the light. The steps can be represented thus:

Ch = chlorophyll
Ch(− 2H) = dehydrochlorophyll

(1) Dark reaction

$$12\ \mathrm{Ch} + 6\ \mathrm{CO_2} + \text{enzyme} \longrightarrow 12\ \mathrm{Ch(-2H)} + \underset{\text{glucose}}{\mathrm{C_6H_{12}O_6}} + 6\ \mathrm{H_2O}$$

(2) Light reaction

$$12\ \mathrm{Ch(-2H)} + \text{light} + 12\ \mathrm{H_2O} \longrightarrow 12\ \mathrm{Ch} + 6\ \mathrm{O_2}$$

This mechanism would appear to account for most of the facts now known about photosynthesis, including Warburg's experiments with a rotating sector. A calculation of the free energy of reduction of carbon dioxide (in the atmosphere) to glucose (in dilute solution) yields information in regard to the necessary reducing intensity of the chlorophyll-dehydrochlorophyll system if it is to function in reaction 1. A reducing intensity of 50 millivolts greater than the hydrogen electrode would be sufficient for reaction 1 to run very far towards completion. A reducing intensity equal to the hydrogen electrode would produce glucose in a thousandth molar solution, if the ratio of chlorophyll to dehydrochlorophyll were kept at about 100 to 1 in a steady state by a combination of reactions 1 and 2. Presumably the glucose or other primary reduction product of carbon dioxide is removed continually from the reaction by a series of irreversible processes. These calculations and a more detailed discussion will be published in full elsewhere.

J. B. CONANT
E. M. DIETZ
S. E. KAMERLING

CHEMICAL LABORATORY,
HARVARD UNIVERSITY

[1] Conant, Hyde, Moyer and Dietz, *J. Am. Chem. Soc.*, 53: 359, 1931.

[2] Robert Emerson, *Jour. Gen. Physiol.*, 12: 609, 623, 1929.

BOOKS RECEIVED

HOUSTOUN, R. A. *A Treatise on Light.* Seventh edition, revised. Pp. xi + 494. Two plates and 340 diagrams. Longsmans, Green. $4.00.

MACKIE, T. J., and J. E. MCCARTNEY. *An Introduction to Practical Bacteriology.* Third edition, revised. Pp. xv + 421. Illustrated. William Wood. $3.50.

MELLO-LEITÃO. *Aphantochilidas e Thomisidas do Brasil.* Pp. 359. 28 plates. Archivos do Museu Nacional, Rio de Janeiro.

RUTHERFORD, SIR ERNEST, JAMES CHADWICK and C. D. ELLIS. *Radiations from Radioactive Substances.* Pp. xi + 588. 12 plates. 138 figures. Macmillan. $6.50.

SLEVIN, JOSEPH R. *Log of the Schooner "Academy" on a Voyage of Scientific Research to the Galapagos Islands.* Pp. 162. 16 plates. California Academy of Sciences, San Francisco. $3.00.

SPEARMAN, C. *Creative Mind.* Pp. xii + 162. 16 plates and 6 figures. Appleton. $2.00.

STEPHENSON, J., Editor. *The Fauna of British India Including Ceylon and Burma.* Cestoda, by T. Southwell. Volume II. Pp. ix + 262. 355 figures. Taylor and Francis, London. 15/.

Tôhoku Imperial University. Science Reports. First series: Mathematics, Physics and Chemistry. Volume XIX. No. 6: Pp. 681–800 + 7. Illustrated. Maruzen Company, Tokyo.